



Comparative Antimicrobial Activities of Neem and *Aloe vera* Extracts against Various Strains of Bacteria and Fungi

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ABSTRACT

This research was carried out in order to identify medicinal plants that are effective against a variety of human pathogens. The study's intension was to test the antimicrobial activity of methanolic extracts of dried neem leaves and fresh gel of *Aloe vera* against various strains of bacteria and fungi such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus brasiliensis* and *Candida albicans*. The extract should be taken in a weight-to-volume ratio of 1:10 (weight/volume). Prepare different concentrations of neem and aloe vera leaf extracts to determine the inhibitory concentration (MIC) of the previously stated bacterial strains.

Keywords: Neem, *Aloe vera*, antimicrobial activity, effectiveness of natural herbs.

INTRODUCTION

Neem (*Azadirachta indica*) and Aloe Vera both are medicinal plants which has been used by ancient times [1]. In addition to Neem and Aloe vera, there are several plants known as Amla, Assam tea, and clove which are used as medicinal plants. [2, 3] Pharmacological studies have recognised the importance of medicinal plants as potential sources of bioactive compounds [1]. The rapid emergence of multidrug resistant pathogens continues to threaten the clinical effectiveness of many existing antibiotics [4, 5]. Illnesses such as this are known to be hard to treat. Throughout human history, herbal or home remedies have been used to treat such diseases [6, 7]. Natural products like these, whether pure, mixed, or formulated with measured constituents of plant extracts, offer limitless opportunities for the development of new drug leads [8]. There is a need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [3,9,10]. The purpose of this study was to identify traditional plants that are effective against human pathogens. In this study, we report on two traditional Indian medicinal plants, 1) *Azadirachta indica* and 2) *Aloe Vera*, and also a



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combination of their methanol extract, to determine their effectiveness in controlling such human pathogens [2,3]. Medicinal plants are a rich source of novel drugs used in traditional medicine, modern medicine, nutraceuticals, food supplements, and modern drugs [11]. Neem is a well-known traditional medicinal plant in India and neighbouring countries for its own versatile role in medical science and broad spectrum of biological activity [12,13,14]. Neem has been extensively used in Ayurveda, Unani, Homoeopathic, and Siddha medicine, as well as skin and hair treatment methods, and has become a modern medicine cynosure [15]. Aloe Vera is another herbal plant for whom the leaves supply water to the plant, designed to allow it to survive long periods of drought. Aloe Vera gel is used for a variety of purposes such as skin care, thermal burn remedy, sunburn relaxation, and wound healing [16]. It also has strong antifungal activity and helps in the activation of the body's immune system [17]. Natural medicinal nontoxic plants are more bioactive than chemicals [18]. Antimicrobial activity of neem leaves extracts against microorganisms such as bacterial strains (*Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp., and *E. coli*) have been reported [19]. The rate of skin infections caused by bacterial strains/organisms is expanding on a routine basis. This has become a significant health problem in both developing and underdeveloped countries [20], and one method to prevent antibiotic resistance in pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents. More than 135 compounds have been isolated from different parts of neem, and its leaves have proved remarkable antimicrobial and fungal activity against a variety of bacterial and fungal strains [21,22]. Synonyms for Aloe Vera Although Aloe barbadensis, also known as Barbados or curacao aloe, is native to northern Africa, it cures a variety of diseases. [22]. The plant possesses over 75 potentially active constituents [23]. The aim of this study is to evaluate the antimicrobial properties of dried fresh neem leaves and aloe Vera gel, and combos of both at various levels, using methanolic extract of both plants.

MATERIALS AND METHODS

MATERIAL

Test organisms: strains of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus brasiliensis* and *Candida albicans*. Stored at -70 °C.

Collection of plant material

Plant sample was collected in November 2021 from a local market on Waghodia Road in Baroda, Gujarat State, India, in which fresh leaves of the plant material *Azadirachta indica* and a sample of fully expanded leaves of aloe vera were purchased. Mrs. Anupama Srivastava of the Parul Institute of Applied Sciences, Microbiology, identified and authenticated the plants.

METHODS

Preparation of aloe vera gel and *Azadirachta indica* leaf extracts

Azadirachta indica (Neem) leaves were collected, washed with distilled water, and dried for 3 to 4 days in the sunlight. They were then powdered further with a sterile mortar and pestle and stored in an airtight container. The plant's fully expanded *Aloe vera* leaves were selected and washed with distilled water. The yellowish liquid is removed from plants and then cut into thin slices with a knife. The small pieces have been dried individually in the sunlight for 96 hours before being finely powdered with the help of a blender. To prevent contamination, powdered is placed in an airtight container. The extraction method was adapted from Athiban et al. (2012), with the following modifications: The dried powder of neem extract was dissolved in methanol at a weight/volume ratio of 1:10. The same method applied for aloe vera (1:10) ratio. To make Neem+ Aloe Vera extract, both powder samples are dissolved in methanol in a 1:1:20 (weight/volume) ratio. For 24 hours, a sample was incubated at 37°C in an incubator with 120-rpm shaking. After 24 hours, the mixture was evaporated at a low temperature in an oven heated to 40–45 °C, and the residue was treated with 10, 10, and 20 ml of methanol, respectively, and incubated as mentioned earlier. It was repeated three times. Again, it was dried at a low temperature in an oven prior to being reconstituted in 5 ml of absolute methanol, packed in separate sterile glass vials, and stored at 4 °C until use [24].



**Nidhi rahul kunjara and Anupama Shrivastava****Investigation of antimicrobial activity**

Antimicrobial activity was evaluated using the agar well diffusion technique, which was adapted from Athiban et al., 2012 with the following modifications. MHA (Mueller Hinton Agar) was used to culture the strains rather than nutrient broth and thioglycolate, and the radii of opaque zones were measured (in mm).

RESULT AND DISCUSSION

In search of an alternative to chemical decontaminants, this experiment appears to test the potential of medicinal plant extracts. Because of their availability, low cost, and lack of known side effects, natural extracts can be a great substitute for diseases and as an effective disinfectant. These natural herbs are inexpensive and readily available, so their use can have a significant influence on society while also helping to minimize soil and air pollution. We can say that they are more secure to utilize than chemical or synthetic one. In this study, we discovered two plants, *Azadirachta indica* (neem) and *Aloe vera* (Aloe), as well as a combination of the two, that are effective against all of the target pathogens, including *E. coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus brasiliensis* and *Candida albicans*. In vitro antimicrobial activity of methanolic extracts of neem leaves, aloe leaves and a combination of both leaves was demonstrated (table 1) against selected gramme-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and gram negative (*Escherichia coli*) bacteria and also against two fungal strains (*Aspergillus brasiliensis* and *Candida albicans*).

The zone of inhibition (in mm) was measured to evaluate the antimicrobial efficacy of neem, aloe and neem+ aloe (Table 1). All these three extracts were proved to be an effective against both *A. brasiliensis* and *E. coli* (Fig.1 & 2). Neem extracts exhibited highest zone of inhibition (23.93 mm) against *E. coli* followed by neem+ aloe vera (21.63 mm) and aloe vera (19.44 mm) respectively. On the other hand, both neem and aloe vera showed equal radius of opaque zones (22.5 mm) followed by neem+ aloe vera (19.3 mm) against *S. aureus* (table 1). This result agrees with the current literature. Methanolic neem extracts demonstrated inhibition of methicillin resistant *S. aureus*, pathogenic *E. coli*, *C. albicans*, *A. brasiliensis*, *B. subtilis* [24,25,26]. According to Habila et al., (2012) [27], crude extracts of neem were effective against *E. coli*, *Salmonella* and *Staphylococcus aureus* In vitro. Ethanolic extracts of aloe vera was effective against methicillin resistant *E. coli*, *E. faecalis* and *Staphylococcus aureus* [24]. *A. brasiliensis* using methanolic extracts Against *E. coli* using agar well of neem and neem + aloe vera. Diffusion method.

The minimum inhibitory concentration levels for each of the microbes mentioned were determined and measured. *A. brasiliensis* showed highest zone of inhibition followed by neem+ aloe (23.3) mentioned in (table 1). One of the most interesting things about neem+ aloe concentration is that it is effective against bacterial (*E. coli*) as well as fungal stains (*Candida* and *Aspergillus* spp.). An agar well diffusion method was carried out using 100 mm petri plates. 0.1 mL of methanolic extracts were added to petri plates through the use of cylinders to test the antimicrobial activities of natural herbs.

CONCLUSION

In conclusion, methanolic extracts of *Neem*, *Aloe vera*, and *Neem+ Aloe Vera* are effective against all tested human pathogens, *S. aureus* and *E. coli*, in controlling their growth in vitro in culture conditions. Bioactive components are also abundant in *neem* and *Aloe vera*, which, as previously stated, are stable over a wide temperature and pH range. These natural herbs, *neem* and *aloe*, help in reducing the contamination of microorganisms which are harmful to the human body. Therefore, these natural extracts can be used for making an effective antibiotic as well as preparing a strong and harmless disinfectant. Further research may be carried out to purify these bioactive components and put them to use as disinfectants. As a result, by incorporating them into an industry, it will be able to generate a good and healthy market.





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Table 1: Result of zones of inhibition (mm) of methanolic extract of neem, aloe vera and neem+ Aloe vera.

organisms	Concentration of neem, aloe & neem+ aloe extracts in (mm).		
	Neem	<i>Aloe vera</i>	Neem+ Aloe
<i>B. subtilis</i>	21.1	19.5	14.64
<i>E. coli</i>	23.93	19.44	21.63
<i>S. aureus</i>	22.5	22.5	19.3
<i>C. albicans</i>	20.1	23.4	22.68
<i>A. brasiliensis</i>	19.6	20.5	23.3



Fig. 1: Antimicrobial Activity Shown



Fig. 2: Antimicrobial Efficacy Test





A Survey on Open Source Tools for Big Data

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ABSTRACT

The age of big data is now coming. But the traditional data analytics may not be able to handle such large quantities of data. The question that arises now is how to develop a high performance platform to efficiently analyze big data and how to design an appropriate mining algorithm to find the useful things from big data. To deeply discuss this issue, this paper begins with a brief introduction to data analytics, followed by the discussions of big data analytics. Some important open issues and further research directions will also be presented for the next step of big data analytics.

Keywords: Big data, Hadoop, Spark, Flink, Hive

INTRODUCTION

As the value of data storage has gone down and superior computers have become a lot of widely accessible, we tend to have seen growth of machine learning (ML) into a host of industries together with finance, law enforcement, entertainment, commerce, and health care. As theoretical analysis is leveraged into practical tasks, machine learning tools are more and more seen as not simply helpful, however integral to several business operations. The goal of machine learning is to alter a system to learn from the past or present and use that data to create predictions or choices relating to unknown future events. Within the most general terms, the advancement for a supervised machine learning task consists of 3 phases: build the model, measure and tune the model, so place the model into production. An example of this progress is in. At the center of machine learning is that the data that powers the models, and therefore the new era of big data is catapulting machine learning to the forefront of research and industry applications. Which means of the term “big data” is still the subject of some disagreement; however it usually refers to data that’s too massive or too complicated to process on one machine. We tend to live in an age wherever data is growing orders of magnitude quicker than ever before. Consistent with International data Corporation’s annual Digital Universe study, the amount of data on our planet is ready to achieve forty four zeta bytes (4.4×10^{22} bytes) by 2020 that would be 10 times larger than it had been in



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2013. Whereas no single entity is working with data at this magnitude, several industries are still generating data large to be processed with efficiency using ancient techniques. Ancestry.com, for instance, stores numerous records totaling regarding ten peta bytes of data. With such a rate in data production, the challenge that we faced here is by the machine learning community is the way to best with efficiency method and learn from big data. Some of the most common machine learning toolkits like R or weka weren't designed for these sorts of workloads. Though weka has distributed implementations of some algorithms on the market, it is not on an equivalent level as tools that were at first designed and designed for terabyte-scale. Hadoop, a preferred frame work for operating with big data, helps to resolve this scalability down side by giving distributed storage and process solutions.

What is Big data

Before discussing about Big data, we should first know what is data. Data is nothing but the characters, symbols, operator which the user gives input to the computer and the computer process upon and them which may be stored and transmitted in the form of electrical signal and recorded on any electrical media. Big data is also a type of data which is very huge in size and growing exponentially over time. The amount of data generated is so huge that no traditional data management tools are able to process the data. There are 3 types of variation in the types of big data that is structured data which is nothing but the working of database. The data which needs to be stored in a fixed format but the unstructured data is the data that hugely generated now a day simply web browsing experience or facebook data, simple text files, images, videos etc. Semi structured data contain both the forms of data the set types of data is represented using an xml file.

Volume is that the most evident of the 3, relating the scale of the data. The large volumes of data that we tend to are presently managing have needed scientists to rethink storage and process paradigms in order to develop the tools required to properly analyze it. Velocity addresses the speed at that data may be received further as analyzed. Within the "Data processing engines" section, we tend to discuss the differences between batch processing, which works on historical information, and stream processing, that analyzes the data in time period because it is generated. This additionally refers to the rate of change of information that is very relevant within the area of stream process. Variety refers to the problem of disparate and incompatible data formats. Data can come in from many alternative sources and take on many alternative forms, and simply getting ready it for analysis takes a major quantity of your time and energy. Big data collections are aggregates of multiple data sets that are singly manageable, however as a bunch are large to fit on disk. The data sets in these collections typically come from completely different sources, are in disparate formats, and are hold on in separate physical sites and in several sorts of repositories. Big information objects are individual datasets that by themselves are too massive to be processed by normal algorithms on obtainable hardware. Not like collections, they typically return from one source. Today, the matter of big data collections is commonly solved through distributed storage systems that are designed to rigorously management access and management in a fault-tolerant manner. One answer for the down side of big data objects in machine learning is through parallelization of algorithms. This is often usually accomplished in one in every of 2 ways that data similarity, within which the data is split into additional manageable item send every set is computed at the same time, or task similarity, within which the algorithm is split into steps that may be performed at the same time. It is not uncommon to encounter big collections of big objects as information grows and becomes more widely obtainable. This, as well as unprecedented access to computing power through more cost-effective high performance machines further as cloud services, is gap up several new opportunities for machine learning analysis. Several of those new directions utilize progressively complicated workflows that need systems designed mistreatment a combination of state-of-the art tools and techniques.

Hadoop Eco System

Many individuals contemplate the terms Hadoop and Map Reduce to be interchangeable, however this can be not entirely correct. Hadoop was initially introduced in 2007 as an open source implementation of the Map Reduce process engine coupled with a distributed file system, however it has since evolved into an immense web of projects connected to each step of a huge data work flow, together with data assortment, storage, processing, and



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abundant a lot of. The amount of projects that have been developed to either complement or replace these original parts has created this definition of Hadoop unclear. For this reason, we frequently hear relevancy the Hadoop scheme instead, that encompasses these connected projects and merchandise. To absolutely perceive Hadoop, one should look at each the project itself and also the scheme that surrounds it.

Apachespark

Spark that was initially developed at the University of California, Berkeley and is currently an Apache top-ranking project, is predicated on Map Reduce however addresses variety of the deficiencies represented higher than. Like Hadoop, it supports unvarying computation and it improves on speed and resource problems by utilizing in memory computation. Spark's approach to process has seen widespread adoption in each analysis and industry. The most abstractions used in this project are known as Resilient Distributed Datasets (RDD) that store data in-memory and supply fault tolerance while not replication. RDDs is understood as read-only distributed shared memory. This model, streamlines the educational method through in-memory caching of intermediate results, considerably lowering on the quantity of read and write operations necessary.

Apachestrom

Storm is used for process data in real-time and was at first planned to over-come deficiencies of different processors in assembling and analyzing social media streams. Development on Storm began at BackType, a social media analytics company and continued at Twitter once a 2011 acquisition. The project was open sourced and have become an Apache superior project in September 2014. The machine learning community has been inserting growing importance on data processing, and as a result, Storm is seeing increased adoption each in production and in analysis environments. The Storm design consists of spouts and bolts. A spout is that the input stream (e.g. Twitter streaming API), whereas bolts contain most of the computation logic, process data within the variety of tuples from either the spout or different bolts. Networks of spouts and bolts, that are depicted as directed graphs, are famous as topologies. The project is primarily enforced in Clojure, however at first used Java for all APIs to encourage a lot of widespread adoption. It currently includes Thrift, a frame work for cross-language development. That permits topologies to be outlined and submitted victimization any programming language. Storm uses real-time streaming, however conjointly offers micro batch via its trident API. Fault tolerance is achieved by method of the topology: Spouts can keep messages in their output queues till the bolts acknowledge them. Messages can still be sent out till they are acknowledged, at that time they can be born out of the queue. A master node, famous as Nimbus as a result of it runs the Nimbus daemon, tracks the heart-beats of employee nodes. If an employee node dies, then Nimbus can transfer the employees to another node. Nimbus conjointly handles the responsibility of assignment tasks to employees, similar to job tracker in Map Reduce. The biggest distinction is if the job tracker dies, all running jobs are lost, however if Nimbus dies, it's automatically restarted. Storm was designed as a complete system independent from Hadoop, however since Hadoop affected to YARN, work has been done to integrate the two comes.

Apache Flink

Flink was developed at the Technical University of Berlin beneath the name Stratosphere. It graduated the Apache incubation stage in January 2015 and is currently a commanding project. It offers capability for each batch and stream process, therefore permitting the implementation of a Lambda design as delineated on top of. It is a scalable, in-memory choice that has APIs for each Java and Scala. It's its own runtime, instead of being designed on high of Map Reduce. As such, it may be integrated with HDFS and YARN, or run utterly independent from the Hadoop system. Flink's process model applies transformations to parallel data collections. Such transformations generalize map and reduce functions, as well as functions such as join, group, and iterate. Also included is a cost-based optimizer that automatically selects the best execution strategy for every job. Flink is additionally totally compatible with Map Reduce that means it will run legacy code with no modification. Like Spark, Flink additionally offers repetitive batch still as streaming choices, although their streaming API is predicated on individual events, instead of the micro-batch approach that Spark uses. This is often identical model that Storm uses for true real-time operation. Connectors are offered that enable for process data streams from writer,



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RabbitMQ (a platform-independent electronic messaging system), Flume, Twitter, and user-defined data sources. The project continues to be in its infancy however machine learning tools are in development. Flink-ML, a machine learning library, was introduced in April 2015.

H2O

H2O is an open source framework that provides a parallel process engine, analytics, math, and machine learning libraries, on with data preprocessing and evaluation tools. To boot, it offers a web-based programme, creating learning tasks a lot of accessible to analysts and statisticians who could not have sturdy programming backgrounds. For those who would like to tweak the implementations, it offers support for Java, R, Python, and Scala. Additionally to its native process engine. They need additionally released a project known as sparkling water that integrates Spark and Spark Streaming into their platform. This is often solely supported in version 3.0. Further efforts are created towards integration with Storm for real-time streaming. H2O's engine processes data fully in-memory victimization multiple execution strategies, counting on what's best for the algorithm used. The overall approach used is Distributed Fork/Join, a divide-and-conquer technique that is reliable and appropriate for massively parallel tasks. This is a methodology that breaks up a job into smaller jobs that run in parallel, leading to dynamic fine-grain load equalization for Map Reduce jobs as well as graphs and streams. They claim to be the quickest execution engine, however as of the time of this writing, no educational studies are published that verify or refute these claims and any analysis is required in this area.

HIVE

Hive efficiently stores huge amounts of data in a given storage space delivering high throughput and fast response time with low latency. Hive is an ecosystem built on top of Hadoop, which supports SQL-like queries and is compiled into Map Reduce jobs executed on Hadoop. Hive supports rich data types to enhance data processing, e.g., Map, Array and Struct, apart from basic data types. Conversely, LOAD statements take less time in Hive. This is because Hive does not verify the data when it is loaded, but rather when a query is issued. This is called schema on read. Hive allows one to query data sets of large sizes (multi-terabyte). The query planner runs the query in parallel across multiple nodes using map/reduce. Hive provides partitioned tables, which allow it to scan a partition of a table rather than the whole table if that is appropriate for the query it is executing. Hive is well-suited for offline reporting, transformation, and analysis of large data sets.

CONCLUSION

In this paper, we reviewed studies on the data analytics from the traditional data analysis to the recent big data analysis. From the system perspective, the KDD process issued as the framework for these studies and is summarized into three parts: input, analysis, and output. From the perspective of big data analytics frame work and platform, the discussions are focused on the performance-oriented and results-oriented issues. From the perspective of data mining problem, this paper gives a brief introduction to the data and big data mining algorithms which consist of clustering, classification, and frequent patterns mining technologies.

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Table: Comparison of Data Analysis Tools

Tool	Characteristics of the tool		
	Processing model	Language Support	Latency
Apache Spark	Mini/micro batches, steaming	Scala, Java, Python	Seconds
Apache Storm	A record at a time	Any	Milli-seconds
Apache Flink	Batch and stream processing	Scala, Java, Python	Seconds
Hive	Streaming	SQL-like	High
H20	Batch processing	Java, Python, R, Scala	Low





The Genetic study, Mapping and Molecular Profiling of Maize Genome (*Zea mays* L.)

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ABSTRACT

Among plant genomes, maize has the most extensively studied genome. Maize has been at the forefront of the development and evaluation of a wide range of molecular markers. The use of molecular markers in the investigation of genetic diversity and differentiation in maize germplasm, especially in tropical and subtropical maize production systems will help the plant breeder to develop new germplasm. Some DNA-based markers are employed for mapping in India and MAS for significant abiotic/biotic factors affecting productivity. The genetic markers of maize genotypes lines established by several public sector institutes in India are presented in this article. 1. The use of markers to examine genetic diversity in India maize genotype, 2. Using QTL mapping, determine the phenotypic and genotypic diversity of traits associated to maize heat tolerance seedlings, as well as their genetic architecture. Some pressures have an impact on maize's agronomic and qualitative attributes, resulting in decreased productivity. It understands genetic complexity features to boost maize productivity in India, which contributes 2% of total world production in 2020-2021. DNA markers are used in traditional linked mapping methods. The advantages and drawbacks of genomic mapping have been highlighted. Finally, we look at the current and future influence of genomic mapping on maize crops, as well as the implications for poor people in developing countries who rely on maize crops for their livelihoods and food security.

Keywords: Crop improvement, Sustainable production, Markers, Genome Mapping, QTLs, etc.





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INTRODUCTION

Maize occupies a prominent position in the global food sector and agricultural output. Developing countries, such as the United States, are more interested in maize production (FAOSTAT, 2020) Maize demand is expected to outpace wheat and rice demand in emerging countries by 2020 (USDA, 2020). However, maize production in a number of developing countries remains low. Maize is grown on about 45 million hectares in the lowland tropics, where productivity is rather high. Severely a number of environmental stresses like biotic and abiotic constraints have inhibited their progress. So, in India, traditional breeding practices have led in significant advancements in maize development and its productivity has a lot of opportunity for improvement (Karp et al., 2018 and Liu, 2020). Using contemporary molecular techniques and procedures, breeders will be able to successfully address important research areas.

The phrase "molecular breeding" is becoming more extensively used to describe the use of molecular (DNA-based) tools like markers to improve the effectiveness of the breeding process. Plant breeding programs could benefit from DNA markers in a variety of ways, including identifying elite genetic stocks, analyzing genetic diversity, and boosting the efficiency of tough trait selection. RFLPs, RAPDs, SSRs and AFLPs are some of the most common DNA-based markers used by plant scientists (Powell et al., 2016a,b). There are excellent studies available to discuss the genetic basis of a variety of DNA-based markers, as well as the methods for detecting molecular polymorphism and the benefits and drawbacks of using these markers for various reasons. The target crop, its breeding behaviour, the experiment's specific aims, the resolution required, and any operational/financial constraints will all influence the optimal marker(s) for a given application.

Different marker systems are compared in terms of their properties and applications. Mogg et al., (2010) studied genetic linkage map can be created using any type of molecular marker. In F2 and backcross generations, codominant markers such as RFLPs, SSRs, and SNPs provide more genetic information than markers that detect mainly presence/absence or dominant polymorphisms, such as RAPDs or AFLPs (Table 1). The use of RFLP as anchor loci is appropriate for comparison mapping within and across crop species because they detect evolutionarily conserved loci in a more predictable manner than hypervariable SSRs and AFLPs. For varied purposes, PCR-based DNA markers are available in a variety of formats (Powell et al., 2011a, b). SSR markers are most commonly used because of their low cost, ease of use, and efficacy, in maize breeding.

SSR markers are abundant, consistently distributed, and codominant in maize genomes, with over 1000 mapped SSR markers available in the public domain. Sequencing by synthesis method (SBS), revealed that an SNP could be discovered at every 40 bp (Mogg et al., 2010). Apart from, maize microsatellites markers' its genome also covers up to 62 million SNPs, based on the size of its genome, which is estimated to be 2.5×10^9 bp (Edwards & Mogg, 2015). While both SSRs and SNPs may be reliably employed on a large scale with only a little amount of DNA required for PCR amplification, SNPs are especially amenable to automation, and so offer significant benefits for plant breeding. SSRs are the ideal alternative when codominant, multiallelic information is required, or when infrastructure and resources are constrained.

Genetic markers a part of ScreeningMaize genome

Researchers working on crop improvement are increasingly using molecular markers as an effective and acceptable tool for addressing a variety of basic and practical research areas pertinent to agricultural production systems (Mohan et al., 2011 and Prioul et al., 2018). In order to effectively maintain germplasm collections, DNA fingerprinting and genetic diversity analyses utilizing molecular markers are quite useful. In addition, a growing emphasis is being made on comprehensive genetic diversity studies in breeding materials for main crops (Warburton & Hoisington, 2012).





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The use of molecular markers to accurately determine the levels and patterns of genetic variation is especially useful in maize breeding; (1). preserving and expanding the elite germplasm's genetic base (2). Lines are assigned to heterotic groupings. (3). choosing the best parental lines for a hybrid combination (4). production of segregating progenies with the greatest genetic variability for subsequent selection. For quick mapping certain areas of the genome, special mapping procedures can be applied.

The use of near isogenic lines is one such method. Throughout the years, back-crossing has been employed by breeders to transfer desirable features from wild relatives to farmed crop plants (Mohammadi & Prasanna, 2012). In each back cross programme, it generates a pair of NILs as follows: the back cross programme recurrent parent in one line, and the back cross programme results, which differs from the recurrent parent solely in the region of its genome that surrounds the gene transmitted by the back-cross. The genome which is highly polymorphic at the level of DNA sequence. It gives a gene that can be used as a target (Devos & Gale, 2012). Typical linkage mapping, on the other hand, would necessitate testing each marker with a complete mapping population to see if it was mapping towards the target gene or not. Individuals homozygous for the gene of interest may also be segregating generation, i.e., F₂, and their genomic DNA could be collected and analyzed for markers close to the gene of interest. When used in conjunction with RAPD markers, it is possible to quickly detect a high number of DNA markers in a given location. As a result, any genomic area having DNA or protein markers that has been mapped can be quickly targeted with new markers. This could come in handy when trying to fill in gaps on a genetic map.

Molecular Profiling of Maize germplasm

Maize breeders in India, like those in most developing countries, have distinguished inbred lines based on major morphological characteristics such as plant height, anthocyanin coloration of various plant parts, tassel type, tassel branching, days to flowering, ear characters, cob coloration, grain colour, and grain types (Virk & Witcombe, 2015). Despite the importance of morphological descriptions in determining the agronomic value of germplasm, they are not very trustworthy due to complicated 'genotype x environment' interactions that necessitate testing in many locations/environments (Smith & Smith, 2010). Methods that exclusively rely on morphological data are not consistent nor successful in unambiguously differentiating elite breeding materials, according to extensive investigations in many crop species, particularly maize. Environmental factors, such as genetic heterogeneity (various combinations of alleles produce similar phenotypes), and genetic heterogeneity (different combinations of alleles produce similar phenotypes) Isozyme and zein chromatographic data have previously been utilized to characterize elite inbred lines and commercial maize hybrids.

In comparison to molecular marker analysis, isozyme analysis is relatively simple and inexpensive; however, insufficient genomic coverage, low levels of polymorphism, developmental regulation, and pleiotropic effects impose major constraints on effectively using isozyme markers in genotype differentiation and genetic diversity analysis. In the recent past. SSR markers based on PCR have been found to be effective. They're employed to distinguish between US and European maize germplasm since they're well-suited to genotyping and discrimination (Stuber & Goodman, 2010; Smith, 2012, Smith & Smith, 2010, 2011; Bar-Hen et al., 2015). In India, no systematic efforts to efficiently employ molecular markers for genetic fingerprinting or characterization of genetic diversity in maize inbred lines established by public sector institutions, including those typically utilized for hybrid maize breeding, have been made (Stuber & Goodman, 2013; Smith, 2015). However, studies have recently been conducted to profile a selected set of Indian maize genotypes, including inbred lines and single-cross hybrids, using morphological and microsatellite markers, as well as to analyses the genetic diversity in maize inbred lines commonly used in public sector institutions.

In 47 Indian inbred lines, observations were made on 20 'categorical descriptors' (qualitative or visually assessed quantitative features) to determine their efficacy in successful genotype discrimination (Smith & Smith, 2010; Dubreuil et al., 2018). The 'categorical' descriptors, on the other hand, revealed relatively little polymorphism in the Indian lines studied, with only 55 variants in total, underlining the serious limits of relying solely on morphological data to determine genotype identity or distinctness (Pushpavalli et al., 2011 and Mohammadi et al., 2012a).





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Molecular profiling of 69 inbred lines, including 58 Indian lines, 6 CIMMYT lines generated in Mexico (used as 'reference genotypes,' and 5 lines from the CIMMYT-Asian Regional Maize Program, was carried out. (CIMMYT-ARMP), Thailand, discovered high levels of polymorphism utilizing 58 polymorphic SSR markers (435 alleles).

The discovery of 109 unique/rare SSR alleles (present in more than 1 or 2 of the genotypes studied) aided in successful genotype discrimination. The average PIC value revealed the high level of polymorphism demonstrated by the SSR loci (0.70). SSR markers like dupssr17, bntg1647, and bntg198 could be utilized to distinguish Indian maize inbred lines since they have high PIC values (>0.75) and discrete allelic size ranges. Smith et al (2011) elaborated multiplexing for improved assay efficiency would be facilitated by distinct and non-overlapping size ranges of the amplification products of SSR loci with high PIC. They proposed a sequential method combining marker information and agro-morphological description, which includes: (i) comparing two lines at marker loci and declaring them distinct only if their genetic similarity (GS) value is below a predetermined threshold; and (ii) comparing two lines for agro-morphological traits only if their GS value is above this threshold. In the second phase, environmental variation for morphological features allows statistical tests to be built to establish the 'minimum distance' between the two inbreds, which is important in the context of plant variety protection. It would be fascinating to see if this suggestion for routine profiling of elite breeding materials has broader relevance and effectiveness.

Fingerprinting has been expanded to the classification of genetically varied materials such as landraces, populations, open pollinated varieties, and germplasm accessions (Fig.1), thanks to the availability of high throughput technologies that can utilize fluorescent-labeled SSR markers through multiplexing. Because the expense and time required to define each line tends to be the limiting factor, previous studies on population characterization focused on only a few people per community. Utilizing a bulking technique for people in a certain population, followed by examination of the bulks using multiplexed SSR primers and semi-automated DNA sequencing technologies, can improve the efficiency and accuracy of population fingerprinting

Molecular markers were used to examine genetic diversity in Indian maize germplasm.

Pedigree information provides a broad approximation of predicted genetic relatedness among lines in allogamous crops like maize, but it's often unavailable or incorrect, especially when inbred lines are generated from a large base population. SSRs and 89 AFLPs, for example, are DNA-based markers that have given valuable tools for assessing genetic variability (Melchinger et al., 2011; Messmer et al., 2013). A group of 23 QPM lines, including 13 inbreds generated by the national program and ten selected tropical/subtropical QPM lines developed by CIMMYT, were investigated for grain quality, agronomic performance, and molecular polymorphism using SSR markers in another recent work at IARI. The QPM inbred lines were effectively differentiated using polymorphic profiles for 36 SSR loci (Pejic et al., 2010 and Vuylsteke et al., 2018).

The investigation identified SSR markers with high polymorphism information richness in the selected QPM genotypes, such as bnlg439, phi037, bnlg125, dupssr34, and bnlgi05. The bulk of the Indians had high levels of heterozygosity, according to SSR markers' lines, as well as CML188, a CIMMYT QPM inbred. (Kassahun & Prasanna, 2012). The Indian QPM inbreds were clearly separated from those generated at CIMMYT utilizing cluster analysis employing SSR data, followed by canonical discriminant analysis. The cluster patterns were mostly consistent with the QPM inbreds study's available pedigree information.

The study confirmed the utility of SSR markers in analyzing genetic links among QPM lines, and it will benefit in the planned use of CIMMYT QPM lines in India's QPM breeding programs (Stuber et al., 2011). One of the potential applications of inbred line molecular marker data is to identify parents useful for breeding. Single-cross hybrid performance is being developed or improved. Although SSRs are unlikely to directly alter the phenotypic expression of the targeted quantitative trait(s), they can be used to identify nearby (linked) genomic sequences. In this situation, inbred line marker divergence can be used to forecast hybrid performance.





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This is especially important in crops like maize, where a lot of time and money is spent in field testing of novel lines in various single-cross combinations to find lines with superior combining potential. A number of experiments in maize have been conducted to determine the relationship between molecular marker divergence and hybrid performance (Mohammadi et al., 2012b), resulting in various outcomes. The majority of research, on the other hand, suggest that genotypic differences may be useful for early identification of loci/alleles for possible hybrid enhancement, but that they are unlikely to accurately reflect hybrid performance.

Mapping QTLs in Maize

The discovery of extensive visualization, variability at the DNA level are combined with the development of statistical packages that can aid in analysing variation in a quantitative trait in congruence with molecular marker data in a segregating population, this led to the mapping of QTL influencing a wide range of agronomically important traits in a variety of crop plants, including maize (Young, 2010 and Ribaut et al, 2012a,b). A quantitative trait locus (QTL) is a region of the genome linked to a quantitative trait (Korte and Farlow, 2013 and Alqudah et al., 2020).

A QTL, in theory, can be a single gene or a group of closely connected genes that affect the trait. There are several excellent reviews available that cover various elements of QTL mapping in crop plants (Xiao et al. 2017). MAS in backcross, pedigree, and population improvement programs can be enabled by QTL mapping and the identification of molecular markers closely connected to QTL with large impacts on a target trait. This is particularly effective for crop traits that are difficult or impossible to select for using traditional methods. Molecular markers have been used to find and define QTL linked to a variety of maize properties, including grain yield, domesticated features, environmental adaptation, disease and insect pest resistance, and drought and heat stress tolerance (Stuber, 1995; Stuber et al, 1999).

The Maize DB (<http://z.agron.missouri.edu>) contains extensive information regarding such experiments. Below is a case study that could be useful in the proper treatment of downy mildew diseases in maize in tropical Asian nations.

Exploitation of Marker Assisted Selection in Maize breeding programme: A way forward to step up genome profile

The goal of MAS is to find connections between markers and alleles of a gene or QTL of interest, then use those associations to produce better lines or populations (White, 2012). Individuals can be backcrossed using marker-assisted backcrossing until they possess the genomic fragment in the recipient's or recurrent parent's genetic background. Recombination between the marker and the gene/QTL must be limited for MAS to be successful. This is accomplished by using flanking markers that are closely related. The main goal of marker-assisted backcrossing is to speed up line conversion and lower the transferred gene's linkage drag (s), (Raymundo, 2010). To recover 99 percent of the recurrent parent genome using conventional backcross (BC) breeding, it would take six BC generations to convey a single dominant gene. For crops like maize, where new lines and hybrids are introduced often, this technique is time-consuming and labour-intensive. The proportion of the recurrent parent genome in a BCI generation would normally be distributed around a mean of 75 percent (the distribution would become increasingly skewed in later BC generations), but given a large enough sample size, it would contain plants with more than 85 percent recurrent parent genome.

To speed up the breeding process, molecular markers can be used to identify these plants (George et al., 2012). It is very impossible to eliminate the linkage drag that comes as "baggage" with the introgressed segment without molecular markers bordering the target gene (Murray et al, 2010). MAS is now widely used in maize breeding programs for (1) locating beneficial alleles in the genomic background of genotypes of interest and (2) identifying individual plants in large segregating populations that have the favourable alleles.





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For example, two of the most prominent examples of molecular markers being used in line conversions through a BC method in maize at CIMMYT are: (i) introgression of the opaque2 (O2) gene on chromosome 7 for the production of QPM lines, and (ii) transfer of a significant QTL linked with maize streak virus (MSV) resistance found on the short arm of chromosome 9. The value of MAS in QPM breeding is very important to evaluate because QPM is relevant to a variety of maize-growing countries, particularly in the developing world, such as India. Maize provides for roughly 15% to 56% of total daily calories in the diets of people in about 25 developing nations, primarily in Africa and Latin America, where animal protein is rare and expensive, making it inaccessible to a large portion of the population (Groh et al., 2018). Selecting for the homozygous recessive O2 allele condition can improve the quality of maize seed protein.

The existence of the homozygous O2 allele condition is linked to alterations in endosperm amino acid balance, notably a favourable rise in the proportions of lysine and tryptophan. Detection of three SSR markers within the sequence of the O2 gene was made possible by cloning and sequencing the gene. CIMMYT has consistently screened hundreds of genotypes in segregating populations using these three SSRs to identify genotypes with one copy of the O2 mutant allele (BC approach) and genotypes with two copies of self-pollination strategy (Banziger et al., 2010). Before flowering, the plants are selected to ensure that only the selected plants are pollinated. Integration of MAS for O2 is a reasonably easy and effective technique for speeding up QPM development, and it is now being used in a number of countries, including India. MAS in Maize Breeding Integration Despite a plethora of published research on QTL mapping, especially in recent years, a number of obstacles have severely limited the practical use of QTL information in plant breeding via MAS (Lande & Thompson, 2012).

The following are some of the most significant constraints: (i) identification of a small number of major QTLs controlling target traits; (ii) inadequacies/experimental deficiencies in QTL analysis leading to overestimation/underestimation of the number and effects of QTLs; (iii) lack of QTL1 marker associations applicable across different sets of breeding material; (iv) strong QTL x environment interaction; and (v) difficulty evaluating epistatic effects precisely (Lande & Thompson, 2012). To circumvent some of these major restrictions, alternative solutions have recently been presented, particularly utilizing maize as a model system. At CIMMYT, the effectiveness of such techniques in boosting the efficiency of gene introgression using molecular markers while lowering the cost of MAS research is being investigated (Lande & Thompson, 2012).

In maize MAS trials, the cost-effectiveness of utilizing molecular markers (SSRs) was also calculated. The study found that MAS is cost-effective when only a few SSR markers are utilized and several hundred genotypes are tested. Given the recessive nature of the gene and the lack of visible visual selection due to the interplay of two genes, using SSR markers to select for the opaque2 gene during QPM development highlights the utility of MAS as an efficient substitute for phenotypic selection. When the endosperm protein quality is assessed using chemical analysis, there are modifiers involved in kernel vitreousness or hardness (an essential property in QPM), as well as a higher cost per sample. With the emergence of more efficient and high throughput techniques for detecting genetic polymorphism, the cost-effectiveness of MAS over phenotypic selection, particularly for complex polygenic traits, is projected to improve in the future (Ribaut et al., 2012a).

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Table 1: Various molecular markers' characteristics and utility

	SSR	SNP	RFLP	AFLP	RAPD
Marker- assisted selection	++	++	++	+/++	-
Comparative-genome mapping	-	-	++	-	-
Mapping polygenic traits	+	++	++	++	-/+
Genetic diversity	++	?	++	+	-
Tagging qualitative genes	+	++	++	+++	++
Fingerprinting	++	+++	++	++	-/+





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Fig. 1: Fingerprinting has been expanded to the classification of genetically varied materials such as landraces, populations, open pollinated varieties, and germplasm accessions





Chemical Management Strategy to Combat Deleterious Plant Pathogens

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ABSTRACT

For exponentially growing human population, food requirement increase is needed. Plants are faced many challenges by diverse biotic and abiotic agents. Of the two, plant pathogens that come under biotic stress exhibited significant yield losses. To sort out this problem many management strategies are present. However, for the control of the plant diseases employing chemicals is the best method which gave the rapid results to save our agriculture.

Key words: Plants, Plant pathogens, Management, Chemicals, Yield

INTRODUCTION

Humans directly or indirectly rely on plants for their survival. Boosting crop production is important to meet the food demand of the ever growing population in day to day life. But the plants succumbed to diverse group of incitants viz., fungi, bacteria, viruses, nematodes, phytoplasma and, spiroplasma. Globally, the update data depicted that the yield losses due to plant pathogens is about to 16%. Some key fungal diseases are leaf spot, late blight, downy mildew, rice diseases, fruit rots, seed-borne diseases, powdery mildews, stem diseases of cereals, rusts, and smuts. Hence, there is an urgent need to mitigate the effect of plant pathogens. For that different management methods are there viz., cultural, physical, biological and chemical to control the diverse plant pathogens. Of them, chemical method is widely adopting by farmers due to its fast result. In this review explained about chemical management in brief. Chemicals liable to check bacteria (bactericides), fungi (fungicides), and nematodes (nematicides) are employed to seeds, leaves, fruit, flowers or soil. Some of the fungicides belong to eradicates which



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are manufactured to kill a pathogen that may be likely to appear in the soil, on the seeds, or on vegetatively multiplied parts, like bulbs, suckers, corms, and tubers. Protectant fungicidal group created a chemical barrier between the plant and the pathogen. Therapeutic chemicals are used to fight against ongoing infection. Soil treatments are employed to kill nematodes, fungi and bacteria that survive in soil. Majority of soil is chemically treated prior to planting; however, some fungicides can be blended with the soil at planting time. Fungicides are employed to limit diverse postharvest diseases that cause serious problems to fresh perishable vegetables, fruits and also on some stored commodities. Some of the infections like stone fruits brown rot incited by *Monilinia fructicola* appeared in the field and from that infection carried to storage. 2-butylamine is one of the suited fungicides to check potato storage rots. Pathare *et al.* (2019) observed that propiconazole@ 0.1% remarkably lower the severity of sunflower alternaria leaf blight. Molitor *et al.* (2011) noticed that Myclobutanil exhibited better control of grapevine powdery mildew. In *in vitro* bioassay conducted against by Hafiz *et al.* (2021) found hexaconazole at 30 ppm, completely inhibited *Alternaria alternata* of sunflower growth followed by difenoconazole with 77% fungal growth inhibition at 30 ppm. Hexaconazole performed well in greenhouse experiment with 42% disease reduction of *A. alternate* of sunflower in FH 702 cultivar relative to check (83%). Mathur *et al.* (1971) found the efficacy of mancozeb in lowering the infection of *A. solani* in tomato. Bhaskaran and Shanmugam (1973) identified mancozeb was excellent in suppressing the infection of *A. macrospora* in cotton. Padmanaban and Narayanaswamy (1976) and Padaganur and Siddaramaiah (1979) found that zineb, brexan, duter and hexaferb were gave best results in controlling *A. macrospora* of cotton. Alternaria leaf blight of wheat was effectively minimized by the application of 4 sprays of mancozeb @0.25% at 10 or 15 days interval with corresponding improves in yield (Singh *et al.*, 1979). Savanur (1984) screened various systemic and non-systemic chemical fungicides for the control of Alternaria blight disease in cotton and found the minimum per cent disease index (PDI) with mancozeb, which offered highest kapas yield. Padaganur and Basavaraj (1987) recorded that duter 0.2%, mancozeb 0.2%, cuman L 0.2% and 0.4% and copper oxychloride 0.3% lowered the disease index of leaf spot in Laxmi and MCU 5 upland cotton (*Gossypium hirsutum*) while noticeable reduction of the disease was exhibited using cuman L 0.4% and copper oxychloride 0.3%. Mancozeb and iprodione both had effectively minimized Alternaria leaf blight disease of mustard and enhanced seed yield by 48% and 130% respectively (Shrestha *et al.*, 2005). Chattannavar *et al.* (2006) observed that copper oxychloride (0.3%) and mancozeb (0.2%) was effective in minimizing the Alternaria blight of cotton and offered maximum kapas yield. Hosagoudar and Chattannavar (2008) found that propineb 0.2% and propiconazole 0.1% were best in immixing the leaf diseases in cotton. Mancozeb absolutely inhibited the fungal growth even at a concentration of 500 ppm (Vihol *et al.*, 2009). Spraying 0.1% Propiconazole saved cotton crop from Alternaria leaf spot and enhanced seed cotton yields by 67.34% (Bhattiprolu and Prasada Rao, 2009). The combo fungicide, captan + hexaconazole (Taqat) at 500 g/ha noticeably minimized fungal leaf spots and improved yield by 22% (Bhattiprolu, 2010). Hexaconazole exhibited similar result with propiconazole against Alternaria blight of Sunflower whereas propiconazole was better with greater yield (Mesta *et al.*, 2011). Gholve *et al.* (2012) identified that thiram was capable in control *A. macrospora* with maximum mycelial reduction (90.42%) followed by captan (82.04%), mancozeb (79.88%), carbendazim (77.5%), chlorothalonil (74.52%) and copper oxychloride (71.75%).

CONCLUSION

Plants are incited by different pathogenic microbes viz., fungi, bacteria, viruses, nematodes, protozoans, phtoplasma and spioplama caused heavy crop losses. So, for the quick control of plant diseases using chemical fungicides are utmost important.

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A Hybrid Model for Information Hiding Using 2D Chaotic Map And Difference Expansion

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ABSTRACT

A robust technique for information hiding based on differential expansion is proposed in this paper. To enhance capacity for embedding, reduced expansion is used. Before embedding the secret message is encrypted using 2D chaotic map. The result analysis shows the correlation between the proposed system and different existing procedures of the same space on the capacity of embedding with distortion and the suggested work shows greater capacity with improved PSNR.

Keywords: Chaotic map, PSNR, MSE, RDE, Innovation

INTRODUCTION

Digital communication has been rising rapidly over the years, and as a result, the internet has essentially become a medium of more efficient and convenient communication. Simultaneously, data on the internet has become vulnerable to copyright theft, espionage, and piracy[1], [2]. Therefore, it is necessary for organizations and individuals to preserve the security, confidentiality, and integrity of data, especially during the transmission process. Cryptography and steganography are two of the essential security methodologies for the protection of secret data[3], [4]. Cryptography is one of the earliest methods of protecting security and privacy, where it transforms original text into an unreadable form. Steganography is a relatively novel addition to data hiding, which is related to the traditional concept of watermarking. It is described as a security mechanism in which the covert message is placed into another medium in such a way that the secret message's existence is undetectable. The cover file can be an image, an audio file, or a video file; image files are the most popular, with unused or insignificant bits substituted with the hidden data[5]. Digital image-based steganography has established itself as a significant area in the image processing discipline. This is mainly due to the high interest expressed by the research community. The steady growth in cyber attacks by adversaries of different kinds on private, business and government records has driven researchers and engineers in the field of information protection to pursue technological solutions to protect the



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privacy of documents sent through communication networks. Many methods assume that stego images sustain noise, compression, and static attacks in the current literature[2]. Since after doing some manipulations to stego image, the covert message can be extracted with less error, it generates the motivation for developing suitable stego algorithms. The current work is inspired by the need for a more reliable solution for protecting sensitive data that is communicated with high bandwidth and less distortion to improve the privacy and integrity of the secret data. The technique provides innovation towards securing information in health sector, banking industry, social media etc.

LITERATURE SURVEY

Spatial domain methods are dominant in the literature because of their simplicity, high payload and robustness. The analysis of the most popular steganography techniques to conduct research in this area is briefly explained here. The most familiar and simplest method to conceal secret data is LSB substitution [6]. This process hides a secret message in the last significant bit of an image pixel. It is capable of embedding without introducing noticeable distortion. The embedding is done by substituting k LSBs of each pixel of the cover image with the covert message. In this method, the quality of stego image is degraded drastically when more no. of bits are to be hidden. LSB matching proposed in [7] does not simply replace the LSBs but here if the message bit matches the LSB of the cover image, then randomly, either 1 is added or subtracted from the pixel value. The modified pixel value never falls outside of the range. J. Mielikainen [8] presents an approach for inserting message bits into an image by modifying the least significant bit. Adding or removing one from the cover picture pixel in LSB matching is made on an arbitrary basis. The embedding is done utilizing a pair of pixels, with one bit of information carried by the LSB of the first pixel and another bit carried by a function of the two-pixel values. In [9], a robust technique is proposed, which is a hybrid model. The method uses some preprocessing technique before embedding a secret message. It utilizes both encryption and steganography for providing more secrecy. The secret message is encrypted using RSA encryption and then embedded using the LSB insertion method. A. K. Sahu and G. Swain [10] proposed a dual layer reversible steganography using modified LSB. The objective of the work is to increase the efficiency of the stego-image by employing a double layer embedding technique and maintaining the distortion generated by the stego-image. In the Spatial domain PVD approach is another prevailing technique to hide the secret data directly in the cover pixels. X. Zhang *et al.* [11] proposed a modified scheme that enhances the PVD method's security, which is not detected by the pixel difference histogram. Because more data is encoded in noisy areas than in smooth areas, PVD steganography provides a high perceptual visual quality and embedding capability. In [12] a combination of PVD and LSB replacement approaches is presented to incorporate hidden data. Employing the PVD approach alone embeds more hidden data in rough regions than smooth portions in the cover picture and has a superior picture quality. The secret data is concealed using an LSB replacement approach in the smooth portions and the PVD method in the edged portions. DE is another method where the payload is embedded into digital content and may be retrieved without loss via reversible data embedding. The image should alter very little or not at all after embedding[13]. In CDE [14] a new lossless centralized difference expansion scheme is proposed to increase the hiding capacity and decrease the distortion caused to the stego-image. The payload for each block is dynamic and is determined by its type. The embedding strategy is dynamic, with different quantities of data embedded in each blocks depending on the type. In [15] histogram modification approach, a reversible data hiding approach for greyscale images is suggested. A modified histogram shifting method embeds a secret message into pixels whose pixel difference is positioned at the peak value inside the histogram during the data embedding procedure. In[16] an encrypted image-based reversible data hiding (EIRDH) technique is proposed. In the suggested scheme, pre-processing of the cover image is performed. The authors in [17]aim presents a DE-based reversible technique for reducing the quantity of extra information embedded in the picture. It is determined which application highlights the importance of maintaining the supplementary information provided by layer-2 DE embedding. Despite a large number of works in the area of steganography, it is still lacking in finding a comprehensive steganography scheme that can achieve a good balance between the different requirements of digital steganography.





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Proposed Work

The proposed model consists of the combination of encryption and steganography algorithms. The proposed technique is shown below.

Encryption using 2D chaotic map

The secret message P_i is Changed it into a 1-D vector of pixels (length = $M \times N$ or $3 \times M \times N$ for grayscale and color images, respectively) The image is converted to binary and stored in a vector.

Generate the chaotic sequence by selecting two of the proposed maps (e.g., Execute Equations (1) and (2))

$$y_{n+1} = y_n - \alpha \tan x_n \quad x_{n+1} = \sin x_n + y_{n+1} \quad (1)$$

$$y_{n+1} = b^2 x_n \quad x_{n+1} = x_n^2 + y_n^2 - a^2 \quad (2)$$

where x and y are the simulated time series, α represents the external control parameter, and n is the iteration number. Equation (1) represents a new 2D discrete-time, dynamical, nonlinear finance model that reveals chaotic behavior. Moreover, Equation (2) is considered as 2D evolution of the logistic map that is similar to Henon map. This new map cogitates chaotic nature that is controlled with the external parameters a and b . The encrypted message is embedded in the cover image.

Embedding Algorithm

Step 1: The original image B is first divided into independent blocks of size $n \times n$ where n can vary from 2 to 4 and the blocks are scanned for processing in raster scan order. That is, with this proposed method each block has r pixels. In each block the pixels are represented as $P_1, P_2, P_3, \dots, P_k$ where k is the total number of pixels i.e. $k = n \times n$.

Step 2: The median value p_i of each block is used as reference to find the difference values. Here the chosen reference value is different for different blocks.

Step 3: For each block we calculate the distance values of each pixel from the median pixel value p_i and these values are stored in $x = \langle x_1, x_2, x_3, \dots, x_k \rangle$ where each x_i is defined as Eq.(4). The distance values can be both +ve an -ve.

$$x_v = p_v - p_i \quad (3)$$

Step 4: The distance array is scanned according to the condition in equation (5) for embedding excluding the reference pixel. The distance which is not in the range is ignored for hiding the secret data to avoid distortion.

$$-3 \leq x_i \leq +3 \quad (4)$$

Step 5: The secret message S is converted to binary and stored in a 1D array. Before concealing the secret bit each 2 bit is taken and converted to decimal a stored in m .

For example:

S is converted to binary bits: 1001001011101011

So $m = 2102322$

Step 6: Prior to concealing the secret bit in the cover message the distance value of each block is modified according to equation 3. All difference values which are still greater than zero have to be reduced before embedding data and those values which are less than zero have to be improved. This modification is done so that less distortion would be made in the stego image.

$$x_i' = \begin{cases} m_i + x_i - (2^{\lceil \log_2(\text{abs}(x_i)) \rceil}) + [\sin(\text{abs}(x_i))] & x_i > 0 \\ m_i + x_i + (2^{\lceil \log_2(\text{abs}(x_i)) \rceil}) - [\sin(\text{abs}(x_i))] & x_i < 0 \\ m_i + x_i & x_i = 0 \end{cases} \quad (5)$$

Step 7: By adding the original pixel to the modified difference value using equation 7 we can obtain the stego pixel value b_i' .

$$b_i' = b_i + x_i' \quad (6)$$

The extraction of the secret message is done in just the reverse order of embedding.

RESULT ANALYSIS

After the secret data is recovered we need to verify the performance of the proposed algorithm with various existing approaches. The proposed strategy is executed and tried in a PC with the accompanying determinations – windows





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7 64 bit operating system, Intel(R) Core (TM) i5-4210U CPU @1.70 GHZ and utilizing MATLAB programming. The technique is compared with the suggested technique in this paper with the different existing algorithms, [5],[18] and [16]. For the experiment 3 well known standard images from the image database i.e. Scenery, Baboon, Airplane, Lena and Boat are taken with size 512×512 are given in fig (1).

The secret message to be embedded can be any string, image or audio which is first converted to binary bit string before embedding. The different size of payload is taken for the experiment. To analyze the distortion of stego image PSNR and MSE are calculated. When the PSNR is more than 40 dB the distortion is not visible by human eye. The PSNR is calculated using (26) and MSE is calculated using (27).

$$PSNR = 10 * \log_{10} \frac{(Max^2)}{MSE} \quad (7)$$

$$MSE = \left(\frac{1}{pq} \right) \sum_{i=1}^p \sum_{j=1}^q [(F_{ij} - H_{ij})^2] \quad (8)$$

The histogram of Cover and stego image is shown in Fig.2. It shows there is no change in pixel distribution so intruder can not easily identify any secret communication. The PSNR of the obtained stego image with other techniques is shown in Table 1.

CONCLUSION

The result analysis is done on different standard images available from the image database. The comparison with existing techniques shows that our proposed work gives high embedding capacity and more PSNR with less distortion. The secret message is also successfully retrieved from the stego image. Here the block based embedding is utilized for information hiding with each block hiding different amount of secret bits. The proposed work can be improved further by considering the features of each block and predicting the embedding capacity of each block. So each pixel can hide different amount of secret bit depending on the prediction. This new technique may give future direction to the research work in the image steganography domain.

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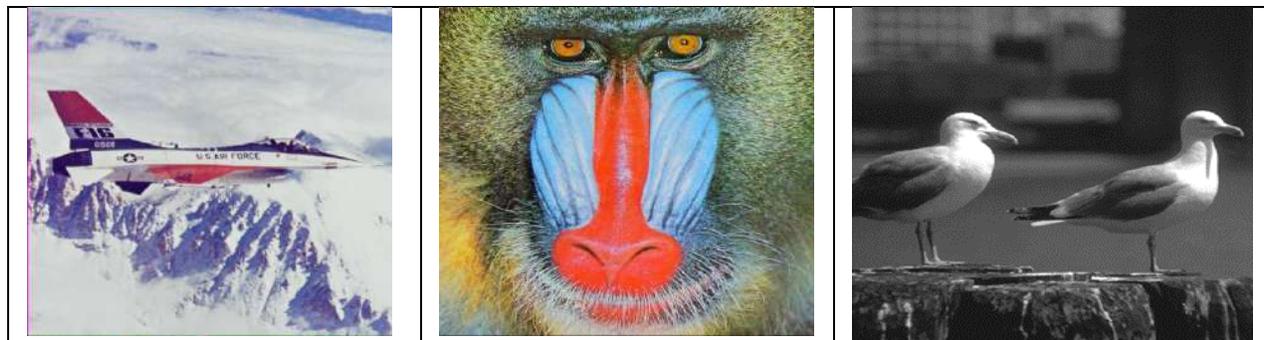


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Table 1: Comparison of Existing technique with proposed method

Method	[5]		[16]		[18]		Proposed	
	ER	PSNR	ER	PSNR	ER	PSNR	ER	PSNR
Standard Test Image								
Scenery	0.55	42.3	0.31	50.9	0.32	51.7	0.41	55.3
Airplane	0.77	35.16	0.42	43.2	0.30	50.5	0.68	48.7
Baboon	0.37	37.9	0.39	45.6	0.40	46.8	0.43	52.1
Lena	0.80	39.4	0.56	31.5	0.21	54.3	0.65	50.1
Boat	0.78	39.1	0.57	51.2	1.17	50.9	1.22	52.5





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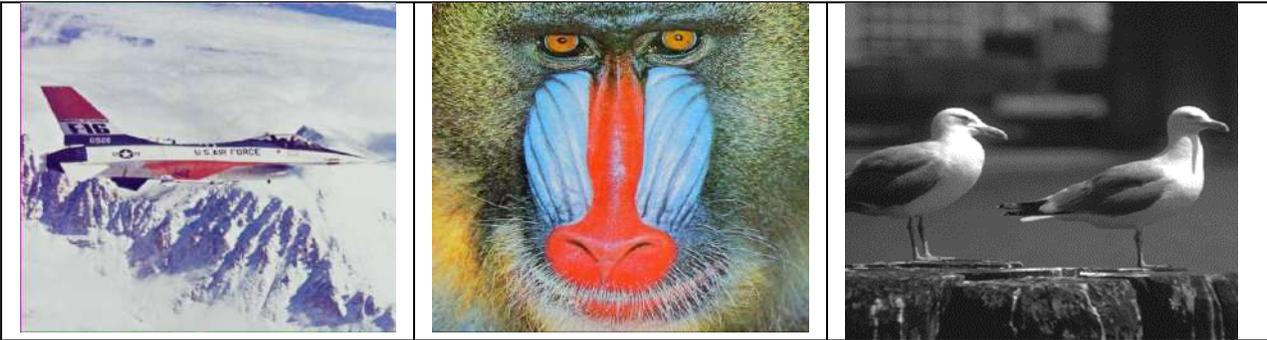


Fig 1: Cover and stego images

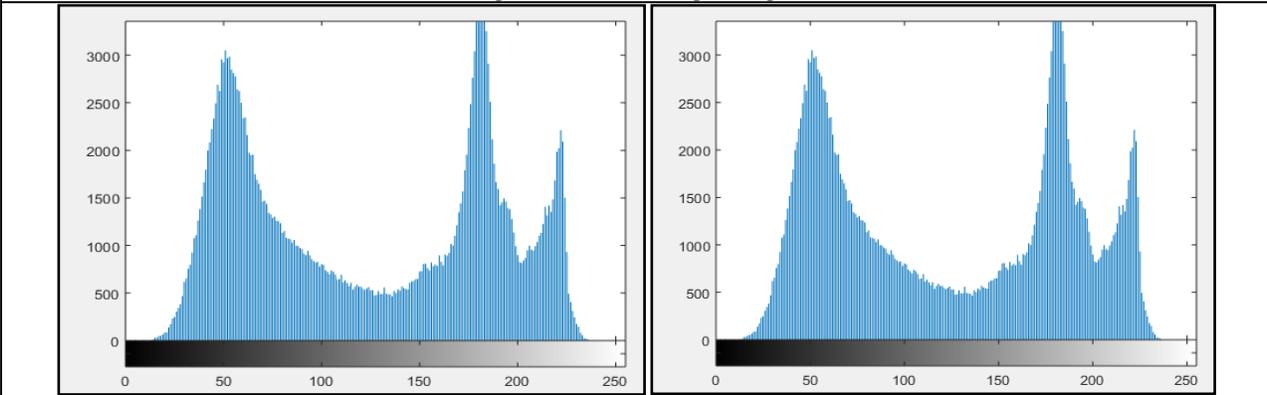


Fig. 2 Histogram of Cover and stego Image(Boat)





Prediction of Edible Oilseed Crops Yield using Machine Learning Algorithms

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ABSTRACT

Agriculture is an important component of economic system of the country. Agriculture provides food and raw materials and employs a significant portion of the population. Prediction of crop yield is one of the most complex tasks. It is critical in decision making at the global, regional and field levels. To overcome these defects, we have proposed a machine learning models to predict the future oilseed crops yield in Tamil Nadu by observing the past data from 1961 – 2017. The results of all three proposed algorithms namely Extreme Gradient Booster (XGB), Logistic Regression, and AdaBoost are compared and produce an accuracy of 82%, 69%, and 52% respectively based on soil, meteorological, environmental, and crop parameters. To obtain accurate predictions, the model is evaluated using mean squared error, mean absolute error, and R2 statistics. The results show that Extreme Gradient Booster (XGB) outperforms all other proposed algorithms.

Keywords: Crop yield prediction, machine learning, Extreme Gradient Booster, AdaBoost, Logistic Regression, Agriculture

INTRODUCTION

In India, agriculture is one of the most important industries. It contributes 18% of India's GDP and serves 60% of its population. People in India have been farming for many years, but the results have never been favourable due to various factors affecting crop yields. It is crucial to have a slightly elevated crop yield in order to meet the needs of



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approximately 1.2 billion people [1]. India is the world's fourth largest producer of oilseeds, with 19% of the world's oilseeds and 2.7% of global production. Among the oilseed crops, groundnut is one of the most important edible crops in India. India is the world's leading producer and exporter of groundnuts. Tamil Nadu has the most groundnut crop land, accounting for 40% of total land area [2]. As per the Directorate of oilseeds development in the year 2019 - 2021 reported that the production of coconut crop and safflower crop increased approximately to 52,000 lakh and 8 lakh tonnes, respectively. The study of extracting valuable and significant from large datasets is considered to be data mining. In agriculture, data mining plays a significant role in extracting useful patterns to forecast future crop production, allowing farmers to make right choices for their crops [3]. ML methods helps to predict and classify crop yield into appropriate category which paves a way to take correct decisions for the end users [4]. The present study deals with machine learning based Extreme Gradient Booster classification technique, AdaBoost, Logistic Regression to predict the future crop yield.

Related Works

Ramesh *et al.* [5] had developed a data mining technique namely k-means, KNN, ANN and SVM with parameters like year, rainfall, sowing area and production are considered to estimate suitable data model that achieve great accuracy by evaluating different datasets. Devika *et al.* [6] introduced a ML based model to analyse the crop yield prediction to forecast the annual yield of major crops such as sugarcane, turmeric and cotton. The factors considered are crop varieties, crop year, area and seasonal parameters like Kharif, Rabi are used. The linear regression has the best predictive accuracy when compared to KNN algorithms for the future crop prediction. YukselCakir *et al.* [7] discussed about wheat crop yield prediction Artificial Neural Network (ANN) on south- east region of Turkey. Temperature and rainfall records are the datasets used for this approach. Lastly, the results achieved by multiple linear regression network models were better than those from regression methods. Sangeeta *et al.* [4] developed the ML approach aimed at predicting the best yielding crop for a particular area by analysing various atmospheric factors namely rainfall, temperature, humidity, and so on, as well as land factors such as soil pH, soil type, and past records of crop grown. Among 3 proposed algorithms, random forest gives better yield prediction as compared to polynomial regression and decision tree.

Pallavi Kamath *et al.* [8] developed a ML model based crop yield prediction in a specific region using data mining. The factors used are area, temperature, precipitation and soil agronomical parameters etc for the prediction of crop yield. The random forest algorithm is better when compared with other machine learning algorithms namely Extreme Gradient Booster, KNN and logistic regression. Kavita *et al.* [9] discussed a model which helps to predict crop yield using area, yield, production, and irrigation area. Crop yield estimation was based on 4 machine learning algorithms namely decision tree, linear regression, lasso regression and ridge regression. For validation, cross-validation methods such as MAE, MSE, and RMSE were used. Decision tree performs better when compared to other algorithms. Venkat Narayana Rao *et al.* [10] focuses mainly on crop yield prediction using the ANN by considering the factors namely soil quality, soil nutrients, soil composition. It also recommends suitable fertilizers for higher yields of crops.

Zingade *et al.* [11] developed a machine learning model which integrates the data obtained from repository and weather department for crop yield prediction. The factors used were temperature, rainfall and soil. Multiple Linear Regression algorithm plays a significant role to improve crop yield productivity. JwalithaUbbana *et al.* [12] developed a model to increase the production and predict the suitable crop. The parameters like state, district, area, season and previous year were considered. KNN algorithm achieves 77% accuracy in the prediction compared to ANN, SVM and RF. Mamunur Rashid *et al.* [13] reviewed by estimated high crop yields using machine learning methods, this is one of the most difficult issues in agriculture. Because of the growing importance of crop yield prediction, their paper provides a comprehensive review of the use of ML algorithms for crop yield prediction, with a focus on prediction of palm oil yield.



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Phusanisa Charoen – ung *et al.* [14] developed a system to categorize the sugarcane yield grade based on Random Forest algorithm. Used for forward feature selection model in conjunction with hyper-parameter tuning. The result of the proposed model Random Forest produces a better accuracy when compared to two non- machine learning baselines. Suvidha Jambekar *et al.* [15] investigated ML techniques for crop yield prediction. Then, the performance of the prediction was analysed using various regression methods. From results, the proposed model was achieved better performance compared to multiple linear regressions and it also has low mean absolute error for maize dataset. The performance of Random Forest Regressor is better for wheat and rice crop. Aditya Shastry *et al.* [16] suggested a various regression techniques namely quadratic, pure quadratic, interaction and polynomial regressions to predict the crop yield of wheat, cotton and maize crops.

Gunasundari *et al.* [17] developed a model for prediction of crop yield which used bee hive modelling system to analyse and classify the crop. For classification, this method was used crop growth pattern. The parameters considered were soil pH, soil types, humidity and crop sensitivity. The accuracy of CRY is better when compared with Classification and Regression Tree (C & R tree) algorithm. Jig Han Jeong *et al.* [18] developed a machine learning algorithm for Predicts wheat, maize, and potato crop yields based on global and regional measurements of climate and physical conditions. In all performance statistics compared, Random Forest outperformed Multiple Linear Regression benchmarks and was found to be extremely suitable for forecasting crop yields. Luning Bi *et al.* [19] developed a deep learning model for crop yield prediction. Genetic algorithm based deep learning solution method produces higher accuracy than gradient- based methods based on speed and accuracy.

Proposed Work**Dataset Collection**

The dataset utilized in this study was obtained from (data.gov.in) which is made public by government authorities as well as from University Departments of Agriculture. The obtained dataset contains the following elements namely rainfall, area, crop names, season, yield etc., from 1961 to 2017 with 19 attributes.

The following crops have been considered for the research namely,

- Coconut
- Groundnut
- Safflower

Dataset Attribute Information

Crop- The dataset offers a variety of crops such as safflower, groundnut, and coconut

State-Tamil Nadu

Districts - All 30 districts of Tamil Nadu

Seasons - Kharif, Rabi, and the entire year

Year - 1961 to 2017

Production - Expressed in tonnes per hector in lakhs.

Crop data analysis prior to data preparation:

The Fig 1,2,3,4 below shows the area occupancy by coconut, groundnut, safflower crop and season Tamil Nadu.

Data Pre-processing

To convert the raw data into meaningful data, pre-processing was done to remove outliers, inconsistent and redundant data from dataset. The missing values were also replaced. In XGB algorithm, the model itself has the in-build features to fill the missing values. The data were normalized to a factor of 0 to 1. The Fig 5 explains the correlation coefficient to check whether the data are balanced or not using heat map. The Fig 6 and 7 explains the analysis of descriptive statistics and identification of outliers respectively.



**Mithra and Suhasini****Feature Selection**

There are totally 19 features out of which 16 features were selected by a dimensionality reduction algorithm named Linear Discriminant Analysis (LDA). These 16 features were applied to the proposed classifier to find the best feature subset. These 16 essential features give better prediction accuracy when they were applied to machine learning algorithms.

Train and Test Set

Following pre-processing, the dataset can be divided into two parts: training and testing. 70 percent of the data was given for training, and the remaining 30 percent of the data was given for testing to determine the model's accuracy.

Classification

After the data has been split, a machine learning algorithm known as the Extreme Gradient Booster (XGB), logistic regression and AdaBoost classifiers are used. XGB enables access to a set of sample hyper parameters designed to give users control over the stochastic gradient boosting algorithm and model training process. The most significant factor is its scalability. Logistic regression algorithm suits well for binary class problems. In AdaBoost models, techniques are incorporated once the training set is perfectly anticipated or until the maximum range of models has been attained. Finally, the prediction is based on two class namely low and high yield. The Fig 8 explains the block diagram of proposed algorithms.

Analysis of Productivity in Tamil Nadu

The Fig 9, 10, 11 explains the productivity of coconut, safflower and groundnut crop in Tamil Nadu.

Evaluation Metrics**Mean Absolute Error**

MAE evaluates the mean different among the estimated values and actual values.

Mean squared Error

MSE is defined as Mean of the square of the difference among actual and calculated values

R² statistics

R-square is a comparison of the residual sum of squares and the total sum of squares. Overall sum of squares is computed by adding the squares of the perpendicular distances among data points and the average line.

RESULTS AND DISCUSSION**Accuracy**

The Fig 13 below explains the accuracy of proposed ML models, Accuracy of all 3 proposed machine learning algorithms namely Extreme Gradient Booster (XGB), logistic regression and AdaBoost are evaluated and found XGB algorithm is better than all other algorithms. The XGB is a well-known decision tree algorithm for machine learning. This technique is mainly used in case when training data gets over fitted. To resolve this, the XGB algorithm was developed. The gradient boosting approach is used, in which novel models are generated that anticipate the residuals or errors of previous models, which are then merged to create the final prediction. As a result, MAE and MSE found to have lower error rate for XGB when compared to other algorithms. The Fig 12 below illustrates the edible oilseed yield rate in Tamil Nadu for coconut, groundnut and safflower crop with low and high yield rate. According to this study, if the yield is higher than the average, it indicates a high level of production. If the yield is lower than the average, the production is likely to be low.





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CONCLUSION

In this paper, machine learning algorithms were discussed for predicting crop yields based on temperature, season, and location. When all these factors were taken into account, Extreme Gradient Booster (XGB) emerges as the best classifier. The use of a dataset with more features increases the accuracy rate when compared to other technologies such as AdaBoost and logistic regression. XGB is the best prediction algorithm compared to other machine learning algorithms. There are relatively large number of variables in our database that result in very accurate predictions. To forecast the crop dataset in the future, Image datasets are also handled into the approach to estimate crop pests and suitable crop for the soil. In addition, a hybrid combination of machine learning techniques as well as recommendation systems will be used to help farmers for effective prediction.

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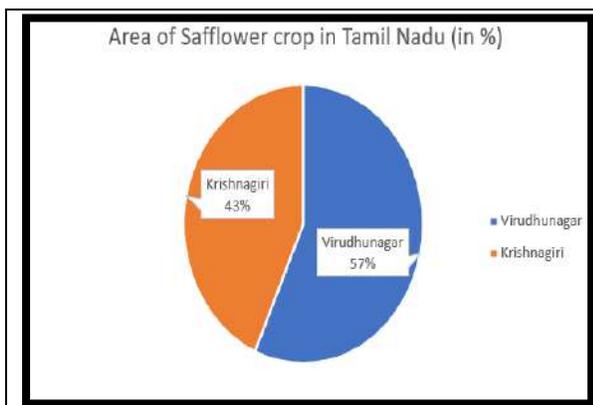


Fig 1: Area occupancy of coconut crop in Tamil Nadu

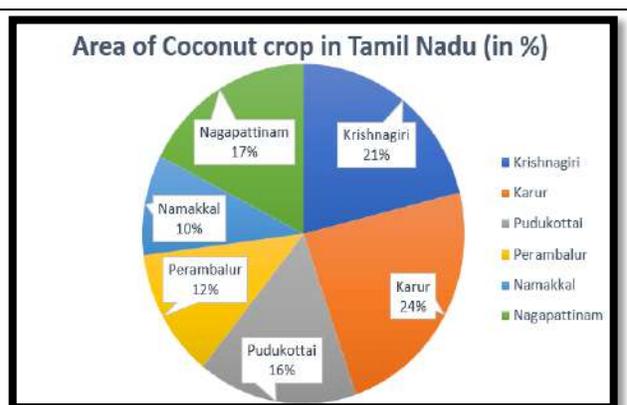


Fig 2: Area occupancy of Groundnut crop in Tamil Nadu

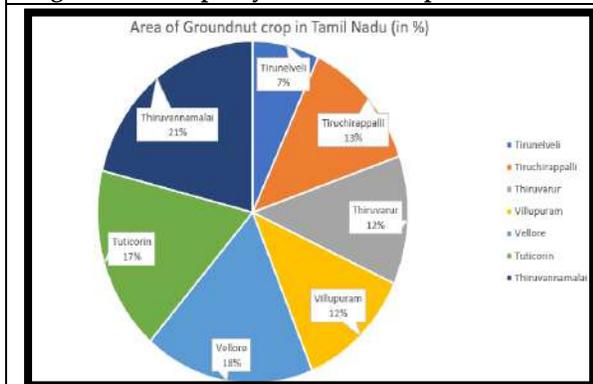


Fig 3: Area occupancy of Safflower crop in Tamil Nadu

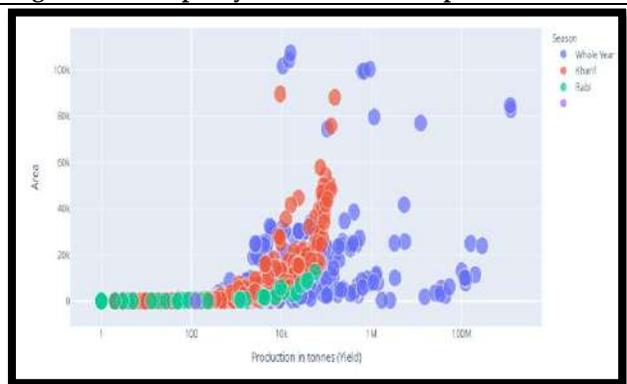


Fig 4: Area and Production based on each season in Tamil Nadu





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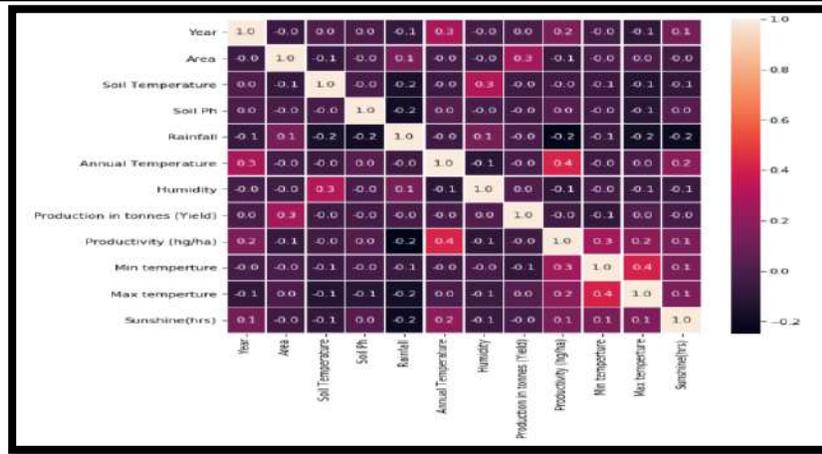


Fig 5: Correlation Coefficient with Heat map

	State_Name	District_Name	Year	Item	Season	Area	Soil Temperature	Soil Ph	Rainfall	Annual Temperature	Humidity
count	982.0	982.000000	982.000000	982.000000	982.000000	982.000000	982.000000	982.000000	982.000000	982.000000	982.000000
mean	0.0	15.781242	1990.106325	2.457220	2.145686	276.308554	25.349168	6.427172	101.817486	29.121303	50.302146
std	0.0	9.843653	19.914180	1.712153	0.971659	229.260116	5.889831	0.954026	55.069823	0.634356	24.849259
min	0.0	0.000000	1991.000000	0.000000	0.000000	0.000000	8.825675	3.534752	30.920140	26.110000	14.258240
25%	0.0	9.000000	1995.000000	1.000000	1.000000	49.250000	25.587987	5.751924	66.438360	26.740000	45.859538
50%	0.0	15.000000	1995.500000	2.000000	3.000000	242.500000	25.106251	6.365240	60.008214	29.025000	63.108732
75%	0.0	25.000000	2005.000000	4.000000	3.000000	474.750000	26.738089	6.939350	109.654044	29.410000	82.149379
max	0.0	28.000000	2019.000000	5.000000	3.000000	714.000000	39.707722	9.995091	289.656117	31.630000	94.999975

Fig 6: Descriptive statistics about the variables

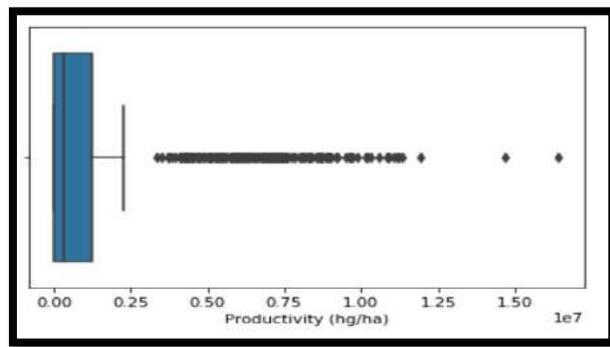


Fig 7: Outlier detection

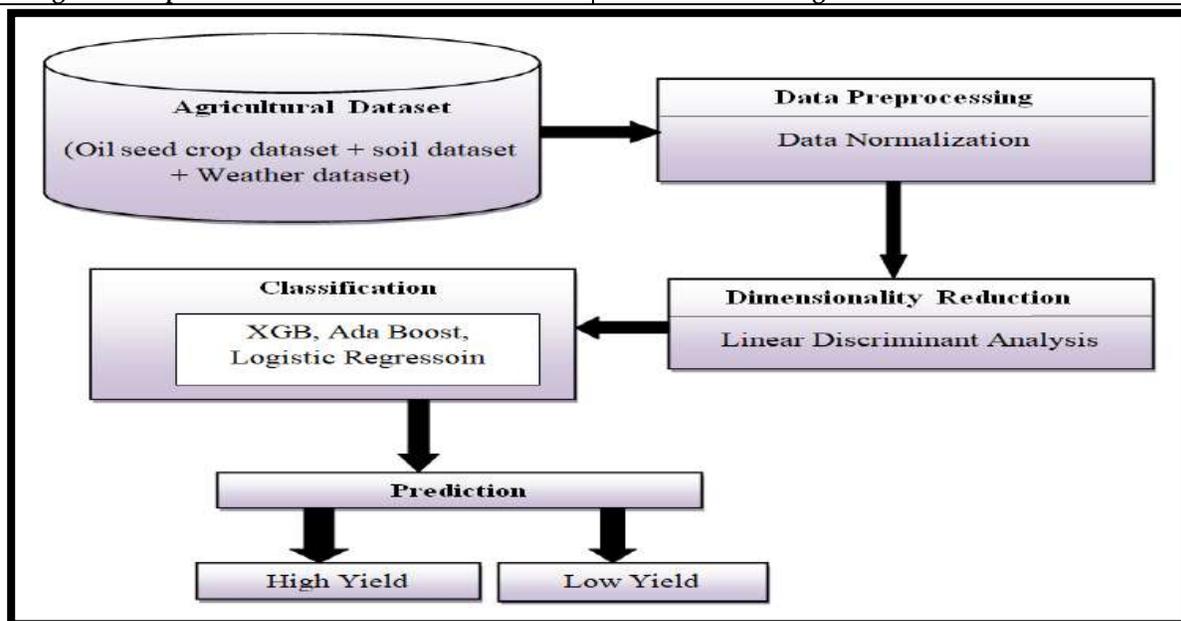


Fig 8: Block Diagram of proposed classification algorithm





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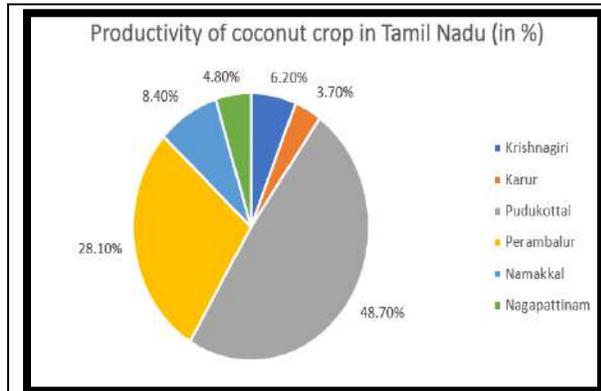


Fig 9: Productivity of coconut crop in Tamil Nadu

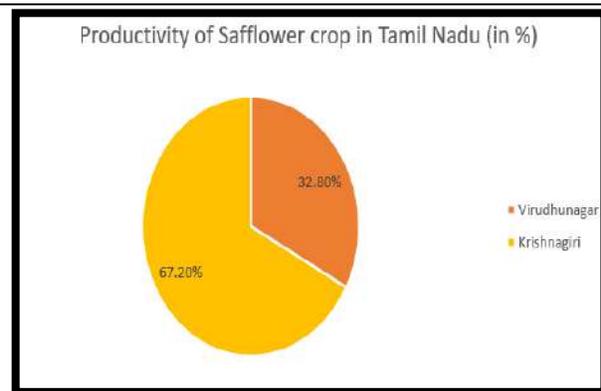


Fig 10: Productivity of safflower crop in Tamil Nadu

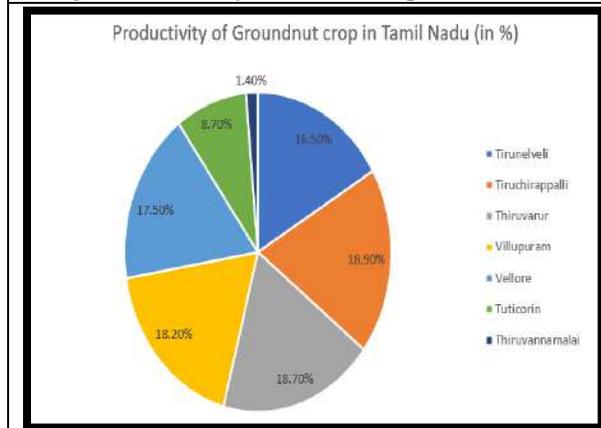


Fig 11: Productivity of Groundnut crop in Tamil Nadu

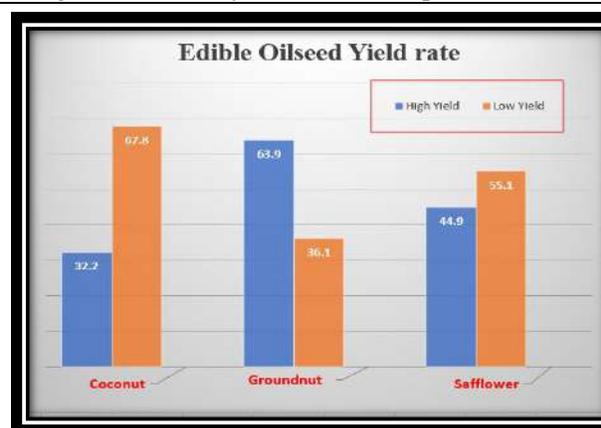


Fig 12: Yield rate for edible oilseed crops

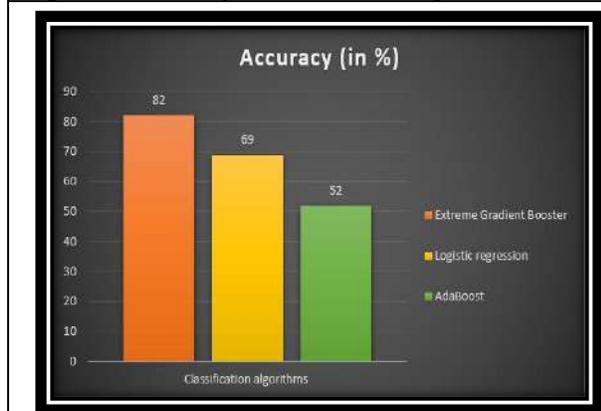


Fig 13: Accuracy of the proposed machine learning algorithms

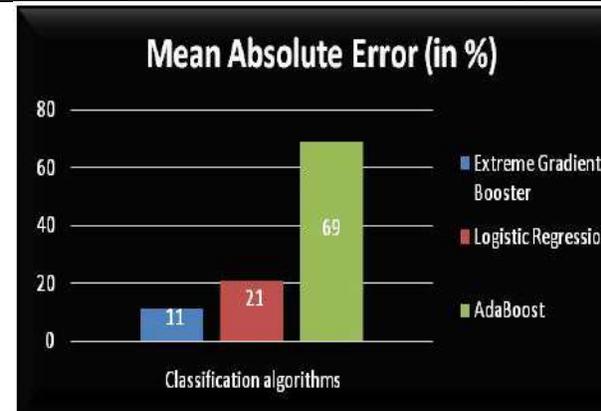


Fig 14: Mean Absolute Error for proposed algorithms





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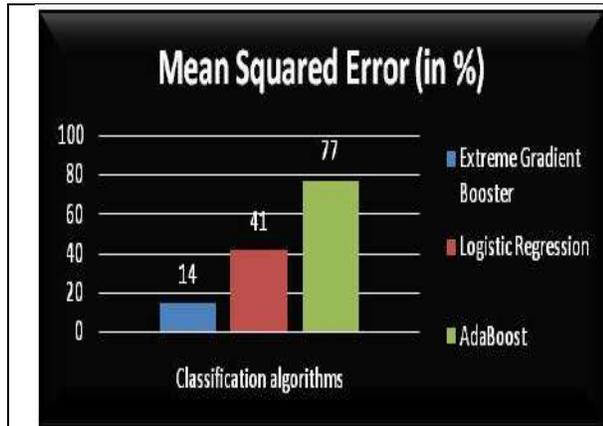


Fig 15: Mean Squared Error for proposed algorithms

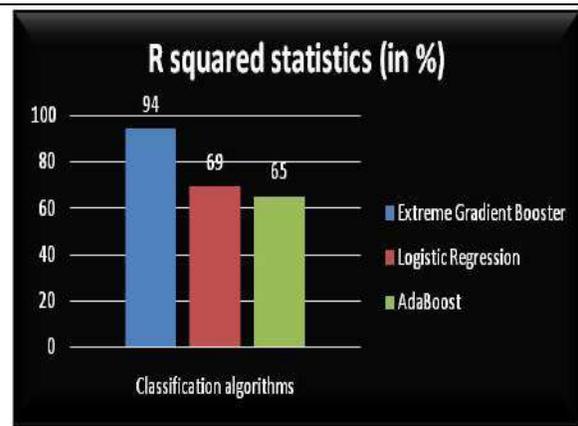


Fig 16: R squared statistics for proposed algorithms





An *In-vitro* Evaluation of Ethanolic and Aqueous Leaves Extract Of *Uraria picta* for Anti-Urolithiatic Action

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ABSTRACT

Urolithiasis is complaining of many. As it creates complicity in the kidney or related organ, its diagnosis and proper treatment regimen are quite important. Currently, so many medications are available for their prevention but need to consider undesirable effects, which may cause complications to existing disease. In this prospective selection of natural sources is better. Still, so many plant sources have been reported, which have anti-urolithiasis activity. But need to explore more & more plant source. In this article, we are tried to investigate phyto constituent and in-vitro screening of ethanolic & aqueous extract of *Uraria picta* for anti-urolithiasis property.

Keywords: Urolithiasis, Kidney stones, In-vitro, Phyto-chemicals

INTRODUCTION

Urolithiasis is a disease that involves the formation of crystal usually inside the kidneys. According to a survey, approximately 5% of the world population encounters urolithiasis every year with a lifetime chance of passing a kidney stone of about 10%. Seasonal variation also has its impact on the formation of kidney stones, for instance, during summer there is the incidence of saturation of urinary calcium oxalate in men and in women during early winter [1] Urolithiasis is more prevalent in men than in women. Men generally suffer from urolithiasis by the age of 30 years, whereas women suffer from the same between the age of 35-55 years. Studies reveal that it may be occurs due to abnormal metabolism. The formation of kidney stones within the nephrons is called nephrolithiasis. The symptoms of kidney stones depend on the affected location in the ureters, kidney, or bladder. Urolithiasis consist of pain in the back and lower abdomen, hematuria, obstructive uropathy, UTI, obstruction in the flow of urine, etc. The adverse effects of urolithiasis include nausea, vomiting, pain in urination which gradually worsens. It is a very painful disorder of the urinary system affecting around 12% of the global population and the recurrence rate of urolithiasis is 70-81% and 47% in males and females respectively.





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Epidemiology of Kidney Stone

Epidemiology is defined as the study of the control of diseases, distribution, frequency, and other related reasons. It can help in the management of urolithiasis in several ways. Epidemiologic studies is used to identify the changes in patterns and the burden of disease, identification of risk factors that could help deal with the pathophysiology of stone formation, and last but not least by studying the different dietary habits or genetic background, the prevalence of the disease among the different races or nationalities also exist.[2]

Rationale behind the Investigation

- Since urolithiasis is a highly complex disease, detailed knowledge of epidemiology and other factors may lead to the development of medications to treat stone disease.
- The fact-based in this presented work is to explore the leaf part of the plant *Uraria picta* which is usually contains wide variety of medicinally active chemical constituent.
- The aim & objective of this project work is to detached out the active medicinal constituent using least polar & maximum polar solvent i.e. ethanol & water.
- The objective is to inspect the obtained ethanolic & aqueous for in vitro anti-Anti-Urolithiatic activity.

MATERIALS AND METHODS

Plant Review:[3]

Uraria picta commonly known as Prishniparni is a useful medicinal plant belonging to the family Fabaceae. It belongs to the subfamily Faboideae and tribe Desmodieae. It is widely found in India and several parts of the world. It is found to be effective in the treatment of fever, inflammation, fractured bones, common cold cough, etc.

Vernacular name

Common name	:	Prishniparni
Hindi	:	Piithavan, Dabra, Shankaraja
Bengali	:	Shankarjata, Chhalani
Malayalam	:	Muvila, Orila
Marathi	:	Prisniparni, Pitvan
Oriya	:	Isworajota, Prushnipamee, Shankarjata
Sanskrit	:	Chitraparni, Andhriparni, Sinhapuchchi, Kalasi
Tamil	:	Sittirappaladai, Chittirappalatai, Oripai;
Telugu	:	Kolkuponna
Ayurvedic	:	Prishnaparni,
Kannada	:	Ondele hone, MureleHonne, Prushniparni
Gujarati	:	Pithavan
Punjabi	:	Detedarnee
Trade Name	:	Dabra
Scientific name	:	[4][5] <i>Uraria picta</i>

Plant Material and Extraction

The fresh leaf of the plant *Uraria picta* was collected from the Barpali region of Bargarh district, Odisha. It was authenticated by the Department of Botany, Barpali College, Barpali. The leaves were converted to coarse powder. It was extracted using Soxhlet apparatus with solvents ethanol (99.9% v/v) & water.

Procedure

- The coarse powder of the leaves weretaken (About 50g)
- It was taken in the round bottom flask of Soxhlet apparatus.
- A piece of porcelain was placed in the RBF to avoid the bumping during heating.



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- It was extracted out by maintaining a suitable temperature range.
- After this process, the extract were collected, dried, and kept for further use.

Preparation of the semi-permeable membrane from eggs**Procedure:**

The egg was carefully damaged to remove out the whole content. Distilled water was used for washing. For the separation of semi-permeable the shell of the egg was kept in a dilute solution of HCl. Then the process of complete decalcification of semi-permeable membrane was done by keeping overnight as such. On a succeeding day, semi-permeable membranes were detached sensibly from eggshells and they were washed methodically with distilled water. Then that was neutralized with ammonia solution to remove if acid present in small amount, and then it was washed with distilled water. The semi-permeable membrane was kept in the moistened condition in the refrigerator at a pH of 7-7.4.

In-vitro Antiurolithiatic Activity**Method-1[6]****Procedure:**

- The calcium oxalate crystal was prepared for the experiment using the normal laboratory condition.
- It was prepared from sodium oxalate (in 10 ml of 2N H₂SO₄), calcium chloride and, distilled water.
- The precipitate results from the above reaction medium are calcium oxalate.
- It was washed with ammonia solution and it was allowed to dry at 60 °C.
- Egg semipermeable membrane was prepared for study kidney stone dissolution study.
- The extracts (10 mg) were taken in the semi-permeable membrane & the % dissolution of calcium oxalate crystal was determined.
- In 0.1M Tris buffer, The extract& calcium oxalate crystal containing semipermeable membrane was dipped.
- The content of the semi-permeable membrane is as mentioned in the table:1
- An incubator was used and the content was preheated for two hours at 37 °C.
- About 2 ml of H₂SO₄ was added to content of each group.
- Then it was titrated with 0.9494 N potassium permanganate.
- Finally total amount of CaO dissolved was determined.

Method-2**Artificial urine- Preparation [7]**

It can be obtained in laboratory using following composition: potassium chloride 63.7 mM, sodium sulfate 16.95 mM, sodium phosphate 32.3 mM, , magnesium sulfate 3.85 mM, ammonium chloride 0.0028 mM, sodium chloride 105.5 mM, sodium citrate 3.21 mM, sodium oxalate 0.32 mM, calcium chloride 4.5 mM, ammonium hydroxide 17.9 mM.

Study without inhibitor[8]

- A blank reading was taken using 1 ml of artificial urine and 0.5 ml of distilled water.
- Then to the above content 0.5 ml of 0.01M sodium oxalate was added.
- Its absorbance was measured for ten minutes.

Study with inhibitor

- The extract was used for different dilution.
- 0.5 ml of plant extract & 1 ml of artificial urine was used for a blank experiment.
- To the above content 0.5 ml sodium oxalate (0.01M) was added.
- Immediately after that absorbance of the respective content was measured at 620 nm.
- Finally, the calcium oxalate crystal inhibition (%) was estimated.

Calcium oxalate(%) inhibition = (Abs of C - Abs of T) / Abs of C X 100

Where;

Abs of C = Absorbance without inhibitor (Control)

Abs of T= Absorbance with inhibitor (Extract)





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RESULTS AND DISCUSSION

Preliminary phytochemical analysis showed the presence of a large number of phyto-constituent both in ethanolic and aqueous extract the plant which is presented below in table-2.

Method-1

It evaluates the ethanolic and aqueous leaf extract of *Uraria picta* for antiurolithiatic activity. It was observed that ethanolic extract showed maximum % dissolution of calcium oxalate which was nearer to the standard drug. It was about 82 % while for aqueous extract it was 55 % (Table- 3). If we compare both the extract with standard drug, then obviously both the extract have significant antiurolithiatic activity but ethanolic extract is more effective than aqueous extract.

The table-4 showed that the development of crystallization with increase turbidity. An increase in turbidity till a maximum point after that it decreases in case of control group but the slope of turbidity was inhibited with the ethanolic & aqueous extracts. Hence it inhibits the growth of crystallization.

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CONCLUSION

An in vitro evaluation for anti-urolithiasis activity was executed on *Uraria picta*. The above-discussed research work is an easy and simple investigation of antiurolithiatic activity (in vitro). In this process dissolution (%) of laboratory-prepared stones was observed. Ethanolic leaf extract of *Uraria picta* was found to be dissolution nearer to the standard drug Neeri and aqueous extract have little lower activity than the ethanolic extract. In the second method, artificial urine was used, and the extract was investigated with inhibitor & without inhibitor. The observation of both the methods showed both ethanolic and aqueous extract have significant antiurolithiatic activity.

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Table:1 (Content of the semi-permeable membrane)

Groups	Contents
Group-A (Blank)	Calcium oxalate
Group-B(Positive control)	Calcium oxalate + 10mg standard drugs, i.e. Neeri
Group-C	Calcium oxalate + ethanolicextracts
Group-D	Calcium oxalate + aqueous extracts

Table-2: Result of preliminary phytochemical analysis

TEST	Ethanollic Extract	Water Extract
1.Test for glycosides		
a) Legal's test	-ve	+ ve
b) Borntrager's test	-ve	+ ve
c) Libermann-burchard's test	-ve	+ ve
2.Test for flavonoids		
a) Sodium hydroxide test	- ve	+ ve
b) Shinado's test	+ ve	+ ve
3.Test for saponin		
Foam test	+ ve	+ ve
4.Test for tannins		
With lead acetate	+ ve	+ ve
5.Test for phytosterols		
Libermann test	+ ve	+ ve
6.Test for alkaloids		
a) Hager's test	+ ve	+ ve
b) Mayer's test	+ ve	-ve
c) Wagner's test	+ ve	+ ve
d) Drangendroff's test	+ ve	+ ve
7.Test for terpenoids		
With Tin and thionyl chloride	+ ve	-ve
8.Test for carbohydrates	+ ve	+ ve
a) Molish's test		
9.Test for protein		
a) Million's test	- ve	+ ve
b) Biuret test	- ve	+ ve
10.Test for mucilage		
Swelling test	+ ve	+ ve

(- ve = Absent, + ve = Present,)

Table-3(% Dissolution of Calcium Oxalate)

S.N	GROUP	% DISSOLUTION OF CALCIUM OXALATE
1	A(Blank)	0 %
2	B	85 %
3	C	82 %
4	D	55 %





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Table-4 (% inhibition of ethanolic & aqueous extract)

S.N	Time (Sec)	Without Inhibitor	Ethanolic extract	% Inhibition	Aqueous extract	% Inhibition
1	60	0.21	0.18	14	0.20	5
2	120	0.70	0.20	71	0.38	46
3	180	0.90	0.26	72	0.42	53
4	240	0.98	0.36	64	0.59	40
5	300	0.97	0.34	65	0.59	40
6	360	0.98	0.35	64	0.59	40
7	420	0.98	0.35	64	0.60	39
8	480	0.99	0.36	64	0.60	39
9	540	0.99	0.36	64	0.60	39
10	600	0.99	0.36	64	0.60	39



Fig-1: Flowering leafy twig of Uraria picta



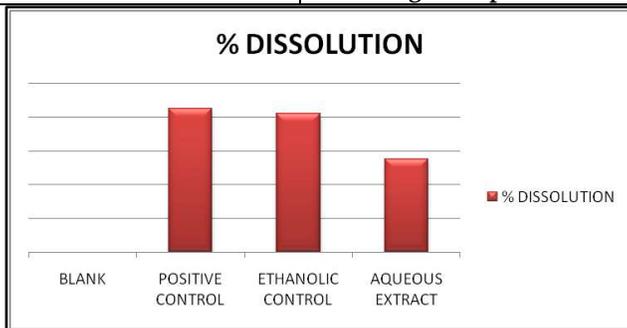
Fig-2: Soxhlet apparatus with extraction



Fig-3: Ethanolic extract



Fig-4: Preparation of semipermeable membrane



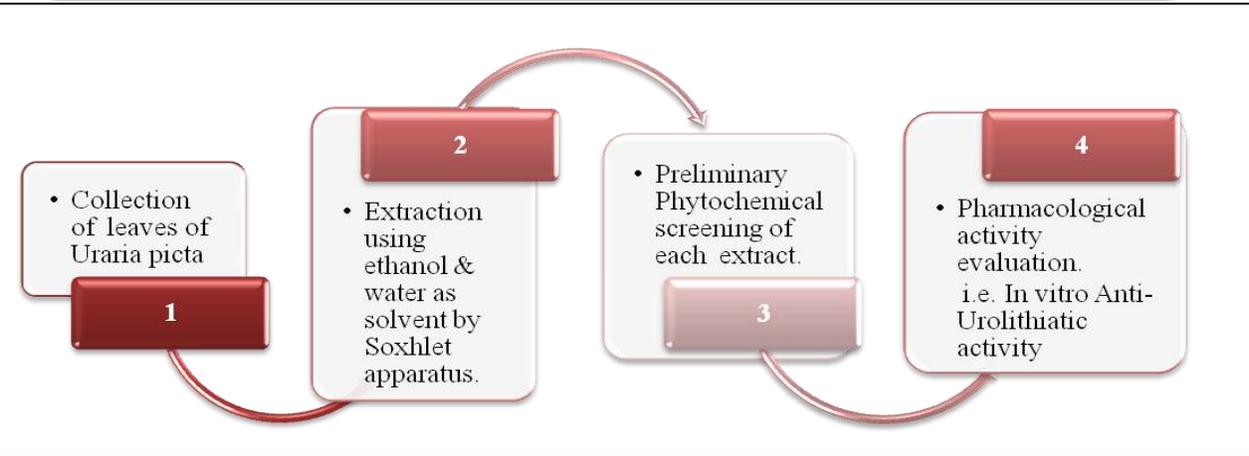
(Fig-5 : Comparision of extract dissolution %)





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Result of *in vitro* study: Ethanolic leaf extract was found to be dissolution nearer to the standard drug, Neeri and aqueous extract have little lower activity (55%) than the ethanolic extract (85%). In another method, artificial urine was used, and the extract was investigated with inhibitor & without inhibitor. The ethanolic extract inhibits crystallization followed by aqueous plant extract. The observation of both the methods showed both ethanolic and aqueous extract have significant antiurolithiatic activity.



Graphical Abstract





A Study on Online Fraud Detection Using Amazon Fraud Detector

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ABSTRACT

Cloud Computing is an emerging, rapidly developing and excellent promising technology. It describes computation, software, data access, and storage services that do not require end-user knowledge of the physical location and configuration of the system that delivers the services. Cloud computing is a natural evolution of the widespread adoption of virtualization, service, autonomic and utility computing. Amazon Web Services (AWS) offers reliable, scalable, and inexpensive cloud computing services and supports many services which are very much useful for the users; Amazon Comprehend is one of them.

Keywords: Fraud Detector, Machine Learning

INTRODUCTION

Fraud entails deception in order to obtain illegal gains; thus, it is mainly evidenced within financial institutions and is a matter of general interest. The problem is particularly complex, since perpetrators of fraud could belong to any position, from top managers to payroll employees. Fraud detection has traditionally been performed by auditors, who mainly employ manual techniques. These could take too long to process fraud-related evidence. Data mining, machine learning, and, as of recently, deep learning strategies are being used to automate this type of processing. Many related techniques have been developed to analyze, detect, and prevent fraud-related behavior, with the fraud triangle associated with the classic auditing model being one of the most important of these.

Amazon Fraud Detector provides a fully managed service that uses machine learning for detecting potential fraud in real time (e.g. online payment and identity fraud, the creation of fake accounts, loyalty account and promotion code abuse, etc.), based on the same technology used by Amazon.com—with no machine learning experience required. With Amazon Fraud Detector, customers use their historical data of both fraudulent and legitimate transactions to build, train, and deploy machine learning models that provide real-time, low-latency fraud risk predictions. To get started, customers upload historical event data (e.g. transactions, account registrations, loyalty points redemptions, etc.) to Amazon Simple Storage Service (Amazon S3), where it is encrypted in transit and at rest and used to



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customize the model's training. Customers only need to provide any two attributes associated with an event (e.g. logins, new account creation, etc.) and can optionally add other data (e.g. billing address or phone number). Based upon the type of fraud customers want to predict, Amazon Fraud Detector will pre-process the data, select an algorithm, and train a model. Amazon Fraud Detector uses machine learning models based on Amazon's 20+ years of experience with fraud to help identify patterns commonly associated with fraudulent activity. This improves the accuracy of the trained model even if the number of fraudulent examples provided by a customer to Amazon Fraud Detector is low. Amazon Fraud Detector trains and deploys a model to a fully managed, private Application Programming Interface (API) end point. Customers can send new activity (e.g. signups or new purchases) to the API and receive a fraud risk response, which includes a precise fraud risk score. Based on the report, a customer's application can determine the right course of action (e.g. accept a purchase, or pass it to a human for review). With Amazon Fraud Detector, customers can detect fraud more quickly, easily, and accurately with machine learning while also preventing fraud from happening in the first place.

Features

- Suits OFFICIAL
- Available in the Ireland region and 5 others internationally
- NCSC Cloud Security Principles aligned, Security Cleared (SC) staff available
- Connectivity options: N3, HSCN, PSN, Police (ex-PNN)
- Deploy into automated Infrastructure-as-a-Service (IaaS), Platform-as-a-Service (PaaS) architectures
- Pre-built model templates
- Trigger actions based on rules
- Real-time fraud prediction API
- A single interface to review and audit your predictions
- Amazon Sage Maker integration'

Benefits

- Build high quality fraud detection ML models faster
- Stop bad actors at the door
- Built-in online fraud expertise
- Give fraud teams more control

Use Cases**Identify suspicious online payments**

Reduce online payment fraud by flagging suspicious online payment transactions before processing payments and fulfilling orders.

Detect new account fraud

Accurately distinguish between legitimate and high-risk account registrations so you can selectively introduce additional checks—such as phone or email verification.

Prevent trial and loyalty program abuse

Spot accounts likely to abuse online services and set appropriate limits on the value of offers to minimize risk.

How it Works**Step 1**

Define the event you want to evaluate for fraud.



**Raj Kumar Mohanta****Step 2**

Upload your historical event dataset to Amazon S3 or stream and store your event data directly in AFD

Step 3

Select a model type and train your model. The service automatically inspects and enriches data, performs feature engineering, selects algorithms, trains and tunes your model, and hosts the model.

Step 4

Create rules to either accept, review, or collect more information based on model predictions.

Step 5

Call the Amazon Fraud Detector API from your online application to receive real-time fraud predictions and take action based on your configured detection rules.

CONCLUSION

Amazon Fraud Detector is a fully managed Machine Learning (ML) fraud detection solution that provides everything needed to build, deploy, and manage fraud detection models. With just a few clicks, fraud analysts can enhance model detection with business rules that help control model behavior then deploy results as production-ready APIs to start generating predictions.

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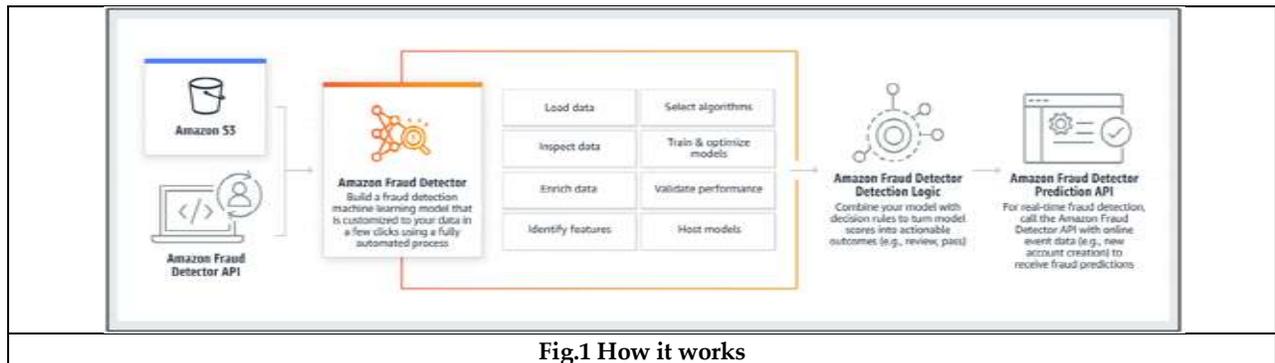


Fig.1 How it works





Role of Doxorubicin in Treatment of Cancer: Review

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ABSTRACT

Cancer is a very complex genetic disorder that is mainly caused by carcinogens. Carcinogens can be present in the air, water, food, chemicals, and sunlight that are exposed to the people. In the case of leukemia, the body produces large numbers of abnormal white blood cells. In the study of blood cancer that is leukemia, blood disorders through visual inspection of microscopic images of blood cells is an important diagnostic tool. From the identification of blood disorders, it can lead to the classification of certain diseases related to blood. Doxorubicin drug most useful for cancer treatment such as Breast cancer, ovarian cancer, Lung cancer, Neuroblastoma cancer, Leukemia, etc. In breast cancer, the efficacy of drug treatment will thus depend on the histology of the tumor tissue. In ovarian cancer, the doxorubicin metabolites accumulated in the ascites and cleared more slowly from the peritoneal compartment than from the serum. Accumulation in the peritoneal cavity with prolonged half-life should be considered when administering medication in patients with ascites. In lungs cancer, the aggregate results of the present series of studies demonstrate that RLIP76 is the predominant doxorubicin transporter in the lung cancer cell. That its transport and ATPase activity is greater in NSCLC than SCLC and that its inhibition by anti-RLIP76 IgG augments doxorubicin cytotoxicity though it's increased accumulation in cells. In neurotumor cells the doxorubicin-induced apoptosis is ceramide-mediated and whether p53 upregulation is necessary for the apoptotic response.

Keywords: Doxorubicin, White Blood Cell, Microscopic Images, Leukemia, Breast cancer, Ovarian cancer, Lung cancer, Neuroblastoma cancer, Health.

INTRODUCTION

Cancer is defined as the abnormal cells division without control and can produce immature cells. These cells can spread to other parts of the body through the blood and lymphatic systems. Cancer is not one disease, but it is a very





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complex form of many diseases. The cancer agents (carcinogens) can be present in the air, water, food, chemicals, and sunlight that are exposed to the people (Malcolm, 2001). Most cancers are named for the organ or type of cell in which they start, for example, cancer that begins in the breast is called breast cancer, cancer that begins in the ovary called ovarian cancer. WHO Cancer Control Programme is to promote national cancer control policies plans and programs, integrated to non-communicable diseases and other related problems. Our core functions are to set norms and standards, promote surveillance, and encourage evidence-based prevention, early detection, treatment, and palliative tailored to the different socioeconomic settings. The global burden of cancer continues to increase. In the year 2000, 5.3 million men and 4.7 million women developed a malignant tumor, and 6.2 million died from the disease. The number of new cases is expected to grow by 50% over the next 20 years to reach 15 million by 2020. World Cancer Report provides a unique global view of cancer. It documents the frequency of cancer in different countries and trends in cancer incidence and mortality as well as describing the known causes of human cancer. The molecular and cellular basis of the multi-step process of malignant transformation is concisely summarized. The report contains an overview of cancer prevention, including screening programs for early diagnosis, as well as advances in surgical and medical oncology, including novel drugs targeting tumor-specific signalling pathways (World Health Organization).

Cause of Cancer

Carcinogens are any substance or agent that is capable of causing cancer – the abnormal or uncontrolled growth of new cells in any part of the body in humans or animals.

Milk adding Chemical: Several milk constituents such as vitamin D, proteins, calcium, CLA, butyrate, saturated fatty acids, and contaminants such as pesticides, estrogen, and insulin-like growth factor I (IGF-I) may be responsible for either a prospective or a harmful association between dairy products and cancers. Dietary fat has been reported to increase the androgen level associated with prostate cancer risk. Dairy foods and their constituents (lactose) have been hypothesized to possibly promote ovarian carcinogenesis.

Obesity: Obesity has been linked to more aggressive characteristics of several cancers, including breast and prostate cancer. The myeloid lineage cells, in the form of myeloid derived suppressor cells (MDSCs) and alternatively polarized M2 macrophages influence almost all types of cancers by regulating diverse facets of immune suppression, angiogenesis, cell proliferation, growth, and metastasis. The different aspects of obesity, namely insulin resistance, increased estrogen, adiposity, and low-grade chronic inflammation from adipose tissue macrophages, may coalesce to promote MDSC induction and M2 macrophage polarization, thereby facilitating cancer development.

Cigarette Smoke: The various carcinogenic compounds have been identified in primary and side-stream tobacco smoke. It is a complex mixture of chemicals in tobacco smoke, including 212Pb and 210Po, react covalently with DNA and produce free radicals causing oxidative damage. Cigarette smoke exhibits very significant synergistic interactions with ethanol to induce oral/ pharyngeal cancers and with asbestos to induce lung cancer.

Alcohol Consumption: The alcohol increases the risk for cancers of the oral cavity and pharynx, larynx, oesophagus, and liver. The biological mechanisms of alcohol induce cancer are not fully understood but may include genotoxic effects of acetaldehyde, production of reactive oxygen or nitrogen species, changes in folate metabolism, increased estrogen concentration. The International Agency for Research on Cancer (IARC) and the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) both published comprehensive reviews of the scientific literature on alcohol have the risk of cancer .

Hair Dye: The permanent oxidant hair dyes have consisted of many chemical components, including ortho-phenylene-diamines (o-PD) and its derivatives, 4-chloroortho-phenylenediamine (Cl-PD) and 4-nitroortho-phenylenediamine. The carcinogenic o-PD and Cl-PD caused Cu(II) - mediated DNA damage, including 8-oxodG formation, and antioxidant enzyme superoxide dismutase (SOD) enhanced DNA damage. This results that SOD





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enhanced the rate of Cu(II) - mediated autoxidation of o-PD and Cl-PD, leading to enhancement of DNA damage and produced cancer

Chronic Infections: The stomach ulcers are due to *Helicobacter pylori*. Mycoplasmas may cause chronic lung disease in newborns and chronic asthma in adults, and *Chlamydia pneumoniae*, a recently identified common cause of acute respiratory infection. These infectious agents that cause or contribute to neoplastic diseases in humans .

Fertilizer: The higher levels exposure of nitrates or nitrites has been associated with an increased incidence of cancer in adults and possible increased incidence of brain tumors, leukemia and nasopharyngeal (nose and throat) tumors in children. The U.S. EPA concluded that there was conflicting evidence in the literature as to whether exposures to nitrate or nitrites are associated with cancer in adults and children .

Environmental Factor: Exposure to Ultraviolet-B-radiation (UVB, 280-320 nm) is known to induce basal and squamous cell skin cancer in a dose-dependent way and the depletion of stratospheric ozone has implications for increases in biologically damaging solar UVB radiation reaching the earth's surface. In humans, arsenic is known to cause cancer of the skin, as well as cancer of the lung, bladder, liver, and kidney.

Medical Drugs: Some drugs used to treat cancer (e.g., cyclophosphamide, chlorambucil, melphalan) have been shown to increase the occurrence of second cancers, including leukemia.

Genetic Disorder: Down syndrome and certain other genetic diseases - some diseases caused by abnormal chromosomes may increase the risk of leukemia.

Leukemia

In the case of leukemia, the body produces large numbers of abnormal blood cells. Leukemia is either acute or chronic. In the case of acute leukemia, produces very immature abnormal blood cells, and it cannot perform their normal functions. In the case of chronic leukemia, some immature cells are present, but in general numbers of mature cells compared to acute leukemia and carry out some of their normal functions. Leukemia arises the main two types of white blood cells. If leukemia affects lymphoid cells, it is known as lymphocytic leukemia and other affected myeloid cells known as myeloid or myelogenous leukemia .Anthracyclins are used in the treatment of various solid tumors and acute myeloid leukemia these agents induce DNA damage in leukemic cancer cells by several ways but the mechanism by which they induce apoptosis is still a matter of debate. Some evidence indicates that the generation of ceramide is an active lipid mediating cell response to various types of stress may provide a key event for anthracycline-induced apoptosis. In this respect the fumonisin B1, a fungal toxin that potently inhibits ceramide synthase can prevent both daunorubicin-induced ceramide accumulation and apoptosis in leukemia cells. In other studies, the apoptotic response elicited by doxorubicin has been related to the accumulation of a ceramide pool produced by sphingomyelinase activation. The considerable apoptotic response elicited by doxorubicin is dependent on the function of p53, a protein that is up-regulated by cell treatment with genotoxic agents that drives cell-cycle arrest or apoptosis by distinct mechanisms. The recent studies have provided evidence for a functional relationship between ceramide and p53. It is shown that p53 up-regulation may be required for the generation of the aceramide pool that mediates the apoptotic effect of some genotoxic agents in leukemia cells.

There are mainly four types of Leukemia

1. Acute Myelogenous (or myeloid) Leukemia (AML).
2. Acute Lymphocytic (or lymphoblastic) Leukemia (ALL).
3. Chronic Myelogenous (or myeloid) Leukemia (CML).
4. Chronic Lymphocytic (or lymphoblastic) Leukemia (CLL).





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Treatment approaches

Role of Doxorubicin in Treatment of Cancer: Doxorubicin trade name Adriamycin, also known as hydroxydaunorubicin is a drug used in cancer chemotherapy. It is an anthracycline antibiotic closely related to the natural product daunomycin and like all anthracycline. It works by intercalating DNA. Doxorubicin is commonly used in the treatment of a wide range of cancers, including haematological malignancies many types of carcinoma and soft tissue sarcomas. Doxorubicin hydrochloride is a cytotoxic anthracycline antibiotic widely used in the treatment of acute lymphoblastic leukemia. The mechanism of cytotoxicity involves the specific intercalation of planar anthracycline nucleus of DH to the DNA double helix resulting in the prevention of further DNA replication. Chemotherapy plays an important role in the management of cancer. As an important example, doxorubicin an anthracycline antibiotic is considered among the most active chemotherapeutic agents. However, the clinical usefulness of doxorubicin in the treatment of cancer is often limited by the development of a type of drug resistance known as multidrug resistance (MDR). MDR is a term used to describe a phenomenon characterized by the ability of some tumors to exhibit simultaneous resistance to several structurally and functionally unrelated chemotherapeutic agents.

Chemistry and Structure-Activity Relationships: The anthracycline antibiotics have a tetracyclic ring structure and attached the unusual sugar, daunosamine. Cytotoxic agents of this class having quinone and hydroquinone moieties on adjacent rings that permit the gain and loss of electrons. Although there are marked differences in the clinical use of doxorubicin, their chemical structures differ only by a single hydroxyl group on C14.

ADME of Doxorubicin

Absorption: Doxorubicin is not absorbed by the gastrointestinal tract. Since the drug is extremely irritating to tissues, it has to be administered by intravenous. It is Soluble in water, slightly soluble in methanol, practically insoluble in chloroform, ether and other organic solvents.

Distribution: Doxorubicin is quickly and widely distributed into the extravascular compartments and half-life 12-18 hours. Binding of doxorubicin to plasma protein is about 75%. However, the doxorubicin does not cross the blood-brain barrier.

Metabolism: Doxorubicin is mainly metabolized in the liver. The major metabolite of doxorubicin is 13-OHdoxorubicinol, produced by aldo-ketoreductases which possess a certain degree of antitumor activity.

Excretion: Following IV administration, plasma levels of doxorubicin follow a multiphasic decline, with a terminal half-life. Doxorubicin is metabolism in the liver and excretion through biliary and fecal excretion. The terminal half-life of 13-OHdoxorubicinol is similar to that of doxorubicin. Plasma clearance is in the range of 324 to 809 ml/min/m². Doxorubicin is eliminated by metabolic conversion to a variety of aglycones and other inactive products. A liposomal doxorubicin product (DOXIL) is available for the treatment of AIDS-related Kaposi's sarcoma. Newer Analogs of Doxorubicin: Valrubicin (VALSTAR) was approved in 1998 for intravesical therapy of bacilli Calmette-Guerin- refractory urinary bladder carcinoma. Epirubicin (4- epidoxorubicin, ELLENCE) was approved by the FDA in 1999 for adjuvant therapy of early lymphnode-positive breast cancer.

Mechanism of doxorubicin

Breast Cancer: In the case of breast cancer doxorubicin shows nuclear fluorescence distinguishable from background fluorescence, which is predominantly from the cytoplasm. Each patient both doxorubicin distribution patterns and CD31 immunohistochemical staining of the same area of the same section are represented. The doxorubicin gradients in tumor islets with high concentrations in the periphery and low concentrations in the center of the tumor islets. The drug gradients were cleared shortly after the injection, but it was detected after 24 h. These doxorubicin gradients were not detected in the connective tissue. Also, no clear gradients were observed in patients with invasive lobular cancer with more connective tissue and strands of tumor cells occasionally; connective tissue showed bands of fluorescence. The dose of doxorubicin in this chemotherapy consisted of moderately high-dose doxorubicin (90





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mg/m² body surface. The efficacy of drug treatment will thus depend on the histology of the tumor tissue. Ovarian Cancer: In the case of ovarian cancer anthracyclines have been in clinical practice since the 1960s and represent one of the most commonly used classes of anticancer drugs. Doxorubicin is highly protein bound and does not cross the blood-brain barrier. Effectiveness and toxicities associated with drugs are partly related to their distribution in the various body compartments. In -vivo, doxorubicin is extensively metabolized and excreted in the bile and produced biotransformation products identified such as doxorubicinol (Dox-ol), 7-deoxydoxorubicinolone (7d-Dox-ol-on) and 7-deoxydoxorubicinone (7dDox-on) were measured using high-performance liquid chromatography. Doxorubicin metabolites accumulated in the peritoneal cavity. The concentrations of the doxorubicin metabolites were initially higher in the serum compared to the ascitic fluid but the following several hours the doxorubicin metabolites became higher in the ascites and remained detectable in the ascites for up to 168 h long after disappearance from the serum. The doxorubicin metabolites accumulate in the ascites and are cleared more slowly from the peritoneal compartment than from the serum. Accumulation in the peritoneal cavity with prolonged half-life should be considered when administering medication in patients with ascites.

Lung Cancer: In this carcinoid, tumors are an uncommon type of tumor that starts in the lungs. They tend to grow slower than other types of lung cancers. They are made up of special kinds of cells known as neuroendocrine cells. RLIP76 (ral interacting protein) function as an ATP –dependent transporter of an amphiphilic drug such as doxorubicin 80 as well as glutathione- conjugates of endogenous electrophonic toxins such as 4- hydroxyonenal (4HNE). Present studies were performed to determine the relationship of the RLIP76 ATPase activity with doxorubicin and 4- HNE resistance in a panel of 13 native human lung cancer cell lines. Results of the present studies show that the specific activity of RLIP76 ATPase correlates with resistance to both an anthracycline and an alkylation agent in lung cancer cell lines and suggest the possible use of RLIP76 ATPase activity as a predictor of chemotherapy sensitivity of lung cancer.

We found that the specific activity of highly purified RLIP76 ATPase from six SCLC (small cell lung cancer) cell line were approximately half that observed for seven NSCLC (non-small cell lung cancer) cell line, including three adenocarcinoma, two squamous cell carcinoma, one bronchioalveolar carcinoma and one large cell carcinoma. The aggregate results of the present series of studies demonstrate that RLIP76 is the predominant doxorubicin transporter in lung cancer cell 86 that its transport and ATPase activity is greater in NSCLC than SCLC and that its inhibition by anti-RLIP76 IgG augments doxorubicin cytotoxicity through its increased accumulation in cells. Neuroblastoma Cancer: In Neuroblastoma is a form of cancer that starts in certain types of very early forms of nerve cells found in an embryo or fetus. The term neuro refers to nerves, while blastoma refers to cancer that affects immature or developing cells. This type of cancer occurs in infants and young children. It is rarely found in children older than 10 years.

Doxorubicin is used in the treatment of neuroblastomas, and a large number of neuroectodermal tumor cell lines has been reported to undergo apoptosis after administration of shortchainceramide. The ceramide generation plays any role in the apoptotic response elicited by doxorubicin in neurotumor cell is unknown. Elucidation of this point is of prominent the chemotherapy could be supported by agents that block ceramide metabolism. Thus, maintaining the active lipid at elevated intracellular concentrations. A further important issue is whether the apoptotic response elicited by doxorubicin is dependent on p53 function. The present study was investigating whether, in neurotumor cells, doxorubicin-induced apoptosis is ceramide-mediated and whether p53 up-regulation is necessary for the apoptotic response. We are used as model systems CHP-100 neuroepithelioma and SH-SY5Y neuroblastoma cells, two lines derived from human neurotumors undergo apoptosis after treatment with exogenous ceramide and respond differently to doxorubicin treatment concerning p53 up-regulation. The drug doxorubicin is successful advent into the pharmaceutical market has introduced to the research community; these drug target for the development of novel anticancer drugs. However, the drug produced cardiotoxicity, but newer alternatives formulation provide advantages of limited toxicity, better activity, activity against different type cancer therapy, which can be used for the benefit of mankind.





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Summary

The aggregate results of the present studies demonstrate that RLIP76 is the predominant doxorubicin transporter in a lung cancer cell that its transport and ATPase activity is greater in NSCLC than SCLC and that its inhibition by anti-RLIP76 IgG augments doxorubicin cytotoxicity through its increased accumulation in cells. The efficacy of drug treatment will thus depend on the histology of the tumor tissue. The doxorubicin metabolites accumulate in the ascites and are cleared more slowly from the peritoneal compartment than from the serum. The drug doxorubicin is successful advent into the pharmaceutical market has introduced to the research community; these drug target for the development of novel anticancer drugs.

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CONFLICTS OF INTEREST

The authors of the article have no conflicts of interest to declare.

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Sushree Sambita Swain *et al.*,

Doxorubicin is efficiently used in chemotherapy, maintenance therapy and recurrence therapy in cancer management.



Doxorubicin intercalate with DNA and inhibit macromolecular biosynthesis.



Inhibits the progression of the enzyme topoisomerase II (which relaxes super coils in DNA for transcription)



Stabilizes the topoisomerase II complex after it has broken the DNA chain for replication.



Preventing the DNA double helix from being resealed and thereby stopping the process of replication.

Mechanism of Doxorubicin





Simulation for Argon Ion Exposure to Layered Calcium Oxide

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ABSTRACT

The Monte-Carlo simulation called SRIM was used to study the damage caused in the regular structure of the material when argon with an energy of 60 keV was irradiated on calcium oxide (CaO). The simulation is done to know the distribution of ions, recoil distribution, energy to recoils, and ionisation data, which will support the experimental data obtained after irradiation.

Keywords: Monte-Carlo simulation, Calcium oxide, Argon ion, SRIM, irradiation.

INTRODUCTION

Calcium oxide is a white coloured powder and is commonly known as quicklime or burnt lime or simply lime. Calcium oxide can be produced by thermal decomposition of materials like limestone or seashells that contain calcium carbonate (CaCO_3 ; mineral calcite) in a lime kiln [1]. It is often used as a desiccant and evolves heat when it is hydrated. CaO is most abundantly found in the nature as limestone and dolomite. CaO is also obtained from biomaterial sources for instance from crab shell, egg shell and oyster shell. Calcium oxide is commonly used in different industries as a catalyst, adsorbent and a remediation agent for toxic-waste[2]. Moura *et al.* derived calcium oxide (CaO) from waste shell materials and used it as catalysts in the transesterification process for biodiesel synthesis [3]. Calcium oxide is also used in the production of porcelain, glass, calcium carbide, calcium cyanamide, refining sugar and bleaching powder. It can be found in water softeners, as well as mortar and cement. It's also utilised to treat soil that's too acidic [4]. In this study the simulation of the irradiation of low energy argon ion on calcium oxide is done in order to know the effect of collision of the ions on its properties.

METHODOLOGY

In this study, A Monte-Carlo simulation known as SRIM was used with an objective to find out the changes in calcium oxide target molecules when bombarded with argon ions. Simulations are made here to understand the effect of argon ions on calcium oxide as a target molecule. The experiment will be performed after gathering sufficient data from the SRIM simulation.





RESULTS AND DISCUSSION

TRIM, a part of the SRIM programme [5], can be used to calculate the stopping and range of ions. The damage is calculated after a detailed analysis of the entire damage cascade. Recoil ions are expected to be distributed in the XY plane. The incidence angle is fixed at 0 degrees. The TRIM setup window is used to provide information regarding ion kinds, targets, and TRIM computations. The target in this case is CaO, and the ion data is argon ion. The collision took place at a very low energy of 60 keV. Argon ions of 60 keV energy collide perpendicularly with target atoms such as calcium and oxygen in this illustration.

Ion Trajectory

Figure 1 shows the trajectory path of the argon ions of 60 keV energy when bombarded on the surface of calcium oxide target. The red dot in the graph denotes the collision between the argon ions and the atoms of the target CaO molecules. The green coloured dots represent the vacancies formed by CaO atoms. About 1000 vacancies are generated by the single recoil atom represented by green dots but ions produce only one vacancy (red dots). When the argon ions bombarded with the CaO target molecules hard, some quantity of energy is lost from the system. From the plot it is clear that some of the ions appear to be leaving the target and deviates its path.

Ion and Atom Distribution

Figure 2 (a) illustrates the ion distribution of 60 keV argon ions and its parameters viz. mean range, straggling, skewness, and kurtosis. Here, the target depth is set to 1 μ m in order to forecast maximum ions in the plot. In the figure, the X-axis represents the target depth and the Y-axis represents the concentration of ions. The figure shows that the average range of ions in CaO target molecule is 1192 Å and the concentration of ions in the target at the target depth 1192 Å is more than 7×10^4 atoms/cm³. A two-dimensional plot of the recoil distribution of the atoms of target molecule CaO is shown in figure 2 (b). This graph illustrates the number of atoms that recoiled back at the depth of the target. In the graph, the orange colour curve represents the recoil distribution of Calcium atoms and the blue curve represents the recoil distribution of oxygen atoms. From the above graph, it can be elucidated that maximum CaO atoms are removed from their lattice site when a 60 keV of argon ions are applied and penetrated through the atoms and resulting vacancies. From the figure 2(b), it can be interpreted that about 30×10^6 atoms / cm³ or more number of Calcium atoms recoiled, and the average number of 25×10^6 atoms / cm³ oxygen atoms are recoiled at target depth of about 1000 Å.

Energy to Recoil Distribution

The recoil distribution to energy for 60 keV energy is shown in Figure 4. This diagram depicts the effects of ion irradiation on the target. The energy transmission from ions to recoils is depicted in this diagram. The energy received by calcium atoms is shown in orange, while the energy absorbed by oxygen atoms is shown in blue. In comparison to the oxygen atom, which has received the maximum energy of 15 eV / ion, the recoil calcium atom absorbs the maximum energy of 22 eV / ion.

Collision Events

Figure 5 depicts the two-dimensional plot for collision events. In the plot, Replacement collisions, target vacancies and target displacements are shown in green blue and red colour respectively and showing that the replacement collision is comparatively less than the target displacements and vacancies. The target displacements and vacancies are almost same at the target depth of about 1000 Å whereas the replacement collisions are very less throughout the target depth.

CONCLUSIONS

In this paper, we present a Monte Carlo simulation for the irradiation of calcium oxide with low energy (60keV) argon ions in order to investigate the changes in CaO characteristics after irradiation. Before conducting an ion





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irradiation experiment on any material, a simulation study is highly important. Here, we have calculated the ion range, ion and atom distribution, energy to recoil distribution, and collision events. This study will be extremely useful in the experimental technique of bombarding CaO molecules with argon ions.

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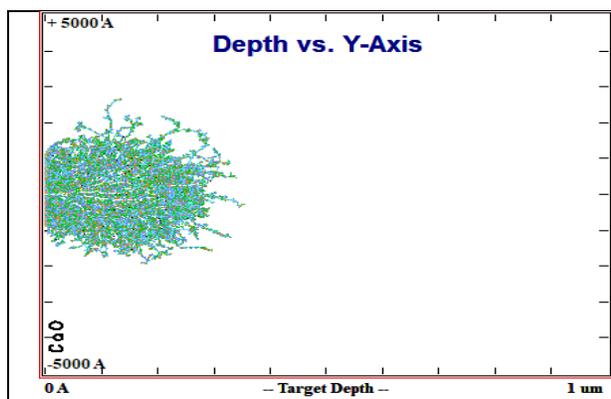


Fig.1 Ion trajectory of 60 keV argon ions on CaO target

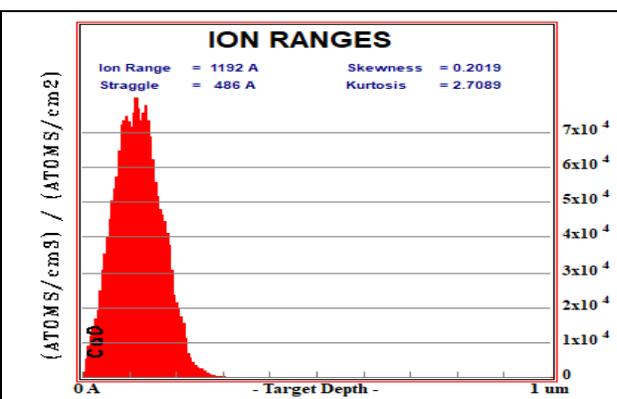


Fig.2 (a) Distribution of argon ions (60 keV)

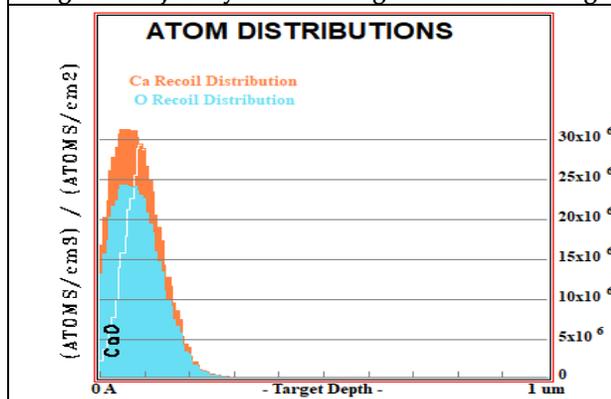


Fig. 2 (b) Atomic distributions of CaO after argon ions (60 keV) exposure

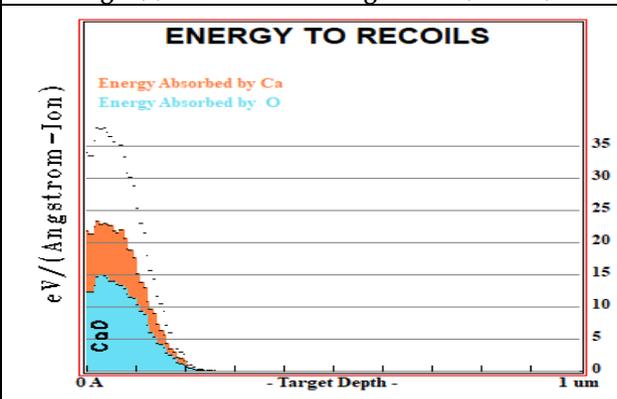
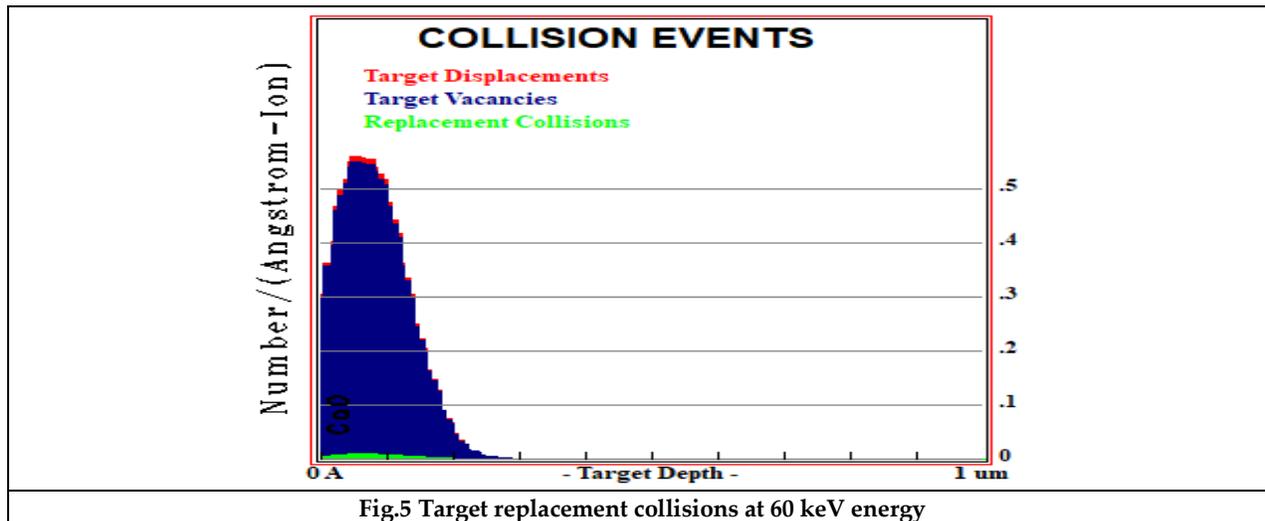


Fig.4 Energy absorbed by different target atoms





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The Effect of Language Club on Speaking Ability of Students in Centurion University of Technology and Management: A Case Study

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ABSTRACT

The present study discusses the effect of the language club established at Centurion University of Technology and Management, Paralakhemundi, Odisha, India. The language club is a platform where students learn with fun and often this platform motivates them to learn by themselves. The activities in the language club were performed beyond study hours, which make students quite comfortable and enjoyable. The present study is an experiment based on which the changes that have occurred with the inmates of the language club over a period of time were thoroughly monitored. Qualitative and quantitative approaches were carried out to analyse the results of the study. The contents of the literature review were used for the qualitative purpose and the results of assessments made on speaking were used for quantitative purposes. A total of 100 students from different schools of the University were taken for the study and these students were regular participants in the activities of the club. The findings of the study have shown that the Language Club had truly made a commendable impact on the English language learning of the students. The students were able to achieve remarkable enhancement in their linguistic skills especially those concerned with reading, writing and speaking in addition to acquiring and improving inter and intrapersonal skills including teamwork and leadership abilities. This micro case study will Surely be helpful towards greater achievement in the area of English language proficiency and will certainly pave a way for building social networks through profound communication skills.

Keywords: Language club, qualitative, quantitative, speaking, communication skill





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INTRODUCTION

Background of the Study

The study was carried out at Centurion University of Technology and Management, Paralakhemundi, Odisha which has a rich eco-system catering to the needs of the present-day education requirements of students. The educational environment of the university is very dynamic in nature. Often students face challenges in their correct way of speaking and writing in English. The students undoubtedly attend their regular class activities but often they remain isolated from each other which may be due to various factors such as their family background, lack of knowledge of the subject, their poor self-regard and poor language skills including ignorance. Other problems include students' inhibition, nothing to say, low participation, and problems in pronunciation, lack of vocabulary and lack of confidence. It is the responsibility of the concerned teacher to find out different strategies and impart new teaching methodologies for improving the speaking skill of the students. The content of course, teacher's knowledge and understanding are not the only requirements in the learning environment but include many other aspects. These problems have prompted the researchers to plan for language club activities beyond the study hours. As Mulyasa (2007) stated that the language club is an extracurricular programme that is held beyond study hours that enhances the students' competence. The researchers thought of introducing this platform in the university with the aim of providing peers' support to students along with recreational and consolidated opportunities further creating awareness among themselves as individuals possessing special talents and capacities. Such an environment can provide ample opportunity to all students to practice English Language skills in many different ways, and equip them in speaking, listening, writing, reading and analytical skills and additionally provide them with a platform to galvanize them into positive energy thereby polishing their innate skills through debating, learning public speaking, writing and even socializing with other members. In the present study, the researchers tried to identify the effect of Language Club on the English proficiency of 3rd semester batch of 2020-2021 students of Centurion University.

Research Question.....?

- What impact does the Language Club programme have on speaking achievement of 3rd semester students of Centurion University of Technology and Management, Odisha, India?
- Are there any remarkable differences between the students who join the English club program and those who do not?

Research Objective

- To examine the effects of Language club program on the students' speaking achievement at the third semester of Centurion University of Technology and Management, Odisha, India ?
- To know the differences between the students who join the English club program and those who do not.

LITERATURE REVIEW

The effectiveness of the language club is presented in various articles. The formation and establishment of language clubs are quite common in university language centres which can be organized in many different ways. It is a casual setting platform that shares the characteristic which involves learners with an opportunity to practice their language comfortably (Ewens, 2013). It is called a "participant-centred" platform. According to Lewis(2001), when learners share responsibility with other colleagues and teachers and have the opportunity to choose their own learning materials, they become more motivated and engaged. Adopting learner-centered activities is a vital way to motivate and engage language learners (Dornyei & Csizer, 1998). Allowing learners to choose the materials according to their own interests will foster intrinsic motivation (Bell, 2010). Language clubs not only motivate high achievers but also weak learners. It will be a great motivating factor for the weak students (Casey, 2008). According to Ewens (2013),



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the language club is a platform where all the activities are flexible, unplanned and interactive, and learners are free from the constraints of exams, syllables, and rubrics. The way language activities are presented generally affects students' level of motivation and interaction.

Language clubs are meant for holding interactive sessions that include various activities such as oral presentations, student intervention and entertainment which are preferred by language learners (Morell, 2004). The important dimensions of language learning are free interactions and learning from each other that motivate language learners and encourage them to be more active participants (Lightbown & Spada, 1993). These clubs act as a powerful vehicle for motivating engaged and interested learners' along with motivating the "disengaged and frustrated" ones (Casey, 2008). According to few other researchers, the club provides ample opportunity to all students to practice English language skills in many different ways, and equip them with speaking, listening, writing, reading and analytical skills, give an opportunity to vent to students' creative talents, provide an encouraging atmosphere for students to express personal views about whatever they choose, enhance students' learning opportunities and contribute towards the development of personality of students.

English Club Program

In this research, English club program is defined as students' extra learning program besides regular English class, which focuses more on mastering students' speaking skill. This program was introduced at 3rd semester of 2020-2021 batch students of Centurion University of Technology and Management and was held every Friday from 6 pm till 8 pm. The following were the details of the club.

RESEARCH METHODOLOGY**Research Design**

Based on the statement of research questions above, the design of this study was an experiment research.

Participants

The participants for this study were taken from the Centurion University of Odisha, India. There were 100 students from different schools like M.S. Swaminathan School of Agriculture, School of Fisheries, School of Applied Science and School of Vocational Education and Engineering etc. from batch 2020 -2021 Academic years.

Research Variable

In this study, the independent variable is the English club program and the dependent variable is the students' speaking achievement.

Instruments of the Research**Observation**

In order to obtain the effectiveness of the English Language Club, the researchers closely observed the active participation of the students during the speaking activities. All the speaking activities were observed on four criteria such as body language, use of vocabulary, correctness of language and fluency.

Opinion Survey

In order to obtain the effectiveness of the club, the researchers did a survey and found valuable opinion from the regular club students. The data from the opinion survey was analysed to describe the effectiveness of English club program.

Speaking Test

The researchers conducted several speaking tests and found the progress of the students and could know about the effectiveness of the Language Club. The data from speaking test was analysed to identify the effects of English club



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program on students' speaking achievement. This data was also used to show the differences between the students who join the English club program and those who do not.

Sharing Of Experience By Old Students

The researchers also gathered valuable opinion of the old students and found about the effectiveness of the Language Club on their career. Their opinions added effectiveness to the case study.

Data Collection

In the study both qualitative and quantitative data were collected. The qualitative data was found by literature review, observation, and interview. The quantitative data was found by analysing two years' speaking test score of the students. The first data was collected from observation. The objective of the exercise was to find out the activities of English club program and to know the teaching-learning activities clearly. The second data was collected from the student's opinion survey. 100 students responded to the questionnaire that was framed by the researchers. The following questionnaire was given to students to collect their opinion.

1. Does the Language club improve the confidence of the students?
2. Does the Language club enhance speaking and reading ability among the students?
3. Does the Language club motivate students to improve themselves?
4. Does the Language club help students to learn from their peers?
5. Does the Language club help students groom themselves?
6. Does the Language club create for learners a fun-filled environment?
7. Does the Language club encourage students to ask questions?
8. Does the language club help students enhance their language ability?
9. Does the Language club help students to enhance their organisational and team spirit abilities?
10. Does the Language club help students to be free from all stresses?

The third data was collected from speaking test. The data from speaking test was also used to show the differences between the students who join the English club program and those who do not. After completion of each speaking session, the participants were asked to appear for the speaking test. Students were asked to participate in the Josh Talk, JAM and GD session. The result of the speaking test showed that students were progressively developing themselves and there have been a remarkable improvement in the scores from the past to the present test.

The fourth data was collected from old students who shared their experience. The students mostly emphasized on the importance of English Club in enriching their speaking and writing skills. During experience sharing, they were asked different questions on the effectiveness of language club and their valuable opinions were collected.

FINDINGS AND DISCUSSION**RESULTS OF THE OBSERVATION**

The performance of students in the clubs was observed very closely. The researchers closely observed the active participation of the entire student and created an assessment rubric and recorded all the performances of the participants. In order to have the right assessment for the speaking activities, the researchers recorded few of the sessions. The observation was recorded based on the following criteria:

Results of the Survey Questionnaire

The participants of the Language Club were asked few questions and the responses were gathered in the following way.



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Figure 1 depicts that out of 100 students, 90 students agree that through language club their confidence have increased, 80 students agree that the language club platform is a fun-filled activity and they always eagerly wait for attending the club, 92 students learn to ask questions, 70 students agreed that language club is a platform to learn from peers, 70 students say that this platform motivates them to learn from themselves and 85 students agreed that through this platform their speaking ability have been enhanced.

The Result of Students' Speaking Achievement

The above figure 2 explains about the comparative result of the speaking activities of the students. According to it, students who were regularly participating were scoring well. As we can understand from the figure that out of 100 students those who were participating club activities regularly 70 students were scoring above average score and 20 students were scoring average and only 10 students were scoring below average score. The result was opposite for the students who did not participate in the language club activities. From the histogram, it is clear that above average performance students were only 8 which was very few, average were 40 and below average students were 52. The assessment was done based on fluency and coherence, grammatical range and accuracy, lexical research and pronunciation. Students who scored above average fulfilled all these criteria, they were having good accent, able to comprehend well, mastered with vocabularies and maintaining fluency and coherence.

Differences Observed Among The Students Who Joined The Language Club Program And Those Who Did not

In order to understand the effectiveness of the club the researchers conducted speaking and writing for the students who joined the Language club and students who did not. The result of the speaking test conducted for both groups showed that there were differences among the students who joined the English club program and those who did not in pronunciation, grammar, vocabulary, fluency, and comprehension. The students of the English club program had better skills than the students who did not join the club.

Experience Shared by Old Students

The old students have shared their experience about the effectiveness of the language club in the following ways: Student 1 shared that language club is a platform to unleash ones talent in the field of English speech, vocabulary, writing abilities and thought process which is therefore practiced in the same place. Language club helped her to build up the confidence that she possesses now and she can work confidently in the corporate world. Students 2 shared that through language club he could learn to express and interact. Initially he was very shy and remaining isolated but after joining the club, he became relaxed and confident. His presentation skill improved a lot and during placement drive he was being selected.

CONCLUSION

Based on the data analysis above, it can be concluded that the introduction of the Language club program at the University has given a tremendous impact on the student's language learning and engagement process. The research tools that were applied in this study gave clear information that a Language club is an appropriate platform for any foreign language. The researchers from their observations have found that the students who regularly participated in the club activities could completely change themselves. Initially, they were remaining isolated and nervous lacking confidence but later they were found to be very relaxed and confident. The old students also shared their experiences of how the club helped them to grow up and how they got jobs in the different corporate sectors. Later, they also expressed how they utilised their learning in the corporate sector to get success. Students also improved their speaking skills by participating in the regular speaking activities. It was clear from the study that the students of the English club program had better English speaking achievement than the students who did not join the English club program. It truly creates a good atmosphere and enjoyable learning activities outside the classroom. It is obvious that an enjoyable and stress-free language learning situation is perhaps all the spark that is needed to kindle a fire of interest. Once students have an interest in learning they can achieve anything. The club could act as the vehicle to





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drive that spark of interest. The club itself cannot become successful without the support of the teachers who can serve as the gatekeepers to plan and execute the activities. With the right mechanisms in place, the language club can be a vibrant and dynamic place for language learning. This small case study could serve as a catalyst for more research in the area.

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Table 1: Result of the observation

1st phase observation			
Body Language	Vocabulary	Correctness of Language	Fluency
Participants of the club were very shy, nervous and not maintaining eye contact during their speaking activities.	Participants lacked vocabulary and they could not frame up proper sentences. Participants were aware of only limited words.	Participants of the club were found committing errors in the use of pronunciations, grammar, subject verb agreements and they were not using appropriate words.	Participants were found to be having lot of pauses and fumbling while speaking.

2 nd phase observation			
Body Language	Vocabulary	Correctness of Language	Fluency
Participants could improve their body languages. They found to be very relaxed, comfortable, maintaining eye contact, used appropriate gesture and posture.	Participants could use wide variety of words and learnt to use them in different context. They learnt to use appropriate words while speaking and writing.	Participants could minimise their errors. They improved their knowledge on grammar, vocabulary and sentence construction.	Participants could deliver their speaking activities without any pauses. All of them improved their pronunciation and accent.





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Image 1: Language Club Activities

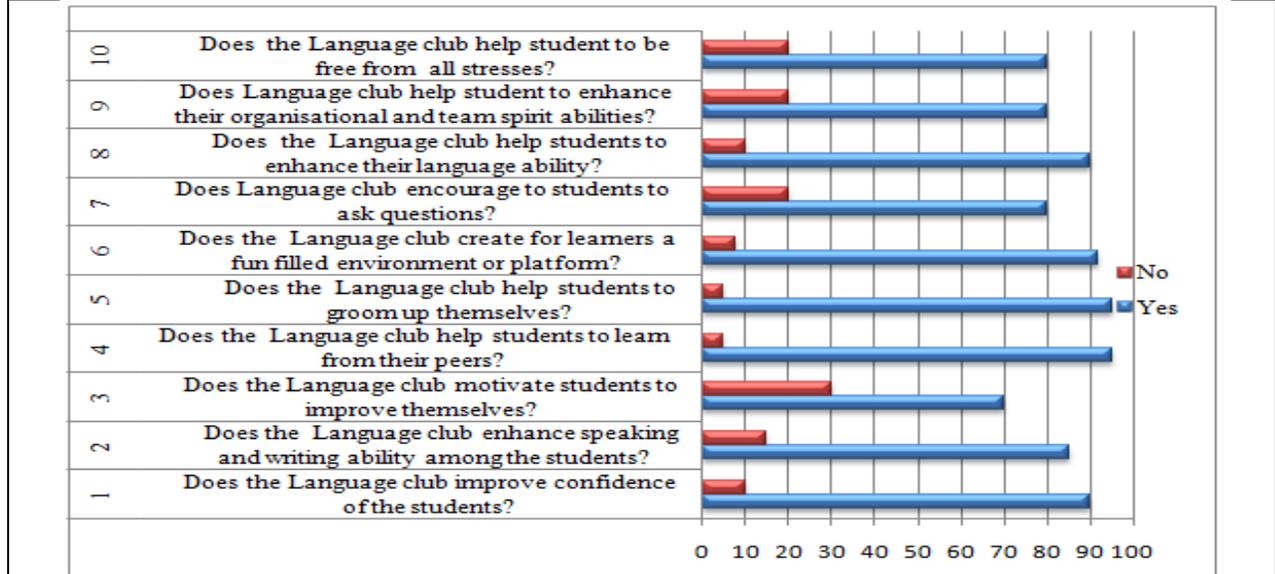


Figure 1: Report on Survey Questionnaire

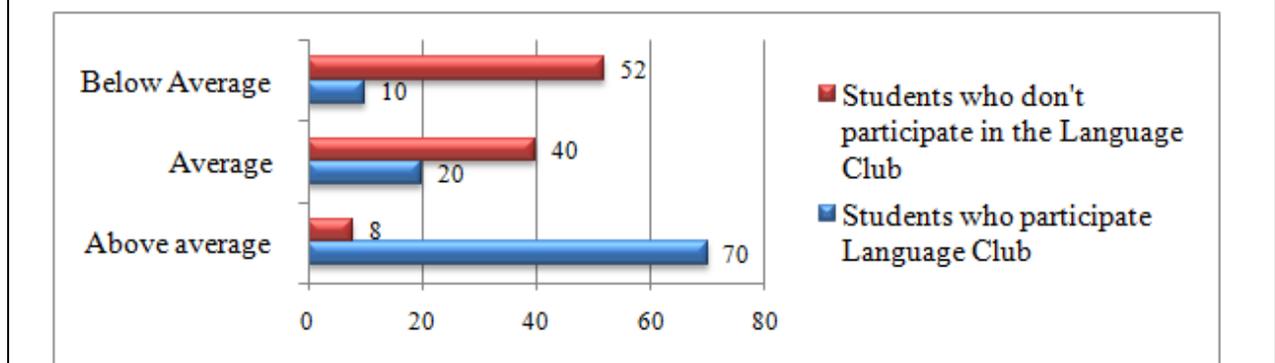


Figure 2: Result of speaking activities





Auditing the Connectivity and Freight Drive in Vijayawada Corridor: Bharatmala Pariyojna, India.

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ABSTRACT

National Highway Authority of India (NHAI) under the jurisdiction of the Ministry of Road Transport and Highways (MoRTH), the Government of India, entrusted to enhance efficiency by investigating the economic corridors, intra-corridors, and feeder routs as part of their endeavor and initiating Project proposals in India. NHAI, Feb 2018, instructed its consultants to prepare a road safety audit for new constructions, upgradation, deletion, and renovation under Bhartamala Pariyojana. The proposals aim at efficient freight movement. The present study envisages improvement of direct connectivity among Indian cities, inter-alia two Greenfield connections from Mancherial to Warangal (120 Km), and Khammam to Vijayawada (Kanchikacherla, 65 Km) under Nagpur – Vijayawada Corridors (NH-65) The road audit has executed through traffic study(Origin-Destination Survey, Axle load Surveys), engineering survey (GIS, RS and GPS), environmental impact assessment, the project's design, drawing has been under formulation with detailed cost estimation; the financial viability of the two corridors investigated considering the base year 2019. The economic, socio-economic profile and social development proposals by screening social and environmental aspects are the focused areas. The feasibility of the audit study reports aided with traffic survey, socio-economic analysis, and environmental and social screening of the feasibility and management of the two projects in corridors of the newly formed, rapidly developing Telangana state amid Naxalites activities. Nagpur to Vijayawada corridor needs to be constructed as fourlanes with paved shoulders(later 6 or 8lanes), build-own-operate-transfer (BOOT) on PPP mode for 30 years of concession period including two construction years.



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Keywords: Connectivity, DPR, Economic Corridor, Freight Movement, NHAI, socio-economic, Telangana state.

INTRODUCTION

MoRT&H (Ministry of Road Transport and Highways), GOI (Government of India), have decided to increase the efficacy of goods in transit movement in India. "The National Highways Authority of India" (NHAI) has been entrusted with the preparation of a road safety audit (RSA) in form of a detailed project report (DPR) to develop the boulevard networks in the newly formed state of Telangana. NHAI entrusted with the development process of National Highways (NH). The Authority has shouldered the responsibility to undertake the Economic, Inter connective corridors, and Feeder Routes principally to develop both intrinsic and extrinsic high freight movement in the India that will enhance GDP (gross domestic product), Verma *et al.*, 2021[1]. The TOR and Technical specifications prepare a draft road safety audit (RSA). The report narrates all that is needed for concurrence of the project for obtaining the administrative approval at the Government level before approval of renovation of the project from 4 lane to 6-lane. The reports include a pre-feasibility report, Survey and Investigation data (S&I), and Road safety audit in form of an appraisal of a DPR along with an EIA (environmental impact assessment) study report for clearance of the proposed project. The traffic studies, design of the road, appurtenant structures, demand forecast, land acquisition, resettlement, and rehabilitation (R&R) cost with the annual rate of return (ARR) and cost-benefit (B/C ratio) ratio. The Project Road bifurcated into the following two packages (Fig 1):

Package I: From Ch. 00 + 000 to Ch. 55+000, Design Length = 55.000 Km

Package II: From Ch. 55+000 to Ch. 111+762, Design Length = 56.762 Km

Proposals for rehabilitation, widening, and renovation of the existing roads and structures including cross-sectional elements, proposed promoters like flyover/underpasses/ service roads, Truck Lay byes, Toll Plazas, etc., are also needed to be designed and cost estimated. The economic & financial analysis and the PERT and GNAT chart need to be prepared for procurement & packaging for the road project. Before the preparation of the audit report, the Regional Officer, NHAI, Hyderabad, and Project Director, NHAI, Warangal visited the site for finalizing the proposed alignment of the Mancherial - Warangal project on 26 Dec 2018, (Govt of Telangana, 2022[2]).

The Study Area

The Project corridor runs North-South between latitude 18°51'4.60"N longitude 79°31'14.24"E and lat. 18° 2'52.11"N, and long. 79°41'5.14"E. Entire length passes through plain and rolling terrain. The project road is part of the Nagpur-Vijayawada corridor, starting on the existing NH-63 (Mancherial – Chinnur) at Km 176+600 (Mancherial – Chinnur) section, overlapping with NH-63 for a length of 3.8 Km and Ending at Km 155+700 on NH - 163 Warangal Eturnagaram Road, near Orugonda village. The total design length is Km 111.762 (Draft EIA. Enviro Infra Solutions PVT. LTD to NHAI [4]). This stretch of the proposed project passes through Bhupalapalli, Jayashankar, Mancherial, and Pedapalli, within the rural district of Warangal in the Telangana State. The Vijayawada Corridor Project runs through Morrurum strata (Lateritic soil) or silty sand, clayey, and at some places, are black cotton soil except for a few places' hard strata. The location map of the project highway shown in (Figure 1).

Objectives of MORT&H and NHAI:

MoRTH and NHAI an apex organization of GOI deal with roads and highways formulation, transport, safety, and administering vehicle standards, conjoint with various central and state organizations to increase the mobility, and efficiency of rising people and urban in India. NHAI is entrusted with the responsibility of planning, developing, and maintaining the international highways and expressways. NHAI formulates the standard specifications for the roads, Vehicles, and bridges in the country and serves as a repository of technical knowledge on roads and bridges.



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The NHAI fixes the accountability of realizing its expanded flagship program, like NHDP (National Highways Development Project) comprises of seven phases of projected financial involvement of outlay of about Rs. 6000 billion INR.NHDP's. The prime focusing areas needs development should be of transnational standard roads with amenities for incessant traffic flow. They are (i) Boosted safety features, (ii) Healthier Riding Surface (iii) Superior Road Geometry (iv) Improved Traffic Administration, and Perceptible Signage, (v) Alienated carriageways, and Service roads, (vi) Over bridges and Underpasses, (vii) Grade separators, (viii) Bypasses, and (ix) Wayside services

Major Economic and Social Benefits of the NHDP are (i) Vehicle operation with cost-saving, (ii) saving of Travel time, (iii) saving of Fuel, (iv) Fast trade especially the carriage of delicate/perished goods, and (v) Saving on running and maintenance costs, (vi) Reduced accidents and (vii) Area development. Considering the flagship, social, and economic benefits, and the status of roads is in (Table 2)

Reporting Structure

Exhibits the various studies & investigations carried out, preliminary proposals, cost estimates, conclusions, recommendations & summaries of the projects in detail. The work involves over viewing of NHAI and MoRTH's activities, project financing & cost recovery mechanism. Auxiliary studies are:

Socio-Economic Profile - Gives the state Geography, administrative, demography & Economic information of the project area.

Engineering Surveys and Investigations – Gives Existing Road Details

Indicative Design Standards - Cites the Proposed Design Standards needed for the adoption of the proposal for the project road.

Traffic Surveys & Analysis – Presents the current traffic scenario, growth rates & demand estimates including, Axle Load surveys, Origin – destination (O-D) Survey, Turning Movement and Delay estimates, etc.

Project Development Proposals – explains the preliminary proposals for the road Section include cross-sectional element rehabilitation / widening of existing structures, proposed truck lay byes, bus stops, preliminary land acquisition etc.

Social Impact assessment - In this section an attempt made to present the social profile of the project area, highlighting administrative units, also the profile of the project affected area population by demographic, social, and economic characteristics.

Environmental Screening & Preliminary Environmental assessment - Environmental Screening Report outlines the methodology used to screen the proposed road development in the two stretches stated above for the requirement to undertake EIA and to suggest (EMP) Environmental Management Plan. It explains the proposals, the assessment of potential environmental effects, and the outcome and conclusions of the screening process.

Preliminary Cost Estimates – The detailed cost estimate for the project road needs to be prepared.

Financial Analysis – The sensitivity analysis that covers the valuation of the Financial Internal Rate of Return (FIRR). Calculation of profitable viability built on costs of various cost components of the project, operation, and maintenance, toll revenue under various financing options

Economic Analysis – Thezest of involvement of the economic costs and an analysis that shall provide the Economic Internal Rate of Return (EIRR).

Reasons for Study

The road network in the State constitutes the National Highways, roads under PWD (R&B), and the Panchayat Raj roads. The distribution of the roads into functional classification, by the year 2014-15 are the Panchayat Roads (PR), Village roads, and ODR (Other District Roads) were about 72.36% of the total road network. The upgraded roads maintained by Roads and Buildings (R&B) under Public Works Department (PWD) are State Highways & Major District Roads (covering 24.99% of the total) and by the National Highway Authority of India are National Highways (NH) and Express Highways (EH) was about 2.72%.



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The Telangana Government has released the (Green Skill Development Programme) GSDP estimates for the year 2016-17 (Advance Estimates). The anticipated GSDP at constant (2011-12) prices for the year 2016-17, apportioned effort (AE) was 51.1286 million INR as against 46.4389 million INR for 2015-16, registering a growth of 10.1 percent. The GSDP at current prices for the year 2016-17 (AE) is anticipated at Rs. 65.4294 million INR as against Rs. 57.5631 million INR for 2015-16 (FRE), growth @ of 13.7 percent. The budgeted estimate in Telangana state from all sectors for the year 2021-22 (Revised) and FY 2022-23 are 47926.529, and 51413.212 million INR respectively (Telangana Budget, 2022-23[4])

Study area

Mancherial District previously known as Adilabad District. The district has Asifabad, Nirmal, Adilabad, Jagtial, Peddapalle, and Bhoopalapallias neighbouring districts, and finally the boundary is the Maharashtra State. The district embraces 18 mandalas and two divisions (revenue) i.e. Mancherial and Bellampalli. The district headquarters is located in Mancherial town.

Mancherial district linked by roadways and railways to many well-known towns. Mancherial has the Secunderabad railway junction along with Bellampalli. The SH-1, NH-63, and Nagpur highways run over and done with the district, linking the entire region. It has one road transport corporation (RTC) depot at Mancherial. Rivers Godavari and Pranahita pass through it. The major crop in the area is Paddy and corn, Millets and Ragi etc. The area housed with number coalmines. The district is home to Colliery at Singareni and accommodate coal consumption of nearby Jaipur Thermal Power Plant. The area possesses several private cement-manufacturing unit, ceramics factories. The largest ceramic pipe industry in the country, 'The Hans India' lies in Telangana state. The district, Mancherial brags the pride of the crocodile sanctuary close to Chennur. The thick forest in and around houses the Kawal tiger biosphere Reserve. At Gudemgutta, the Sri Satyanarayana Swami Temple is one of the famous attraction to pilgrims in and around the state.

The district have good connection with the NH (National Highways), SH (State highways), major and other district roads (ODR's) as well. The NH-563, NH-63, and SH-1, SH-7 pass through the Mancherial region. Telangana State Road Transport Corporation (TSRTC), operates, and provides services with well-connectivity to various major towns like Manchuria, Bellampalle, and Mandamarri and even to remote villages in the area.

REVIEW OF LITERATURE

The burgeoning population and rising vehicular possession led to traffic crowding, environmental pollution, green or coastal corridor deterioration, and surging road accidents that contribute to the national budget but welcoming the climate change, particularly the CO₂, MoRTH yearbook 2016-17[5], Mishra et al., 2020[6], Vijjarapu et al., 2020[7] Verma et al., 2021[1], Ciarlantini et al., 2022[8], Chakrabarti et al., 2022[9]. The distresses caused to roads in India due to heavy traffic wear and tear are cracking, rutting, raveling, potholes, patching, etc., Mukherjee et al., 2012[10], Das et al., 2019[11], Vijay et al., 2022. The rapid growth of urbanization demands roads network growth, the major cities and cosmopolis occupies only 16% total population of India, Rathi 2017[12], Behera et al., 2019[13], Various NH (National Highways) -rules and acts in India are the National Highways Act, 1956. The National Highways Authority of India Act, 1988, and erstwhile The Central Road and Infrastructure Fund Act, 2000 are in implementation with CRF Rules, 2014- Repealed w.e.f. 31.03.2018, MoRTH A-R 2021-22[14], <https://morth.nic.in/node/10352>

The state, Telangana, has 4926 km length of 30 numbers NH roads. As per the MoRTH annual report for 2022-23, about 20 numbers of the pipeline, projects are under progress and annual expenditure by Dec 2021 was 49.65 million INR whereas the project costs are about an 118 million INR. (MoRTH annual report 2022-23[15]). The state has only 970 km, 1564 km, and 921 km of single roads, two-lane roads, and four and more lane roads (Total 3455 km) NH road infrastructure, 25553 km SH, and 22303 km ODR in Telangana state (Basic road transport yearbook, 2017[16]). The newly developed Telangana state needs anastomosed road network with upgradation, renovation, new formations and maintenance created on the type of pavement, and prioritization, Modinpuroju et al., 2016[17], Mishra et al.,





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2021[18], Hatab *et al.*, 2021[19]. Extension of roads network augments flexibility, trade, hospital care, connectivity, Socio-economic growth, and integration but has adverse impacts are health impacts due to accidents, environmental degradation, and emissions due to pollutants, Reshma *et al.*, 2015[20], Solanki *et al.*, 2016[21], Carbon Brief 2020[22]. The newly formed states in India have portions of NH, SH, or express highways are lack maintenance and are accident-prone. The surge in population demands more traffic and transportation of goods. New urban areas growing for administrative reasons. The connectivity needs guarantee for the urban growth and traffic. The audit of the NH-roads profile needs attendance in the Indian scenario.

METHODS AND METHODOLOGIES

Telangana has planned for two RRTs (regional rapid transit) as a commuter system from Hyderabad to Vijayawada, and Hyderabad to Warangal. That is for a safe, economic, fast, and comfortable journey, for 651.73 km in Telangana at a projected cost of INR 1839.123, to be completed by 2025, published on 18th Feb 2022; The New Indian Express, <https://urbantransportnews.com/news/telangana>, Telangana Today May 3, 2022. The expressway from Hyderabad to Vijayawada is 247 km long and four-lane that opened on Oct. 2012 proposed for 6-lane to be completed by 2024, The Hindu, April 1, 2022. The present investigation focuses on the flaws observed in the project road considering the function, efficiency of structures, and safety necessities of the predicted traffic Jothula *et al.*, 2022[23]. The findings from various surveys and investigations, audit results, and experiences gained from similar projects have utilization. Identification of the requisites of improvement matching the ARC design norms, cost and benefits have been pointed. By assessing the financial viability of upgraded four-lanes with paved shoulders standards are the prerequisites. The methodologies of draft road safety audit report as a DPR (Detailed Project Report), in Fig-3.

- a. Traffic investigations
- b. Engineering reconnaissance survey and investigations
- c. EIA/ EMP with Social Screening
- d. Design procedures
- e. Detailed estimates of cost
- f. Financial feasibility

The approaches agreed in booming out numerous errands for the draft detailed project report or RSA discussed briefly. Outlining the various proposals agreed upon the outcomes can have link with surveys & investigations, planning, formulation and designs for the improvement of project road.

Collection of Available Data:

Secondary statistics specifically concerning socio-economic outline, former traffic trends, and other hydrology and climate parameters gathered from various Government Units/other project bodies, and reviewed whether significant and indispensable. The accidents statistics and the black spot area send to end the road within the road extent, if available, have been taken from required offices and engineering divisions.

Socio-Economic Profile

The socio-economic profile of the area, state and the domain of the Project have carried considering the stakeholders, local people and of various professions. The traits consists of aboriginal tribes, indigenous individuals, contagious diseases predominantly HIV/infectious diseases /AIDS, poverty mitigation, gender, child labour, industry, agriculture, health, education, employment, land acquisition, and resettlement. The Secondary data collected from diverse sectors of the federal institution for analysis and preparation of socio-economic profile (Balaji *et al.*, 2018).

Evaluation Methodologies

Greenfield project corridor starts near Mancherla on existing NH-63 (Mancherla – Chinnur road) Chainage Km 176.600, continues on NH-63 for a length of 3.8 Km before taking diversion towards Warangal. The Corridor ends near Warangal on NH-163 Chainage 155.700km (Warangal – Eturnagaram Road).





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The alignment is passing through four districts, namely, Mancherial, Pedapalli, Jayashankar BhupalaPalli, and Warangal (Rural), in the Telangana State. The HCM 2010 (*Highway Capacity Manual*) elucidates the roundabout model for tanner capacity, which is an analytical model, based on gap acceptance theory. But the HCM 2010 model needs the design and field data about the roundabouts like the configuration and number of approaching lanes, quarter-hourly or hourly lane wise traffic volume distribution, the crossing of heavy/ light vehicular movement, by-cyclists, and pedestrians on each approach,

Town & Villages:

The important enroute Towns / Villages along the Project corridor are in Table 3

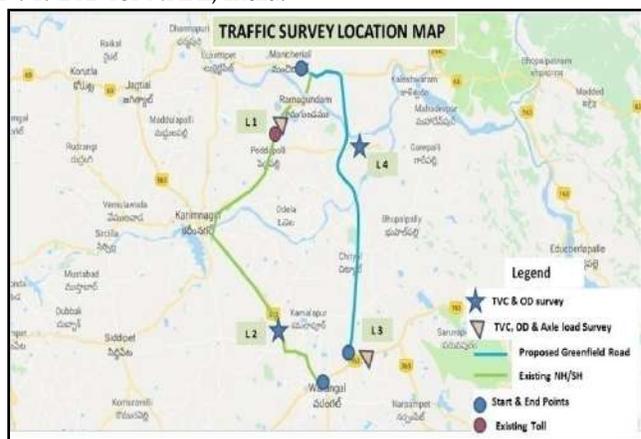
Environmental and Social Screening

An EIA assessment embarked on, along with preparation of a report that comprises of a moderation plan that sets feasibility and cost-effective optimization. By doing so, that shall condense potentially environmental implications significantly to a satisfactory level. An introductory ecological study conducted about the assessment of environmental relied on key impacts, issues, and substitutions, together with information essential for the development.

Traffic surveys: type, locations, and duration: To capture and assess the traffic characteristics, and travel patterns the Consultants have conducted the following primary traffic surveys. The under mentioned project profile and various survey results

- Classified Traffic Volume Count (TVC) Survey
- Commodity movement survey
- Speed and delay surveys
- Origin-Destination Survey
- Axle load Surveys

The classified volume count survey of various roads in the Vijayawada corridor at Gurralapadu (NH 365A), Kethankot (NH-65), Venkatapuram (NH 30), and Kallur (SH-42) from 5 Jan to 12 Jan 2019. The O-D survey was conducted at Gurralapadu (NH 365A), and at Kethankot (NH-65) on the eighth, ninth and 10th of January 2019. Similarly, the survey for the axle load was conducted from 8 to 10 Jan 2019, by ENVIRO INFRA SOLUTIONS PVT. LTD for NHAI, India.



From TVC data and analysis, the projected traffic was 5055 vehicles with, and with PCU as 10264 where Go-cart as 6PCU, Tractor/trailer as 4.5PCU, bus/ truck are PCU is 3, Minibus/LCV as 1.5PCU, and two-wheelers have 0.5 PCU considered.

O-D Survey Analysis

The axle load survey conducted in both directions for 24 hours on a random sample basis simultaneously with the O-D survey using Axle Load Pads. The axle load surveys piloted at two locations along the project corridor. The range



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of loads from the axles and the numbers of equal 8.16 t standard axles for the different categories of commercial vehicles have been determined based on the axle load survey.

Proposed Right of Way (PROW)

As per NHAI guidelines for Bhartamala Pariyojana projects, the Proposed Right of Way (ROW) is 70m, considering future requirements. However, where it becomes unavoidable and necessary to keep the alignment through such Reserve Forest/Restricted areas, the land needs the acquisition with a ROW of 40-70 m. As far as possible, the improvement schemes can be proposed like Geometric improvements, bypasses, major junctions, rest areas, toll plazas; wayside amenities, etc. additional land requirements are proposed to manage the project by public-private Partnership.

Economic Costs

The standard conversion factor of 0.90 based on IRC SP: 30-2009 guidelines used for transforming market rates of road construction aspects (material & Labour) and maintenance inputs into economic costs. For other inputs, like the fuel, vehicles, and vehicle components, economic costs estimated by net of transfer payment e.g. taxes, fees, charges, subsidies, etc.

Financial and Economic Cost of the project

The capital costs (financial) of the project road converted into economic costs by using a standard conversion factor (SCF) of 0.9, as suggested in the IRC code for highway projects in India. The financial and economic costs presented in Table 4 for various improvement sections. The capital cost has been phased over 24 months from 2019 to 2022 with phasing as 50% and 50% for the section.

Traffic Volume and Composition**Base Year Traffic**

The base year traffic estimate for the project road for homogenous sections by composition is the basic input for the HDM Model application. The Traffic data presented in the Traffic Study Chapter are considered in the analysis.

Traffic Forecasts

Traffic forecasts for 25 years have been given in the earlier chapter on "Traffic Surveys Analysis and Forecast" of this report. An annual growth rate of 5% considered for performing Economic Analysis.

ECONOMIC ANALYSIS – RESULTS

Economic analysis results for each section given in Table 6. The summary attached to the report.

CONCLUSION**General**

The preceding chapters of this report give detailed proposals on the various items of the study carried out by the Consultants for the Feasibility for Mancherial - Warangal Section (Design length- 111.762 Km.) in the State of Telangana needs to estimate and proposed for allocation of funds. The design proposal and recommendations summarized below:

Proposed Alignment

Greenfield alignment option from Mancherial to Warangal designed as an access-controlled highway with closed tolling and to needs to operate by (Public-Private Partnership) PPP mode. Safety maintenance on roads can have enhanced results maintained by Highway managers if attended from the stage of planning, design, bid procurement, construction, signage and super- vision and operation stages. The major conclusions from RSA Study Report for the above packages given below.





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The base year (2019) traffic was observed to be more than 10,000 PCU for the entire stretch; hence, the project road is proposed to be fourlanes with a paved shoulder configuration.

The entire project stretch proposed to have a Cement-treated base & subbase (CTB) Pavement.

Design speed of 100Km/h to maintain along with road safety signboards and road furniture.

Five nos. of Toll Plazas are proposed along the project corridor, with closed tolling, out of which 2 are Standard 16-lane plazas near the start and endpoint of the project and the remaining are three-lane exit and entry toll plazas.

Four nos. Truck lay-byes proposed for the project corridor at design Ch. 15+300 for Package- 1 and at design Ch. 99+200 for Package 2.

Four nos. Rest Areas proposed for the project corridor at design Ch. 28+500 on RHS and design Ch. 31+000 on LHS for Package 1 and at design Ch. 83+700 on LHS and design Ch. 87+300 on RHS for Package 2.

Five no's Major bridges, 47 nos. Minor bridges, 170 nos. box culverts and 34 no's pipe culverts are proposed.

Six numbers Flyovers/interchanges are proposed with exit and entry facility; 24 no's VUPs and 14 nos. LVUP/SVUP is proposed.

The projected shortfall in the execution of the project may be (i) facing a law and order state of affairs, (ii) adequate in time funding under Pandemic expenditures, (iii) shortfall of adequate security forces, and (iv) Land acquisition constraints. The strategy can have solution of fast LA, design and in time procurement, and finalization of tenders at a higher rate to allure the executant for early completion of the project. The conclusion is "GOOD and efficient roads are strengths, and inefficient roads are obligation to the nation"

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12. R&B Department, NH Circle, Hyderabad
13. MORT & H





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Table 1: Highway Details in India:

Sl. No	Name of the Road	Length in km
1	National Highways (NH) / Expressway	115435
2	State Highways (SH)	179287
3	Other Roads (Major and Village)	5322900
Total length in kilometers		5617622 km

Table 2: Status of various Road Sector programs:

(Source: extract copy of NHDP phase IV approval)

NHDP & Other NHA I Projects						
Road Details		Total Length	Already 4/6Laned	Under Implementation	Contracts Under Implementation	Balance length for award
		(Km.)	(Km.)	(Km.)	(No.)	(Km.)
NHDP	GQ	5,846	5,846 -100.00%	0	0	-
	NS - EW Ph. I & II	7,142	6,568	300	28	274
	Port Connectivity	435	383	52	7	-
	NHDP Phase III	11,809	7,621	2,161	71	2,027
	NHDP Phase IV	13,203	4,058	6,050	105	3,095
	NHDP Phase V	6,500	2,564	1,428	33	2,508
	NHDP Phase VI	1,000	-	184	9	816
	NHDP Phase VII	700	22	94	4	584
	NHDP Total	46,635	27,062	10,269	257	9,304
	Others (Ph.-I, Ph.-II & Misc.)	2,048	1,743	305	18	-
SARDP -NE	110	110	0	1	-	
Total by NHA I		48,793	28,915*	10,574	276	9,304

*Total 20,000 km was approved under NHDP Phase IV Out of which 13,203 Km. as assigned to NHA I remaining km with MORTH. (as on 31st May 2017)

Table 3: List of Town and Villages within the Project Road

Sl. No.	Village Name	Sl. No.	Village Name
1	Ramraopeta	27	Muthram
2	Indharam	28	Adavisirampur
3	Maddikunta	29	Odedu
4	Narva	30	Kalvapalle
5	Takmatla	31	Giddemutharam
6	Yelkanti	32	Raghavapur
7	Shetpalle	33	Pangidipalle
8	Narasingapuram	34	Tekumatla
9	Bijjal	35	Ramkrishnapuram(T)
10	Kundaram	36	Ankushapur
11	Rommipur	37	Somanpalle
12	Kistapur	38	Navabpet
13	Velal	39	Mogullapalle
14	Gopalapur	40	Medaramatla





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15	Nagaram	41	Issipet
16	Vilochavaram	42	Rangapuram
17	Pandulapalle	43	Laxmipuram
18	Kannala	44	Venkatapuram
19	Puttapaka	45	Malkapet
20	Adivarampeta	46	Madharam
21	Begumpet	47	Vellampalle
22	Lakkaram	48	Pocharam
23	Sarvaram	49	Gutlakaniprthy
24	Mydambanda	50	Pulkurthy
25	Potharam	51	Pasaragonda
26	Kesanpalle	52	Oorugonda

Table 4:Capital Cost for Economic Analysis (in Rs. Crores)

S. No.	Section	Improvement	Pavement Type	Financial Cost per km	Economic Cost per km
1	Mancherial to Warangal Section	Realignment	Cement Treated Base	16.74	15.065

Table 5:ProjectedTraffic inputs to the 4-lane Vijayawada corridor until 2035

S. No.	Year	Diverted Traffic in vehicles
1	2019	6011
2	2022	7497
3	2025	8929
4	2030	11949
5	2035	15990

Table 6:Economic Analysis Results (INR, Rs. millions)

S. No.	Section	Scenario	EIRR (%)	NPV @12%
1	Mancherial to Warangal section	Cement Treated Base	40.2	70,543.96

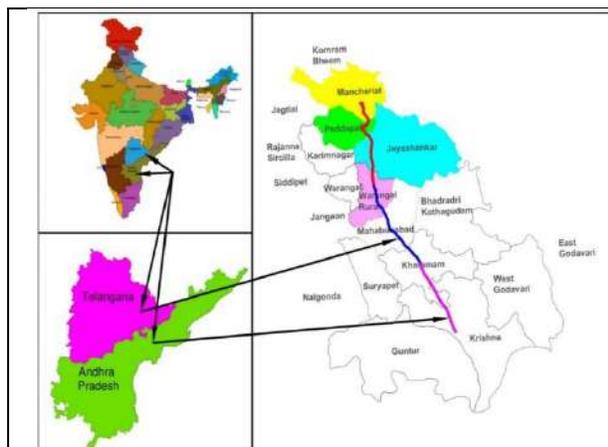


Fig 1: The index map of the study area in Telangana state (Mancherial to Warangal (120 Km) and Khammam to Vijayawada (Kanchikacherla) (65 Km);(Nagpur – Vijayawada Corridors)



Fig 2: The proposed upgrading to NH in the Nagpur-Vijayawada Corridor of 112km (4 to 6laning)





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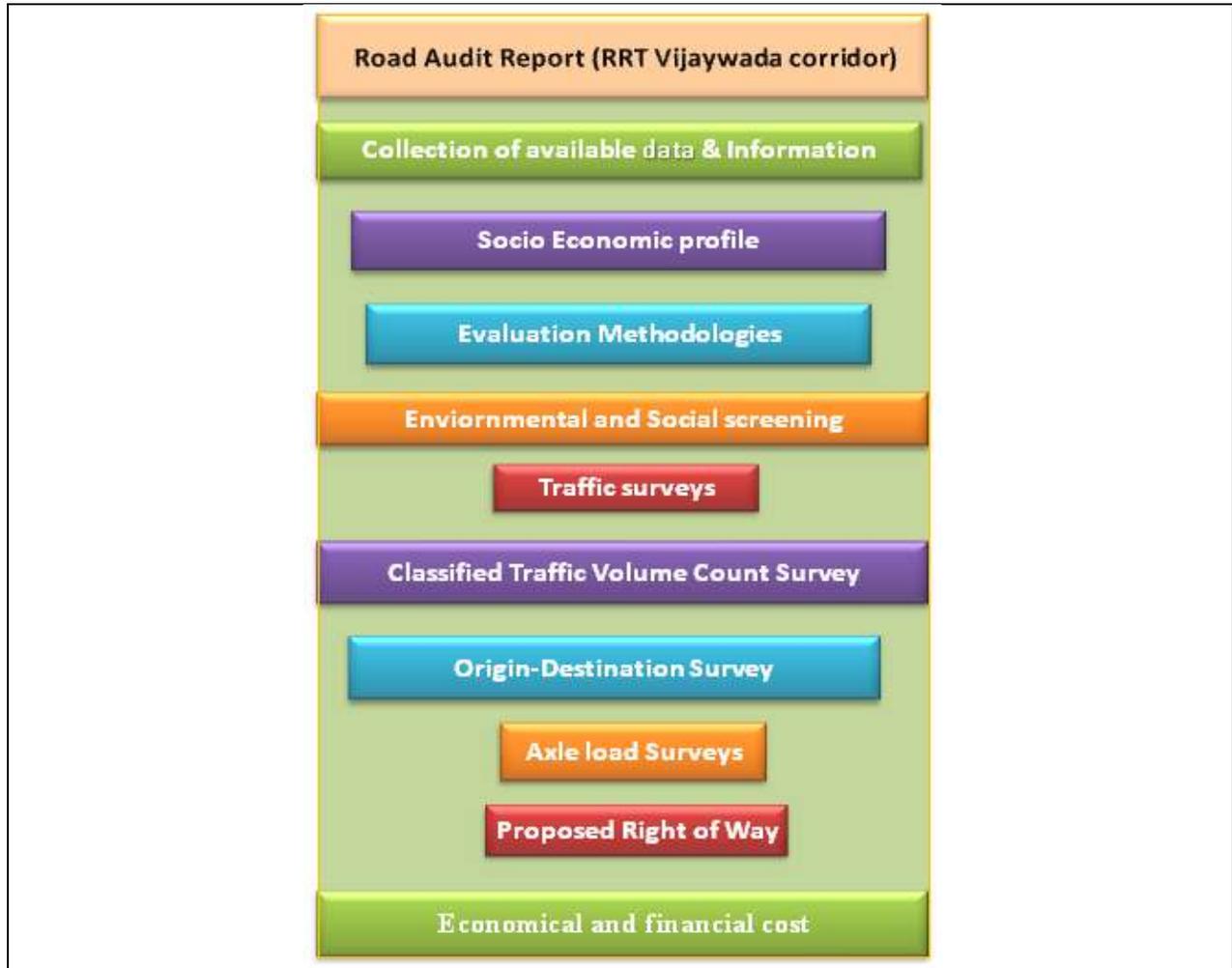


Fig 3: Methodologies adopted in the road audit of the Vijayawada Corridor



Fig 4(a): Project Starts at starts near HKR road (SH – 1)on NH – 63 (Mancherial – Chinnur road)



Fig 4(b) Project ends at on NH-163 (Warangal – Eturnagaram Road)





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Fig 5(a): OD survey at HKR Toll Plaza



Fig 5(b): OD survey at Eklaspur location



Fig 5(C):OD survey at Oglapur location



Fig 5(D): Axle load survey at HKR Toll plaza location

Fig 5: The traffic flow location, O-D,axial load survey with toll plaza for PPP mode of operation.





Traditional uses, Phyto-chemistry and Antimicrobial Activity of *Pongamia pinnata*(L.) Pierre: A Review

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ABSTRACT

Pongamia pinnata(L.)Pierre.is known for its diverse medicinal activities. It is used in the traditional medicine in the treatment of a number of ailments across the globe. This review focuses on the traditional uses; biological activities and phytochemistry to bridge the gaps require in future research possibilities. All the plant parts have medicinal effects on the treatment of different ailments such as piles, wounds and skin diseases. It also exhibits antimicrobial, anti- inflammatory and antiulcer activities.

Keywords: Anti- inflammatory, antimicrobial, antiulcer, phytochemistry, *Pongamia pinnata*

INTRODUCTION

Plant contains phytochemicals that shows toxic activities against pathogens. These bioactive compounds are the valuable sources for humankind because of their medicinal value. These phytochemicals have the medicinal and antimicrobial properties (Gurjar *et al.* 2012). Alkaloid, tannins, flavonoids, and phenolic compounds are the most important compounds. Specific plants have the unique medicinal properties due to different active constituents and their variation in concentrations. Plants have gained attention for their antimicrobial properties because of less toxicity as well as it is also cost effective as compared to costly synthetic drugs. Microorganisms are becoming antibiotic resistance; therefore, plants have been recognized as valuable resources to be used for antimicrobial activities (Farjana *et al.* 2014). Medicinal plants are used by the local people as traditional medicine to get rid from infectious diseases (Kunwar *et al.* 2008; Manandhar *et al.* 2019). World health organization reported that the medicinal plants would be the chief sources to obtain a variety of drugs. Traditionally medicinal plants are used all over the globe as remedies for curing various type of diseases such as asthma, respiratory disorder ,skin infections ,hepatic, gastrointestinal symptoms and cardiovascular disease because of harboring different potential bio active compounds (Egamberdieva *et al.* 2017). *Pongamia pinnata*(L.)Pierre. is commonly known as 'karanja', belongs to

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family Fabaceae and is recognized as a valuable resource to treat various diseases of human beings as scientifically proved for its pharmacological properties (Ghumare et al., 2014). The name 'Pongamia' is derived from the Tamil word 'pinnata' which refers to pinnate leaves and is a medium sized fast growing, evergreen, glabrous, deciduous ornamental nitrogen fixing tree producing non edible oil seeds containing 30 to 40 % oil (Chopade et al., 2008). 'Karanja' is used in Ayurvedic and Siddha medicinal system mainly to treat skin diseases. All the plant parts are used drug preparation for the treatment of various disease such as tumors, piles, skin diseases, itches, painful rheumatic joints, ulcers and diarrhea etc. 'Karanja' twigs are as tooth brush by rural people. It plays an important role in fixing atmospheric nitrogen and significantly contributed for management of soil health. *P. pinnata* (L.)Pierre. is also the source of biomedicine and specially used as antimicrobial agents (Ghumare et al., 2014). *P. pinnata* (L.)Pierre. is widely distributed in the tropical Asia and Seychelles island, Australia, South Eastern Asia. In India particularly in Maharashtra this plant is locally distributed along the river banks. This tree is cultivated along the road in India and is the potential source of biodiesel. (Ghumare et al., 2014; (Halder et al., 2014).

Traditional use of *P. pinnata* (L.) Pierre.

Traditionally different parts of the trees are mainly used for curing whooping cough, bronchitis, joint pains and diabetes (Kirtikar et al. 1995). The leaves are used to treat piles, used as laxative and boost digestion. Leaves immersed in hot water are used as bath to relieve the rheumatic pain, for cleaning ulcer, infectious skin diseases such as leucoderma, leprosy, lumbago, muscular pain and also used in articular diseases. The plant is itself a versatile plant (Sangwan et al., 2010). The seeds oil is used for itches and various type of skin disease (Wagh et al. 2007), flowers are used for diabetes (Carcache et al. 2003), and bark is consumed internally for bleeding piles and treatment of beriberi (Mumcuoglu et al. 1990; Akhtar et al. 1996). Leaf juice is active against *Micrococcus* and is used to cure cold cough, diarrhea and dyspepsia. Seed oil used in treating scabies, piles, leprosy, ulcers, chronic fever and reducing liver pain. Roots are also used to clean gums and treat ulcers.

Phytochemistry of *P. pinnata* (L.) Pierre.

P. pinnata (L.) Pierre. seeds reported to contain six compounds viz., two sterols, three of them are sterol derivatives and one is disaccharide along with eight fatty acids out of which three are saturated and five are unsaturated. The metabolites, stigma sterol, galactoside and sucrose are also being reported. Oleic acid occurred in highest amount of 44.24%, stearic acids with 29.64% and palmitic (18.58%) acids are present. Glabrachalcone isopongachromene is another flavonoid found isolated from seeds (Tanaka et al., 1992; Shameel and Usmanghani, 1996). Several flavones and chalcone such as galbone, pongone, pongagallone and pongalabol are also derived from the leaves and stem (Shameel et al., 1996; Chopade et al., 2008). Another study reported 18 flavonoid compounds isolated from its root bark including few pongamones iii-ix and pinnatin which is a furanoflavone (Tanaka et al., Chopade et al. 2008).

Pharmacological properties of *P. pinnata* (L.) Pierre.**Antimicrobial activity**

P. pinnata (L.) Pierre. seed oil reported to show antimicrobial activity against *Aspergillus niger*. This plant can contribute to develop new pharmaceutically potent antimicrobial drugs (Wagh et al., 2007; Ghumare et al., 2014). Significant antipyretic action of extract against brewer's yeast induced pyrexia was also reported. Antifungal and antibacterial activities of *P. pinnata* (L.) Pierre. depends on the concentration of seed oil against *Aspergillus niger*, *A. fumigatus* (Mdee et al., 2009), *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Antiviral activity has also been reported (Sharma et al., 2009). Gas chromatography and mass spectroscopy shows the presence of fatty acids which is used for developing antimicrobial drugs (Farjana et al., 2014; Egamberdieva et al., 2017).

Anti-inflammatory activity

It was reported that *P. pinnata* (L.) Pierre. leaves extracted using 70% ethanol showed potent anti-inflammatory activity in different phases of inflammation such as acute, sub-acute, and chronic phases on gastric mucosa without any side effects (Srinivasan et al., 2001).





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Antiulcer activity

Aqueous extract of *P. pinnata* (L.)Pierre. roots significantly decrease the quantity of gastric juice without showing any side effects on activity of mucin in acetyl salicylic acid induced ulcerated rats (Akhtar *et al.*, 1996). A change is noticed in hexose and fructose content of carbohydrate. It decrease ulcer index significantly ulcer protective effect. The roots also prevent lipid peroxidation and proliferation of cells rather than the offensive secretion of pepsin.

CONCLUSION

Further study on the phytochemical constituents and mechanisms associated with definite biological activities are required to know the pharmacological activity associated with the phytochemical constituents. Toxicity of other toxic compounds is to be assessed to check their eligibility to be included in the preparation of drugs.

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Nano Particle-Based Drug Delivery System for Cancer Therapy: Review

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ABSTRACT

This review article discusses about the current cancer treatment techniques and the extensive recent research studies done on nanoparticles as carrier systems for the delivery of anticancer drug molecules in cancer treatment. A variety of nanoparticles of different structural and chemical formulations have been tested for their target-specificity and as drug carrier systems. Numerous scientific research works have been performed to test the use of magnetic nanoparticles in the treatment of carcinogenic brain tumour cells and breast cancer cells; colloid gold nanoparticles, liposomes and polymeric micelles as drug delivery systems to target tumour cells and deliver anticarcinogenic drug in a controlled manner. The article also discusses about ceramic nanoparticles and its applications in photodynamic therapy for cancer treatment. The article thus reviews the subject in brief with suitable references to original research articles and review articles discussing the earlier and current research findings about various types of nanoparticles as drug delivery systems in cancer therapy.

Keywords: Nanoparticles, drug delivery systems, cancer therapy, magnetic nanoparticles, colloid gold nanoparticles, liposomes, polymeric micelles.

INTRODUCTION

Nanotechnology is the ability to work at the atomic, molecular, supra molecular levels (on a scale of ~1-100nm) in order to understand, create and use material structures, devices and systems with fundamentally new properties and functions resulting from their small structure [1]. Nanotechnology has offered us the ability to design materials with totally new desirable characteristics. Extensive researches are being done worldwide to understand the advantages and scientific limitations of nanoparticles as drug delivery systems. There has been a remarkable progress over the last decade in the development of nanoparticles as effective drug delivery carriers. The various types of nanoparticles that are currently studied for their use as drug delivery systems are





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polymeric biodegradable nano particles that include nanospheres and nanocapsules; ceramic nanoparticles; polymeric micelles; dendrimers; liposomes [2]. The carrier can also be a carbon nanotube[3], a carbon nanohorn[4] or a silica nanoparticle with drug molecules bound to its surface [5]. A magnetic nanoparticle which can be drawn to a particular part of the human body under the influence of magnetic field can serve as an excellent drug delivery system [6]. It can also be an implantable nanoscale device filled with drug molecules encapsulated by nano porous membrane which can act as tiny turnstiles for releasing the drug [7]. To make these nanoparticles to function as effective drug delivery systems, specific biological molecules like antibodies, enzymes, hormones and pharmaceutical drugs can be coupled structurally to these particles. This review will give an overview on current cancer treatment methodologies and address the recent research works on nanoparticle- based drug delivery systems with a focus on nanoparticle-drug formulations that have been specifically tested for their target-specificity towards cancer cells.

Cancer treatment techniques

Currently, there are different methodologies available for cancer treatment. But each technique has its own disadvantages and adverse side effects [8, 9]. Surgical treatment (excision of the tumor) is usually the first choice of treatment preferred by physicians. However, it is not effective when the cancer cells have infiltrated the nearby vital organs or have spread to distant parts of the body (metastasis). Surgical excision is preferred for the removal of large tumors. Cryosurgery is another surgical technique used for freezing and killing the abnormal tumor cells. It is an alternative to surgical excision and is used to treat tumors that have not spread and for the treatment of some precancerous or non-cancerous conditions. Chemotherapy is the use of anti-cancer drugs. The drugs are administered as pills, intravenous injection or topically application on skin. Chemotherapeutic drugs may destroy healthy tissue along with destroying the cancer cells and carcinomatous tissue (cytotoxicity). The cytotoxic effect of chemotherapeutic drugs is greatest in organs like bone marrow, gonads, hair follicles and digestive tract which contain rapidly proliferating cells. The adverse effects of chemotherapy include fatigue, nausea, vomiting, alopecia (loss of hair), gastrointestinal disturbance, impaired fertility, impaired ovarian function and bone marrow suppression resulting in anemia, leucopenia and thrombocytopenia [10, 11].

Another technique of cancer treatment is the radiation therapy which uses radiation energy to destroy cancer cells and reduce the size of tumors. Bone marrow transplantation and peripheral blood stem cell transplantation are done to restore stem cells that were destroyed by high doses of chemotherapy or radiation therapy. Immunotherapy (sometimes called biological therapy, biotherapy, or biological response modifier therapy) is a treatment technique that utilizes human body's immune system to destroy cancer cells [12]. The immune system is stimulated by an outside source, such as an antibody, or synthetic immune system proteins or biological response modifiers (BRMs). BRMs include interferons, interleukins, colony-stimulating factors, monoclonal antibodies, vaccines, gene therapy and non-specific immune modulating agents. Recent research works have been concentrating on studying gene therapy for cancer treatment. Gene therapy is an experimental treatment that involves introducing genetic material into the cancer cells to destroy them [13]. Angiogenesis inhibitors are also currently being evaluated in clinical trials. These are chemicals which inhibit the formation of blood vessels (angiogenesis). Angiogenesis plays an important role in the growth and spread of cancer cells [14].

New blood vessels act as a source of oxygen and nutrients to the cancer cells allowing these cells to grow, invade nearby tissue, spread to other parts of human body and form new colonies of cancer cells. Angiogenesis inhibitors are used to prevent the formation of blood vessels and thereby depleting the cancer cells of oxygen and nutrients and thereby resulting in the destruction of cancer cells. Hyperthermia (also called thermal therapy or thermotherapy) is a type of cancer treatment technique in which the cancer cells are exposed to high temperatures (up to 113°F). Research has shown that high temperatures can damage and kill cancer cells, usually with minimal injury to normal tissues [15]. By killing cancer cells and damaging proteins and structures within cells, hyperthermia destroys cancer cells [16]. Hyperthermia may make some cancer cells more sensitive to radiation or harm other



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cancer cells that radiation cannot damage. It can also enhance the effects of certain anticancer drugs. So, it is almost used with other forms of cancer therapy, such as radiation therapy and chemotherapy [17]. Hyperthermia is under study in clinical trials. Laser therapy uses high-intensity laser to treat cancer [18]. Laser can be used to shrink or destroy tumors. Laser therapy is most commonly used to treat superficial tumors on the surface of the body or the lining of internal organs. Photodynamic therapy (PDT) is a type of cancer treatment that uses a drug called a photosensitizer or photosensitizing agent [19]. Photosensitizer is activated by light of a specific wavelength. When photosensitizers are exposed to this specific wavelength of light, they produce singlet oxygen which destroys cancer cells. Targeted cancer therapy uses target-specific drugs that invade cancer cells and block the growth and metastasis of cancer cells by interfering with specific molecules involved in carcinogenesis and tumor growth [20].

Anti-carcinogenic chemotherapeutic agents

In general, anti-carcinogenic chemotherapeutic agents can be divided into three main categories based on their mechanism of action [21].

Prevention of Synthesis of preDNA Molecule Building Blocks

DNA building blocks are folic acid, heterocyclic bases, and nucleotides, which are made naturally within cells. All of these agents work to block some step in the formation of nucleotides or deoxyribonucleotides (necessary for making DNA). When these steps are blocked, the nucleotides, which are the building blocks of DNA and RNA, cannot be synthesized. Thus, the cells cannot replicate due to impaired DNA synthesis. Examples of drugs in this class include Methotrexate, Fluorouracil, Hydroxyurea and Mercaptopurine.

By Chemical Damage of DNA in the Cell Nuclei

Some chemotherapeutic agents destroy DNA and RNA of cancer cells. They disrupt replication of the DNA and totally halt the replication of DNA or RNA that may stimulate cancer cell formation. A few examples of drugs in this class include Cisplatin, Anti-biotics – Daunorubicin, Doxorubicin and Etoposide.

Disruption of Synthesis or Breakdown of Mitotic Spindles

Mitotic spindles serve as molecular railroads with 'north and south poles' in the cell when it starts to divide. These spindles are very important because they help to split the newly copied DNA such that a copy goes to each of the two new cells during cell division. These drugs disrupt the formation of these spindles and therefore interrupt cell division. Classic examples of drugs in this class of mitotic disrupters include Vinblastine, Vincristine and Paclitaxel.

Nanoparticles in cancer treatment

Nanoparticles are currently studied for their use in detection of cancer at its earlier stage and in targeted anti-cancer drug delivery of the above mentioned drugs. The critical step in cancer treatment is the detection of cancer at its initial stage of carcinogenesis. Results of the numerous researches done in nanotechnology are inspiring the scientific community to discover new innovative non-invasive tools at the nanoscale level for such purposes. Nanoscale cantilevers [22] and quantum dots [23, 24] are being studied as cancer detection tools at the cellular level. If the tumor has not been detected in its early stage, treatment methods should be devised to eradicate the fully developed cancer cells without harming the normal healthy cells of human body. Targeting of nanoparticles can be divided into 'active' and 'passive' targeting [25].

Active targeting can be further subdivided into 'chemical/biological' and 'physical' targeting. Chemical/biological targeting involves modification of a nanoparticle surface by chemical/biological tumor specific ligands. Physical targeting involves directing the nanoparticles to tumor cells under the influence of an external magnetic field.



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Passive targeting involves modifying the nanoparticle itself without the addition of any ligands or physical methods, thereby increasing the circulation time. This enables accumulation of nanoparticles in tumors by an effect called 'enhanced permeability and retention effect' (EPR) effect. The EPR effect utilizes the property by which certain sizes of molecules, typically liposomes or macromolecular drugs, tend to accumulate more in tumor tissue than in normal tissues [26, 27]. In order for the tumor cells to grow quickly, they must stimulate the production of blood vessels (angiogenesis). Tumor cell aggregates of size as small as 150-200 μm , start to become dependent on blood supply for nutritional and oxygen supply. These newly formed tumor vessels are usually abnormal in form and architecture. Furthermore, tumor tissues usually lack effective lymphatic drainage. All these factors lead to abnormal molecular and fluid transport dynamics especially for macromolecular drugs. The EPR-effect is even more enhanced by many pathophysiological factors like more vascular endothelial growth factor (VEGF) production by the newly developing capillaries. The EPR effect also provides a great opportunity for more selective targeting of lipid or polymer-conjugated anticancer drugs [28, 29].

The various types of nanoparticles that are currently studied for their use as drug delivery systems are polymeric micelles, magnetic nanoparticles, colloidal gold nanoparticles and ceramic nanoparticles [30-32]. These nanoparticle-based drug delivery systems can be characterized for their localization in tumor cells by coating them with tumor-specific antibodies, peptides, sugars, hormones and anti-carcinogenic drugs, to mention a few. These nanoparticles have been effectively coupled with the above mentioned anti-carcinogenic chemotherapeutic agents and have been tested for their target-specificity. These nanoparticles are superior over the conventionally available drug delivery systems as the chemotherapeutic agents can be targeted to a specified area of the human body by adding nanoscale surface receptors. These receptors specifically recognize the target tissue and bind to it and release the drug molecules [33]. Thus, healthy cells can be spared from cytotoxic effects of the drug. Drugs can also be protected from degradation by encapsulating them with nanoparticle coatings [34]. As nanoparticles are extremely small, they can penetrate through smaller capillaries and are easily taken up by cancer cells. This causes efficient drug accumulation at the target site. Use of biodegradable nanoparticles allows sustained drug release over a period of time [35]. Thus, nanoparticles as drug delivery systems with enhanced target specificity can overcome the limitations of conventional cancer treatment techniques.

Gold Nanoparticles for Anti-Carcinogenic Drug Delivery

Colloidal gold nanoparticles are the most commonly used nanoparticles for anti-carcinogenic drug delivery. Colloidal gold nanoparticles are more biocompatible than other nanoparticles [36]. The physical and chemical properties of colloidal gold nanoparticles allow more than one protein molecule to bind to a single particle of colloidal gold. The use of colloidal gold nanoparticles as drug delivery vectors of tumor necrosis factor (TNF) has been tested in a growing tumor in mice [36]. Although TNF has been evaluated in cancer treatment, it causes adverse effects like hypotension and in some cases causing organ failure resulting in death. But recent researches have shown that when coupled with colloid gold particles, therapeutic amounts TNF can be successfully delivered to destroy the tumour cells in animals [37]. The use of laser to destroy the tumour cells in human breast cancer tissue has been described by a technique of selective nanothermolysis of self-assembling gold nanoparticles [38]. These gold nanoparticles were coated with secondary Ab goat anti-mouse IgG. This structural configuration showed specific localization in the adenocarcinomatous breast cells targeted with primary Ab. Colloidal gold nanoparticles can also function as safe and efficient gene delivery vehicles in gene therapy and immunotherapy of cancer. Plasmid DNA encoding for murine interleukin-2 was complexed with gold nanoparticles [39]. Gold nanoparticles showed significantly higher cellular delivery and transfection efficiency than other gene delivery vehicles.

Liposomes in Cancer Treatment

Liposomes are small artificial spherical vesicles made from naturally occurring non-toxic phospholipids and cholesterol [40]. There are four major types of liposomes. Conventional liposomes are either neutral or negatively charged. Sterically stabilized 'stealth' liposomes carry polymer coatings to obtain prolonged circulatory duration. Immunoliposomes have specific antibodies or antibody fragments on their surface to enhance target specific bind-





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ing. Cationic liposomes interact with negatively charged molecules and condense them to finer structure thereby carrying them externally rather than encapsulating the molecules within [41]. Due to their size, biocompatibility, hydrophobicity and ease of preparation, liposomes serve as promising systems for drug delivery. Their surfaces can be modified by attaching polyethylene glycol units (PEG) to enhance the circulation time in blood stream. Liposomes can also be conjugated with ligands or antibodies to improve their target specificity. Anti-estrogens solubilized within the oily core of liposomes incorporated high amounts of 4-hydroxy tamoxifen (4-HT) or RU58668 [42]. This combination was used in the treatment of multiple myeloma as the cancer cells express estrogen receptors and is of particular interest in the treatment of estrogen dependant breast cancer. In 20-30% of breast cancer cells there is a high amount of human epidermal growth factor-2 (HER2) expression. Anti-HER2 antibodies conjugated immunoliposomes with magnetic nanoparticles were used to treat breast cancer cells with hyperthermia [43]. Such studies demonstrate the potential of liposomes as a drug delivery system in breast cancer treatment.

Magnetic Nanoparticles in Cancer Treatment

Carbon magnetic nanoparticles (CMNP) are made up of spherical particles of 40-50 nm in diameter with iron oxide particles dispersed in a carbon-based host structure [44]. Doxorubicin molecules (DOX) immobilized on activated CMNP formed CMNP- DOX conjugates which were demonstrated effective in cancer cell cytotoxicity assays [45]. This showed that CMNPs can be used as effective drug delivery systems. Iron oxide magnetic nanoparticles can also be sheathed with sugar molecules [46]. Therefore these are not recognized by the immune system. When these particles are brought under the influence of an external magnetic field, they heat up the tumour cells and destroy them without affecting the surrounding healthy tissues. A group of researchers have synthesized biodegradable magnetic nanoparticles using organic polymers and nanosized magnetite's [47]. After the characterization studies, an external magnetic field was used as a guidance system to direct the magnetic nanoparticles to the specified part of the experimental setup. The results of such studies substantiate the theory of targeting magnetic nanoparticles to specific areas of the human body using an external magnetic field.

The magnetic nanoparticles can be targeted to specified tumour cells by adding nanoscale surface receptors (targeting moieties). These receptors specifically recognize the target tumour tissue and bind to it and release the drug molecules. Different tumour cells exhibit different cell receptors. Iron oxide nanoparticles can also be coated with amino groups to achieve cell specific delivery of therapeutic agents, for example, to carcinomatous brain cells, without unselectively invading the whole brain. This concept has been demonstrated in a study about functionalized superparamagnetic iron oxide nanoparticles and their interaction with the brain cells [48]. An approach of localizing the iron oxide nanoparticles to specific cell receptors has been studied by functionalizing them with glycoproteins like lactoferrin and ceruloplasmin [49]. In breast cancer tissue, luteinizing hormone release hormone (LHRH) receptors are expressed predominantly. So, to localize the iron oxide nanoparticles to the cancerous breast tissue the magnetic nanoparticles can be conjugated with luteinising hormone release hormone. Such an approach [50] has demonstrated the target specificity the iron oxide nanoparticles in breast cancer treatment. These approaches prove that magnetic nanoparticles can be functionalized with suitable targeting moieties to localize them specifically to tumour cells under the influence of an external magnetic field.

PEG Polymeric Micelles as Drug Delivery Systems

Polymeric micelles serve as a novel drug delivery system due to their target specificity and controlled release of hydrophobic anti-cancer drugs [51]. Poly (ethylene glycol) PEG-based micelles are biocompatible and biodegradable. Effective drug delivery of cytotoxic drugs to cancer cells using PEG polymeric micelles has been demonstrated by conjugating Doxorubicin with poly (ethylene glycol)-poly (α , β -aspartic acid) block copolymer [52]. Doxorubicin also known as Adriamycin was physically entrapped and chemically bound to the core of the polymeric micelle. Due to reduced uptake by the reticuloendothelial system (RES), this drug carrier had a prolonged circulation time in the blood stream. Localization to the cancer cells can be achieved by linking monoclonal antibodies, sugars and biotin or tumour specific peptides to the polymeric micelles [53]. PEG-coated biodegradable nanoparticles can be coupled with folic acid to target folate-binding protein which is a soluble form of folate protein which is over expressed on the surface of many tumour cells. These folate linked nanoparticles have





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been tested and confirmed for their selective target binding [54]. These studies demonstrate the potential of PEG polymeric micelles as a novel drug delivery system in cancer treatment.

Ceramic Nanoparticles in Photodynamic Therapy

Ceramic nanoparticles are made from calcium phosphate, silica, alumina or titanium. These ceramic nanoparticles have certain advantages like easier manufacturing techniques, high biocompatibility, ultra-low size (less than 50nm) and good dimensional stability [55]. These particles effectively protect the doped drug molecules against denaturation caused by changes in external pH and temperature. Their surfaces can be easily modified with different functional groups and can be conjugated with a variety of ligands or monoclonal antibodies in order to target them to desired sites [56]. These nanoparticles can be manufactured with the desired size, shape and porosity. A ceramic nanoparticle does not undergo swelling or porosity changes caused by fluctuations in temperature or pH and are small enough to evade the reticuloendothelial system (RES). The application of ultrafine silica-based nanoparticles which are ~35 nm diameter with photosensitive anti-carcinogenic drugs encapsulated within has been described [57]. These ceramic nanoparticles have been used to destroy cancer cells by photodynamic therapy. When activated by light of suitable wavelength of 650 nm, the drug produces singlet oxygen which necroses the tumour cells. This concept of using silica nanoparticle platforms which can attach to the external surface of tumour cells and deliver photosensitizer like m-THPC (meta-tetra (hydroxyphenyl)-chlorin) and singlet oxygen induced cancer cell apoptosis has also been demonstrated [58].

Nanobiotechnology-based approach towards drug delivery in cancer treatment

Discussed above are the few most promising groups of nanoparticles and their applications in drug delivery for cancer treatment. There are numerous other nanobiotechnology-based approaches being developed to formulate nanoparticles as carriers of anti-carcinogenic agents. These include dendrimers, chitosan nanoparticles, low density lipoproteins (LDL), nanoemulsions, nanoliposomes, nanoparticle composites, polymeric nanocapsules, nanospheres and nanovesicles. Their applications in nanoencapsulation and targeted drug delivery of anti-cancer drugs in combination with radiotherapy, laser therapy, thermotherapy, photodynamic therapy, ultrasound therapy and nanoparticle-mediated gene therapy have been extensively reviewed in the earlier literature [59].

Nanoparticle-based delivery of specific anti-carcinogenic drugs

Methotrexate, a potent anticancer drug has been coupled with polybutylcyanoacrylate nanoparticles of different sizes from 70-345 nm and tested for their ability to overcome blood-brain barrier in the treatment of brain cancer. This study showed that polysorbate 80-coated polybutylcyanoacrylate nanoparticles of diameter below 100 nm can effectively overcome the blood-brain barrier [60]. Research studies done on experimental rats have demonstrated that the nanoparticle formulation consisting of poly (amidoamine) modified with PEG-500 had the ability of sustained release of 5-Fluorouracil and was target specific [61]. PLGA-mPEG nanoparticles were used as a carrier for Cisplatin and this study [62] showed that PLGA- mPEG effectively delivered the drug to human prostate cancer cells. PLGA nanoparticles have been shown as effective carriers of Doxorubicin [63]. Positively charged polysaccharide chitosan nanoparticles have also been tested for their target-specificity to deliver doxorubicin [64].

PLGA nanoparticles containing vitamin E have also been tested for their drug-carrying potential for Paclitaxel, an anticarcinogenic drug which interferes with mitotic spindles and therefore inhibiting cell division [65]. Nitrocamptothecin, an alkaloid drug belonging to a class of anticancer agents called topoisomerase inhibitors have also been target-specifically delivered to the cancer cells by PLGA nanoparticles [66]. Trastuzumab (more commonly known under the trade name Herceptin) is a humanized monoclonal antibody that acts on the HER2/neu (erbB2) receptor which are over expressed in breast cancer cells. Human Serum Albumin (HSA) nanoparticles were used as drug delivery systems and this study showed that a stable and biologically active system like albumin can be utilized in cancer treatment [67]. Another drug belonging to the same category and used in breast cancer treatment is Tamoxifen. Poly (ethylene oxide)-modified poly (caprolactone) polymeric nanoparticles (PEO-PCL) have been tested for their target specificity as a carrier for tamoxifen. These research studies prove that a wide variety of nanoparticles





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can effectively function as drug delivery systems for anti cancer drugs and thereby eliminating the adverse effects of these pharmaceutical agents.

Limitations of nanoparticles as drug delivery systems

The science and knowledge that the scientific community has today about nanotechnology and its potential versatile applications is only based on the researches done in the laboratories. These researches are being conducted to understand how matter behaves at the nanoscale level. Factors and conditions governing the behavior of macrosystems do not really apply to the nanosystems. The major limitations and technological hurdles faced by nanotechnology and its applications in the field of drug delivery should be addressed [68, 69]. Scientific community hasn't yet understood completely how the human body would react to these nanoparticles and nano- systems which are acting as drug carriers. Nanoparticles have larger surface area when compared to their volume. Friction and clumping of the nanoparticles into a larger structure is inevitable which may affect their function as a drug delivery system. Due to their minute size these drug carriers can be cleared away from the body by the body's excretory pathways. When these are not excreted, larger nanoparticles can accumulate in vital organs causing toxicity leading to organ failure.

Polymeric micelles were reported to cause acute hypersensitivity reactions in a few animal studies. Liposomes have certain drawbacks like being captured by the human body's defense system. The drug loading capacity of liposomes is being tested by researchers and still remains inconclusive. A few studies have shown post-treatment accumulation of the nanoparticles in skin. Studies on crystalline silver nanoparticles in therapeutic application raised the possibility of cytotoxicity in lesioned skin or growing human fibroblasts and keratinocytes [70, 71]. Recent study in mice revealed that tissue distribution of gold nanoparticles is size-dependent with the smallest nanoparticles (15-50 nm) showing the most widespread organ distribution including blood, liver, lung, spleen, kidney, brain, heart and stomach [72]. Once the nanoparticles are administered into the human body, they should be controlled by an external control preventing them from causing adverse effects. These drug delivery technologies are in various stages of research and development. It is expected that these limitations can be overcome and the discoveries to come into practical use within the next 5-10 years.

Summary

Discussed in this review are the research works done in the past decade in targeting novel nanoparticles towards the treatment of cancer. This study has expanded tremendously in the past few years as new nanoparticle carrier systems and anti-cancer drugs are being discovered. The uses of nanoparticles for early diagnosis of cancer and in gene therapy have been extensively reviewed in the literature [73-77]. A few of these innovative treatment techniques have made their way into clinical trials. There is a lot more to be done in order to treat or perhaps prevent advanced cancer by treating it in an early stage. This will require superior detection and targeting methods which many of the researchers are pursuing on nanoparticle-based drug delivery systems. These research studies in nanotechnology will definitely pave the way for early detection and prevention of cancer thereby improving the life and quality of cancer patients.

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CONFLICTS OF INTEREST

The authors of the article have no conflicts of interest to declare.





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Secondary Qualitative and Quantitative Data Analysis of Formulation Optimization of Drug in Transdermal Drug Delivery System

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ABSTRACT

The *transdermal drug delivery system* avoids the route of non-invasive treatment, thus solving the issues of needle phobia in the patients, thus allowing them to comply with the entire procedure of the drug administration. The process of the *transdermal drug delivery system* avoids the system of the pre-systemic metabolism and thus provides improvisation in the bioavailability of the drug. The research philosophy that is used in this research study is realism. Realism is used as the research philosophy because realism solely depends on the real values that are obtained from the part of the research. The research approach that is used in this research study is the deductive research approach. The significance of the study revolves around the overall formulation of the drug to be administered, the optimization of the drug administered to the patients and the evaluation of their effectiveness when they are administered to the patients who opt for a non-invasive method of drug administration due to problems such as needle phobia and others. Other forms of research philosophy such as interpretivism, positivism and pragmatism are not used as the research philosophy in this case because the effects of the drug administration cannot always be positive, and hence positivism cannot be used as research philosophy.

Keywords: Transdermal drug delivery, skin patch. Film-forming Gel, Formulation, Drug administration.

INTRODUCTION

Background of Study

The transdermal drug delivery system was initially designed for the overall sustenance of the release and improving the bioavailability of the drug that is being injected into the patient and for the better compliance of the patient with the application of the drug. The transdermal drug delivery system is basically the painless method that is implemented on the patients when the drug is injected into them through the skin by the process of drug formulation (Ahmed *et al.* 2019). The main focus of the transdermal drug delivery system is to make sure that the



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drug is injected into the target area that is the bloodstream without accumulating the layers of the skin. The drug is generally injected with the help of the needles into the stratum corneum, then it is passed on to the epidermis and the dermis. When the drug spreads the dermal layer, then it becomes obtainable for absorption into the system through the “dermal microcirculation”. The process of transdermal drug delivery system has much more advantages over the other conventional methods present to administer the drug into the system of the patient. This transdermal drug delivery system avoids the route of non-invasive treatment, thus solving the issues of needle phobia in the patients, thus allowing them to comply with the entire procedure of the drug administration (Dave *et al.* 2017). The process of the transdermal drug delivery system avoids the system of the pre-systemic metabolism and thus provides improvisation in the bioavailability of the drug. The requirement for a non-invasive mode of drug administration has led to the development of the transdermal drug delivery system.

Significance of Study

The significance of the study revolves around the overall formulation of the drug to be administered, the optimization of the drug administered to the patients and the evaluation of their effectiveness when they are administered to the patients who opt for a non-invasive method of drug administration due to problems such as needle phobia and others (Opatha *et al.* 2020). The process of the transdermal drug delivery system is significant in the provision of a reliable method of introducing the drug into the body of the patient when the case of rapid onset is not significant. The transdermal drug delivery system provides an effective bypassing of the enterohepatic circulation in the human body. The entire significance of the study revolves around the formulation, optimization and the evaluation of the transdermal drug in the human system. The report specifically enhances the formulation procedure of the drug Fentanyl. The transdermal drug Fentanyl is one of the most used drug in the healthcare sector due to its property of reducing acute pain in the patients who are undergoing surgical procedures. This action leads to a much more effective clinical action. With the availability in the market, the transdermal drug delivery system provides the same efficacy is the transdermal delivery of the drug which is equivalent in comparison to the infusion of IV drugs, despite being non-invasive in nature (Choudhury *et al.* 2017). The study proves the transdermal use of opioids may prove to be advantageous over any other drug administration method such as situations where a long-term pain control may be required, but the side effects of the transdermal drugs are the same as the other opioids are almost the same with a little less intensity. The study revolves around the process by which these transdermal drugs are formulated, the methods by which they are optimized and the level of effectiveness they produce when they are administered in the blood system.

MATERIALS AND METHODS**Research Philosophy**

The research philosophy that is used in this research study is realism. Realism is used as the research philosophy because realism solely depends on the real values that are obtained from the part of the research. The research study is based on scientific study and hence realism acts as the foundation of the scientific reasoning used in this research study to evaluate the effects of the transdermal drug delivery system (Opatha *et al.* 2020). Other forms of research philosophy such as interpretivism, positivism and pragmatism are not used as the research philosophy in this case because the effects of the drug administration cannot always be positive, and hence positivism cannot be used as research philosophy. Interpretivism cannot be used as the effects of the transdermal drug delivery system cannot be interpreted without administering the drug to the patients (Ahmed *et al.* 2019).

Research Approach

The Deductive research approach is used in this research study for further discussion on this particular drug components related topic. A deductive research approach is used in this research study because the research deals with the formulation of any drug in the transdermal drug delivery system, their evaluation and their effectiveness when they are administered to the patients. Another reason for using the deductive approach is that the effects of the drug administered have to be recorded and their effects have to be thoroughly analyzed to deduce the final outcome



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(Souto *et al.* 2020). An exploratory and descriptive research approach is not used in this research study because the study does not explore the effects of the drug, rather it focuses on the effectiveness of the administration procedure (Zhou *et al.* 2018).

Research Design

The experimental research design that needs to use in this research study for further discussion. The main reason for using the experimental research design is that the drug administration given to the patients is given for recording their effects and feedback from them (Gu *et al.* 2019). The entire form of the research is based on the process of carrying out the objectives of the research objectives so that the precision of the research study is maximized as possible. The main reason for using the experimental research design is because this design will help the researchers conclude the effects of the drug administration on the patients. Descriptive and exploratory research design is not used in this case study due to the sole reason that the research study does not require to describe of the side effects of the “transdermal drug delivery system” and it does revolve around exploring the components of the “transdermal drug delivery system” (Chaurasiya *et al.* 2019).

Data Collection Method**Secondary Qualitative Data Collection**

The data collection method used in this research study is secondary qualitative and secondary quantitative. A secondary qualitative data collection technique is used in this research data because the research study deals with previously done literary works such as journals, articles, online articles and websites to collect the data regarding the evaluation of the transdermal drug delivery system in the human system (Kathe & Kathpalia, 2017).

Secondary Quantitative Data Collection

A secondary quantitative data collection technique is employed in this research study to gather the numerical data regarding the previously done work on the assessment of the optimization of the transdermal drug delivery system, in order to assess the efficacy of the transdermal drug delivery system. The advantages of conducting secondary quantitative data collection are vast in regard to the topic of the research study. The advantages of conducting the secondary quantitative data collection method also include the exploring of the dosage in which the transdermal drug is administered to the patient. Transdermal drugs such as Fentanyl is can he explored properly by the tables and the numerical values that are obtained through the process of data collection. The secondary quantitative data collection is economical in nature and saves a lot of time and cost in drawing a final conclusion to the overall case study. Primary quantitative data collection is not used in this research as the patients to whom the drugs are administered are not allowed to reveal any such information (Qindeel *et al.* 2019).

Reliability and Validity

Reliability and validity of the research are the furthestmost important aspects which determine the quality of entire research. The process of reliability and validity are used as an indicator of the test, method or the techniques employed in this to actually measure the optimization, evaluation and effectiveness of the transdermal drug delivery system (Cherukuri *et al.* 2017). Reliability of the research study also deals with consistency of the research study while the validity of this research study contracts with the accuracy with which the entire research study is conducted. Reliability and validity of research are the most important components to be considered while creating the research design and planning the methods of research design execution.

Ethical Consideration

All the data used in this research study is completely authentic and the information gathered in this research study is purely for academic use and not used for various commercial purposes. In this research study, a legal provision of the “Data Protection act 2018” is considered in order to ensure that the collected data is safe and protected from any kind of mishandling. In this research, the “General Data Protection Regulation” has also been implemented to ensure



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that the collected data is used only for academic purposes and not for any sort of commercial use (Jagdale & Pawar, 2017).

RESULTS**Secondary Qualitative Data Collection****Theme 1: Optimization of “transdermal drug delivery system”**

The various methods used to figure out the optimization of the “transdermal drug delivery system” is the incorporation of the various kinds of transdermal patches in the circulatory system through the skin. The patches have been found to be highly effective and have large advantages in comparison to the conventional drug delivery system. The overall advantage that the transdermal drug delivery system offers consists of controlled absorption of the drug in the circulatory system, improved bioavailability and painless application (Babaie *et al.* 2020). Another piece of evidence that has been collected about the optimization of the transdermal drug delivery system is the presence of uniform levels of plasma present in the body of the patients in whom the transdermal drug delivery system is administered. The effectiveness of the transdermal drug delivery system is proved by the reduction of the side effects of the drug administration when they are applied to the skin of the patient. The system of transdermal drug application deals with another benefit of flexibility of terminating the entire procedure without any potential downside to the treatment procedure by the process of simple removal of the transdermal patch from the skin surface of the patient (Ng & Gupta, 2020).

Theme 2: Factors affecting the absorption of transdermal drug delivery system

The factors that highly affect the efficacy of the transdermal drug delivery system in the human system are the differences in the content of the lipid molecules, and the thickness of the skin of the patient. The patient skin plays a huge role in the successful administration of the transdermal drug delivery system. The other factors that play a huge role in the successful administration of the drug are the age, gender, the amount of skin hydration of the patient in cases such as Fentanyl and the existent disease of the patient (Babaie *et al.* 2020). The skin of the patient varies with the age group a person is in and is a considerable factor. All these factors result in the variation in the drug absorption rate across the skin of the patient, as they are part of the transdermal drug delivery system. These factors are counted and the variations are calculated to overcome the hindrances in drug administration.

Theme 3: Drug Selection of “transdermal drug delivery system”.

The drug that is selected in this research study order to exploit the drug selection process of “transdermal drug delivery system” is Fentanyl (opioid). Fentanyl is a “transdermal drug” that is used as remedy to treat patients in order to cure them of their pain using the non-invasive measures. Generally Fentanyl is administered to the patients using the intravenous route of drug delivery in order to treat their acute pain, but the intravenous treatment requires the lodging of the intravenous ports into the skin of the patients that cause the infection of the skin such as skin infections, dislodgments of the intravenous equipments, and utter distress to the patients during their treatment methods. Fentanyl is an opioid drug that can be administered to the patients who are suffering from acute pain by a variety of methods. Fentanyl can be administered intravenously by the method of IV port, intramuscularly, or transdermally in the form of film-forming gel. Fentanyl is also available in the form buccal soluble film, which is meant to dissolve in the mouth. This opioid can be administered in the form sublingual tablet. Though Fentanyl is a “transdermal drug”, the side effects of the transdermal drug are various. The administration of Fentanyl slows down the hepatic elimination of the drug. The drug when administered transdermally can cause the fumes of the drug to cause respiratory depression and sleep apnea. Some patients are even found to be intolerant towards the application of Fentanyl, as the drug causes the hypersensitivity in the patients.

Secondary Quantitative Data Collection

In the secondary quantitative data collection resources, a set of recordings has been collected in order to understand the formulation and evaluation of any drug in the transdermal drug delivery system.



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The tables collected as a part of the secondary quantitative data collection show that the transdermal drug delivery system is mainly done by formulating in the form of gels and applying it to the transdermal skin patches applied to the patients. The tables also indicate the methods of administering the drug can be divided into three forms which are patches, film-forming system and semisolid induction. The tables show that drugs such as Fentanyl, are non-sticky and non-greasy which means that the drug is very much susceptible to be transferred from one location to the other by the process of touching. The above table indicates that most of the “transdermal drug delivery system” is administered in the form of solutions, sprays or emulsion gel that forms a film after application of the drug on the skin surface of the patient. The transdermal drug delivery system is mainly evaluated on the basis of the eradication of pain in the patients who are undergoing the treatment and the positive outcomes the “transdermal drug delivery system” brings to the treatment procedure (Jain & Kumar, 2017).

DISCUSSION

In the secondary qualitative data collected in the research study, it has been found that there are various advantages to the application of the transdermal drug delivery system as an alternative form of drug administration for the patients who are suffering from the phobias such as needle phobia. Not only does the transdermal drug delivery system have its advantage in eradicating the pain from the entire drug administration procedure but also provides a certain set of perks over the conventional system of drug administration, but also provides an easy withdrawal technique to end the procedure (Wake & Kshirsagar, 2017). The transdermal drug delivery system has a certain set of disadvantages as well which is limited to the size of the molecules that are being absorbed over the surface of the skin. Transdermal drugs are highly dependent on the hydrophilic structure of the drug molecules. Studies suggest that the hydrophilic nature of the transdermal drug molecules causes their permeation in the human skin too slowly, to produce any therapeutic effect (Zhou *et al.* 2019). Another downside of the transdermal drug delivery system is that the drug such as Fentanyl delivered by the process of transdermal drug delivery is delivered by the formation of gel, which may stick to the surface of the skin of the patient and are highly likely to cause problems such as erythema, severe itching and causation of local oedema. The transdermal drug delivery system also comes with a disadvantage in that the effect of the drug is dependent on the site of the skin on which the drug is applied. The barrier function of the skin varies in different areas in the patient body, and also varies from patient to patient according to their demography. Drugs like Fentanyl, should not be used as concomitant with certain types of medications. These medications are of the category of CYP3A4 inhibitors like macrolide antibiotics and antifungal agents. The protease inhibitors in the patients may cause the plasma concentration of the drug, Fentanyl to rise in the blood stream with every dosage administered to the patient. From the secondary quantitative data collection, it is highly evident that the formulation of the transdermal drugs is based on the polymer matrix of the drug. Tables suggest that the drugs are formulated on the basis of certain factors of the skin that plays a huge role in determining the absorption rate of the drug. The first factor is the percentage moisture content of the film-forming solution spray or solution (Das & Ahmed, 2017). The percentage moisture content is counted by the following formula:

$$\frac{\{(initial\ weight - final\ weight) * 100\}}{final\ weight}$$

Another factor that is counted in the final evaluation of the transdermal drug delivery system is the percentage of moisture uptake in the transdermal drugs. The percentage of the moisture that uptake is calculated in the formulation process of the transdermal drug by the following formula:

$$Percentage\ moisture\ uptake = \frac{\{(final\ weight - initial\ weight) * 100\}}{initial\ weight}$$

All such formulation techniques are used in the overall formulation, optimization and evaluation process of the “transdermal drug delivery system” (Agrawal *et al.* 2020). The formulation of the drug Fentanyl is prepared by the infusion or the dilution of infusion fluids that contain 6% of glucose or the concentration of sodium chloride, before they are administered to the patients by PVC infusion.



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CONCLUSION

It can be concluded from the research study that the transdermal drug delivery system is purely evaluated on the basis of the therapeutic advantage it provides to the patient while bypassing all the invasive methods possible to lessen the amount of pain caused to the patient. The in vitro permeation study of the transdermal drug delivery system that is Fentanyl results vastly vary from the actual application of the transdermal drug in the patients who receive the drug treatment. The prime factors that play a huge role in the efficacy or the optimization of the effects of the transdermal drugs are skin permeation and sensitization. The overall positive effects of the drug surpass the minor problems caused by the retention of the drug on the surface of the skin for a long duration. The stability studies have also concluded that the transdermal drugs need to be stored at a 75+6% RH for at least about 7 months to produce the maximum effect when applied in the “transdermal drug delivery system”. The overall formulation, optimization and evaluation of the “transdermal drug delivery system” is a dynamic process and is highly subjective to the patient undergoing the drug treatment.

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	Patches	Film forming system	Semisolids
Visual appearance	Highly visible	Almost invisible	Visible
Skin feel	Non-sticky, non-greasy	Non-sticky, non-greasy	Sometimes sticky, greasy
Administration	Convenient	Convenient	Sometimes messy
Dose adjustment	Low	High	High
Dosing frequency	1–7 d	1–2 d	1 d or less
Sustained release	Yes	Yes	No
Occlusive properties	Yes	No	No
Wipe off resistance	Yes	Yes	No
Residual remains	Possible	No	No

Figure 1: comparison of Transdermal drug delivery system(Source: Jain & Kumar, 2017)

Product	Drug	Company	Formulation type
Lamisil Once® [61]	Terbinafine hydrochloride	Novartis Consumer Health, Australasia, Pty Ltd	Film forming Solution
Axiron® [62]	Testosterone	Lilly USA, LLC	Film forming spray
Medspray® the Patch-in-a-Can® [63]	Terbinafine hydrochloride	MedPharm Ltd, UK	Film forming spray
Liqui-Patch technology [64]	Testosterone hydrocortisone	Epinamics GmbH, Germany	Film forming spray
Durapeel Technology [65]	Ropivacane	Crescita Therapeutics, Inc	Film forming gel
PharmaDur®Technology [66]	Hydroquinone	Polytherapeutics, Inc	Film forming emulsion-gel

Figure 2: The commercialized film-forming system of transdermal drug delivery(Source: Sarheed *et al.* 2020)





Phytopharmacological Properties and Commercial Value of *Madhuca longifolia* (L.) J. F. Macbr. for Sustainable Applications

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ABSTRACT

Madhuca longifolia (L.) J. F. Macbr. plant is from Sapotaceae family found in India. Local tribes rely on it for both food and survival. Three fundamental requirements are fulfilled by this tree (3F's) i.e. Food, Fodder, and Fuel. Mahua flowers are harvested and dried by Indian tribes to prepare "mahuadaaru," handmade alcohol containing 20-40% alcohol. Edible oil seed cakes are mostly utilized as fodders because of their excellent nutritional value and protein content. Non-edible oil cakes can also be utilized as fungal growth substrates at a minimal cost. Mahua flower extracted in methanol exhibited possible protective effects by reducing SGOT, SGPT, ALP, and total bilirubin levels raising total proteins and albumin levels in the blood. Mahua flowers are high in calcium and phosphorus, protein, and lipids. Medicinal efficacy towards anthelmintic, antibacterial, analgesic, hepatoprotective, antioxidant and anticancer activity of Mahua flowers is also known. The Mahua flowers are utilized as a biomass feedstock in the manufacturing of alcohol as well as in the creation of alcoholic beverages. It is nutrient rich and is useful for a variety of uses, and it may also be used after detoxification from poisonous saponins). Flowers are valued as supplementary food and contribute towards food security and played an important role in mitigating rural poverty and also contributing towards zero hunger and curing various diseases to maintain the health and wellbeing for sustainable development. Additionally, the seed cake may also be utilized for mushroom cultivation.

Keywords: Anthelmintic, health and wellbeing, hepato protective, *Madhuca longifolia*, sustainable development, zero hunger





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INTRODUCTION

Madhuca longifolia (L.) J. F. Macbr. is an indigenous plant that belongs to the Sapotaceae family, found in subtropical zones of India. It is used by local tribes for their food and survival for fulfilling the three fundamental needs (3F's) i.e. food, fodder and fuel (Patel *et al.*, 2012). *M. longifolia* is an annual bearing tree whose flower-bearing period is March-April and flowers shed after maturity. Flowers are sweet in taste and are a good source of vitamins, fats, proteins and mineral nutrients. Flowers are used in making traditional dishes like *halwa*, cakes and *kheer* in mahula production belt of India because of high sugar content (Patel *et al.*, 2012; Gopalan *et al.*, 2007). Fresh flowers are also known for its nutritional properties (Suryawanshi and Mokate, 2019). The uses are many starting from the production of alcohol, ethanol (Swain *et al.*, 2007), dietary supplements, bio-pesticide, fungicide (Shanmugasundaram *et al.*, 1989), dermatitis curing properties, enhancing lactation (Patel, 2008; Ramadan *et al.*, 2010), cooling properties on burns (Patel *et al.*, 2012) and biodiesel production (Kapilnand Reddy, 2008; Ghadghe and Rehman, 2005; Puan *et al.*, 2005). Flowers are valued as supplementary food and contribute towards food security and played an important role in mitigating rural poverty and also contributing towards zero hunger and curing various diseases to maintain the health and wellbeing for sustainable development.

Ethno Medicinal Uses

In Ayurveda, various uses of the underutilized tree are mentioned where the flower could be used as tonic for heart diseases, skin diseases and to cure eye diseases because of its high protein content (Amia and Ekka, 2014). Mahua flower works as a cooling agent. Local people consume the roasted flowers to cure bronchitis and cough. The flower juice is rubbed on the skin to get rid from itching, used in the treatment of diarrhea, treats piles and colitis (Amia and Ekka, 2014; Sinha *et al.*, 2017). *M. longifolia* flowers can cure general infirmity and impotency when consumed with milk. (Acharya and Srivastava).

Traditional Utilization of Mahua

The flowers and seeds are used by tribal people because of its invaluable composition. Tribal people use mahua flowers as the natural sweetener because of high sugar content in preparing various traditional and local dishes (Behera *et al.*, 2016). Fresh flowers are plucked and dried for two to three days under direct sunlight and stored in gunny bags. The mahua flowers are used as the raw materials in production of alcohol and alcoholic beverages. Local tribes prepare "mahuadaaru", the homemade alcohol containing 20-40% alcohol (Acharya and Srivastava, 2008). In Odisha, tribal people prepare the country liquor 'mahuli' from mahua flowers (Behera *et al.*, 2016). In this process of fermentation, mahua flowers are kept in water and jaggery and navshar (Ammonium chloride) was added and kept for fermentation. Traditional distillation pump is used to procure the alcohol. About 300 to 400 ml of alcohol is produced from one kilogram of dried flowers (Kumari *et al.*, 2016). Both mahuli and mahuadaaru preparation methods are similar, but the difference lies in the process of distillation and the addition of ingredients at the time of fermentation. In the preparation of 'mahuadaaru' bakhar tablets consists of *Asparagus racemosus* (roots), *Cissampelos pareira* L. (roots), *Clerodendrum serratum* (roots), *Homalium nepalense* (bark), *Dipteracanthus Suffruticosus* (roots), *Elephantopus scaber* L. (roots), *Lygodium flexuosum* (roots), *Ochna obtisata* (roots), *Phoenix acaulis* (roots), *Holarrhena pubescens* (bark), *Woodfordia fruticosa* L. (flower), *Xantolis tomentosa* (fruit), *Madhuca indica* (seeds)] was added during fermentation.

Phytochemicals Composition of Flower

M. Longifolia flowers are used to prepare modern and traditional alcoholic beverages because of their higher sugar content. Mahua flowers show antioxidant activity because of high vitamin C content (Indu and Annika, 2014; Palani *et al.*, 2010). Flowers also contain carotene, the precursor of vitamin A, minerals viz., phosphorus and calcium and very lesser quantity of proteins and fats. A certain briefing was done on the composition, medicinal properties of the mahua flower based on previous studies (Table 1). Pharmacological and antimicrobial activity of mahua flowers Various pharmacological properties and antimicrobial activity of Mahula flowers was depicted in Table 2.





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Value Addition and Commercial Utilization of Mahua Flowers and Oil Seed Cakes

Nowadays underutilized crops are gaining attracting researchers, pharmaceutical industries and food industries aiming to produce new products which are healthy as well as sustainable. Due to its higher sugar content, it is used as sweetener and also in preparation of butter, jelly, cakes, candies which add a healthy initiative towards a healthy diet. The seeds contain 50-60% fat giving more than 50% oil yield. The leftover oil seed cake is used as fertilizer. Apart from these, it can also be used as cattle feed to increase in milk production and improve in the health condition in cattle. (Sinha *et al.*, 2017). Oil seeds produce two types of oil seed cakes viz., edible and non-edible. Edible seed cakes are mainly used as animal feeds because of high protein content and enriched with nutrition. Non edible cakes obtained from Mahua (*M. longifolia*(L.) J. F. Macbr.), neem (*Azadiracta indica*), karanja (*Millettia pinnata*) and jatropa (*Jatropha curcas*) is not used as animal feed despite of their high nutritional content because of the presence of toxic compounds. These cakes can be used as biopesticides because of the toxicity. For growth of the fungus, non-edible oil seed cakes could be utilized as low-cost substrates (Satawati *et al.*, 2017).

CONCLUSION

M. longifolia flowers, seed oil and seed cake has several uses. The studies highlight and explore the sustainable usage and nutritive significance which gives a prime purpose to awareness at the capability usage for economic, pharmaceutical, and industrial benefits. The flowers can be used as the new assets for antibacterial, antifungal, and antiviral agents. After alcohol manufacturing or any fermentation system, the leftover may be used as biofertilizer. New sustainable methods of oil extraction are preferred for price-saving. The nutrient-wealthy composition of seed cake makes it beneficial for numerous applications, which may be used after detoxification of poisonous saponins. The seed cake may be used for mushroom cultivation. The leftover seed cake or the flower pulp can be used as feed for farm animal for its high nutritional contents.

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Table 1: Composition of fresh flowers

Constituents	Fresh flowers (%)	Constituents	Fresh flowers(mg/100g)
Moisture	73.6-79.82	Reducing sugar	36.3-50.62
Ash	1.5	Starch	0.96
Fibers	10.8	Total sugars	47.35-54.06
Proteins	6.05-6.37	Calcium	45
pH	4.6	Phosphorus	22
Fats	1.6	Vitamin C	40
Cane sugars	3.43	Carotene	307(µg/100 g)

(Gopalan *et al.*, 2007; Swain *et al.*, (2007); Hiwale, 2015; Patel *et al.*, 2011)

Table 2: Pharmacological and antimicrobial activity of mahua flowers

Pharmacological activity	Solvent extract	Remarks	References
Hepatoprotective activity	Methanol	Showed lowering of total bilirubin, ALP, SGPT, SGOT with increase in total serum proteins and albumins	Patel <i>et al.</i> , (2012); Yadav <i>et al.</i> , (2012); Sinha <i>et al.</i> , (2017)
Anthelmintic activity	Methanol and ethanol	Significant anthelmintic activity against Indian earthworm by methanolic extract than ethanolic extract	Yadav <i>et al.</i> , (2012); Sinha <i>et al.</i> , (2017)
Antibacterial	Methanol	Showed significant antibacterial activity	Verma <i>et al.</i> , (2010); Patel <i>et al.</i> ,





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activity	and aqueous	for Bacillus subtilis and Klebsiella pneumonia than methanolic extract	(2012); Yadav <i>et al.</i> , (2012); Sinha <i>et al.</i> , (2017)
Anti-inflammatory activity	Aqueous and alcohol	Showed analgesic effect on mouse as per recommended dose value	Verma <i>et al.</i> , (2010); Chandra, 2011; Patel <i>et al.</i> , (2012); Yadav <i>et al.</i> , (2012); Sinha <i>et al.</i> , (2017)
Antioxidant activity	---	Ferric reducing antioxidant power increases with the increase in the concentration of flower extract	Indu and Annika, (2014)
Anticancer activity	---	Decrease in viability of cells increases in response to concentration of floral extract	Indu and Annika, (2014)

(ALP: Alkaline phosphatase; SGPT: Serum glutamic pyruvic transaminase; SGOT: Serum glutamic oxaloacetic transaminase)





A Critical Overview on Drone Algorithms Performing Agricultural Way

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ABSTRACT

Our country's backbone is agriculture. The majority of food grains are transported to cities from rural areas. Traditional farming methods are difficult to come by these days. As a result, it is necessary to adapt to current agricultural methods in order to reduce labor costs, time consumption, and other factors that lead to an improvement in crop output. In the end, the profit will go to the farmer. The goal of this study is to give a general overview of one of the most recent forms of agriculture, Agricultural Drones, and their application in several agricultural disciplines. Drones are causing a significant shift in the way we grow crops. Precision agriculture, which utilizes GPS and big data to manage crops, has been touted by drone proponents for years as a method to boost crop productivity while addressing water and food shortages. Drones delivering agricultural intelligence for both farmers and agricultural consultants are changing agricultural practices, from the ability to image, recreate, and analyze individual leaves on a corn plant from a height of 120 meters to getting information on the water-holding capacity of soils to variable-rate water applications.

Keywords: Drone analysis, Precision agriculture, Traditional farming, GPS, Crop management

INTRODUCTION

A DRONE (Dynamic Remotely Operated Navigation Equipment) is a device that may fly on a predetermined course using autopilot and GPS coordinates, or manually using radio signals via a remote control or smartphone app. Drones can detect things that are outside the visible range of human sight thanks to the abundance of sensors available. As a result, drones can provide real-time, more precise, dependable, and objective data with more precision and fewer errors. Drones are widely regarded as humanity's greatest innovation. Drones have a wide range of applications and are also known as unmanned aerial vehicles (UAV)(Maddikunta *et al.* 2021). Land vehicles were employed in old agriculture methods to monitor various agricultural processes, which took a lot of human labor and time. Drones in agriculture are more useful than traditional methods for agricultural chores. Due to its most amazing qualities, the use of drones in agriculture provides a tremendous benefit in terms of economy and time(Dileep *et al.*



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2020). Many studies have shown in recent years that drones can cover nearly 10 to 15 times the area that traditional land-based approaches can cover (Rani *et al.* 2019). A drone has an advantage over a satellite in that it may be controlled at any time, but a satellite can only pass at a certain period. As a result, when compared to satellite data, drones give real-time data with more detail and less error. To avoid drones flying over and over in the field, the data collected by drones can be combined with satellite data, increasing the accuracy of satellite data. As a result, data collected by drones can be used to supplement satellite data (Frankelius *et al.* 2019).

Drone Algorithm Involvement In Agricultural Ways:

Crop Health Tracking: - Drones equipped with Multispectral camera sensors can detect disease and stress early on, sometimes even before it is visible from the ground or with ordinary color cameras. Drone surveys also give real-time field imagery for precision farming. Irrigation Monitoring, Crop Health Monitoring, and other key parts collect and analyze the data obtained to assist farmers to focus on treatment strategies. The capacity to inspect in-progress crops from a using Normalized Difference Vegetative Index (NDVI) or near-infrared (NIR) sensors has so far been the most popular use of drones in agriculture.

Crop spraying Management: -Drones may spray fertilizers, insecticides, and other chemicals based on the spatial variability of the crops and land. Depending on the crop circumstances or the severity of the insect-pest assault, the amount of chemicals to be sprayed can be changed. Drones pave the door for precision agriculture in this way.

Crop Damage assessment: -Drone pilots can collect high-resolution data that can be used to measure and quantify agricultural damage caused by unforeseen catastrophes such as floods, fires, pests, and weather disasters. Farmers and government agencies can use data from drones with remote sensing capabilities and Photogrammetry as evidence to claim crop insurance or get an estimate.

Irrigation visibility and analysis: - Drones equipped with Thermal Cameras and Remote Sensing capabilities, can assist in resolving irrigation concerns, as well as locations that receive too much or too little rainfall. RGB image visualization in field topography can be helpful in positioning and segregating crops to enhance drainage, follow natural land flow, and avoid waterlogging. Farmers will be able to adapt to various situations with ease with our services.

Soil and Field monitoring: - Drones implement the role of sensors in analyzing soil and terrain conditions, surface soil wetness, nutrients content, and soil fertility levels, which can then be used to plan the pattern of sowing different crops, irrigation scheduling, and fertilizer application while taking into account spatial variability of crop growth and field conditions.

LITERATURE REVIEW

(Rani *et al.*, 2019) has adopted modern agricultural technologies, such as the use of drones, which can dramatically improve risk and damage assessments and alter the way people plan for and respond to disasters that affect the lives of vulnerable farmers and fishermen, as well as the country's food security. (Dileep *et al.*, 2020) has studied the Various types of agricultural drones based on feature, capacity, range, cost, and the areas of agriculture where they are most useful, as well as a statistical analysis of drone use in agriculture. Thus, Agriculture will provide a significant amount of GDP (Gross Domestic Product) for the country. (Colloredo-Mansfeld *et al.*, 2020) focused on the use of high-resolution imagery from an unmanned aerial vehicle (UAV) in the Galapagos Islands for on-farm qualitative study that intends to investigate the visible and invisible aspects of farming methods. Thus, UAV imagery helps agricultural heritage studies not just by recording agricultural landscapes but also by disclosing agrarian knowledge and practices, according to the findings.



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(Esposito *et al.*, 2021) has utilized UAVs and machine learning approaches which can increase weed management sustainability by accurately identifying weed areas in cultivated fields. Thus, UAVs can help with integrated weed management (IWM), reducing both the selection pressure against herbicide-resistant weeds and the spread of pesticides in the environment. (Dutta & Mitra, 2021) in their work, they used IoT to emphasize the design of a unique drone idea with 3D mapping, as well as resolving post-COVID19 challenges in agriculture and proposing monitoring in a comparative comparison. As a result, implementing IoT (Internet of Things) in agriculture has the potential to improve the environment while also increasing the efficiency of companies.

(Kulbacki *et al.*, 2018) in their study drones are undertaken, with a focus on remote sensing applications in Smart Farming. According to assessments from around the world, there is interest in and a need for using remote sensing technologies with drones to boost farm production and harvests. (Ahrwar *et al.*, 2019) described on the uses of smart technology in agriculture, such as drones, has the potential to address a number of major and minor difficulties. Irrigation, crop monitoring, soil and field studies, and bird control are some of the most common uses of drones in agriculture. (Manikandan *et al.*, 2019) in their study integrated the mobility of a UAV (Unmanned Aerial Vehicle Drone) with a GSM monitoring system and an Arduino interface, real-time data may be collected. The drone, which is based on the Arduino programme, is intended to improve agricultural chores such as spraying pesticides and water. (Panday *et al.*, 2020) presented a better knowledge of how drone-based data solutions might aid in the fight against food insecurity caused by pandemics, zoonotic diseases, and other food shocks by increasing cereal crop productivity in low-income nations' small-scale farming systems. (Hafeez *et al.*, 2022) presented an examination of drone technology for crop monitoring and pesticide spraying in Precision Agriculture (PA) and their evolution through time in the agriculture sector to fulfil increased food demand due to population growth.

(Daponte *et al.*, 2019) proposed a study of strategies for precision agriculture monitoring using drones equipped with multispectral, thermal, and visible cameras. The key limits of each application are noted, as well as the criteria to consider before performing a flight. (Bruscolini *et al.*, 2021) described an algorithm for detecting the location and quantity of plants in vineyards for precision monitoring using drone RGB data. The first results reveal an 87 percent accuracy in plant detection. (Saha *et al.*, 2018) adopted Drones with the suitable cameras, sensors, and integrated modules will make precision agriculture simple, efficient, and cost-effective. hence It will not only save time, but it will also result in improved cultivation as a result of the data analysis. As a result of the systematic monitoring, crop management will be more efficient. (Faiçal *et al.*, 2014) described on unmanned aerial vehicles (UAVs) that can be used to create a control loop for agricultural applications, such as spraying pesticides on crops. In addition, they assessed an algorithm for adjusting the UAV route in response to variations in wind (strength and direction) as well as the impact of the amount of messages exchanged between the UAV and the WSN.

Critical Appraisal Of Reviewed Literature:

Advanced drone technology and Machine Learning for Precision Agriculture have enhanced agricultural yields and profitability, according to the literature reviewed above. As a result, farmers were forced to use less standard input to grow crops and manage land, water, fertilizer, herbicides, and insecticides. Crop yield projections, accurate crop count, crop emergence analysis, irrigation monitoring, crop health, crop damage assessment, field soil analysis, and other applications using a combined strategy of UAV Aerial Imagery and Machine Learning systems. Drone data and photogrammetry of high quality protect crops, ensuring productivity and providing farmers with all available benefits.

CONCLUSIONS

Drones can transform Indian agriculture into a new field level experience. Its production is likely to become more cost-effective in the future. Due to some unavoidable obstructions still involved in farming, today's youth are not much interested in agriculture. Drones' implications may interest and encourage young people to pursue careers in agriculture. Drones can play a key part in the next agricultural revolution, which will be data-driven. Appropriate





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data use can help farmers improve their lifestyles while also increasing agricultural yield without harming the environment. Drones may thus become an integral element of agriculture in the future, assisting farmers in better and more sustainable management of their crops and resources. The following noted points are consequences of drone application in agriculture:

- Drone inspections provide higher-resolution visual inspections than ground-based examinations.
- Thermal/4k capabilities increase efficiency by providing high-resolution data in a timely manner.
- Aerial mapping of crop fields helps farmers make better decisions and avoid costly mistakes.
- When compared to a ground-based approach, Drone Mapping for Crop Fields saves money, time, and resources.
- Inventory information, crop emergence, drive replanting decisions, overall yield projection, and other predictions are highly accurate.

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Osmotically Controlled Drug Delivery Systems: A Review

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ABSTRACT

The current review sought to develop an osmotic drug delivery system for regulated drug release. The oral route of drug delivery is the most prevalent and appropriate approach. Because recurrent dosage administration is necessary for the treatment of chronic illnesses, the osmotic drug delivery system serves as a tool for controlling drug release in these conditions and avoiding repetitive administration. The following review explains the fundamentals of osmotic drug delivery systems, types with a special emphasis on controlled porosity osmotic pumps, osmosis mechanism, ideal drug candidate, formulation techniques, various osmotic agents, pore formers, coating materials, and marketed preparations based on osmotic drug delivery systems. These factors may be useful in designing dose for modifying the release of different drugs that have a difficulty in their conventional form.

Keywords: Osmosis, Osmotic pressure, Osmotic drug delivery, Osmotic Pump

INTRODUCTION

Conventional oral drug delivery methods are known to offer rapid drug release, with no control over drug release and effective concentration at the target location. This type of dosage strategy results in unpredictability of plasma drug concentration, which leads to significant adverse effects. The bioavailability of the drug from these formulations can vary greatly depending on factors such as the drug's physicochemical properties, the presence of excipients, and various physiological factors such as the presence or absence of food, the pH of the gastrointestinal (GI) tract, and GI motility[1]. Oral medication delivery techniques are well recognised for providing fast drug release with no control over drug release and effective concentration at the target site. This sort of dosing approach causes unpredictability in plasma drug concentration, which might have serious consequences. A superior distribution pattern is to dispense the agent from a sustained release delivery system, which slowly distributes the active agent during the delivery time[2]. Several technological advances have recently been developed. They have resulted in the creation of novel medication delivery methods. These methods are capable of regulating the pace of medication delivery in the following ways:

1. capable of regulating medication delivery rate



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2. Maintain therapeutic action over an extended period of time.
3. Targeting medication delivery to tissues.

These improved techniques have resulted in the creation of many Novel Drug Delivery Systems (NDDS), which have revolutionised medicine and therapeutic benefit.

The release of drug molecules from the drug delivery system is initiated by some physical, chemical, or biological processes in this type of Control drug delivery system (CDDS)[3].

Classifications[4]**1. Physical Methods**

- a. Drug delivery method triggered by osmotic pressure.
- b. Hydrodynamic pressure drug delivery system
- c. Vapor pressure drug delivery system
- d. Mechanically activated drug delivery system
- e. Magnetically activated drug delivery system
- f. Sonophoresis activated drug delivery system
- g. Ionotrophoresis activated drug delivery system
- h. Hydration activated drug delivery system

2. Chemical methods

- a. pH-activated drug delivery method.
- b. Ion exchange drug delivery system.
- c. The drug delivery mechanism was triggered by hydrolysis.

3. Biochemical methods

- a. A medication delivery device that is triggered by enzymes.
- b. Drug delivery device that is biochemically triggered.

An adequately designed osmotic drug delivery system (ODDS) is unaffected by physiologic variables, and drug release may be easily anticipated based on the drug's and dosage form's known characteristics. Among controlled-release devices, osmotically driven systems stand out due to their dependability and capacity to distribute materials at a specified zero-order rate over an extended length of time.[5] Osmotic drug delivery methods, with their flexibility and highly predictable drug release rates, provide numerous biological benefits when administered parenterally, such as decreased dosage, site targeting, avoiding gastrointestinal stability, and hepatic bypass of drug molecule. As a result, the objective of this review article is to investigate numerous parameters influencing drug release from ODDS and to conduct a critical analysis of diverse techniques in the design of ODDS[6]. The most dependable controlled drug delivery systems (CDDS) are osmotically controlled drug delivery systems (OCDDS) or osmotic devices, which may be used as oral drug delivery systems. These devices employ osmotic pressure as a driving factor to deliver the medication in a regulated manner. Osmotic pump tablet (OPT) is made up of a core that contains the medication, an osmotic agent, additional excipients, and a semi permeable membrane cover[7].

Osmosis

The process of moving solvent molecules from low concentration to high concentration through a semipermeable barrier is referred to as osmosis. Osmosis is the phenomena that allows for regulated medication administration. The osmotic pressure generated by fluid imbibitions from the external environment into the dosage form governs medication delivery from the osmotic device. The rate of medication distribution from an osmotic pump is proportional to the osmotic pressure created by fluid imbibitions by osmogen[8]. Osmotic pressure is a colligative characteristic of a solution in which the magnitude of the solution's osmotic pressure is independent of the number of discrete solute entities present in the solution. As a result, the rate of medication release from osmotic dispensing devices is determined by the solute's solubility, molecular weight, and activity coefficient (osmogen)[9]. Abbe Nollet described the first osmotic effect in 1748. Later that year, in 1877, Pfeffer conducted an experiment in which he





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separated sugar solution from pure water using a semi-permeable membrane. He demonstrated that the osmotic pressure of a sugar solution is related to its concentration and absolute temperature. Vant Hoff discovered an underlying relationship between osmotic pressure, concentration, and temperature in 1886[10]. He discovered that osmotic pressure is proportional to concentration and temperature, and that the connection can be represented using the equation below,

$$\pi = \varnothing C R T$$

Where, \varnothing = Osmotic pressure,

π = osmotic coefficient

C = molar concentration of solute in the solution

R = Ideal gas constant

T = Absolute temperature

Osmotic pressure for concentrated solutions of soluble solutes commonly used in controlled release formulations is typically high, ranging from 30 atm for sodium phosphate to 500 atm for a lactose-fructose mixture, as their osmotic pressure can generate high water flow across a semipermeable membrane[11]. The equation describes the osmotic water flow through a membrane,

$$dv/dt = A Q \Delta \pi / L$$

Where, dv/dt = water stream across the membrane of area A in cm^2 , L = thickness, Q = permeability, $\Delta \pi$ = the osmotic pressure dissimilarity between the two solutions on either side of the membrane.

This equation applies only to fully permeable selective membranes that are permeable to water but entirely impermeable to osmotic agents[12].

History of Osmotic Drug Delivery System

There was a breakthrough in the design of medication delivery devices around 75 years after the discovery of the osmosis principle. The Australian scientists Rose and Nelson pioneered osmotic medication delivery. In 1955, they created an implanted pump with three chambers: a medication chamber, a salt chamber containing surplus solid salt, and a water chamber. The first osmotic pump for oral medication administration was introduced in 1975[13]. The pump is made up of an osmotic core that houses the medication and is surrounded by a semipermeable membrane with a delivery hole. When this pump is submerged in water, the core imbibes water osmotically at a regulated pace dictated by the membrane's water permeability and the osmotic pressure of the core formulation. Because the membrane is non-expandable, the increase in volume produced by water imbibitions causes the formation of hydrostatic pressure inside the tablet[14]. The passage of saturated solution out of the device through the delivery hole relieves this pressure. Implantable osmotic pumps were a key development in the 1970s, allowing a wide range of medicines and hormones, including peptides, to be delivered at a steady and controlled pace[15].

Advantages[16]

1. Easy to formulate and easy to use.
2. Reduced frequency improves patient compliance.
3. Long-lasting therapeutic action with consistent blood concentration.
4. After an initial latency, they generally provide a zero order release profile.
5. If desired, deliveries can be delayed or pulsed.
6. Drug release is unaffected by stomach pH or hydrodynamic conditions.
7. They are properly defined and comprehended.
8. The release mechanisms are not drug-dependent.
9. In osmotic systems, there is a high degree of in-vitro and in-vivo correlation (IVIVC).
10. The justification for this method is because the presence of water in G.I.T. is pretty constant, at least in terms of the quantity needed to activate and regulate osmotically base technologies.
11. When compared to traditional diffusion-controlled drug delivery systems, osmotic systems can achieve higher release rates.
12. The presence of food in the gastrointestinal tract has little effect on osmotic system release.



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13. The osmotic system's release rate is very predictable and may be controlled by varying the release control settings.

Disadvantages[17]

- Expensive
- There is a danger of film flaws resulting in dosage dumping if the coating process is not adequately managed.
- The size of the hole is essential in the case of elementary osmotic pressure.system.
- Drug release from osmotic systems is influenced.the availability of food helps to some extent.
- Therapy retrieval is not feasible in the event of unanticipated negative occurrence
- Rapid tolerance development
- The coating process's integrity and uniformity are not guaranteed,there is dose dumping when everything is in control, so beads on the film or particles must be induced to join to form a film having consistent characteristics
- Laser drilling requires a significant investment.
- A hypersensitivity response may develop after implantation

Limitations[18]

1. It may cause irritation or ulceration as a result of the drug's saturated solution being released.
2. Making an opening in the system necessitates the use of specialised equipment.
3. The system's residence duration in the body changes with stomach motility and food consumption.

Basic Components Of Osmotic Systems[19]

Drug

Coating agent

Wicking agent

Semipermeable membrane

Pore forming agent

Plasticizers

Osmotic agent

Surfactants

Solubilising Agents

Flux Regulators

Hydrophilic and hydrophobic polymers

Channeling Agents

Drugs[21]

Drugs with a short biological half-life (2-6 hours) and utilised for long-term therapy are excellent candidates for osmotic systems. Osmotic delivery is used for a variety of medication candidates, including Diltiazem hydrochloride, Carbamazepine, Metoprolol, Oxprenolol, Nifedipine, Glipizide, and others.

A drug with the following properties is appropriate for formulation:

1. It should have a short half-life.
2. Prolonged drug release should be sought.
3. It must be powerful in nature.
4. Drug solubility should not be extremely high or extremely low.

Coating Agent[22]

Inert inorganic and organic solvents that do not damage the core, wall, or other materials are ideal for producing polymeric solution that is utilised to manufacture the wall of the osmotic device. Methylene chloride, acetone, methanol, ethanol, isopropyl alcohol, butyl alcohol, ethyl acetate, cyclohexane, carbon tetrachloride, water, and other common solvents. Solvent combinations like acetone-methanol (80:20), acetone-ethanol (80:20), acetone-water (90:10), methylene chloride-methanol (79:21), methylene chloride-methanolwater (75:22:3), and so on can be utilised.



**Nihar Ranjan Kar****Wicking Agent[23]**

A wicking agent is a substance that has the capacity to pull water into the porous network of a delivery device.

A wicking agent might be either swellable or non-swellable.

They are distinguished by their capacity to perform physiosorption with water.

Physiosorption is a type of absorption in which the solvent molecules attach to the surface of the wicking agent by Vander-Waals interactions between the wicking agent's surface and the absorbed molecule.

The wicking agent's role is to transport water to surfaces inside the tablet's core, where it creates channels or a network of enhanced surface area.

The wicking agent's role is to transport water to surfaces inside the tablet's core, where it creates channels or a network of enhanced surface area.

Colloidal silicon dioxide, kaolin, titanium dioxide, alumina, niacinamide, sodium lauryl sulphate (SLS), low weight poly (vinyl pyrrolidone) PVP, m-pyrol, bentonite, magnesium aluminium silicate, polyester, and polyethylene are examples of materials that can be used as wicking agents. Non-swellable wicking agents include SLS, colloidal silica, and PVP.

Semi Permeable Membrane [24]

The semipermeable membrane housing is a key component of the osmotic drug delivery system.

As a result, the polymeric membrane selection is critical in the osmotic delivery formulation.

Certain features of the membrane should be present, such as

- Wet strength and water permeability are sufficient.
- It must be biocompatible.
- Non-swelling and rigid
- Should be thick enough to resist the pressure within the gadget.

As a coating material in osmotic devices, any polymer that is permeable to water but impenetrable to solute can be utilised. Some polymers that can also be utilised.

Pore Forming Agent [25]

The pore-forming chemicals are responsible for the development of micro porous membranes. A pore-former may build the micro porous wall in situ by leaching it during system operation. Pore-formers can be inorganic or organic, solid or liquid in nature. Alkaline metal salts such as sodium chloride, sodium bromide, potassium chloride, potassium sulphate, potassium phosphate, and so on, alkaline earth metals such as calcium chloride and calcium nitrate, carbohydrates such as sucrose, glucose, fructose, mannose, lactose, sorbitol, and mannitol, and diols and polyols such as poly hydric alcohols, polyethylene glycols and polyvinyl pyrrolidone can be used as pore forming agents.

Plasticizers[26]

The different types and amounts of plasticizers used in coating membranes play an important role in the formulation of osmotic systems. They can alter the viscoelastic behaviour of polymers, which can impact the permeability of polymeric films. The following are some of the plasticizers that have been used:

Polyethylene glycols are a kind of polyethylene glycol.

Monoacetate of ethylene glycol

Diacetate is used for reduced permeability.

Diacetin or Diethyl tartarate- for more permeable films

Osmotic Agent[27]

These are osmogens or osmogens that are used to produce osmotic pressure within the system. When a drug's solubility is poor, it will exhibit zero order release but at a sluggish rate. An osmotic agent is added to the formulation to speed up the release rate. The osmotic agent generates a very high osmotic pressure gradient within





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the system, which enhances the rate of medication release. Sodium chloride, Fructose, Sucrose, Potassium chloride, Xylitol, Sorbitol, Citric acid, Dextrose, Mannitol, and Lactose are some of the commercially available osmotic agents.

Some Osmotic Agent Combinations:

Dextrose +Fructose,

Lactose +Fructose

Sucrose+Fructose

Lactose+Dextrose

Sucrose+Fructose

Mannitol and Dextrose

Dextrose with Sucrose

Sucrose + Mannitol

Surfactants[28]

Surfactants are particularly useful when added to wall forming material because they produce an integral composite that is useful for making the device's wall operational. Surfactants act by regulating the surface energy of the material to improve their blending in to the composite and maintain their integrity in the environment of use during the drug release period. Typical surfactants include polyoxyethylenated glyceryl recinoleate, polyoxythlenated castor oil including ethylene oxide, glyceryl laureates, glycerol, and others.

Solubilising Agents[29]

There are three types of swellable solubilizing agents.

- Agents that inhibit drug crystal formation or otherwise act by drug complexation (e.g., PVP, PEG, cyclodextrins),
- A high HLB micelle forming surfactant, particularly anionic surfactant (e.g., Tween 20,60,80,polyoxyethylene or polyethylene containing surfactants and other long chain anionic surfactants such as SLS or alkyl esters particularly triethyl citrate

Flux Regulators[30]

When flux regulating agents, flux boosting agents, or flux lowering agents are introduced to a wall forming material, they help to control fluid permeability across the membrane.

As flux regulators, poly hydric alcohols such as poly alkylene glycols and low molecular weight glycols such as poly propylene, poly buylene, and poly amylene, among others, can be added.

Hydrophilic And Hydrophobic Polymers[31]

Hydrophilic and hydrophobic polymers: These are used in the formulation creation of osmotic systems with drug matrix cores. The polymer is chosen based on the following criteria: drug solubility the amount and pace of medication release from the pump. HEC (hydroxyl ethyl cellulose) HPMC (hydroxyl propyl methyl cellulose) CMC (hydroxyl propyl methyl cellulose) are examples of hydrophilic polymers (carboxy methyl cellulose). Examples of hydrophobic polymers include EC (ethyl cellulose), wax compounds, and others.

Channeling Agents[32]

Osmotic pumps are composed of water-soluble components that aid in the controlled release of drugs. Upon contact with semipermeable membranes, the dissolution medium dissolves the channeling agent and creates pores in the semipermeable barrier. The dissolving fluid then enters the osmotic system and, via the process of osmosis, releases the medication in a regulated manner over a lengthy period of time. Polyethylene glycol (PEG) 1450, mannitol, bovine serum albumin (BSA), diethylphthalate, dibutylphthalate, and sorbitol are some examples of channelling agents.

Drug Delivery Devices Used For Osmotic Delivery

In general, osmotic drug delivery devices are classified into two broad categories: implants, oral and specific pumps. These are described below.



**Nihar Ranjan Kar****Implantables**

Osmotic pumps implanted in an animal or man usually deliver drugs through the orifice upon contact with body fluids. Implantable osmotic pumps are outlined below.

The Rose and Nelson pump

The first osmotic pump was invented in 1955 by two Australian physiologists, Nelson and Rose. They inserted the pump into sheep and cattle's guts to administer drugs. The apparatus consists of three chambers: a drug chamber with an orifice, a salt chamber with an elastic diaphragm that contains excess solid salt, and a water chamber (Fig.1). A semipermeable rigid membrane separates the drug chamber and water chamber[33]. The difference in osmotic pressure across the chamber causes water to move from the water chamber to the salt chamber. This water flow causes an increase in the volume of the salt chamber, separating the salted drug chamber and pumping drugs out of the device. Rose and Nelson pumps work on the basis of the following equation:

$$dm/dt = dv/dt * c$$

where dm/dt is drug release rate, dv/dt is volume flow of water into salt chamber and c is the concentration of drug in the drug chamber[34].

Higuchi Leeper pump

A simplified version of the Rose Nelson pump developed by Alza Corporation in 1970 was the Higuchi Leeper pump. It is advantageous because there is no containment chamber in the pump, and it is activated by water ingested from the surrounding environment when swallowed or implanted in the body. The pump is housed in a rigid enclosure with a perforated frame that supports it (Fig.2). After the pump is prepared, it is then loaded with drug and can be stored for weeks or months until it is needed [35].

Higuchi Theeuwes pump

In the early 1970s, Higuchi and Theeuwes developed another, indeed simpler variant of the Rose-Nelson pump. As with the Higuchi-Leeper pump, water to spark the bibulous action of the pump is attained from the girding terrain. In the Higuchi-Theeuwes device, still, the rigid casing is allocated with and the membrane acts as the external covering of the pump. (Figure 4) This membrane is relatively sturdy and is strong enough to repel the pumping pressure developed inside the device[36]. The device is loaded with the asked drug previous to use. When the device is placed in a waterless terrain, release of the drug follows a time course set by the salt used in the salt chamber and the permeability of the external membrane covering. Utmost of the Higuchi-Theeuwes pumps use a dissipation of solid salt in a suitable carrier for the salt chamber of the device (Fig.-3)[37].

Implantable mini osmotic pump

It is made up of three concentric layers: a drug reservoir, osmotic sleeves, and a semipermeable membrane that controls the rate of the drug. The flow moderator is a separate component that is put into the osmotic's body. An osmotic sleeve, a cylinder holding a high concentration of osmotic agent, surrounds the innermost compartment of the drug reservoir[38]. A semipermeable membrane covers the osmotic sleeve. Water enters the sleeve through a semipermeable membrane, compresses the flexible drug reservoir, and displaces the drug solution through the flow moderator when the system is put in an aqueous environment. These pumps come in a variety of supply rates ranging from 0.25 to 10 ml per hour, with delivery times ranging from one day to four weeks (Fig-4)[39].

Alzet osmotic pump

ALZET pumps work because of an osmotic pressure difference between the salt sleeve, a compartment within the pump, and the tissue environment in which it is implanted. The salt sleeve's high osmolality leads water to flow into the pump through a semipermeable membrane that forms the pump's outer surface. The water compresses the flexible reservoir as it enters the salt sleeve, displacing the test solution from the pump at a controlled, predefined rate. Pumps are designed for single-use only because the compressed reservoir cannot be refilled (Fig.-5)[40].

LiRIS®



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It's a single-compartment osmotic system that can move around freely in the human bladder. Interstitial cystitis and painful bladder syndrome (IC/PBS) are treated with the LiRIS® Lidocaine Releasing Intravesical System. The gadget is made of a medical-grade PDMS tube with two lumens. The lidocaine tablets are in one portion of the lumen, while the shape memory wire constructed of nitinol is in the other. The device is inserted and removed from the bladder using nonsurgical techniques (catheter or cystoscopy)[41]. The extremely elastic effect of the wire, together with the interstices breaks between the lidocaine tablets, allows the system to flex into a linear shape for insertion and then revert to its pretzel-like structure after insertion. Following insertion into the bladder, the entire silicone tube acts as a semi-permeable membrane, with a small laser-drilled orifice within its wall acting as a lidocaine release outlet[42].

Ivomec SR®Bolus

Merck & Co., Inc., NJ, USA introduces the pump, which has a diameter of 20 to 30mm and a length of roughly 100mm. It's mostly utilised in veterinary medicine. It's made to inject ivermectin directly into cattle's intestines. Because of its increased density (up to 3g/cm³), the device settles in the animal's lumen. The device's osmotic agent compartment and medication compartment are separated by a wax-based piston. Push melt™ technology causes the thermoresponsive medication formulations to melt at the body temperature of cattle and be pushed out by the piston. With the pump, the drug can be kept in a steady state for 135 days[43].

Acuros

Humboldt University in Berlin, Germany, is the first to introduce the pump. A salt chamber (osmotic agent), a water chamber (solvent), and an extrusion chamber with a moveable barrier displacing drug from the drug reservoir make up the osmoregulatory micro pump. The salt chamber and the extrusion chamber are connected via a semi-permeable hollow fibre flowing through the water room. This allows water to osmosis into the fibre, causing a convective flow through the fibre towards the extrusion chamber. The fiber's diluted salt solution travels through a salt chamber containing a more concentrated solution[44]. The displacement of salt solution at this particular site from the salt chamber causes a convective flow inside the osmotic agent chamber. As a result, a traditional recirculation system is maintained, which continuously supplies the fibre with highly concentrated salt solution. By displacing the movable barrier into the liquid drug reservoir, the majority of the diluted salt solution within the hollow fibre flows into the extrusion chamber and promotes the outflow of the drug solution[45].

Hydrogel Pump

As osmotic agents, it uses poly(N-isopropyl acrylamide) or other powerful swelling super absorbent polymers. Switching a trigger activates the pump. The reservoir opens after activation, and the liquid-based swelling agent is delivered to the hydrogel actuator. The supply of swelling agent is independent of the device's spatial orientation since the reservoir is pressed by a spring force. Before it may begin to dislodge the self-locking system, the expanding hydrogel must first fill a predetermined volume. As a result, the volume defines a time delay that may be changed by an external screw, allowing for individual delays[46].

Oral Osmotic Pumps**Single Chamber Osmotic Pump (Elementary Osmotic Pump)**

The EOP is a revolutionary drug delivery mechanism. An active substance with an appropriate osmotic pressure is compressed into a tablet, which is then covered with a semi-permeable material and a small hole is produced in the membrane of this device. Because of the osmotic pressure gradient, the agent inside the tablet draws water through the semi-permeable membrane and creates a saturated solution inside the device when it comes into touch with the watery environment of the GI tract[47]. Because the membrane is non-extensible, the increase in volume generated by water imbibitions causes hydrostatic pressure to develop inside the tablet. This osmotic water imbibitions results in the creation of a saturated drug solution within the core, which is delivered at a controlled pace via the membrane's delivery orifice[48]. Though 60-80 percent of the medication is released at a steady rate from the elementary osmotic pump, the system hydrates for 30-60 minutes before zero order delivery from the EOP occurs in most circumstances. Only water soluble medications can be delivered using this technique. The kind and thickness of



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the coated membrane influenced drug release, while orifice size, static and stirred conditions of the release medium had no effect (Fig.-6)[49].

Multiple chamber Osmotic pumps**Push Pull Osmotic Pump**

A bilayer or trilayer tablet core with one push layer and one or more drug layers makes up the push pull system. Poorly soluble drugs, osmotic agents, and suspending agents make up the drug layer. Other ingredients in the push layer include osmotic agents and water-swellaible polymers. As in the EOP method, a semi-permeable membrane surrounds the tablet core, with an aperture drilled into it on the drug layer side[50]. These push pull osmotic pumps swell and push the drug layer after coming into contact with the aqueous environment, releasing the drug in the form of fine dispersion or solution via the orifice at a constant zero order rate. The hydration kinetics of both the membrane and the tablet core are thought to regulate drug release kinetics in these systems(Fig.-7)[51].

Osmotic Pump with Non Expanding Second Chamber

The second chamber in such systems is non-expanding. However, such systems can be classified into two groups based on the function of the second chamber. The second chamber is used to dilute the drug solution before it leaves the device in one category. This is beneficial since saturated drug solutions might induce GI tract discomfort in some circumstances. The second type of EOP tablet combines two independent EOP tablets into a single tablet[52]. Both drugs are released at the same time by the device. Another variation on the original model: one chamber contains an osmotic agent, while the other houses the drug. Both chambers imbibe water when the system comes into touch with the surrounding watery environment. Through the connecting hole, the osmotic agent solution created in the first chamber is transported to the drug chamber, where it mixes with the drug solution before exiting the membrane. Drugs that are somewhat insoluble can be given using this type of device (Fig.-8)[53].

Specific Types Of Osmotic Pump**Osmotic Bursting Osmotic Pump**

This system is identical to an EOP, with the exception that there is no delivery orifice and the size may be smaller. Water is imbibed and hydraulic pressure is built up inside until the wall ruptures and the contents are expelled into the environment when it is placed in an aqueous environment. The semipermeable membrane's thickness and area can be adjusted to control medication release. This method works well for pulsated release drug delivery system[54].

Liquid Oral Osmotic system

They are of three types: a) L OROS hard cap b) L OROS soft cap c) Delayed liquid bolus delivery system

a) OROS Liquid Soft Cap

Soft cap has a liquid drug formulation in a soft gelatin capsule that is surrounded by a barrier layer, an osmotic layer, and a release rate-controlling membrane (Fig.-10)[55].

b) Oros Liquid Hard Cap

Composition- A liquid drug layer and an osmotic engine are encased in a hard gelatin capsule and coated with a semi permeable membrane in the hard cap.

Mechanism of Action:-The buildup of hydrostatic pressure is caused by the expansion of the osmotic layer, causing the liquid formulation to burst through the hydrated gelatin capsule shell at the delivery aperture. The osmotic engine expands as water is ingested across the SPM, pushing against the barrier and releasing the drug through the delivery orifice (Fig.-11)[56].

Advantages:-APIs will be delivered as liquid formulations, combining the advantages of prolonged release and high bioavailability. Lipophilic APIs can be delivered in a controlled manner.

Delayed Liquid Bolus Delivery System

A liquid medication layer, an osmotic engine or push layer, and a semipermeable membrane covering are all included in each of these systems. Water seeps across the rate-controlling membrane and activates the osmotic layer when the system is in touch with an aqueous environment[57]. The buildup of hydrostatic pressure inside the system is caused by the expansion of the osmotic layer, forcing the liquid formulation to be delivered through the delivery



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orifice. The L OROS delayed liquid bolus drug delivery system is meant to deliver a pulse of liquid medicine, whereas the L OROS hard cap or soft cap system is designed to give continuous drug delivery[58]. A placebo delay layer, a liquid drug layer, and an osmotic engine are all enclosed by a rate-controlling semi-permeable membrane in the delayed liquid bolus delivery system. On the placebo layer end of the capsule-shaped device, the delivery hole is drilled. The placebo layer is released first when the osmotic engine expands, delaying the release of the medication layer. The release of a drug might be delayed for up to ten hour(Fig.-12)[59].

Monolithic Osmotic Systems

It's a simple water-soluble agent dispersion in a polymer matrix. Water imbibition by the active agents occurs when the system comes into contact with the aqueous environment, rupturing the polymer matrix capsule encapsulating the drug and releasing it into the outer environment[60].

Initially, this process happens in the polymeric matrix's outer environment, but it eventually moves in a serial fashion towards the matrix's interior. However, if more than 20–30 volume per litre of active agents are introduced into the device, the system fails because above this level, significant contribution from simple leaching of the material occurs(Fig.-13)[61].

Delayed Delivery Osmotic Device

An osmotic device inherently has a lag time before medication distribution begins due to its semipermeable membrane. Although this trait is normally considered a drawback, it can be leveraged to your benefit. Certain drugs (drugs for early morning asthma or arthritis) may benefit from a delayed release. Other methods to further postpone drug release are described in the following paragraph(Fig.-14)[62].

Telescopic Capsule for Delayed Release

This device is divided into two chambers: the first contains the drug and an exit port, while the second houses the osmotic engine. A coating of waxy-like material is utilised to divide these two compartments from one another. If the desired medication release is delayed, the reservoir volume containing the active agent is kept constant, resulting in a low pressure differential between the environment of application and the reservoir interior. As a result, the net flow of ambient fluid forced by pressure into the reservoir is very low, and no medication is released during that time period (Fig.-15)[63].

OROS – CT

OROS-CT is a twice-daily or once-daily formulation to deliver medications to the colon in a targeted manner. The OROS-CT is a diagnostic tool that can be used to diagnose a variety of conditions. It can be made up of a single osmotic agent or up to five. As the system hits the small intestine, the enteric coating breaks, allowing water to absorb into the core, causing the push compartment to enlarge. At the moment, in the drug compartment, a flowable gel is created at the same time, is pushed out of the orifice at a perfectly controlled rate, by the rate at which water passes through the semipermeable membrane(Fig-16)[64].

Multi Particulate Delayed Release Systems (MPDRS)

MPDRS are drug-filled pellets with or without an osmotic agent that are coated with a semi permeable membrane. Water penetrates the core of this system when it comes into touch with an aqueous environment, forming a saturated solution of soluble component. The osmotic pressure difference causes the membrane to expand rapidly, resulting in the creation of holes. According to zero order kinetics, the osmotic agent and the medication are released through the pores. The coating level and osmotic characteristics of the dissolution media were found to affect the lag time and dissolution rate(Fig.-17)[65].

OSMAT

It's a new osmotically driven matrix system that uses hydrophilic polymers to swell and gel in an aqueous medium, forming a semipermeable membrane in the process. In-situ releases from such a matrix system containing an osmogen could thus be modulated by the osmotic phenomenon. As a result, OSMAT carefully balances both matrix



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osmotic properties, resulting in a quantum improvement in drug delivery from a swellable matrix system. In an agitation-dependent manner, OSMAT produces regulated medication release with acceptable delivery rates. As a result, osmat stands for a low-cost osmotically driven controlled drug delivery system that is simple, adaptable, and straightforward to build(Fig.-18)[66].

Sandwiched Osmotic Tablets (SOTS)

A polymeric push layer is placed between two drug layers, each with two delivery orifices. When the tablet is placed in an aqueous environment, the centre push layer holding the swelling agent swells, and the medication is released from two orifices on opposing sides of the tablet, making SOTS appropriate for drugs that cause local stomach mucosa irritation(Fig.-19)[67].

Effervescent Osmotic Tablet (EOT)

Effervescent compounds are included into dosage forms in this technique, which react with acid in the environment to produce carbon dioxide. This gas swells and disperses the precipitate medication, preventing the aperture from becoming blocked. This technique is useful for poorly soluble drugs that precipitate at low pH and clog the delivery aperture. In this approach, sodium bicarbonate is frequently used[68].

Self Emulsified Osmotic Tablet

Self-emulsifying agents have been added to the tablet-core composition in the case of slightly soluble or practically insoluble medicines. Approximately 40% of medications available have a low aqueous solubility. By using a self-emulsifying method, the drug's bioavailability, regulated release rate, and plasma concentration stability are all improved. The hydrophobic medicines are emulsified. Typical surfactants used for this purpose include polyoxyethylenated glyceryl recinoleate, polyoxyethylenated castor oil with ethylene oxide, glyceryl laureates, glycerol (sorbiton oleate, stearate, or laurate), and others[69].

Controlled Porosity Osmotic Pump

The pump can be built with a single or multicompartiment dosage form; in any case, the delivery system contains a core containing the medication surrounding by an asymmetric membrane, which consists of a thin dense skin layer supported by a porous substructure. A phase inversion process controlled by the evaporation of a mixed solvent system forms the membrane. Water permeates the membrane, but it is impervious to solutes and insensitive pore-forming additives scattered throughout the wall. Low quantities of water-soluble additive are leached from polymer materials that were permeable to water but insoluble when exposed to water. The sponge-like structure that resulted established the desired regulated porosity walls and was highly permeable to both water and dissolved pharmacological ingredients[70].

Osmotic Pump for Insoluble Drugs

A single layer tablet core is wrapped by a semi-permeable membrane in the EnSoTrol technology. An exit hole is bored through the membrane. To dissolve the medicine, the tablet core contains the drug, an osmotic agent, a wicking agent, and solubility enhancers. Water enters the core through the membrane when the EnSoTrol tablet is eaten. The solubility enhancer in the core aids in the dissolution of the medication, and the drug solution is given to the GI lumen through the exit orifice, where it can be absorbed into the systemic circulation. Because the poorly soluble substance is supplied from this tablet in a dissolved condition, inter-patient variability in plasma concentrations from the EnSoTrol system is projected to be lower (Fig.-21)[71].

Pulsatile delivery based on expandable orifice

Alza's patents 5318558 (1994) and 5221278 (1993) claim pulsatile delivery of agents from osmotic systems using expandable orifice technology. The system consists of a capsule from which the medicine is given via osmotic infusion of moisture from the body by the capsule. To generate a pulsatile delivery effect, the delivery orifice opens and closes intermittently. The orifice created in the capsule wall, which is made of a flexible substance. As the osmotic infusion advances, pressure within the capsule builds, stretching the capsule wall. When the elastic wall



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relaxes, the flow of the medication through the aperture virtually stops; however, when the elastic wall stretches above the threshold due to increased pressure, the orifice widens sufficiently to allow the drug to be released at the required rate. Styrene-Butadiene copolymer and other elastomers can be employed[72].

Pulsatile delivery by a series of stops

Alza was also granted patent 5209746, which specified an implanted capsule for pulsatile administration. The medication and absorptive osmotic agent engines are each positioned in compartments separated by a moveable partition in the capsule. A set of stops along the capsule's inner wall allow for pulsatile delivery. These inhibit the partition from moving, but as the osmotic pressure climbs above the threshold level, they are overcome one by one. The number of stops and their longitudinal arrangement along the length of the capsule determine the number and frequency of pulses, while the partition configuration determines the pulse intensity. This technology has administered porcine somatotropine, according to reports[73].

Miscellaneous Devices

A mixed diffusion osmotic pump medication delivery system is described in Patent 6352721 (2002), which was assigned to Osmotica Corporation (Tortola, British Virgin Islands). The gadget includes a centrally situated expanding core that is totally encircled by an active substance-containing layer and a membrane. An expanding hydrophilic polymer and an optional osmogen make up the core. An active ingredient, an osmogen, and an osmopolymer make up the entire composition that surrounds the core. The membrane has a delivery hole and is microporous in nature. The device can deliver drugs that are Insoluble, Slightly Soluble, Sparingly Soluble, Or Very Soluble To The Environment(Fig.-21)[74].

Factors Influencing The Design Of Osmotic Controlled Drug Delivery Systems**Solubility[75]**

In osmotic controlled drug delivery systems, drug solubility plays an important role in drug release kinetics. As a result, medications should be soluble enough to be administered via osmotic delivery. The solubility of a model drug for osmotic delivery should be between 50 and 300 mg/ml. Various solubility improvement strategies are used to improve medication solubility. They're listed below.

1. Application of wicking agents
2. Use of a different type of salt
3. Employing polymorph
4. Resin modulation technique
5. Swellable polymers are used
6. Utilization of effervescent mixtures

Size of Delivery Orifice[76]

To achieve a zero-order delivery profile, the orifice area must be large enough to prevent osmotic pressure build-up in the system. Otherwise, the hydrostatic pressure will distort the membrane, lowering the zero-order delivery rate. As a result, the orifice's cross sectional area should be kept within the minimum and maximum values; orifice sizes range from 600 microns to 1 mm.

The following are some methods for creating a delivery orifice in osmotic systems:

1. Mechanical drilling: This is accomplished by manually drilling the orifice using specialised benchtop equipment or by using a needle to get the desired diameter of the delivery orifice.
2. Laser drilling: This method is well-known for generating sub-millimetre holes in tablets. Drilling is usually done with a CO2 laser beam, which is quite reliable.
3. Indentation: For this sort of core tablet, customised punches with a needle on the upper punch are used. During the coating process, the hole created by the indentation is not covered, acting as a channel for drug release in the osmotic system.
4. Pore-forming chemicals in the semipermeable membrane coating: regulated porosity osmotic pump, for example.



**Nihar Ranjan Kar****Membrane Thickness[77]**

The thickness of the membrane is a key aspect in limiting the rate of water penetration into the dispenser. The permeability of water through the membrane can be improved by selecting the right membrane material. The thickness of the membrane can readily change the period of release of the active ingredient by a factor of 1000. In general, changing the membrane material can increase the rate of drug release, while changing the thickness of the membrane can increase the rate of drug release by up to 5%.

Osmotic Pressure

The osmotic pressure differential between the inside of the section and the external environment is the next release-controlling factor that must be optimised. The rate of drug release from an osmotic system is directly proportional to the core's osmotic pressure relative to the core's osmotic pressure. Maintaining a soaked solution of osmotic agent in the compartment is the simplest and most expected technique to establish a constant osmotic pressure[78]. If the osmotic pressure of a soaked solution of the medicine is insufficient, an extra osmotic agent must be added to the core formulation. The addition of carbonate or bicarbonate salt to the drug chamber has the advantage of preventing the precipitated drug from overwhelming the tablet's delivery aperture. Polymeric osmogens are mostly utilised to make PPOPs and other specialised devices for the controlled release of medicines with low water solubility. These are hydrophilic, swellable polymers that interact with aqueous fluids to swell or expand to a balanced condition[79].

Membrane Type

The release rate of a semipermeable membrane is essentially independent of the pH of the environment since it is permeable to water but not to ions. The medication disintegration takes place entirely within the delivery system, away from the outside world[80].

Evaluation***In-vitro* Dissolution**

The traditional USP paddle and basket type equipment was used to examine the in vitro release of medication from the oral osmotic system. The temperature of the dissolving media was maintained at $37 \pm 0.5^{\circ}$ Celsius. Distilled water, as well as stimulated stomach fluid (for the first 2-4 hours) and stimulated intestinal fluids (for the subsequent hours), have been employed as dissolving media. At various time intervals, 10 ml of the samples were removed and replaced with 10 ml of fresh medium. The same standard specifications that apply to oral controlled medication delivery systems are also applicable to oral osmotic pumps. A UV spectrometer was used to examine the samples[81].

Scanning Electron Microscopy

The porous morphology of coating membranes acquired before and after complete dissolution of core contents can be investigated using a scanning electron microscope. Membranes were dried for 12 hours at 45°C and stored in a desiccator between wax paper sheets until examination[82].

Effect of pH

On vitro release studies in dissolving media with varying pH can be conducted to explore the influence of pH on drug release and ensure that the produced formulations perform reliably regardless of pH. 0.1 N HCl, pH 4.5 acetate buffer, pH 6.8 phosphate buffer, pH 7.5 phosphate buffer, and distilled water were used for dissolution. The drug release investigation was conducted using a dissolution equipment (USP-II). The samples (10ml) were taken at regular intervals and examined with a UV spectrometer[83].

Effect of Agitational Intensity

The effect of agitation intensity on drug release was investigated by dissolving the optimised formulation at various rotation speeds. Dissolution was done in the USP-II (Paddle) at different rotational speeds of 50, 100, and 150 rpm. The samples (10ml) were taken at predefined intervals and evaluated and compared using a UV spectrometer[84].



**Nihar Ranjan Kar****Effect of Osmotic Pressure**

Release experiments of the improved formulation in medium of various osmotic pressures can be done to confirm the principal mechanism of drug release. Osmotically effective solutes (mannitol, sodium chloride, etc.) with varied concentrations can be added to enhance the osmotic pressure of the release media (pre-equilibrated to 37°C 1°C). Drug Release Kinetics We can know the kinetics of drug release by fitting the data collected in different models at different time intervals and using statistics. The medium's osmotic pressure was calculated using the Van't Hoff and Morse equation.

$$\pi V = nRT$$

Where, π is the osmotic pressure, V is the volume of the solution in litres, n is the number of moles of solute, T is the absolute temperature, and R is the gas constant (0.082 lit atm/mol deg.)[85].

Market Status[86]

As indicated in Table 1, many osmotic devices are introduced to the market by businesses such as Alza, Novartis, Merck, Janssen, and others. As a result, the ODDS is successful not just in R&D but also on a broad commercial scale. Over the previous 35 years, the ODDS have progressed from the EOP to a variety of specialised OPs with modulated and programmed drug delivery. In the coming years, it is expected to have a significant market share in the controlled drug delivery system. Table 2 shows the most recent patenting systems for osmotic medication delivery systems.

Current & Future Developments

Osmotic drug delivery formulations have been identified as a promising therapeutic approach for drug delivery control. The high score of patent literatures in diverse osmotic delivery applications, such as oral, implantable, and parental distribution, has proven its worth in drug delivery. The development of multiple chamber systems (Push-pull osmotic pump and Osmotic pump with non-expanding second chamber) is of particular importance. Osmotic pumps have also been created recently for the regulated administration of water insoluble medicines. Because of the rising interest in understanding the basic technology of osmotic systems in the pharmaceutical industry, numerous systems have already been approved by the USFDA[89].

CONCLUSION

The driving mechanism for drug release in the osmotic delivery system is osmotic pressure. The medicine is released from the system as the pressure inside the dosage form rises due to water intrusion. Precision control of zero-order or other structured release over an extended time period is one of the main benefits. Osmotic drug delivery has come a long way since its inception, and it has undergone numerous modifications. Although osmotic drug administration is a little more expensive drug delivery technology, it is nevertheless employed because it ensures a high rate of drug release, which helps it gain acceptability in the pharmaceutical industry[90].

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Table-1. Marketed Products of Osmotically Controlled Drug Delivery Systems[87]

Product Name	Drug	Type of System	Developed by
Acutrim	Phenylpropanolamine	EOP	Alza Corp.
Cardura XL	Doxazosin	PPOP	Pfizer Inc.
Covera HS	Verapamil	PPOP with time delay	Pfizer Inc.
Ditropan XL	Oxybutinin chloride	PPOP	Alza Corp.
Dynacirc CR	Isradipine	PPOP	Novartis
Efidac/24	Pseudoephedrine	EOP	Alza Corp.
Glucotrol XL	Glipizide	PPOP (bilayer)	Pfizer Inc.
Minipress XL	Prazosin	EOP	Alza Corp.
Procardia XL	Nifedipine	PPOP	Pfizer Inc.
Volmax	Albuterol	EOP	Alza Corp.
Sudafed 24®	Pseudoephedrine	EOP	Pfizer Inc.
Invega®	Paliperidone	PPOP	Janssen Pharmaceutica
Viadur®	Leuprolide acetate	Implantable osmotic systems	Alza Corp.
Chronogesic™	Sufentanil	Implantable osmotic systems	Durect Corp.
Minipress XL	Prazosin	EOP	Alza Corp.
Procardia XL	Nifedipine	PPOP	Pfizer Inc.
Alpress LP	Prazosin	Push –Pull	Alza/Pfizer
Calan SR	Verapamil		Alza/GD Searle &Co
Concerta	Methyl phenidate		Alza
Covera HS	Verapamil	Push -Pull with time delay	Alza/GD searle
Efidac 24	Chlorpheniramine meleteate	Elementary Pump	Aza/Novartis
Teczam	Enapril and Diltiazem	Elementary Pump	Merck/Aventis
Tiamate	Diltiazem	Push-Pull	Merck/Aventis
Tegretol XR	Carbamazepine		
Ditrophan XL	Oxybutynin Chloride		
Evomac SR Bolus	Ivernectin		
Tiamate	Diltiazem		

PPOP- Push-pull Osmotic Pump; EOP- Elementary Osmotic Pump

Table-2:Recent Patenting Systems for Osmotic Drug Delivery Systems[88]

Sl. No	Rationality for Invention of the Patent	Title of the Patent	Patent number
1	A core containing a homogeneous mixture of glipizide, a	Oral osmotic controlled	US 20030219485





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	<p>hydrophilic polymer, and other pharmaceutically acceptable excipients, A semipermeable wall around the core that is impermeable to the contents of the core but permeable to fluids present in the used environment. A route through the wall for releasing the core's contents into the use environment.</p>	drug delivery system	
2	<p>A poorly soluble medication and at least one alginic acid derivative are combined in a monocompartment osmotic controlled drug delivery system.</p>	Mono compartment osmotic controlled drug delivery system	WO/2003/092660
3	<p>To provide a dosage form for administering a medicine that is made as an osmotic caplet and that overcomes the constraints of the prior art.</p>	Osmotic caplet	US6773721
4.	<p>Multiple osmotic pumps and/or semipermeable membranes to extend the medication delivery system's useful life cycle and functioning. Different medications can be delivered from the same implanted system using an implantable system with numerous implantable osmotic pumps.</p>	Osmotic pump drug delivery systems and methods	US 6471688
5	<p>The osmotic module is designed to be put into the cavity so that fluid entering the cavity through the inlet port can activate it.</p>	Osmotic Intraosseous Drug Delivery System	US20070005043
6	<p>Devices are made up of a mixture of medicine with a specified average particle size dispersed in a polymer with a specific water permeability, tensile strength, and Young's modulus in specific volume proportions, so that the particles are surrounded by the polymer substantially individually.</p>	Osmotic bursting drug delivery device	US4177256
7	<p>The current invention is a monocompartment osmotic controlled drug delivery system that includes at least one alginic acid derivative and a poorly soluble medication.</p>	Monocompartment osmotic controlled drug delivery system	WO/2003/092660
8	<p>The piston is movable in relation to the capsule's internal surface and forms a movable seal with it. The osmotic agent is separated from the beneficial agent by the moveable seal. The piston's length-to-total-diameter ratio is approximately 1.1:1, and its core-diameter-to-total-diameter ratio is approximately 0.9:1. The piston allows for more beneficial and/or osmotic agent payload without increasing capsule size.</p>	Minimally compliant, volume- efficient piston for osmotic drug delivery systems	US7112335
9	<p>The flow modulator assembly for the osmotic delivery system reduces the likelihood of air or gas pockets forming in the enclosure of the system during assembly. Flow modulator assembly also reduces the likelihood of beneficial agent waste during osmotic delivery system assembly.</p>	Osmotic delivery system flow modulator apparatus and method	US 7407500
10	<p>Devices that distribute one or more active chemicals through diffusion through multiple micropores in the membrane or osmotic pumping through one or more prepared passageways in the membrane.</p>	Combined diffusion/osmotic pumping drug delivery system	US 6352721





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11	Anhydrous carbamazepine in fine particles. A polymeric swelling agent consisting of one or more swellable hydrophilic polymers selected so that the polymeric swelling agent exhibits controlled swelling and the wall does not rupture or burst, (iii) a crystal habit modifier in which the anhydrous carbamazepine is transformed into cuboidal or rod-shaped crystals of the dihydrate of carbamazepine, or mixtures thereof and iv) Osmosis-inducing water-soluble chemicals	Oral osmotic controlled drug delivery system for a sparingly soluble drug	US 6534090
12	Osmotic module with an osmotic agent and a medication formulation suited to be put in the cavity in order to activate the osmotic module with fluid received through the inlet port.	Osmotic intraosseous drug delivery system	WO/2007/005680A1
13	The osmotic agent absorbs liquid from the surrounding environment through a semipermeable body, moving the piston and allowing the beneficial agent to be released from the capsule.	Osmotic delivery system having space efficient piston	US 6544252
14	A liquid or gel addition is an incompressible lubricant that fills any air gaps between the osmotic agent and the chamber walls, reducing start-up times significantly.	Osmotic delivery system and method for enhancing start-up and performance of osmotic delivery systems	EP1066081

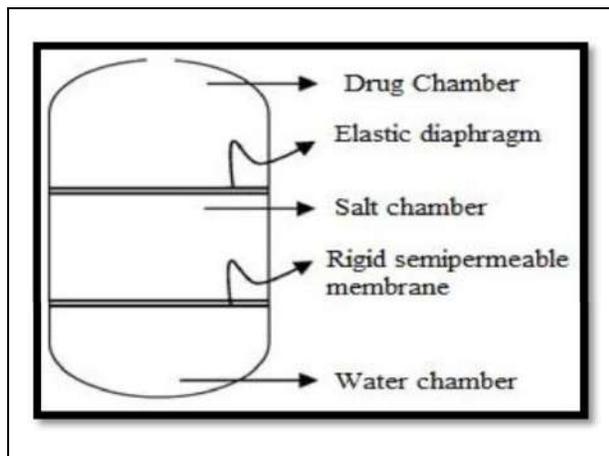


Fig. 1: Rose Nelson pump

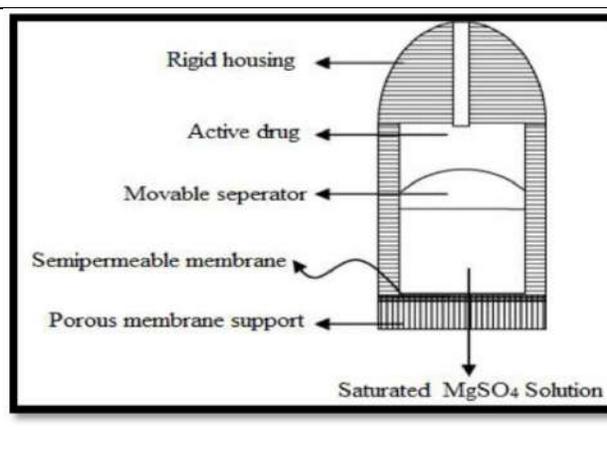


Fig. 2: Higuchi Leeper Pump

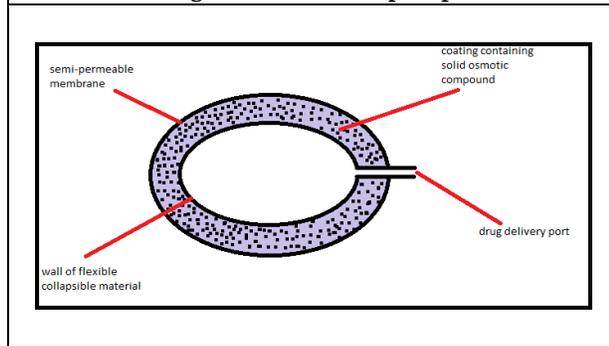


Fig-3: Higuchi-Theeuwes pump

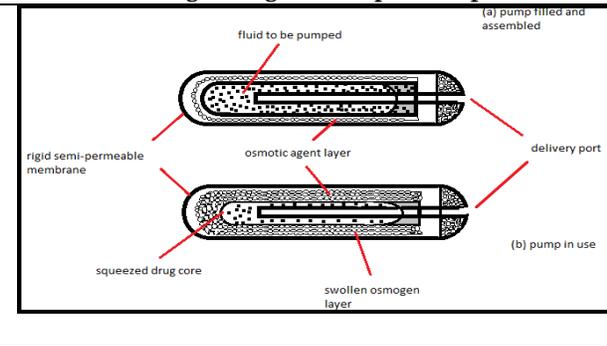


Fig-4: Theeuwes miniature osmotic pump





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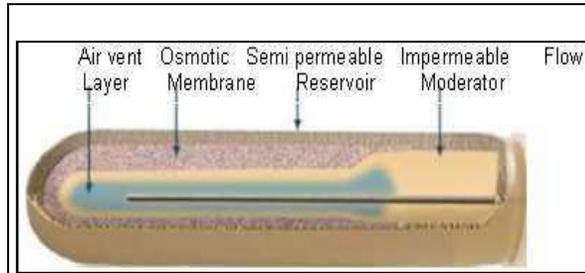


Fig.-5: Alzet Osmotic Pump

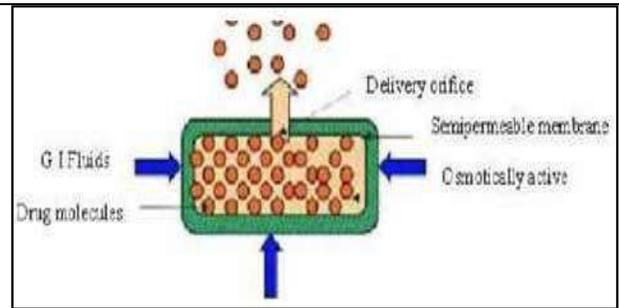


Fig.-6: Elementary Osmotic Pumps (EOP)

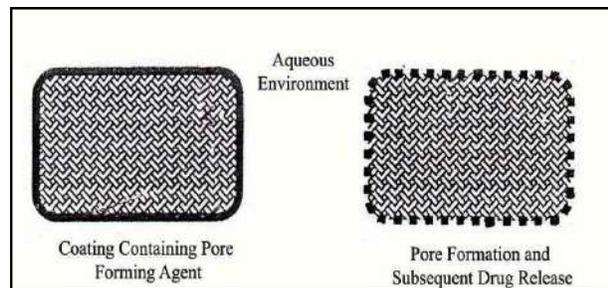


Fig.-7: Push Pull Osmotic Pump

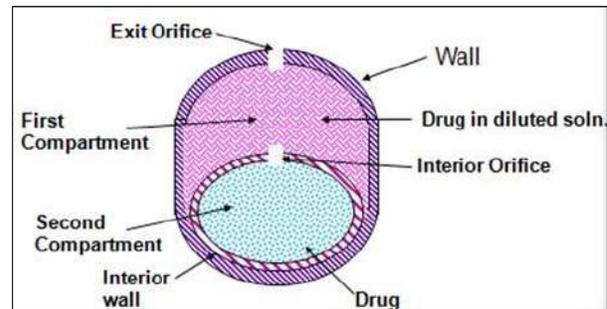


Fig.-8: osmotic pump with non- expanding second chamber

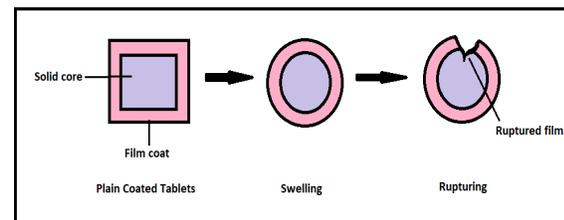


Fig. - 9: Osmotic Bursting Osmotic Pump

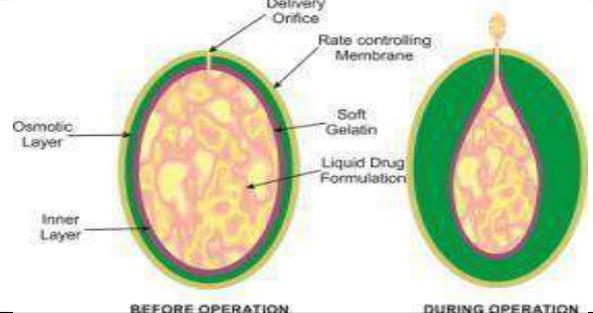


Fig.-10- Liquid OROS Soft cap

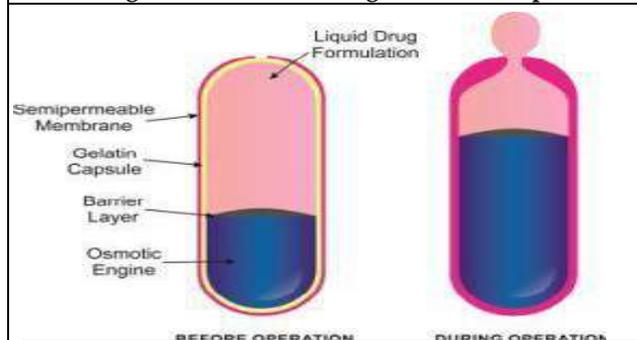


Fig.-11- Liquid Oros Hard Cap

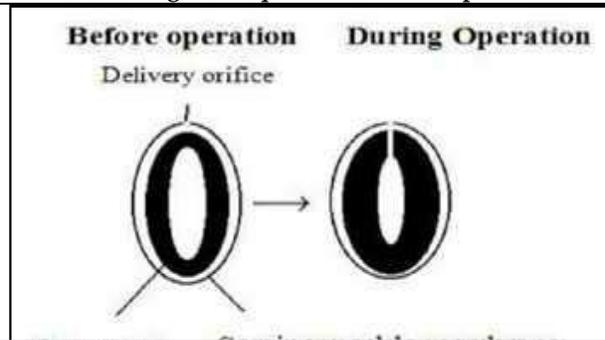


Fig.-12: Delayed liquid porous delivery system

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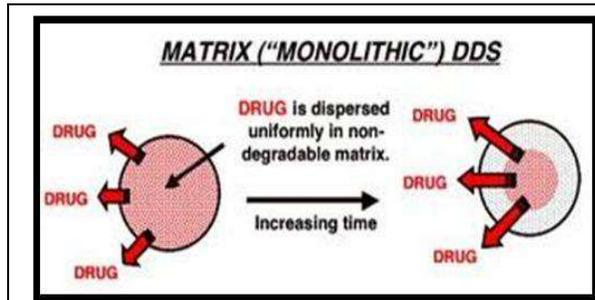


Fig.-13: Monolithic Osmotic Pumps

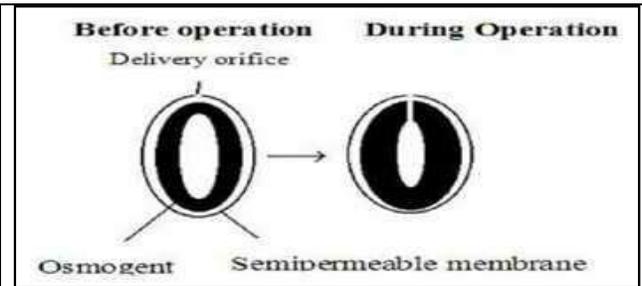


Fig.-14: Delayed Delivery Osmotic Device

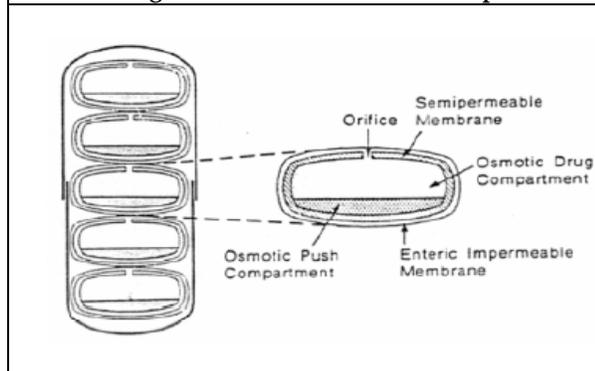


Fig.- 15: Telescopic capsule

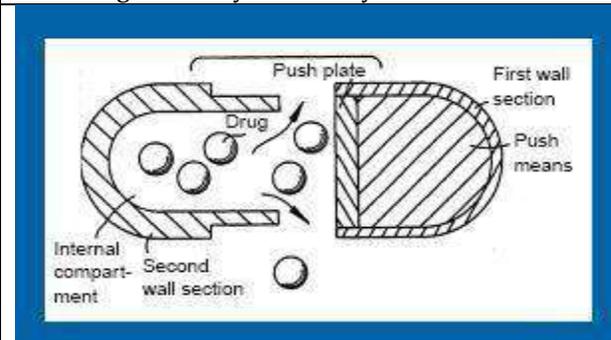


Fig.-16: OROS-CT

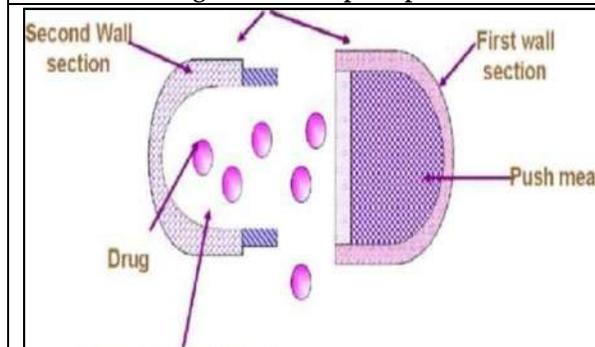


Fig.-17: Multi-particulate Delayed Release System

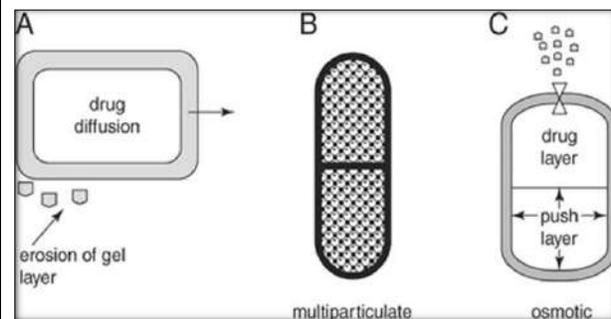


Fig. 18: Osmotic Matrix Tablet (OSMAT)





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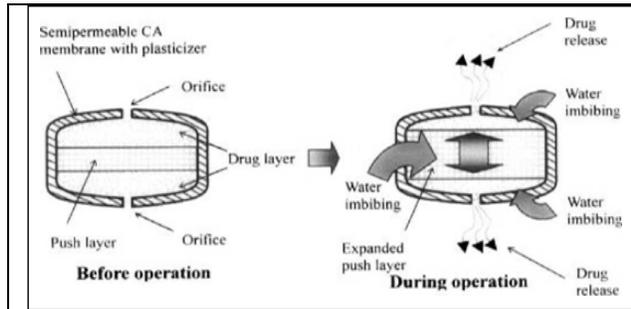


Fig.-19: Sandwiched Osmotic Tablet

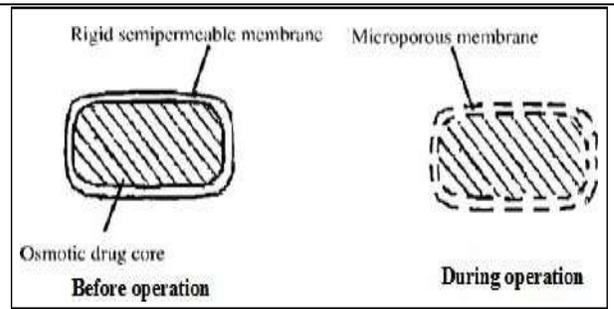


Fig.-20: Schematic diagram of controlled porosity osmotic pump

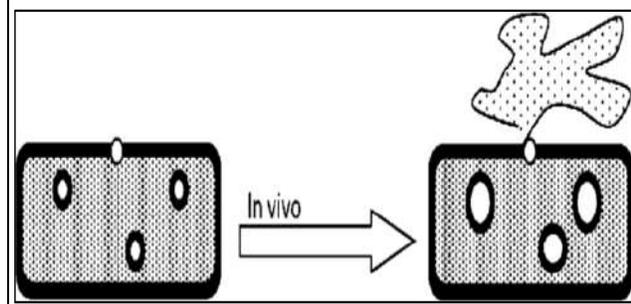


Fig.-21: Osmotic pump for insoluble drugs

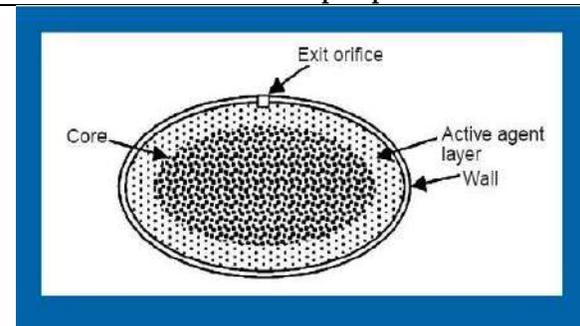


Fig.- 22: Miscellaneous device





Network Pharmacology Based Investigation Explores the Anti-Tumor Potential of Lupeol against Glioblastoma Multiform”

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ABSTRACT

Glioblastoma multiform is an aggressive tumor that has a higher percentage of mortality. Although researchers worldwide obtained vast knowledge with decades of work on malignant tumor mechanisms, treatment is still out of reach. Recent studies suggest that network pharmacology is a unique method to identify therapeutic features of compounds against the tumor. Current studies follow the anti-tumor potential of lupeol on glioblastoma multiform: compound targets and tumor targets extracted from Pubchem and DisGnet databases. The current study identifies 50 correlative targets for lupeol and GBM. use of STRING analysis in constructing protein-protein interaction and cluster networks for correlative targets. The ESR1, MAPK3, JAK2 proteins act as core proteins in the cluster network. These proteins validate the cytotoxicity potential of lupeol using *In vitro* methods and *In silico* analysis of lupeol docking to core target proteins.

Keywords: Network pharmacology, Gene Ontology, STRING, Glioblastoma multiforme.



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INTRODUCTION

Glioblastoma multiforme (GBM), sometimes termed Astrocytoma Grade IV, is rapidly growing and aggressive. It also invades the nearby tissue of the brain. GBM can arise from lesser-grade astrocytoma. GBM prevails in the cerebral hemispheres in adult patients, mainly in the brain's temporal lobes. GBM can be graded into primary and secondary tumors. Primary tumors manifest de novo (i.e., low-malignant precursor lesion)—the secondary GBM progress from malignant low-grade astrocytoma [1]. The molecular characteristics of primary GBM constitute loss of heterozygosity (LOH) of chromosome 10 holding phosphatase and tensing homolog PTEN and TERT promoter mutation, the mutation in the epidermal growth factor receptor gene, and amplification. Secondary tumors constitute loss of heterozygosity of 19q and mutations of IDH1/2, TP53, and ATRX [2]. Identifying various alterations at the genetic and epigenetic levels has adversely influenced GBM prognosis. Although despite this heterogeneity, three major signaling pathways are identified as the major player in deregulation: inhibition of p53, retinoblastoma protein (Rb) signaling pathways, and receptor tyrosine kinase (RTK) activation, phosphoinositide 3-kinase (PI3K) pathway [3]. In the adult, most mutated genes are EGFR and TP53, but ATRX is identified as the most affected gene in the young group. In addition, the somatic mutations in the genes related to chromatin remodeling, MGMT, IDH1, ATRX, and DNA repair were identified as unique distribution in the young adult population [4].

It is highly recognized that Network pharmacology is widely exploited to determine the novel targets and their interaction with unknown signaling pathways. The Network pharmacology analysis provides a new understanding of the systemic interlinkage within therapeutic targets and widespread disease. It also acts as a holistic tool for identifying disease mechanisms and potential bioactive components [5]. Network pharmacology methods led to a unique understanding of disease therapeutics to reveal the common modular association of drug-target-pathway-disease, predicting the target distribution and pharmacological properties of herbal compounds [6]. Network pharmacology acts as a powerful tool by combining systems biology, bioinformatics, and polypharmacology, which helps in clarifying the complicated interactions at the molecular level such as proteins, genes, metabolites associated with disease, and drugs [7]. The bio-active ingredients from natural sources are found intact with their original pharmacological properties to enhance the efficacy of the medication in treating diseases. Thus, the active components can act as a reliable therapeutic source also as selective molecular targets against various cancers [8]. Due to higher risk factors involving traditional cancer chemotherapies, there is a vital principle in using alternative medicine from natural sources for cancer prevention [9]. The studies found that AKT can increase the cell viability level in prostate cancer using various ways, such as activating rapamycin (mTOR) targets to induce the cell resistance to apoptosis within androgen-deficient conditions. Some research studies also found that 5'adenosine monophosphate-activated protein kinase (AMPK) can reduce the mTOR functions by phosphorylation of TSC2 and raptor interference within the AKT signaling mechanism. Inhibition of AMPK activity can initiate cell proliferation and the malignant activity of cells [10].

The current aim of the studies is to identify the active pharmacological targets for lupeol using the network pharmacology approach and molecular docking. The overall plan is divided as below:

- (i) To Identify the potential targets of lupeol based on its association with Glioblastoma.
- (ii) Investigate the critical role of the identified targets through functional enrichment and analysis of pathways by Gene Ontology (GO) terms.
- (iii) To Construct the Protein-Protein interaction (PPI) network of the correlative targets using STRING analysis.
- (iv) To Validate the molecular docking analysis of lupeol binding with potential targets.

MATERIALS AND METHODS

Data Collection and Preparation to Predict Core Targets

Based on database analysis screening, targets were identified against GBM and relative targets for lupeol. Swiss Target Prediction (www.swisstargetprediction.ch) was accessed to identify the putative targets towards lupeol and



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DisGeNET database (<http://www.disgenet.org>) to screen the target hits associated with Glioblastoma multiforme. The correlative targets for GBM and lupeol were predicted using FunRich software (Venn diagram), and the extracted data was utilized for further studies.

Gene Ontology (GO) and Pathway Enrichment Analysis

Analysis of Gene Ontology and functional pathway enrichment of the correlative targets were carried out by FunRich software v.3.1.4 (<http://www.funrich.org>). The functional pathway enrichment was performed using the Reactome database for annotations in FunRich software. The false discovery rate (FDR) < 0.01 was adjusted during the GO terms and pathway analysis, detailed as the enriched terms and pathways. Each category of gene ontology: biological process, molecular function, and cellular component plotted against the graph. Significantly, leading ten enriched pathways were obtained with the help of Reactome pathway database analysis, and resultant pathways were constructed using a doughnut chart in the FunRich software.

Construction of STRING Network and Module of Glioblastoma Multiforme Targets

Protein-protein interaction (PPI) network for identified correlative targets was constructed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING, <https://stringdb.org>). Based on confidence scoring, the PPI network database produces the interaction between different proteins to protein co-relation and its interactive level. The correlative 50 anti-GBM targets were all inserted into the STRING interaction database. The *Homo sapiens* category was selected for visualization of an interactive network. The interaction network was constructed by maintaining a medium score (confidence score) of 0.4 to 0.7, respectively. Furthermore, the PPI network was clustered into distinctive clusters using the K-means algorithm. The clusters were processed to module construction through FunRich software.

In silico analysis by Molecular Docking

The molecular docking analysis was performed to evaluate the putative targets using bioinformatics tools Schrodinger-Maestro v.8.5. Lupeol's three-dimensional (3D) structure was initially extracted from PubChem (PubChem.NCBI.Nlm.nih.gov) database. The 3D structure of the identified protein targets was obtained from the PDB database (www.rcsb.org). All protein structures were processed with receptor grids to get various binding poses for the ligand on its active sites. The best ligand-receptor interaction can be obtained with the least Glide score and calculation of binding energy by Schrodinger Prime using generalized Born surface area (MM-GBSA). Furthermore, Lig Plot was used to visualize amino acid interaction with the protein target complex Schrodinger-Maestro v.8.5 using Schrodinger-Maestro v.8.5, New York, NY, USA.

In vitro Cytotoxicity Assessment

U-87MG glioblastoma cells were seeded at a density of 1.0×10^6 cells per well in the 96 healthy plates. The incubation period was maintained for 24 hours and 48 hours. After the 24 hours and 48 hours of incubation, cells were treated with different concentrations (5, 25, 50, 75, 100 $\mu\text{g}/\text{m}$) of lupeol. Further, each well was added with 10 μl MTT (Sigma, MO, USA). After another 4 hours of incubation, the leftover media was removed, and 150 μl dimethylsulfoxide (DMSO) (Sigma) was added to dissolve the Formosan crystal formed in each well by stirring. The absorbance of the sample was read at 490 nm using a Spark 10 M microplate reader (Tecan, Mannedorf, Switzerland).

RESULTS**Prediction of Candidate Targets against Glioblastoma multiforme**

Swiss target prediction and DisGeNET databases were accessed to identify the targets for anti-GBM. Overall, 4049 hits screened for Glioblastoma multiforme using DisGeNET databases and 104 targets for lupeol using Swiss target prediction. The Venn diagram (Fig.1) was constructed using FunRich software to identify the co-relative targets for GBM and lupeol. The total number of correlative targets observed was 50 between GBM and lupeol.





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Functional Enrichment and Pathway enrichment

The total 50 correlative anti glioblastoma targets of lupeol were enriched with FunRich software's help. The Gene ontology studies/analysis are mainly based on cellular components, biological function, and molecular function. The significantly enriched leading targets as observed in tables. The cellular component enrichment in Fig.2(A) was observed higher in the plasma membrane (60%), cytosol (54%), nucleus (48%), cytoplasm (48%), and nucleoplasm (44%). Fig.2(B) shows the biological process was linked with positive regulation of transcription from RNA polymerase two promoter and negative regulation of transcription from RNA polymerase. The higher GO term-molecular function in Fig.2(C) enriched was zinc ion binding (32%), identical protein binding (28%), and enzyme binding (26%).

The total 50 potential targets of pathway enrichment analysis were identified using FunRich software. Based on data screened, leading pathway enriched were Nuclear Receptor transcription pathway, SUMOylation of intracellular receptors, Regulation of IFNG signaling, Growth hormone receptor signaling, ESR-mediated signaling, Response to elevated platelet cytosolic Ca²⁺, RHO GTPases Activate NADPH Oxidases, Disinhibition of SNARE formation, Calmodulin induced events, Oncogene Induced Senescence represented in Table.1.

Network Interaction of the Glioblastoma Multiforme

The functional protein network was obtained by introducing 50 correlative targets of lupeol and Glioblastoma multiforme into the STRING database to understand the mechanism of the protein-protein interaction network. Fig.4A represents the PPI network of correlative target proteins constructed using the K-mean algorithm by adjusting the medium confidence score at 0.500 and *p*-value enriched at $<1.0 \times 10^{-16}$. The network consists of 77 nodes and 412 edges. Further, the PPI networks were clustered into four distinctive networks. The Fun Rich software was utilized to construct modules from unique clustered network proteins, ESRI, MAPK3, JAK2 (Fig.4B), as a core target.

Molecular docking analysis of target compound and target genes

The core target proteins of lupeol were processed using molecular docking analysis. The PDB ID of core proteins was downloaded from Protein databank RCSB to extract a three-dimensional molecular structure. The PDB.ID of core target proteins JAK2(PDB.ID-2B7A), MAPK3(PDB.ID-6GES). The glide docking score of target proteins was measured based on Standard Precision(SP). The final output of the docking showed the decent score level: JAK2: -3.432kcal/mol, MAPK3: -2.271kcal/mol denoted in Fig.5.

Molecular docking analysis has been a valuable tool in drug discovery. However, the post docking process should improve certain limitations like poor scoring function. The post docking analysis involves tools like Prime/Molecular Mechanics-Generalized Born Surface Area (MMGBSA) which includes Free binding energy. The Free binding energy is calculated: JAK2: -21.94 kcal/mol, MAPK3: -6.43. The other factors like Coulomb energy, lipophilic energy, and van der Waals energy were denoted in Table.3

Cell Viability analysis by MTT assay

The results obtained confirm the decrease in the U-87MG tumor cell population with an increase in the concentration of lupeol compared to the control cells. The cells were treated for 24 hours and 48 hours at the concentration 5 μ g, 25 μ g, 50 μ g, 75 μ g, 100 μ g. After treatment, the half-maximal inhibitory concentration value was enumerated at 100 μ g with IC₅₀ (60.1). The cell viability observed at 96%, 89%, 77%, 66%, and 56% for specific concentrations. Thus, the proliferation of the cells is inhibited by Lupeol in a dose-dependent manner represented in Fig.6.

DISCUSSION

Glioblastoma multiforme (GBM) is found to be a debilitating disease & highly invasive with many mutations at genetic and epigenetic levels. The mutations are essential factors to distinguish and classify to gather knowledge on the tumor environment and its resistance to therapeutics. Due to various triggering mutations and the significant



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mutations in the GBM stem cells, glioblastoma multiform is differentiated into primary tumors, which arise from neural stem cell precursors and secondary tumors from the mutations in mature neural cells astrocytes [11]. Chemotherapy (majorly Temozolomide) is a widely used therapeutics system for treating GBM. TMZ's ability to travel across the blood-brain barrier with more minor myelotoxic characteristics than any other anti-GBM drug. However, mutant TP53 initiates the resistance to TMZ through up-regulation of MGMT expression in GBM cells. Due to the disadvantage factors of chemotherapy, various naturally derived compounds are exploited, which enhances the destruction of cancer cells by blocking the specific phase G0/G1, G2/M in the cell cycle. The obstruction of the cell cycle leads to a reduction of cancer cell proliferation and causes cell death. Thus, believe in the natural substance's ability to restore the standard biological mechanism[12].

The natural compounds exhibit anti-cancer abilities by directly acting on the mechanism, ROS generation, Anti-angiogenesis, Anti-metastasis, miRNA regulation [13].The network pharmacology concepts provide extensive knowledge to identify the core targets involved in the biological mechanism within the network perspective, thus helping drug design therapeutics [14].

The present studies explore the potential of bioinformatics tools (Web-based database) used to investigate the therapeutic mechanism of terpenoidslupeol against Glioblastoma multiform cells. The DisGeNET platform is built to access freedom in exploration and understanding the genetic underpinnings of the full spectrum of human diseases like the Mendelian, rare and complex, and it also includes the symptoms, signs, and other phenotypic features of diseases. The data from the platform are collected from the popular repositories across the field, which are enriched and expanded with information extracted from the scientific literature through modern text mining tools [15]. The Database analysis tools such as Swiss target prediction and DisGeNET were used to identify therapeutic targets for Glioblastoma and lupeol. Further, Correlative analysis rendered 50 therapeutically valuable targets related to GBM and lupeol. These targets play a significant role in the subsequent investigative studies.The identified correlative targets were analyzed for functional enrichment using Gene Ontology (GO) through FunRich software tools.

The GO terms are cellular components, biological processes, and molecular mechanisms. The maximum number of identified targets enriched at the plasma membrane in Cellular component, regulation of transcription from RNA polymerase II promoter in biological process, zinc ion binding from the molecular mechanism. Mitochondria being a powerhouse of cells, speculation can be that more anti-cancer activity occurred at the plasma membrane[16].Cancers possess an extensive range of DNA mutations and induce rearrangements to promote oncogenes and the transformation of malignant cells through silencing the tumor suppressor factors. The significant features of these changes are the regulation of transcription from RNA polymerase II promoter[17,18]. Zinc has a catalytic role in neutralizing free radicals in the body. Zinc plays a significant role in controlling cell survival and cell death (apoptosis).The deregulation of zinc leads to the pathogenesis of many diseases like various types of cancer. In cancer, depletion of zinc results in irreversible DNA damage and initiates free radical generation and loss of DNA repair mechanism[19].The top 10 enriched pathways identified with a higher percentage in the nuclear receptor transcription pathway based on Reactome database studies.

The network construction uses string analysis to study the protein-protein interaction of identified targets. The network clustered to form a sub-cluster; each cluster shows the distinct module formation of target interaction with core gene MAPK3,ESR1,JAK2. The over expression of transcription factor ESR is one of the vital conditions in cancer cells. Binding of Dimeric ESR to a promoter, distant enhancers of E2-sensitive genes regulate their over expression. The post-translational modification (such as phosphorylation) of the receptors by plasma membrane ESRs, the protein complex formation with Specific membrane-associated proteins. Proto-oncogene Proteins from the tyrosine-protein kinase Src family (SRC) are involved in various molecular processes of cancerous cells [20]. The MAPK3 pathway acts as a controlling factor in the regulation of cancer cells through roles in proliferation, differentiation, and apoptosis. Some research studies exploited the possibility of targeting the MAPK3 pathway with novel inhibitors, which significantly reduced gliomacells [21]. The current analysis also provides an outcome in inhibiting cancer cells by lupeol through MAPK3 targeting.





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JAK2 has primary functions as a signaling factor in cell proliferation (growth and division). The mutations in the JAK2 lead to instability in proliferation with dire consequences cancers. Various inhibitors manipulate the JAK2 function in cancer, which plays a vital role in targeted therapy in cancers[22]. Further, *In silico* studies of identified targets of lupeol and Glioblastoma show significant interaction/binding between ligand and receptor. The Docking analysis recognized the residue to hydrogen bond to JAK2 (GLU-1006, ASN-1111, VAL-1110), MAPK3 (GLU-343, HIE-197, ARG-96, LYS-220). *In vitro* studies on tumor cells, inhibition of cell proliferation was observed with response to raise in dose concentration (5µg, 25µg, 50µg, 75µg, 100µg).

CONCLUSION

In the conclusion of the current studies, the molecular mechanism of lupeol against Glioblastoma was identified using network pharmacology and molecular docking as the core source. Also, correlative target hits were identified for lupeol and Glioblastoma. Gene ontology enrichment terms were analyzed for correlative targets and the regulation of nuclear receptor transcription pathways and other pathways identified using the Reactome database. Protein-protein interaction (PPI) network was constructed, and ESRI, MAPK3, JAK2 were identified as core targets in clusters. Finally, *invitro* studies and molecular docking analysis concluded the ability of lupeol to inhibit glioblastoma cells by forming a complex with target proteins.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 1. Top enriched pathways and their percentage of genes

Pathway	Percentage of genes
Nuclear Receptor transcription pathway	14
SUMOylation of intracellular receptors	10
Regulation of IFNG signalling	8
Growth hormone receptor signalling	8
ESR-mediated signalling	6
RHO GTPases Activate NADPH Oxidases	6
Oncogene Induced Senescence	6
Response to elevated platelet cytosolic Ca ²⁺	4
Disinhibition of SNARE formation	4
Calmodulin induced events	4





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Table 2.Molecular docking analysis of target protein interacted with lupeol and binding energies.

Target protein	PDB.ID	SP (kcal/mol)	Hydrogen bond residues
JAK2	2BA7	-3.432	GLU-1006, ASN-1111,VAL-1110
MAPK3	6GES	-2.271	GLU-343,HIE-197 ARG-96,LYS-220

Table 3.Prime / Molecular Mechanics - Generalized Born Surface (MMGBSA) for Ligand-Protein complex.

Complex (Protein)	Free Binding Energy (ΔG_{Bind})	Coulomb's Energy (ΔG_{Coul})	Lipophilic Energy (ΔG_{Lipo})	Vander Waals Energy (ΔG_{vdw})	Solvation Energy (ΔG_{solGB})
JAK2	-21.94	-1.44	-20.56	-31.13	31.2
MAPK3	-6.56	-4.06	-13.54	-19.02	30.55

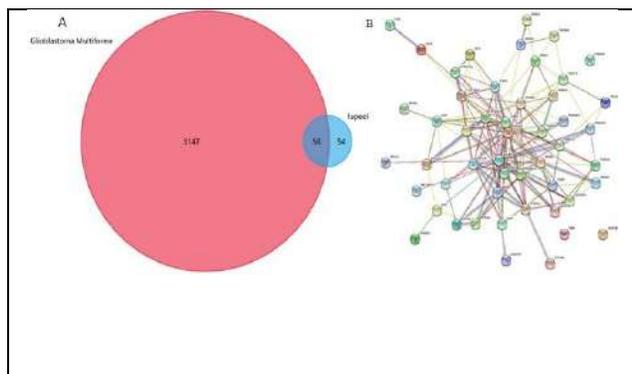


Figure 1: (A) Venn Diagram: the targets of glioblastoma targets and lupeol. The Swiss-target prediction database and DisGenet database were used as sources for identifying targets for glioblastoma and lupeol. (B) Protein-protein interaction (PPI) network of fifty correlative targets constructed using STRING database

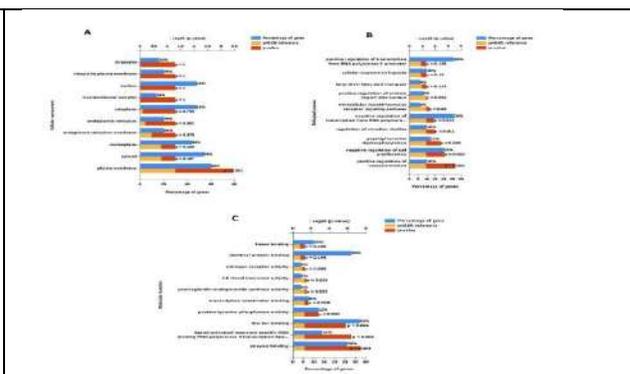


Figure 2: Gene ontology enrichment of identified targets. The GO features involve (A)cellular components,(B) biological processes, and (C) molecular function of correlative targets of Glioblastoma and lupeol

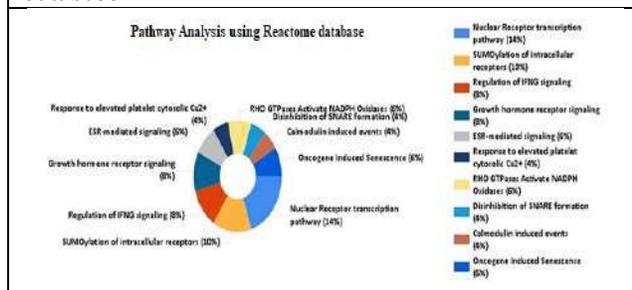


Figure 3: Pathway enrichment analysis of lupeol and glioblastoma: Doughnut chart represents Top 10 enriched pathways of targets identified using Reactome pathways database.

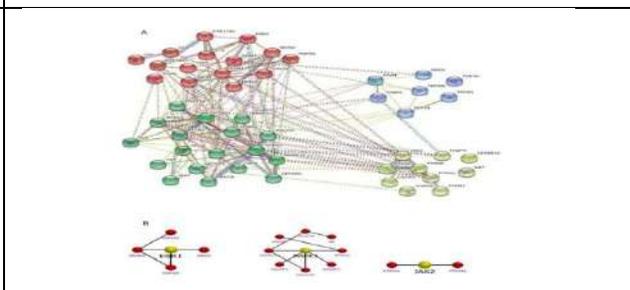


Figure 4: Analysis of protein targets clustering using STRING database: (A) the protein-protein interaction network of lupeol constructed. Based on the K-mean algorithm, the PPI network is clustered into three distinctive networks. (B) Module constructed to each core target protein.





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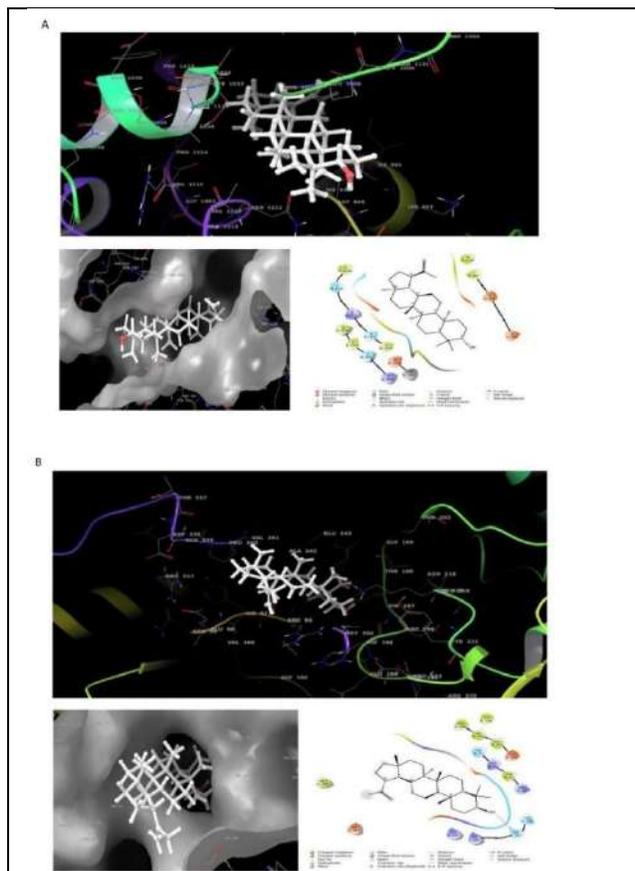


Figure 5: Molecular Docking Analysis of Core Targets: two-dimensional representation of lupeol docking with core target proteins (a) 2MA7 (b) 6GES

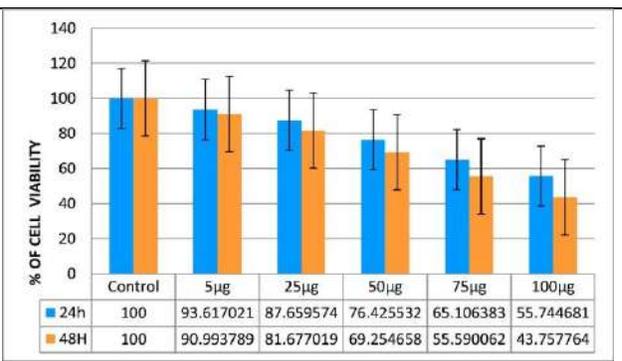


Figure 6: cell viability assay: Viability of U-87MG cells with a dose-dependent response. The graphical pattern denotes the Decrease in cell viability percentage with an increase in dose-response at two different time periods.





Population, Diversity and Phylogenetic Analysis of Freshwater Mussels (Bivalvia: Unionidae) In Cauvery River, Srirangam, Tiruchirappalli, Tamil Nadu

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ABSTRACT

Freshwater mussels are bivalve inhabitants of inland waters represented on all continents except Antarctica. They are among the most endangered animal taxa. In the present study population estimation and phylogenetic study of freshwater mussels (Unionidae) was conducted. Totally 3019 animals in 5 different species of freshwater mussels were recorded in Cauvery river, Srirangam, Tiruchirappalli district, Tamil Nadu, India. They were belonging to two genera, *Lamellidens* and *Parreysia* and one family Unionidae. *Parreysia favidens* was recorded in maximum number and minimum numbers of *Lamellidens corrianus* were recorded in Cauvery river. Phylogenetic study result revealed that there was close relationship between *Lamellidens corrianus* and *Lamellidens marginalis* and distantly related species were *Lamellidens corrianus* and *Parreysia corrugata*.

Keywords: Freshwater ecosystem, Freshwater mussels, Bivalves, Unionidae, Diversity and Phylogenetic.



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INTRODUCTION

Freshwater environments are some of the most fragile and highly threatened ecosystems in the world. These ecosystems have been constantly manipulated by humans to satisfy their needs with little or no thought to the long term effects on them [1]. Freshwater bivalves of the order Unioniformes represent the largest bivalve radiation in freshwater. This diverse group is under 6 families, 181 genera and 800 species. These families are distributed across 6 of the 7 continents and represent the most endangered group of freshwater animals alive today [2,3]. Molluscs are an important group for freshwater biodiversity and where abundant play an important role in ecosystem functioning [4]. They form an important biological monitor that rate quality of water and status of aquatic systems [5,6,7]. In particular Bivalves are accumulate toxic substances to the greater extent than other organisms and are used to detector water quality [8]. Molluscs show a great specialization of ecological niches in freshwater environments, making them more vulnerable to modifications in their environment [9,10]. Consequently, freshwater molluscs have severe decline in diversity and distribution due to human induced alteration of habitats, pollution, siltation, deforestation and poor agricultural practices [11,12].

Relationships among populations of freshwater organisms have been formed by major geological changes in aquatic systems [13,14]. Genetic relationships within and among populations are not only formed by historic geological events, but also affected by intrinsic effects [15], including habitat preferences and dispersal abilities among habitats [16,17,18]. Dispersal ability appears to be major determinant of population genetics of freshwater organisms [17,19]. Freshwater mussels are globally in decline and are among the most endangered aquatic species [20]. [21] recorded 224 (44%) of the 511 freshwater mussel species as Near Threatened or Threatened in the 2015 IUCN Red List of Threatened Species. Despite having highly important ecosystem roles, gaps exist on biology and population structure of freshwater bivalves still standing as the primary obstacle to management and conservation. Non-marine molluscs, which includes land and freshwater molluscs, comprise the largest number of recorded extinctions in the last 300 years [22]. Hence, conservation efforts are urgently needed to maintain and recover these unique components of aquatic biodiversity. Results of the present study will help in the conservation of freshwater mussels Fauna in human dominated freshwater ecosystem.

METHODOLOGY

Study Area

Srirangam is an island and a part of the city of Tiruchirappalli, Tamil Nadu, India located at latitude 10°51'44 and longitude 71°N 78°41'23.9"E. It is bounded by the Cauvery River on one side and its distributary Kollidam on the other side. Srirangam is home to a significant population of Sri Vaishnavites. Srirangam is Tropical region and average temperature range in summer is maximum 37.1°C (98.8 °F) and minimum 26.4 °C (79.5 °F) and in winter maximum temperature is 31.3 °C (88.3 °F) and minimum 20.6°C (69.1 °F). Average rainfall is 835 millimetres per year. The flow of wind E is at 14 km/h and humidity is 62%. The Cauvery River is India's fourth largest River, draining about 89,600 sq. km. The River originates at Talacauveri, Kodagu district of Karnataka and flows generally southeast and finally emptying into the Bay of Bengal. The river basin covers one Union ([Puducherry](#) 148 square kilometres [57 sq mi]) and three states (Tamil Nadu [43,868 square kilometres (16,938 sq mi)], Karnataka [(34,273 square kilometres 13,233 sq mi)] and Kerala [2,866 square kilometres). The river bifurcates into two large branches at Mukombu Trichy District in the name of Kollidam (Coleroon) and Cauvery and re-joined at Grand Anicut (Fig. 1).

Sample Collection

The collection of specimens was done at five different collection sites in Cauvery River Srirangam, Tiruchirappalli District, Tamil Nadu, India. Sites were distanced among 1 KM and 2 feet in depth. Healthy freshwater mussels were hand collected from selected sites of Cauvery River at one month interval for one year and recorded samples were Identified and returned them into their habitats. The identification of molluscan species was carried out at Zoological Survey of India (ZSI), Southern Regional Station, Chennai followed by [23,24,25].





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Population Estimation

Freshwater mussel's population was estimated in every month for 12 months. Population of freshwater mussel's belongings to family Unionidae in Cauvery River, Srirangam area, Tiruchirappalli District, Tamil Nadu was estimated by the method of Stout and Vandermeer [26].

Diversity Index Study

Species diversity was calculated by Shannon Wiener diversity index (H'). Diversity index was calculated by using following formula.

$$\text{Diversity index } (H') = -\sum[(pi) * \ln(pi)]$$

$$\text{Evenness } (E) = H/H_{max}$$

$$H_{max} = \ln(S)$$

Where, (H')

Sum = Summation

P_i = Number of individuals of species, i /total number of samples

S = Number of species or species richness

H_{max} = Maximum diversity possible

E = Evenness

Phylogenetic Study

The mitochondrial COI gene of the different species such as *Lamellidens consobrinus* (KT869147) *Lamellidens corrianus* (MT111611), *Lamellidens marginalis* (MK879801), *Parreysia favidens* (KT869142), and *Parreysia corrugata* (KJ872817) was retrieved from GeneBank (<http://www.ncbi.nlm.nih.gov/>). Multiple sequence alignment and Phylogenetic tree construction were done by using online software Clustal Omega.

RESULTS AND DISCUSSION

Population Estimation of Freshwater Mussels

The data on the distribution of freshwater mussels (Unionidae) in Cauvery River, Srirangam area is depicted in Tables 1, 2 and 3. Totally 3019 specimens were recorded throughout the experimental period. There were five different species such as *Lamellidens consobrinus*, *Lamellidens corrianus*, *Lamellidens marginalis*, *Parreysia favidens*, and *Parreysia corrugata* belongings to the two genera, *Lamellidens* and *Parreysia* with one family Unionidae (Fig. 2). Maximum (695) numbers of *Parreysia favidens* were recorded throughout the experimental period and minimum (567) numbers of *Lamellidens corrianus* were recorded in the experimental period (Table 1). In these, *Parreysia favidens* was recorded maximum (22.40 ± 3.36) number in the month of May at 2018 and *Lamellidens corrianus* was recorded in minimum (3.00 ± 1.00) number at November 2018 (Table 2). This different may be depend on the level of water flow in the Cauvery River. Maximum (20.20 ± 6.69) numbers of specimens were recorded in site 1 in the month of June 2018 and minimum (2.60 ± 0.89) number of specimens was collected in site 4 at October 2018 (Table 3).

In past decades, many research had effort to understand mussel abundance and diversity are affected by environmental processes operating at various spatial scales [27,28,29,30]. Few studies have been conducted about associations of mussel species distribution with climate variables [20], because of broad-scale mussel surveys are rare. However, together with laboratory-based thermal-tolerance tests [31,32], is essential for the prediction of impacts of climate changes. Several geological variables were found to be important for species richness, mussel presence-absence, total mussel abundance and species abundance. Geology, together with climate and topography, shapes substratum composition, stream channel morphology and flow regimes [33,34,35], are known to be important for mussel assemblages at certain spatial scales [36,37]. In the management perspective, the strong effects of natural environmental variables on mussel species and assemblages attainable for ecological restoration and conservation targets. In spite of the effects of natural variables, many mussel species were associated with land use and connections to dams/ponds. Those species tended to increase with distance from a dam or pond. Total mussel



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abundance responded similarly to the connection measures. These findings are largely supported by previous field studies [38,39,40,41].

Diversity Study Of Freshwater Mussels

The diversity of indices is showed in Table 4. Simpson index and Shannon index showed a good diversity and the value of the evenness index (0.999) indicated even distribution of freshwater mollusc in the Cauvery river. Diversity results of the samples showed that no remarkable changes (Shannon index H) and evenness (ln E). In India, the diversity studies of freshwater molluscs are limited. Mostly diversity; distribution and biology study were confined to its southern peninsula as well as in the Indo-Burma region [42,43,44,45,46]. [47] reported that 112 species of gastropods and 74 species of bivalves (186 species in total) from the Eastern Himalayan region of which about one third (i.e. 32.6%) of the total population is reported to be DD or LC due to lack of information on their current status. [48] also recognized 112 freshwater bivalve species from the Indo-Burma region. Out of which 5.2% were under critically endangered and 8.0% EN. This data deficient that freshwater bivalve species are generally suffers from critical extinction tendency from their natural habitat.

Phylogenetic Study

Phylogenetic is the study of evolutionary relationship among various groups of organisms (e.g. species and populations), which is discovered through morphological and molecular data matrix. Since last two decades, phylogenetic of various molluscan taxa studied by use of ribosomal marker gene, those are reliable tool in phylogenetic relationships study below the class level [49,50]. Phylogenetic guide tree indicated that the *Parreysia favidens* and *Parreysia corrugata* were closely related animals and *Parreysia corrugata* and *Lamellidens corrianus* were distance related species. Based on the phylogenetic tree, *Parreysia favidens* and *Parreysia corrugata*, and *Lamellidens corrianus*, *Lamellidens marginalis* were closely related species. Species *Lamellidens consobrinus* was intermediated that had equal distance between *Parreysia favidens* and *Parreysia corrugata*, and *Lamellidens corrianus* and *Lamellidens marginalis* (Fig. 2). However, all the five species were confirmed relationships among them.

The enzyme cytochrome C oxidase is a well-known protein of electron transport chain and is found in both bacteria and mitochondria. The COI and COII genes code for two of seven polypeptide subunits in the cytochrome C oxidase complex. The COI gene consists of approximately 894 bp. COI and/ or COII sequences have been applied to phylogenetic problems at a wide range of hierarchical levels in insects, from closely related species to genera and subfamilies, families, and even orders [51]. It is a good performer in recovering an expected tree [52]. So sequencing both the genes represents one of the largest sequence data sets generated for phylogenetic study of any group and also fulfils the putative phylogenetic accuracy. The phylogenetic tree among the four different groups of freshwater mussels showed the closely relationship. The rooted phylogenetic tree was proved the closely relationship among the four groups of freshwater mussels. The unrooted phylogenetic tree also supported to the relationship among the all species. However, *Lamellidens corrianus* and *Lamellidens marginalis* were closely related species and, *Lamellidens corrianus* and *Parreysia corrugata* were distantly related species.

The inference of phylogenetic with computational method has numerous important applications in biological and medical research, such as drug discovery and conservation biology. A result published by [53] that times the evolution of the HIV-1 virus, demonstrates that ML techniques can be effective in solving biological problems. Phylogenetic trees already have witnessed applications in numerous practical domains, such as in conservation biology [54] epidemiology [55] (predictive evolution), forensics [56], gene function prediction [57]. Other applications include multiple sequence alignment [58,59] protein structure prediction [60] and drug design [61].



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The close relationship between *Lamellidens corrianus* and *Lamellidens marginalis* may be due to same habitat. The distance relationship of the two species *Lamellidens corrianus* and *Parreysia corrugata* may be depending on the habitat characterization and also morphological and biochemical characterization. [2,62] gathered combined matrix that included both 28S and COI (as well as 59 morphological characters and compared relative merits of the two molecular character sets to resolve family-level phylogeny of Unionidae. The consensus of both of these studies was that COI is of limited utility. The problem is well known and the solutions widely discussed [63,64,65]) and applying probabilistic models that account for unobserved character transformations along with the relatively long branches, and breaking up long branches by including additional taxa.

CONCLUSION

From the study, it is concluded that totally 5 species of freshwater Mussels belonging to two genera such as *Lamellidens* and *Parreysia* and one family Unionidae were recorded in Cauvery River, Srirangam Area Tiruchirappalli district, Tamil Nadu, India. *Parreysia favidens* species was collected maximum number and *Parreysia corrugata* was collected in minimum numbers. Phylogenetic study concluded that closely relationship was observed in *Lamellidens corrianus* and *Lamellidens marginalis*, *Lamellidens corrianus* and *Parreysia corrugata* were distantly related species.

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Table 1. Total Population of Freshwater mussels (Unionidae) in Cauvery River Srirangam Area, Tiruchirappalli District, Tamil Nadu, India.

S.No	Species	Total Population (Nos)
1	<i>Lamellidens consobrinus</i>	568
2	<i>Lamellidens corrianus</i>	567
3	<i>Lamellidens marginalis</i>	613
4	<i>Parreysia favidens</i>	695
5	<i>Parreysia corrugata</i>	576
	Total Population of Freshwater mussels	3019

Table 2. Species wise distribution of Freshwater mussels (Unionidae) in Cauvery River Srirangam Area Tiruchirappalli District, Tamil Nadu, India.

S. No	Year	Month	Species	Mean \pm SD (Numbers)
1	2018	4	<i>Lamellidens consobrinus</i>	10.00 \pm 2.83
		4	<i>Lamellidens corrianus</i>	12.40 \pm 3.91
		4	<i>Lamellidens marginalis</i>	11.00 \pm 3.74
		4	<i>Parreysia favidens</i>	21.40 \pm 3.21
		4	<i>Parreysia corrugata</i>	17.00 \pm 2.92
2		5	<i>Lamellidens consobrinus</i>	15.60 \pm 4.56
		5	<i>Lamellidens corrianus</i>	15.00 \pm 4.06
		5	<i>Lamellidens marginalis</i>	21.80 \pm 3.70
		5	<i>Parreysia favidens</i>	22.40 \pm 3.36
		5	<i>Parreysia corrugata</i>	14.60 \pm 3.78
3		6	<i>Lamellidens consobrinus</i>	15.60 \pm 5.13
		6	<i>Lamellidens corrianus</i>	16.40 \pm 3.85
		6	<i>Lamellidens marginalis</i>	18.80 \pm 4.97
		6	<i>Parreysia favidens</i>	21.00 \pm 5.15
		6	<i>Parreysia corrugata</i>	16.20 \pm 6.57
4	7	<i>Lamellidens consobrinus</i>	14.00 \pm 4.69	
	7	<i>Lamellidens corrianus</i>	14.80 \pm 3.70	
	7	<i>Lamellidens marginalis</i>	12.40 \pm 3.98	
	7	<i>Parreysia favidens</i>	12.60 \pm 4.04	
	7	<i>Parreysia corrugata</i>	11.60 \pm 2.61	
5	8	<i>Lamellidens consobrinus</i>	6.80 \pm 3.63	





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		8	<i>Lamellidens corrianus</i>	8.20 ± 1.79
		8	<i>Lamellidens marginalis</i>	7.80 ± 3.77
		8	<i>Parreysia favidens</i>	7.20 ± 3.42
		8	<i>Parreysia corrugata</i>	5.40 ± 2.30
6		9	<i>Lamellidens consobrinus</i>	7.60 ± 2.88
		9	<i>Lamellidens corrianus</i>	7.60 ± 3.65
		9	<i>Lamellidens marginalis</i>	5.40 ± 2.07
		9	<i>Parreysia favidens</i>	6.20 ± 1.09
		9	<i>Parreysia corrugata</i>	6.40 ± 2.61
7		10	<i>Lamellidens consobrinus</i>	4.80 ± 3.83
		10	<i>Lamellidens corrianus</i>	3.00 ± 1.87
		10	<i>Lamellidens marginalis</i>	3.20 ± 1.30
		10	<i>Parreysia favidens</i>	4.80 ± 1.92
		10	<i>Parreysia corrugata</i>	4.40 ± 1.52
8		11	<i>Lamellidens consobrinus</i>	4.00 ± 1.58
		11	<i>Lamellidens corrianus</i>	3.00 ± 1.00
		11	<i>Lamellidens marginalis</i>	4.60 ± 0.89
		11	<i>Parreysia favidens</i>	3.60 ± 1.34
		11	<i>Parreysia corrugata</i>	3.60 ± 2.30
9	2018	12	<i>Lamellidens consobrinus</i>	5.80 ± 1.92
		12	<i>Lamellidens corrianus</i>	4.00 ± 1.87
		12	<i>Lamellidens marginalis</i>	4.20 ± 1.30
		12	<i>Parreysia favidens</i>	5.80 ± 1.30
		12	<i>Parreysia corrugata</i>	5.00 ± 1.87
10		1	<i>Lamellidens consobrinus</i>	9.00 ± 3.39
		1	<i>Lamellidens corrianus</i>	6.60 ± 0.89
		1	<i>Lamellidens marginalis</i>	8.80 ± 0.84
		1	<i>Parreysia favidens</i>	9.40 ± 4.83
		1	<i>Parreysia corrugata</i>	6.20 ± 3.42
11	2019	2	<i>Lamellidens consobrinus</i>	9.80 ± 1.09
		2	<i>Lamellidens corrianus</i>	11.20 ± 2.05
		2	<i>Lamellidens marginalis</i>	12.00 ± 4.64
		2	<i>Parreysia favidens</i>	13.20 ± 3.35
		2	<i>Parreysia corrugata</i>	10.80 ± 3.35
12		3	<i>Lamellidens consobrinus</i>	10.60 ± 2.88
		3	<i>Lamellidens corrianus</i>	11.20 ± 2.28
		3	<i>Lamellidens marginalis</i>	12.60 ± 2.96
		3	<i>Parreysia favidens</i>	11.40 ± 2.88
		3	<i>Parreysia corrugata</i>	14.00 ± 4.18

Table 3. Sitewise distribution of Freshwater mussels (Unionidae) in Cauvery River Srirangam Area Tiruchirappalli District, Tamil Nadu, India

S.No	Year	Month	Site	Mean ±SD (Numbers)
1	2018	4	1	15.20 ± 6.98
		4	2	13.60 ± 6.31
		4	3	15.20 ± 6.57
		4	4	14.60 ± 4.72





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		4	5	13.20 ± 3.35
2		5	1	16.00 ± 4.89
		5	2	18.00 ± 5.92
		5	3	20.20 ± 4.15
		5	4	18.60 ± 6.88
		5	5	16.60 ± 3.78
3		6	1	20.20 ± 6.69
		6	2	15.60 ± 5.68
		6	3	18.60 ± 1.82
		6	4	19.20 ± 5.36
		6	5	14.40 ± 4.62
4		7	1	13.80 ± 3.70
		7	2	13.20 ± 4.09
		7	3	12.40 ± 3.51
		7	4	13.00 ± 3.54
		7	5	13.00 ± 5.15
5		8	1	8.80 ± 2.78
		8	2	7.20 ± 3.42
		8	3	5.60 ± 2.07
		8	4	5.60 ± 3.78
		8	5	8.20 ± 2.17
6		9	1	3.40 ± 1.34
		9	2	6.00 ± 1.58
		9	3	7.60 ± 2.07
		9	4	9.00 ± 2.24
		9	5	7.20 ± 1.64
7		10	1	5.20 ± 3.42
		10	2	4.40 ± 1.67
		10	3	4.80 ± 1.92
		10	4	2.60 ± 0.89
		10	5	3.20 ± 2.17
8		11	1	3.20 ± 0.84
		11	2	3.60 ± 1.14
		11	3	3.20 ± 1.92
		11	4	4.40 ± 1.67
		11	5	4.40 ± 1.67
9	2018	12	1	5.00 ± 1.87
		12	2	4.00 ± 2.12
		12	3	6.00 ± 1.58
		12	4	4.60 ± 1.52
		12	5	5.20 ± 1.48
10	2019	1	1	7.20 ± 3.27
		1	2	9.40 ± 3.65
		1	3	9.80 ± 2.17
		1	4	7.20 ± 3.42
		1	5	6.40 ± 2.70
11		2	1	12.20 ± 3.03
		2	2	11.60 ± 5.03





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		2	3	11.20 ± 2.86
		2	4	10.80 ± 2.28
		2	5	11.20 ± 2.86
12		3	1	12.20 ± 4.32
		3	2	12.60 ± 4.39
		3	3	11.40 ± 2.07
		3	4	12.00 ± 2.55
		3	5	11.60 ± 2.70

Table 4. Different diversity indices of freshwater mussels in Cauvery River, Srirangam, Tiruchirappalli, Tamil Nadu, India.

Sites	Total No. of Organisms	Average Population size	Simpson index (D)	Shannon index (H)	Evenness (E)
Site 1	612	122.4	0.207	1.594	0.991
Site 2	596	119.2	0.203	1.601	0.995
Site 3	630	126.2	0.200	1.609	1.0
Site 4	608	121.6	0.204	1.600	0.994
Site 5	573	114.6	0.201	1.608	0.999

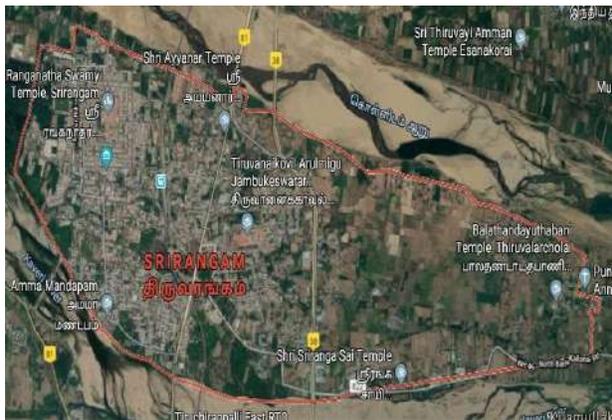


Fig. 1a) Study Area Srirangam. Tiruchirappalli District, Tamil Nadu, India.



Fig. 1b) Cauvery River, Srirangam Area, Tiruchirappalli District, Tamil Nadu, India.



Lamellidens consobrinus

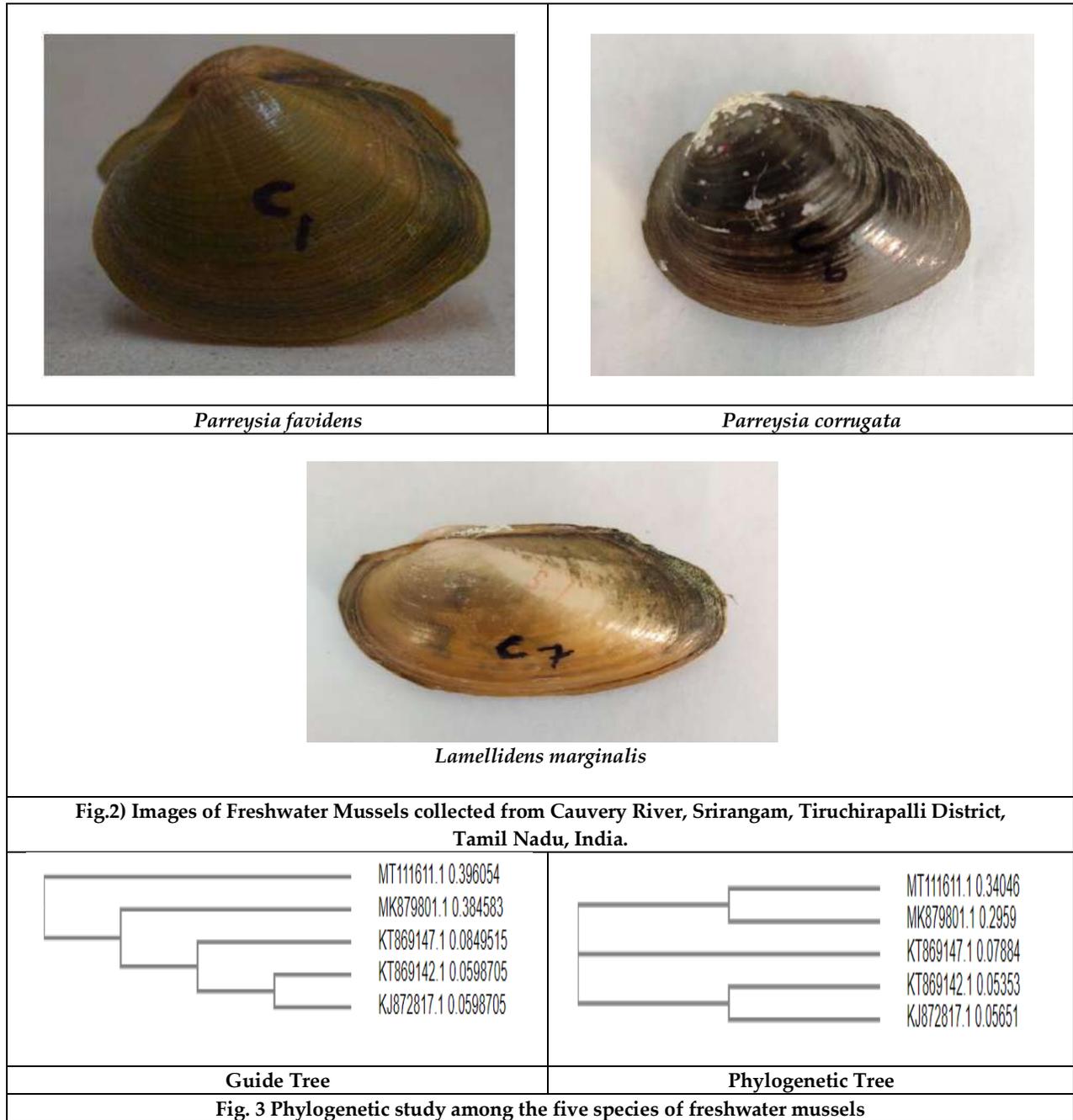


Lamellidens corrianus





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Review of Research and Development in Concrete Containing Waste Materials

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ABSTRACT

Concrete is very extensively used construction matter in the world, and its manufacture necessitates a large number of aggregates and cement as the primary component materials. The energy-intensive process of getting these concrete components causes a slew of environmental issues, including natural resource depletion, pollution, a waste disposal challenge, and carbon dioxide emissions. As a result, an alternative eco-friendly, long-lasting Concrete is required. Industries, agriculture, bio-waste, building & demolition trash, and marine debris can all be recycled and used as a supplemental sustainable concrete material for this. This will have a lower environmental impact while also lowering energy use. As a result, the current work attempts to outline the potential uses of various waste products as well as their impacts on the properties of concrete in order to raise awareness of the importance of repurposing discarded resources in order to achieve concrete production sustainability.

Keywords: Sustainable concrete, Green concrete, Industrial pollution, Greenhouse gases.

INTRODUCTION

Any country's economy grows as a result of its infrastructure. Transportations, flyovers, houses, airstrips and docks are all examples of infrastructure. With rapid necessity for urbanization mounting, the demand for concrete, resulting in an increase in cement demand. Cement concrete is in high demand in the building business, but it is also a worry for the environment because it emits harmful gases at various stages of manufacture. We all know that any concrete mix contains cement, aggregates, sand, water, and additives according to the design specifications. This is an endeavor to switch conventional ingredients with different replacements in order to manage greenhouse gasses and treat discarded supplies from various sectors (Vishwakarma et al., 2016). It includes information on the effects on compressive strength, setting time, cost effectiveness, and pollution reduction. Because natural resources are finite, we must discover alternatives to replace them while maintaining the quality and effectiveness of the final product. Cement kilns emit toxic gases that contribute to global warming. Pollution from cement plants has a negative impact on animals as well. Cement bags are subjected to wear and tear during transportation from the



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manufacturing site to the end user, and cement dust drifts into the environment. It affects those who used to transmit, landfill, and redeploy the stockpiled cement stacks, causing breathing problems. Due to an increase in the number of waste materials and industrial by-products, solid waste management has become a serious challenge (Devi et al., 2017).

This exacerbates the problem of land filling and raises the expense of garbage recycling. The only way to avoid the disposal issue is to use these materials as Green Concrete. Green Concrete is created from environmentally friendly waste materials and, thanks to its technology, heralds in a revolution in the concrete industry (Vishwakarma & Ramachandran, 2018). Waste materials can be utilised directly as a partial replacement for cement, reducing energy usage during cement manufacture. Pozzolan characteristics can be found in some waste products. Pozzolans are silica and alumina-rich materials that have little or no cementing characteristics on their own, but in the presence of water, they chemically react with calcium hydroxide at room temperature to provide cementitious qualities (Hossain et al., 2016). The entire cement manufacturing process is polluting, from the crushing and delivery of lime stone to the heating of kilns and crushing.

River sand, which is acquired from riverbeds and erodes valuable top soil, is used as fine aggregate in concrete mixtures. Because sand mining is a profitable business, it does not benefit the government financially and disrupts river ecosystems. The riverbed is damaged and the water flow is erratic. Gravel powdered and pounded to size from granite and blue stones is coarse aggregate. Mining and blue metal firms are also compelled to chip and blast mountains as a result of this. The majority of the mountains have been entirely chiselled away, affecting the rainfall pattern. Blue metal quarrying has a number of drawbacks, including pollution and ash dust in the air (Vishwakarma et al., 2016). It's also pulverised to make M sand, which is a less expensive alternative to river sand. As fine aggregates, natural environment such as igneous rocks and thermally modified materials can be employed. Water is a fundamental element, and for cement concrete mix manufacture, potable water is usually the best option. Because fresh water is in short supply, recycled water must be fed to the system to assure a constant supply. Mortar mix pollutes the environment by releasing atmospheric CO₂ gases. Concrete is the most commonly used material in the building sector, and it deteriorates in a variety of ways as a result of environmental factors. Every day, a large volume of waste materials from various settings, environments, and industries are produced (Qian et al., 2018).

Trash items such as rice husk ash (RHA), saw dust ash (SDA), rubber crump, plastic waste, coconut husk and shell, textile waste (sludge and fibre), and other waste materials contribute to a waste disposal dilemma. Such wastes can be recycled and utilised as an additive in the creation of Green Concrete constructions. This will lower the amount of cement required, as well as CO₂ emissions and global warming (Benhelal et al., 2013). The purpose of this study is to explain how waste materials can be used as an additive to improve the strength and durability of concrete. This not only solves environmental and ecological problems, but also dramatically improves the microstructures and durability properties of concrete. The demolished building debris is blended into concrete, which reduces the amount of space needed to dispose of them while also recycling them and eliminating the need for new materials.

Types of wastes**Industrial wastes****Fly Ash**

Fly ash is a by-product of coal combustion that is mostly obtained from thermal power plants. Because there is a supply of Lignite and coal in India, we are still highly reliant on thermal power plants. When coal is burned, it produces a residue that must be disposed of. The replacement of fly ash with cement provides a disposal solution as well as a cost-effective alternative. Fly ash combines with cement's calcium hydroxide to generate stronger, more lasting compounds (Yao et al., 2015). It prevents hazardous chemicals from polluting natural resources while also lowering energy consumption and carbon dioxide emissions. It minimizes water consumption, enhances workability, minimises bleeding, segregation, and hydration heat, and improves sulphate and acid resistance, as well as long-term strength improvement. CSH gel is a by-product of interactions between fly ash and calcium hydroxide



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that acts as excellent filler, boosts strength, lowers permeability and corrosion, increases sulphate resistance, and reduces alkali-aggregate reaction (Amran et al., 2021).

Ground granulated blast furnace slag (GGBFS)

It is a by-product of iron and steel manufacture, as well as ground iron slag from blast furnaces, and its composition is determined by the raw materials used in iron production. It is regarded as a recyclable and environmentally friendly material. Its slag cement made up of silicates, aluminates, CaO, and MgO that's utilised in concrete mixes. Dehydration, permeability, thermal expansion, and cracking difficulties plague concrete structures that are exposed to high temperatures. As a result, concrete structures lose strength and become more porous. Concrete loses 15–20 percent of its strength when exposed to high temperatures. The compressive and flexural strengths of slag cement applied as a partial replacement for cement (up to 40%) were higher than regular concrete modified with Portland cement. This modified concrete structures made with iron slag are a long-term building material for pavements, pipes, foundations, and marine applications (Pal et al., 2003).

Silica fume

Because of their great fineness and high silica content, silica fumes are an extremely effective pozzolanic substance. It is a by-product of the industries' smelting process for producing silicon metal and ferrosilicon alloy. It's an ultrafine powder that's spherical in shape and has an average particle size of 150 nanometres. Silica fumes have a silica content of 85–90%, which is 100–150 times smaller than cement particles (Mazloom et al., 2004). It is used in concrete as a filler and as a chemical admixture. It adds strength to concrete by reacting with fresh concrete's calcium hydroxide to form extra CSH gel, which reduces permeability and refines pore structure, resulting in increased resistance to sulphate attack in harsh conditions. The silica fume combines with the calcium hydroxide in Portland cement to generate calcium silicate hydrate, which is identical to the calcium silicate hydrate in Portland cement (Siddique, 2011).

Foundry sand

Foundry sand is a high-quality silica by-product of the ferrous and nonferrous metal casting industries with consistent physical qualities. WFS has a high thermal conductivity when utilised as a moulding material. Foundry sand is sand that has been extracted from a foundry. Many studies have shown that waste foundry sand may be used in a variety of purposes, including highways and other concrete products like bricks, blocks, and paving stones (Siddique et al., 2009). WFS in concrete not only makes it more cost-effective, but it also helps to reduce disposal worries. When fine particles were substituted for 30% of the coarse aggregates, the increase in compressive, splitting tensile, and flexural strength was compared to standard concrete (Siddique & Singh, 2011). Foundry sand was also used as fine aggregates in structural grade concrete with varying percentages of replacements such as 0, 10, 20, and 30% by weight of fine aggregate, with experiments lasting 7 and 28 days. After 28 days, the M20 grade cured concrete reached a strength of 20 N/mm². The M20 concrete grade has a volume ratio of 1:1.5:3 for cement, sand, and aggregates (Manjunatha & Rakshith, 2021).

Construction and demolition waste**Recycled coarse aggregate**

Every year, activities such as rehabilitation, reconstruction, and demolition of existing concrete structures generate a significant amount of construction and demolition (C & D) trash. Many countries have been using C&D waste as an alternative to construction resources such as natural fine and coarse aggregates (NFA and NCA) for manufacturing concrete to reduce various environmental problems. Recycled coarse aggregates are aggregates made from leftover concrete that have been screened, crushed, and sieved (RCA). Although using RCA as a substitute to NCA decreases energy consumption and pollutants, RCA's physical and mechanical properties are inferior to those of NCA due to the associated mortar (Kwan et al., 2012). The quantity of RCA in the concrete and the strength of the old concrete from which the RCA was produced are two major parameters that determine the behaviour of recycled aggregate concrete (RAC). When NCA is replaced up to 30% with RCA in creating RAC, most previous investigations found no substantial drop in concrete compressive strength. However, when different percentages of NCA are replaced with



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RCA, even up to 100%, compressive strength, split tensile strength, and flexural strength of concrete are lowered by 30%, 10%, and 23%, respectively, when compared to normal aggregate concrete (NAC) (Chakradhara Rao et al., 2011). Furthermore, due to poor RCA performance and a weak transition between old and new mortar, the drop in elastic modulus is more dramatic (up to 45 percent) than the other mechanical parameters. Water absorption of RCA is significantly higher than that of NCA, which has a negative impact on the mechanical and durability of RAC as the substitution level of RCA in the concrete increases. Drying shrinkage and creep coefficients are two properties that are directly related to RCA content.

Agricultural waste**Oil palm shell**

Palm fiber and shells are obtained from oil palm shell, which is an agricultural by-product of palm oil leftover. They are burned at temperatures ranging from 800 to 1000 degrees Celsius, generating electricity thermally. It is one of the environmental risks since it is disposed of as landfill materials due to its inability to be reused. It was employed as a supplemental cementitious ingredient in mortar or concrete by numerous researchers (Munir, 2015). The silica oxide in oil palm shells can react with calcium hydroxide ($\text{Ca}(\text{OH})_2$) produced during the hydration process to form additional calcium silicate hydrate (C-S-H). 20% replacement of oil palm shell may be the ideal level for achieving concrete strength, which will steadily decrease beyond this level of replacement. (Sata et al., 2004) investigated the compressive strength of concretes including oil palm shell at ages of 7, 28, and 90 days.

It was observed that 10, 20, and 30% of ground oil palm shell concretes and found that the highest strength was in the 20% substitute of oil palm shell at the age of 28 days. (Liu et al., 2014) observed the use of palm oil fuel ash as a binder in lightweight oil palm shell geopolymer concrete was investigated, and it was discovered that a combination of up to 20% oil palm shell can be classified as structural lightweight concrete. (Andalib et al., 2014) did experimentation instead of cement, oil palm shell with fly ash was used in reinforced concrete beams, which was highlighted. In concrete buildings with increased compressive strength, adding a superplasticizer is important for achieving workability, high filling ability, fluidity, reducing inter-particle friction, maintaining deformation capacity, viscosity, and self-compacting ability. The environmentally friendly manufacture of sustainable concrete from oil palm shell is possible. Based on the findings, it is possible to conclude that oil palm shell could be utilised as a successful supplemental cementing ingredient in concrete and mortar, replacing 20% of the cement.

CONCLUSIONS

Every year, millions of tonnes of waste are created around the world, the majority of which is not recyclable. Furthermore, garbage recycling consumes energy and emits pollution. Furthermore, waste accumulation in the suburbs and rubbish removal are extremely hazardous to the environment. Using waste material in concrete production is an effective way to accomplish two goals: waste reduction and the addition of good characteristics to concrete. Because the green concrete business is growing, it's more important than ever to assess waste-contained concrete from all angles in order to determine its suitability. This literature review is divided into two sections: waste as a substitute for cement and waste as a substitute for aggregates. As an alternative, leading waste material has been employed. The qualities of the produced concrete are tested using leading waste materials that have been employed as alternatives. Rubber was discovered to improve fire resistance and ductility in concrete, as well as agricultural and PET wastes being successfully employed in non-structural concrete, and glass was found to aid improve thermal stability.

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The Requirement for Resilience in Order to Deal with the Stress that Comes with Entrepreneurship

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ABSTRACT

Due to the inherent unpredictability of starting a new firm, entrepreneurs are particularly vulnerable to stress. The entrepreneurial process is full with unpredictability and adversity over which the person has little control. Entrepreneurs must be able to withstand, handle, and overcome major and unique work-related problems in order to keep their businesses afloat. As a result, it's no surprise that the notion of resilience has spread to the realm of entrepreneurship. Financial difficulty is one of the most common sources of stress for entrepreneurs. Entrepreneurial difficulty can lead to good coping on one side, or maladaptive coping, despair, and desperation on the other. Because psychological capital is flexible, entrepreneurs may use it to increase their own and their workers' psychological capital.

Keywords: Stress, Resilience, Entrepreneurship, Psychological capital, Quality of life

INTRODUCTION

Entrepreneurs are frequently seen as a valuable economic resource (McMullen & Shepherd, 2006). They generate income and progress for the entire community. However, for entrepreneurs, the risks of beginning a firm and the consequences of failure are significant. Entrepreneurs are described as passionate and enthusiastic about their firms on the one hand (Baum & Locke, 2004); on the other hand, they are significantly exposed to negative emotional states such as worry, fear, and stress (Chen et al., 2009). Entrepreneurial judgement, opportunity awareness, decision-making, and innovation are all influenced by affect. Entrepreneurs who choose to confront the adversity, hazards, and stress of entrepreneurship have particular dispositional features and psychological capital that allow them to withstand high levels of uncertainty and other sorts of entrepreneurial hardship (Brandstätter, 1997).





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Entrepreneurs must be able to withstand, handle, and overcome major and unique work-related problems in order to keep their businesses afloat. It may include various types of problems including those of financial in nature. Goal 3 of SDG advocates providing universal health coverage including financial risk protection (Envision 2030, Goal 3: Good Health and Well-being). Through the provision of adequate financial risk protection many of the entrepreneurs would be relieved of some pressures while passing through the turmoil of either starting or running a business. It is no surprise that the notion of resilience has spread to the realm of entrepreneurship. Due to the obstacles given by highly variable, complex employment demands, entrepreneurs might endure severe stress. Mood swings, anxiety, changes in sleep patterns, changes in food, exhaustion, lack of interest in family, friends, and hobbies, and alcohol dependency are all symptoms of stress (Padhy. et. al. 2020).

Entrepreneurial sources of stress

The antecedents of stress for entrepreneurs, according to Ahmed et al. (2022), comprised certain elements such as job characteristics, family-work conflict, business/financial issues, and life difficulty. Some of such characteristics are discussed below.

Job features: The majority of studies on entrepreneurship stress have focused on the kinds of the work that entrepreneurs do as a cause of stress. Using Karasek's (1979) Job Demand-Control (JD-C) Model, researchers looked at job demands, job control, autonomy, and role ambiguity, claiming that stress is a result of high job demands and poor job control. The nature of an entrepreneur's labour differs from that of a salaried employee. However, it might be difficult to identify employment characteristics particular to entrepreneurship that can be a substantial source of stress when comparing two highly different groups.

Work-family conflict: Stress can have a negative impact on one's family and social life (Padhy. et. al., 2020). Conflicts emerging from the family role influencing the job role (family-to-work conflict) and the work role interfering with the family role (work-to-family conflict) (Greenhaus&Beutell, 1985) can be stressful for entrepreneurs' careers and well-being (Parasuraman et al., 1996). Nowadays farmer families are also subjected to different types of stresses. Farmers get crippled when they are unable to cope with the stresses of life (Padhy et al., 2020). Entrepreneurs face strain-based work-to-family conflict because they are distracted by thoughts about their business, and time-based family-to-work conflict because there is a family push to be more available, according to Konig and Cesinger (2015). According to Werbel and Danes (2010), the entrepreneur's spouse's stress might exacerbate the entrepreneur's stress from work and family conflict.

Business and financial challenges: Entrepreneurs' resources are depleted and their stress levels are elevated when they face financial difficulties (Chadwick & Raver, 2019). Shepherd (2003), on the other hand, looked at how mourning, which is akin to the feeling of losing a loved one, may be an intense negative emotional response to business failure that causes stress and is followed by other secondary stressors like attempting to find a job or selling a property. Similarly, entrepreneurs frequently personalise and internalise firm loss, connecting it with personal failure, so exacerbating grief and stress (Jenkins et al., 2014).

Entrepreneurial resilience

Entrepreneurial resilience refers to an entrepreneur's ability to persevere in the face of adversity. It is a dynamic adaption process that enables entrepreneurs to look ahead despite severe financial failures (Windle, et al., 2011). Entrepreneurs are able to confront an unknown future with a positive attitude rather than sentiments of helplessness, dread, indifference, and desperation as a result of this approach (Heinze, 2013). Entrepreneurial resilience may be defined as sustained happiness in the face of hardship. The less unfavourable entrepreneurial conditions have on entrepreneurs' well-being, the more resilient they are. Positive affect is thought to indicate coping skills and a tolerance for high stress levels.

For two reasons, the resilience construct is important in entrepreneurship study. To begin, researchers frequently use the terms resilience, readiness, hardiness, perseverance, and self-efficacy interchangeably to describe why certain





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entrepreneurs and their businesses outperform their non-resilient counterparts. Second, cognitive and behavioural entrepreneurial skills, as well as specific kinds of entrepreneurship such as social entrepreneurship, are claimed to improve a company's ability to adapt to changing conditions and contribute to long-term sustainability via innovation (Biggs et al., 2010). In that perspective, farmers also need someone who can talk to and seek advice from when they are under a lot of stress. It may make the difference when it comes to figuring things out during difficult situations. Farmers' mental health should be understood by extension workers and advisors (Padhy and Raju, 2020).

Individual Entrepreneurial Well-Being and Entrepreneurial Resilience

Entrepreneurs are a valuable resource for the economy since they enable wealth generation and economic prosperity. They, on the other hand, are exposed to a slew of dangers and consequences if they fail. Despite the fact that entrepreneurs are typically represented as energetic, passionate, and risk-takers, they are subjected to a variety of negative affective states such as stress, worry, and anxiety, all of which have a detrimental impact on their well-being. Entrepreneurial resilience, which includes characteristics such as entrepreneurial judgement, decision making, opportunity recognition, stress management, and overall innovation, is hampered by poor health (Corner et al., 2017). The various components of resilience are indicators of an individual's ability to adjust positively in the face of adversity. Dijkhuizen et al. (2017) came to the conclusion that an entrepreneur's resilience is a predictive value for business success and is a product of an entrepreneur's well-being and ability to mediate the effects of stressors. Entrepreneurs' well-being is frequently jeopardised as a result of a variety of obstacles, including anxiety, indifference, despair, financial failures, powerlessness, and uncertainty.

Dijkhuizen et al. (2017) define well-being as "the good impacts and contentment with life". As a result, it is a comprehensive assessment of an individual's quality of life based on the criteria specified, with a primary focus on self-realization and full functionality. Entrepreneurial resilience has a positive impact on an individual's well-being. Higher levels of well-being, according to Foo et al. (2009), might recharge entrepreneurs' resilience and motivate entrepreneurs to continue persisting in difficult undertakings that others typically think unachievable. The entrepreneur's journey can be stressful, and this can have a negative impact on their psychological well-being and resilience. Despite the fact that various programmes addressing distress and well-being have been established, little attention has been devoted to the function of entrepreneurs' basic psychological needs, which can have an impact on their well-being and resilience in the entrepreneurial environment.

Challenges

Naik (2012) defines perceived entrepreneurial stress as damaging emotional or physical responses when business or employment requirements do not meet the entrepreneurs' capabilities, resources, or demands. Entrepreneurial research often focuses on firm-level outcomes such as performance and growth, but idiosyncratic and personal components of entrepreneurship are also crucial. Decades of research into the effects of stress on entrepreneurs' well-being has yielded equivocal and contradictory results. Entrepreneurs' basic psychological requirements have been generally disregarded in studies, despite the fact that they may be critical to their well-being.

Increased stress among entrepreneurs has a negative impact on both mental and physical health, leading to decreased performance and burnout. Stress isn't necessarily bad, but too much of it can be harmful to one's health. What causes stress depends on how we perceive a situation. For example, entrepreneurs may experience stress due to financial strain, work overload, and role uncertainty, among other things. Entrepreneurship entails a lot of responsibilities and commitments. Entrepreneurial stressors are physiological and behavioural factors that are harmful not just to entrepreneurs' businesses but also to their well-being, given the intense, uncertain, stressful, and complex nature of their work (Bliese, et al., 2017).

The Road Ahead

Workplace stress has been labelled a "global epidemic" by the World Health Organization, with no end in sight as the rate and scope of change experienced by employees continues to accelerate at an incredible rate (Jensen,





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2012). Individuals working in established businesses confront several problems, but those participating in the process of launching and establishing a new company endeavour are likely to be under considerably more stress owing to the inherent uncertainties and demands. Recognizing the particular needs and skills of individual employees appears to be critical as firms look for strategies to help employees navigate an ever-changing work environment. The evolving positive organisational behaviour approach (POB) (Luthans, 2002) provides such viewpoint and acts as a framework. Self-efficacy, optimism, hope, and resilience are among the positive psychological abilities linked to POB thus far. These abilities are referred to as psychological capital, or "PsyCap," when they are combined (Luthans et al., 2007).

An individual's positive psychological state of development is characterised by: (1) having confidence (self-efficacy) to take on and put in the necessary effort to succeed at challenging tasks; (2) making a positive attribution (optimism) about succeeding now and in the future; (3) persevering toward goals and, when necessary, redirecting paths to goals (hope) in order to succeed; and (4) When confronted with obstacles and hardship, persevering and rebounding back (resilience) to achieve achievement. This operational definition distinguishes "PsyCap's" core construct from the widely recognised aspects of human capital (i.e., what you know in terms of knowledge, skills, abilities, and experience) and social capital (i.e., who you know, including your network of relationships) by focusing on who you are (Luthans et al., 2007).

A facilitator can give "PsyCap" training interventions to the entrepreneur and/or workers in a traditional classroom setting or via web-based delivery (Jensen, 2012). These training sessions generally run one to three hours, depending on the number of participants, and involve exercises to improve self-efficacy, optimism, hope, and resilience, as well as PsyCap overall. In the hope component, for example, participants begin by identifying essential goals that they will employ throughout the session. The facilitator then goes over the importance of having (1) concrete end points to measure success; (2) an approach (rather than avoidance) framework that allows participants to work toward rather than away from desired goals; and (3) using a "stepping" method of identifying sub goals to reap the benefits of even minor accomplishments. The participants are then asked to come up with numerous paths to the objective and discuss the feasible (and unrealistic) choices that have been discovered.

The PsyCap training intervention helps participants build resilience by asking them to identify recent personal setbacks in their work domain, which can range from major to minor setbacks. Following the participants' instant reactions to the observed setback, the facilitator elaborates on instances of a firm perspective of reality and an ideal resilient method for framing a setback, in accordance with the expand and build positivity principle. Each participant then assesses the true impact of the setback, including what is in (and out of) their control, as well as action alternatives. Participants exercise learned cognitive processes that help them develop resiliency and realistic optimism by anticipating and dealing with subsequent setbacks. Maintaining a happy attitude, enough rest and sleep, regular exercise, humour and recreation, counselling, remaining connected with others, being active, eager to learn, accepting responsibility, and giving to others are all possible remedial measures for facing the psychological challenges (Padhy et al., 2020). In that perspective, digital alignment can help for overcoming global poverty and hunger (Padhy et al., 2022).

CONCLUSION

Eliminating the stress that entrepreneurs and their founding workers face in the workplace is not a feasible, or even desirable, organisational goal. However, assisting businesses and their employees in properly managing stress is (and will be) an important goal for good human resource management. The moment has come to realise the potential power of psychological capital development as a beneficial stress management resource. Efforts to build resiliency are also based on realistic appraisals of setbacks and the development of appropriate coping mechanisms for such setbacks. While scientific research on the influence of resiliency in new companies is still sparse, there are numerous anecdotal accounts demonstrating how stressful events and early failures often fail to deter entrepreneurs from pursuing their entrepreneurial dreams.





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Polymer Nanocomposites in Packaging Applications

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ABSTRACT

When nanoparticles are dispersed in a polymer matrix the resulting material is called as a polymer nanocomposite. The addition of these nanoparticles results in the improvement of certain properties which are valued highly in the packaging industry. Properties like modulus strength, thermal stability, gas permeability, water resistance, vapour resistance are improved upon the addition of nanoparticles and because of this improvement these polymer nanocomposites can be utilised as a packaging material instead of the conventional packaging materials. And addition of biologically active substances will impart the packing materials with the appropriate functional qualities. As a result, bio-functional polymer-based nanocomposites packaging materials have a significant potential for use in the active food packaging industry. In this review preparation characterization of polymer nanocomposites(PNC) and the applications of these PNC in the packaging applications are discussed.

Keywords: Polymer nanocomposites ,Packaging material

INTRODUCTION

Particles whose size range is between 1nm-100nm can be called as nanoscale or nano materials Polymer nanocomposites are phase separated materials which consist of two or more phases which are chemically distinct in which one or more phase is in the nanoscale with a polymeric matrix phase. The nanoparticles that are used as dispersed phase can be classified based on the dimension, they display in the nanoscale regime as 0D (Quantum dots),1D(Platelets),2D and 3D. The nanoparticles possess certain unique properties that are given in figure-1.

The polymer nanocomposites are polymeric materials (thermoplastics, thermosets, elastomers biopolymers) that are reinforced with a small amount (less than 5%) of nanoparticles with a high aspect ratio. The main difference between



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the conventional composites and PNC is the size of the dispersed phase particles which are in the order of few microns in conventional composites and of the order of few nanometres in PNC because of which the PNC have got various functional properties and this has led to the increase in usage of the PNCs in the packaging industry in the recent years.

Polymer Nanocomposites

Polymer nanocomposite (PNC) is a composite material in which a polymeric material (thermoplastics, thermosetting, or an elastomer) is present as the matrix phase and the dispersed phase or the filler consists of nanoscale material (nanoparticle) and at least one of the dimensions of the nano particle is in the size range of 1nm-100nm. The most pivotal requirement for a polymer nanocomposite is the amalgamation of excellent interaction or good compatibility of the nanoparticle surface with the polymer matrix with a stellar dispersion of the particles and to achieve this the requirements needed are same surface energy of the polymer and the particle surface, lesser agglomeration energy, lower polymer viscosity and higher mixing efficiency during the process. The final properties of the polymer nanocomposites depend upon a) Types of nanoparticles and their surface treatments b) Nature of the polymer matrix b) Matrix morphology and dispersed phase morphology d) Polymer nano composites synthesis methods, e) Polymer matrix crystallinity and glass transition temperature, and f) Nature of the interaction between the polymer matrix and the nanoparticles dispersed. The most commonly utilised nanoparticles include nano clay, carbon nanotubes, nano silica, nano aluminium oxide (Alumina, Al₂O₃), nano titanium dioxide (TiO₂), nano zinc oxide (ZnO), metal nanoparticles, and other nanoparticles. Table1. Gives a brief summary of the different nanoparticles used as fillers or dispersed phase and the improvement in the properties of the PNC reported by different researchers.

The nanoparticles used as the dispersed phase in the polymer nanocomposites are synthesised by using various methods which are classified under two approaches [12] namely top-down approach in which the reduction of a bulk material to nanosized particle is done and bottom-up approach in which the build-up of material from atom to clusters to nanoparticles is achieved and the different types of nanoparticles synthesized by these two approaches is given in the below table2.

Preparation of polymer nanocomposites [13,14]

Various methods have been developed for the preparation of polymer nanocomposites and few of them are given below.

- i) **In-situ polymerization:** -This method is used to synthesize polymer nanocomposites which consist of layered silicates. In this method the polymer or pre-polymer is dissolved in this technique, and the silicate layers swell. The degree of penetration of the polymer chains into the silicate galleries determines the structure of nanocomposites made using this approach, which ranges from intercalated to exfoliated. As a result, making polymer-layered silicate nanocomposites has become a regular procedure.
- ii) **Melt mixing method;** - In melt mixing method the polymer is melted first, and the nanofiller is then combined with the polymer melt using a shear extruder. In most cases, it's done in the presence of an inert gas like argon, nitrogen, or neon. It is a safe approach due to the lack of organic solvents, and it has gained popularity due to its compatibility with industrial processes such as extrusion and injection moulding.
- iii) **Solution Mixing method;** - This method involves dissolving of a polymer in a suitable solvent and suspending the nanofiller in the same or another compatible solvent after which high-speed shear mixing, ultrasonication, or stirring is usually employed to mix the filler suspension with the polymer solution followed by the composite film casting due to the solvent evaporation. The use of the solvent depends upon the polymer solubility in the solvent. Solvents used in this method include toluene, xylene, tetrahydrofuran (THF) chloroform, dimethyl formamide (DMF), acetone, and cyclohexane etc.

Characterization of Polymer nanocomposites

The prepared polymer nanocomposites are characterized by various techniques [14]. The structure of polymer nanocomposites is characterized by a combination of transmission electron microscope (TEM) and wide-angle X-ray



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diffraction (WAXD). The dispersion of the nanoparticles in the polymer matrix can be studied by using WAXD. Transmission electron microscopy is used to characterize polymer nanocomposites as it is allowing a qualitative understanding of the internal structure, spatial distribution of the various phases, and direct visualization of defect structure. Scanning transmission electron microscopy (STEM) is also used for studying the degree of dispersion of the nanoparticles in polymer nano composites. Scanning electron microscopy (SEM) is used to investigate the surface morphology of the polymer nanocomposites. Atomic Force Microscopy (AFM) is used to measure many different forces, including adhesion strength, magnetic forces and mechanical properties of the PNC. FTIR (Fourier transform infrared spectroscopy) is used to detect functional groups and understand the structure and differential scanning calorimetry (DSC) to determine the kind of crystallisation taking place in the matrix.

Properties of Polymer nanocomposites

The Polymer nanocomposites display an improvement in the mechanical, physical properties as compared to the conventional polymers and composites. Properties like Higher modulus, greater strength and heat resistance, reduced gas permeability and flammability, and increased biodegradability of biodegradable polymers are among the improvements. The main reason for these improvements lies in the fact that the dispersed nanoparticles surface area is very high that leads to a better interfacial attraction between the dispersed nanoparticles and the polymer matrix. The various properties of PNC used for packaging applications are shown in figure 2

Packaging technology

The technology that deals with the process of designing, production, and distribution of products from the industry to the markets to consumers houses is known as packaging technology. Packaging technology plays an active role in the storage of the product, safety of the product, hygiene of the product and marketing of the product [14]. Packaging technology also helps in the information transmission in the form of Package labelling which is a written, electronic and graphic communication on the package which gives details about how to use, transport, recycle or dispose of package or products. This packaging technology finds applications in almost every sector like medical device packaging, chemical packaging, food packaging, pharmaceutical packaging. Packaging materials provide physical protection [14] and generate the required physicochemical conditions for items or products to achieve a long shelf-life packaging materials prevent physicochemical or biological influences from causing product deterioration and preserves overall quality of the product throughout storage, handling and delivery of the product to the consumer. And the basic properties of the packaging material that govern the safety and quality are mechanical properties, barrier properties, chemical reactivity. In this way, polymer nanocomposites-based packaging materials have various useful qualities as packaging materials, such as increasing the product quality and extending shelf life by reducing microbial growth in the product. They can act as both a barrier to moisture, water vapour, gases, and solutes, as well as a carrier of some active compounds. The function of PNC in packaging applications are shown in figure3.

Polymer nanocomposites in Packaging applications [14,15]

Because of the improvement in their properties like gas barrier properties, flame retardant properties, antimicrobial properties, mechanical properties lead to a wide range of property profiles and they can even compete with synthetic polymeric materials in packaging, both in terms of price and performance. And it is even feasible to create packages with improved mechanical, barrier, and thermal performance by incorporating appropriate nanoparticles. And the various applications of the PNCs in packaging are as follows. Because of their nanoscale size dispersion, polymer nanocomposites, particularly natural biopolymer-layered silicate nanocomposites, have significantly better packing qualities. Higher modulus and strength, lower gas permeability, and increased water resistance are among the gains. Additionally, biologically active substances can be added to provide the packing materials the appropriate functional qualities. As a result, biopolymer-based nanocomposite packaging materials with bio functional qualities have a lot of potential in the active food packaging market. Emamifar *et al.* [14] investigated the use of ZnO and Ag nanoparticle-filled LDPE nanocomposite packaging as a new way to preserving and extending the shelf life of orange juice by employing an antibacterial nanocomposite.





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Recent studies have revealed that the presence of montmorillonite (MMT) in the polymer film reduced lipid oxidation in processed meat products, extending the shelf life of the film and potentially reducing the risk of contamination. And few properties and some more applications of PNC materials in packaging are given below in table 3

CONCLUSION

The area of nanocomposites have inspired and motivated to create very new and amazing materials with enhanced properties and performance as compared to the conventional composites and among these polymer nanocomposites find a unique place because of their display of enhanced mechanical, thermal, gas barrier, properties along with improved fire retardancy and they can even compete in applications such as packaging, both in terms of price and performance when compared with the existing packaging materials and can replace them in the near future.

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Table 1. Nano fillers and their contribution in the polymer nanocomposite

Nanoparticle	Property enhanced	Reference
Nano clay	Mechanical, thermal,	[2 ,3]
Zinc oxide (ZnO)	Photocatalytic and rheological	[4,5]
Silica (SiO ₂)	Thermal and electrical conductivity	[6,7]
Alumina (Al ₂ O ₃)	Mechanical & Thermal	[8]
Carbon nanotubes	Thermal stability and tensile strength	[9]
Calcium carbonate (CaCO ₃)	Hardness and impact strength	[10]
Graphene	Mechanical and electrical	[11]

Table2. Types of nanoparticles synthesized by top down and bottom-up approaches [12].

Approach	Method	Nanoparticles
Top-down	Mechanical milling	Metal, metal oxide and polymer based
	Laser ablation	Carbon based
	Nano Lithography	Metal based
Bottom-up	Sol-gel	Metal and metal oxide based
	Chemical vapour deposition	Carbon based
	Combustion	Metal oxide based

Table3.Properties and applications of PNCin packaging [15]

Properties	Applications
Optical property	Transparent packaging, UV-Resistance packaging
Active packaging	Shelf-life prolonging
Mechanical property	Improved shelf life
Thermal property	Improved shelf life, Heat resistance
Biodegradation	Improved biodegradation

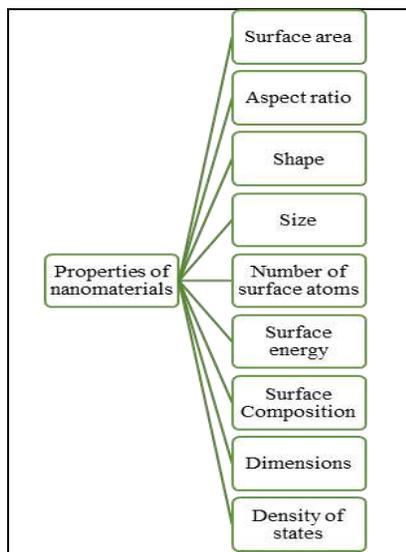


Figure1.Unique properties of Nanomaterials [1]

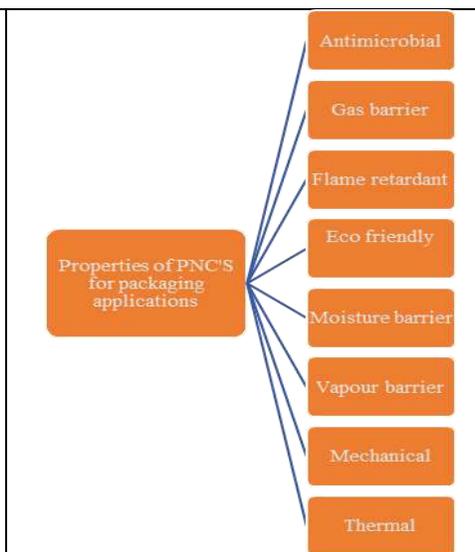


Figure2.Properties of PNC for packaging applications [15]

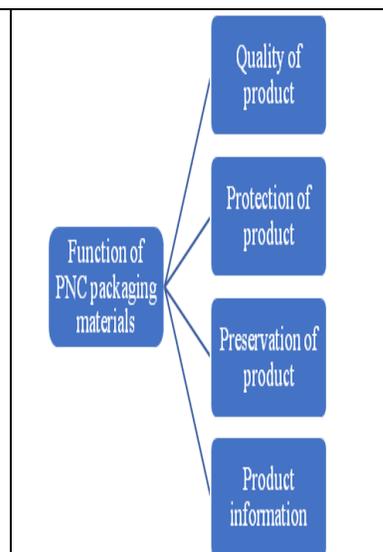


Figure3.Functions of PNC in packaging applications [15]





Di-pyridine Containing Macrocyclic Cu(II) Complex: Synthesis and Characterization

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ABSTRACT

Di-pyridine containing macrocyclic ligand 3,7-diaza-1,5(2,6)-dipyridinacyclooctaphane (L) is reported here. The ligand was synthesized through multi steps organic synthesis and its Cu(II) complex $[\text{Cu}(\text{L})]\cdot\text{Cl}_2$ was also successively synthesized. Both the ligands and complex were characterized by standard spectroscopic technique in both solid phase as well as solution phase. Crystal data reveals that the $[\text{Cu}(\text{L})]\cdot\text{Cl}_2$ is six coordinated distorted octahedral geometry with four nitrogen donor of macrocyclic ring and two chlorine atom.

Keywords: Di-pyridine, Cu(II) complex, ligand

INTRODUCTION

In the past few decade macrocyclic ligands occupy a unique segment to the recognition in the field chemistry and biochemistry. Macrocyclic rings having nine or more hetero atomic members with proper binding site (Lewis base) like Nitrogen (N), Oxygen (O), Sulphur (S) and phosphorus (P) can easily bind with different metal (Lewis acid) ions. The great challenge for synthesis of macrocyclic compounds were, multi steps synthesis, low yield, low solubility, difficulties in purifications and different open chain intermediates. Coordination chemistry get exploded after synthesized of macrocyclic ligands which are used for mimic of porphyrins and related systems [1]. In research a large numbers of articles and reviews are published in the field of coordination chemistry of the macrocyclic ligands. Macrocyclic ligands have several benefits in the field of coordination chemistry, it forms highly stable complexes which reduce the leaching of metals from the pocket. Furthermore it reduces the entropy of the system due to cyclization as compare to the linear system. Macrocyclization used as a tool in drug delivery, mimic of different enzymes[2], target for different analytes, luminescence properties,[3] inclusion properties, [4] chiro-optical properties [5, 6], gelation properties, catalytical properties [7, 8]. In this article we have explored the synthesis of Cu(II) complex of 3,7-diaza-1,5(2,6)-dipyridinacyclooctaphane ligand (L) (Scheme 1). Both the ligand and complex are synthesized



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by using standard synthetic procedure, and characterized by different standard spectroscopic techniques. Further the solid state structure has been characterized by using single crystal X-ray diffraction.

METHODOLOGY

All reagents were utilized for the syntheses were of analytical grade and spectroscopic grade and used without further purification. Most of the chemicals were bought from Sigma-Aldrich Co. Other chemicals were supplied by Spectrochem (India), Alfa-Aesar (India), Spectrochem (India). DMSO-d₆ and CDCl₃ were used as available in Sigma-Aldrich Co. whereas double distilled water was used for absorption and emission studies. Melting points were measured using a BUCHI M-500. Fourier transform infrared (FT-IR) spectra were recorded on a BRUKER ALPHA-T FT-IR spectrometer with KBr pellets. ¹H and ¹³C NMR spectra were recorded on a BRUKER AVANCE 400 NMR spectrometer using CDCl₃ and DMSO as solvent and analysed by taking the solvent peaks as reference.

RESULTS AND DISCUSSIONS

Synthesis of Ligand

Compound **L** involved the Synthesis of (**1**) (Scheme 1) which was synthesized from commercially available dipicolinic acid, which was esterified in presence of conc. H₂SO₄ followed by reduction in presence of NaBH₄ to obtain pyridine-2,6-diyldimethanol by using published procedure, [9,10]. The asymmetric (6-(bromomethyl)pyridin-2-yl)methanol was then synthesized with aqueous HBr by using the published literature procedure. [11] Further reaction with *p*-toluenesulphonamide in acetone solvent using K₂CO₃ as a base to form *N,N*-Bis[[6-(hydroxymethyl)pyridine-2yl]methyl]-*p*-tosylamide which was brominating in presence of PBr₃ to form compound **1**.

Synthesis of Macrocylic Compound 2

In a two neck round bottom flask, *p*-toluene sulphonamide (0.237 g, 1.39 mmol) and K₂CO₃(0.76 g, 5.5 mmol) were taken and 30 ml of dry DMF was added in presence of N₂ atmosphere and heated at 100 °C for 1 h. Then compound **1** was dissolved in 15 ml of dry DMF and was added drop wise over 30 minutes. The temperature was reduced to 60 °C and continued to 24 hr. The progress of the reaction was monitored by TLC. After completion the total reaction mixture was poured into 100 ml of ice cooled water, the white ppt was formed which was separated by vacuum filtration and dried under high vacuum. The white crude was purified by column chromatography using eluent Chloroform/ Ethyl acetate (3:1) to get pure product as white solid. Yield: 0.42 g, (75%). ¹H (400 MHz, CDCl₃): 7.62 (d, 6 H), 7.38 (t, 3 H), 7.24 (d, 6 H), 7.09 (d, 6 H), 4.24 (s, 12 H), 2.40 (s, 9 H). ¹³C NMR (ppm, CDCl₃): 154.5, 142.1, 136.4, 134.2, 128.1, 127.1, 120.9, 52.1, 20.1.

Synthesis of ligand (L)

The compound **2** (0.65 g, 1.18 mmol) was dissolved in 10 ml of concentrated sulphuric acid in a 25 ml round bottom flask. Then the reaction mixture was refluxed at 100°C for 2 hours. After the completion of reaction the reaction mixture was transferred into a 100 ml beaker containing crashed ice and neutralised the solution by using 4N NaOH solution. The product was extracted with chloroform by using separating funnel, after dried the organic layer under vacuum to get the desire product (**L**). Yield: 0.230 g, (79%).

Characterisation of Ligand(L)

The synthesized ligand was characterized by different spectroscopic techniques. The ¹H NMR data for the ligand shown below the figure 1. ¹H NMR (400 MHz, CDCl₃): 7.28 (s, 2H), 7.09 (t, 2H), 6.51 (t, 4H), 3.95 (s, 8H). The [¹³C] NMR data for the ligand shown below the figure 2. ¹³C NMR (ppm, CDCl₃): 159.5, 135.8, 119.8, 56.1. ESI-MS *m/z*: calcd for C₁₄H₁₇N₄⁺, 241.14 ([MH]⁺), Found: 241.18 ([MH]⁺).





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Synthesis of complex [Cu(L)].Cl₂

The ligand, L (50 mg, 0.21 mmol) was dissolved in 10 ml of dry ethanol and CuCl₂.2H₂O (71 mg, 0.42 mmol) was added and the total reaction mixture was stirred for 2 hours at room temperature. After that the solvent was removed under vacuo and the blue ppt was collected and washed with diethylether and dried under vacuo for 12 hours to give the desired compound [Cu(L)].Cl₂. Yield: 66 mg, (85 %).

Characterisation of complex [Cu(L)].Cl₂

The complex was characterised by different standard spectroscopic technique. The mass analysis confirmed that the complex was formed. ESI-MSm/z: calcd for C₁₄H₁₈N₄CuCl₂⁺, 374.00 ([MH]⁺), Found: 374.021 ([MH]⁺). Presence of aromatic pyridine ring the maximum absorption of complex in Uv-Vis spectroscopy (Fig. 3) in MeOH was found at 265 nm. FT-IR (Fig. 4) (KBr pellet, cm⁻¹): 3396 (s, broad); 2926 (w) 1653 (m); 1594 (m); 1456 (s); 1116 (w); 791 (m).

Crystal structure of Complex [Cu(L)].Cl₂

Single crystal X-ray diffraction data were collected on a Bruker Kappa APEX III charge-Coupled Device (CCD) using Mo-K α radiation ($\lambda = 0.71073 \text{ \AA}$). Diffraction images are solved using program SADABS¹², and the SHELXTL package [13]. The crystal details are given in table 1. The block shaped blue crystal were formed by slow evaporation of mixture of solution of MeOH and CHCl₃. X-ray diffraction reveals that [Cu(L)].Cl₂ complex adopted Orthorhombic unit cell with a space group Fddd. In the complex [Cu(L)].Cl₂ the copper metal coordinated to four nitrogen atoms N1, N1-i, N2 and N2_i of the macrocyclic backbone and two chlorine atoms Cl and Cl_i (Fig. 5). Two pyridine rings were twisted to one direction such that they formed umbrella like structure and two chlorine atoms were bounded opposite direction to form distorted octahedral structure. The complex has two types of metal-nitrogen bond and one types of metal-chlorine bond. The bond distance of metal and pyridine nitrogen (Cu1-N1) is shorter than that of metal-amine nitrogen (Cu1-N2) with in the macrocyclic backbone, and the metal-amine nitrogen (Cu1-N2) bond is greater than that of metal –chlorine (Cu1-Cl1). The bond bond lengths of Cu1-N1, Cu1-N2 and Cu1-Cl1 are 2.051(3), 2.343(3) and 2.3205(11) respectively. According to Jahn teller distortion it was found that the two amine nitrogen (N2 and N2_i) are present in the axial position. The overall charge of the complex is neutral by binding with two chlorine atoms.

CONCLUSIONS

The macrocyclic ligand 3,7-diaza-1,5(2,6)-dipyridinacyclooctaphane and its corresponding Cu(II) complexes were successfully synthesized. Both ligand and its complex were characterised by different standard spectroscopic techniques both in solid states as well as solution states, such as NMR, Mass, IR and UV-Vis spectroscopy. Crystal data confirmed that the complex [Cu(L)].Cl₂ was coordinated with all nitrogen atoms of macrocyclic ring and two chlorine in a distorted octahedral geometry with a space group Fddd. Red shift was observed in UV-Vis spectroscopy when Cu(II) coordinated to with ligands. The paper can motivate the reader to design of new macrocyclic compound and their synthesis.

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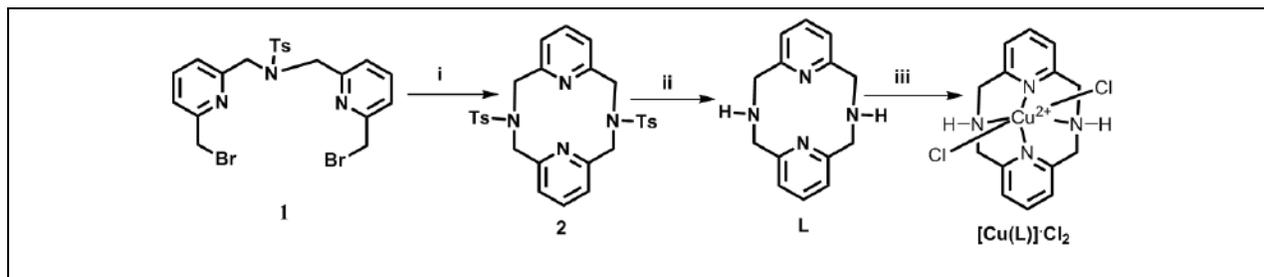
Table 1: Crystal data and structure refinement details.

Parameters	[Cu(L)].Cl ₂
Empirical formula	C ₇ H ₉ ClCuN ₂ O
Formula weight	236.15
Temperature (K)	110(2)
Wavelength (Å)	0.71073
Crystal system, space gr.	Orthorhombic, F d d d
Unit cell dimensions	a = 8.6755(4) Å, α = 90.0° b = 28.2559(15) Å, β = 90.0° c = 29.4486(16) Å, γ = 90.0°
Volume (Å ³)	7218.9(6)
Z, Calculated density (g/cm ³)	32, 1.738
Absorption coefficient (mm ⁻¹)	2.667 mm ⁻¹
F(000)	3808
Crystal size (mm ³)	0.4 x 0.3 x 0.2
Theta range for data collection	2.551 to 28.323°.
Limiting indices	-10 ≤ h ≤ 11, -37 ≤ k ≤ 36, -39 ≤ l ≤ 39
Reflections collected / unique	17907 / 2252 [R(int) = 0.0490]
Completeness to theta	99.7 %
Absorption correction	Empirical
Refinement method	Full-matrix least-squares on F ²
Data / parameters	2252 / 101
Goodness-of-fit on F ²	1.092
Final R indices [I > 2σ (I)]	R1 = 0.0522, wR2 = 0.1693
R indices (all data)	R1 = 0.0776, wR2 = 0.2039
Largest diff. peak and hole (e.Å ⁻³)	1.276 and -0.626





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Scheme 1. Synthetic Scheme of [Cu(L)].Cl₂

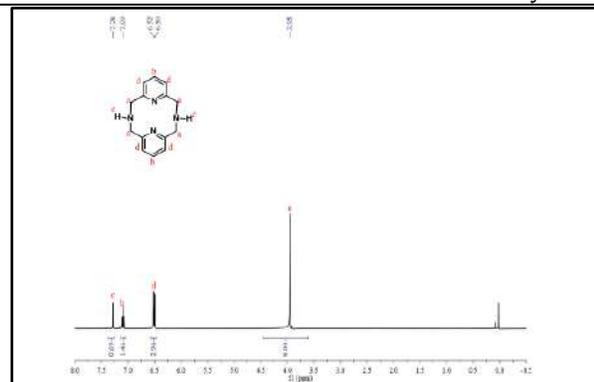


Fig. 1 [1]H NMR spectra of ligand L in CDCl₃.

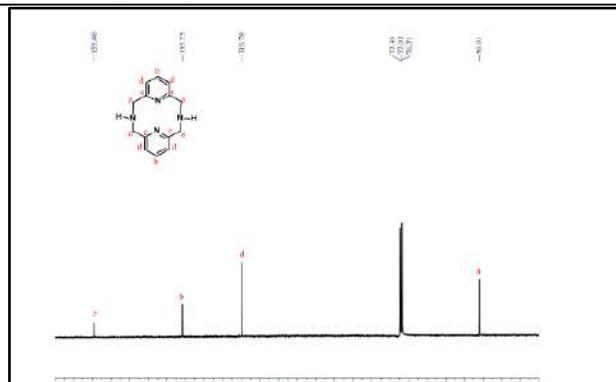


Fig. 2 ¹³C NMR spectra of ligand L in CDCl₃.

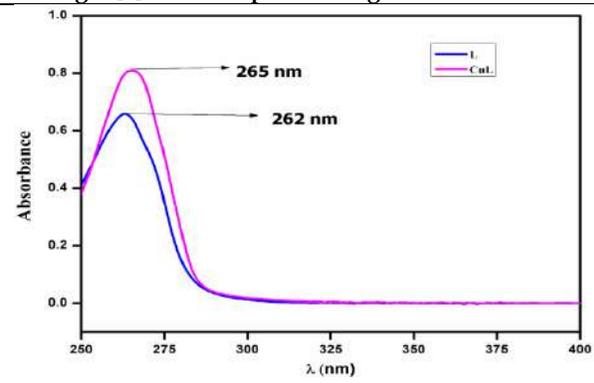


Fig. 3 UV-Vis spectra of ligand L and its [Cu(L)].Cl₂ in MeOH.

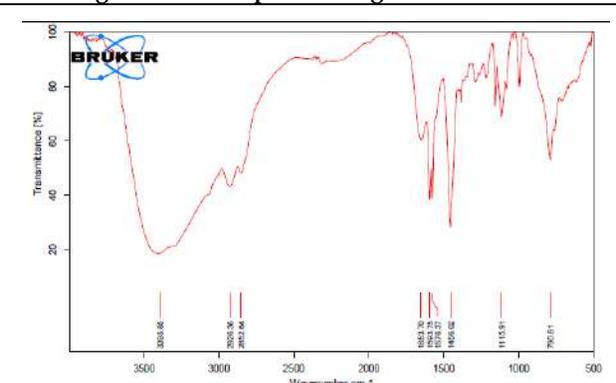


Fig. 4 IR spectra of [Cu(L)].Cl₂ in KBr pallet.

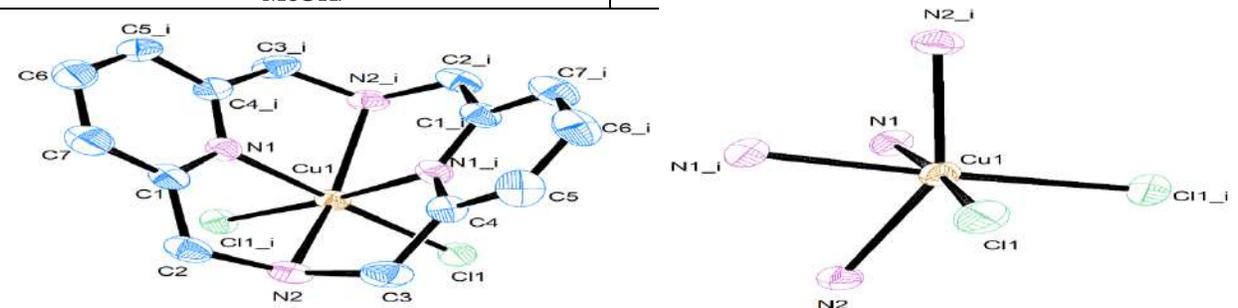


Fig. 4 X-ray crystal structural of the macrocyclic complex [Cu(L)].Cl₂ (Right) and binding site of Cu(II) ion (left)





A Short Account on Supercritical Fluid Extraction of Rosemary (*Rosmarinus officinalis* L.) Essential Oil

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ABSTRACT

Supercritical fluid extraction process (SFE) has emerged as an environmental friendly, economic and efficient extraction process. It has various advantages over conventional organic solvent extraction, hydro-/ steam-distillation and soxhlet extraction processes as it is nontoxic, no residual solvent in the final extract, operates at lower temperature, prevents the extracts from oxidation and decomposition and retains most of the original characteristics. Rosemary essential oils have been proved to have numerous bioactive properties owing to the presence of different types of terpenoids, sesquiterpenes, polyphenolic compounds. This work provides a concise overview of supercritical carbon dioxide (SC-CO₂) extraction process in rosemary essential oil extraction

Keywords: Rosemary, *Rosmarinus officinalis* L., Essential oil, extraction, supercritical fluid, supercritical CO₂, SDG 3

INTRODUCTION

The modern day food industry, pharmaceutical and cosmetic industries use different active compounds such as antioxidants, anti-bacterial agents and so on. Most of these compounds are procured via synthetic or semisynthetic methods to meet the vast demand. However, these synthetic agents pose various adverse effects, environmental hazards as well as emergence of resistant microorganisms. Thus, in recent times researchers have become more concerned about procurement of natural alternatives of these synthetic active compounds in a sustainable way. Super critical fluid extraction process has various advantaged over conventional solvent extraction methods [1] Though organic solvents can effectively be used to extract phyto-components, their application becomes limited when it comes to food and pharmaceutical applications owing to their toxicity, as well as environmental hazards.



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Apart from that, removal of an organic solvent requires heating which can destroy many volatile components or cause oxidative degradation. Super critical fluids on the other hand, are gaseous at room temperature and can be separated easily without any residual solvent in the extracts. Supercritical CO₂ is the preferred solvent for SFE process due to its non toxicity, non-inflammability, and lower cost hence more economically viable in an industrial set-up [2] Moreover, studies have found that extracts obtained from supercritical fluid extraction (SFE) exhibit better yield and bioactivity than solvent extracts, though there are exceptions where solvent extracts have higher bioactivity than SFE products [3].

Rosemary (*Rosmarinus officinalis* L.) is a widely cultivated herb. It has shown various bioactive properties, such as antioxidant, antifungal, antibacterial as well as anxiety relieving properties [4]. Different components present in rosemary have different bioactivities. The nature and amount of bioactive compounds in rosemary depends on its cultivation conditions, geographical location [5]. The extraction process also determines the overall yield and composition of the extracted oil which in turn is responsible for different activities. To maximize the yield as well as to study the composition and activity, various research groups have worked on rosemary extraction in SFE process. Supercritical CO₂ extraction of rosemary. In the early nineties various groups performed the SFE extraction for rosemary and compared the outcome with commonly used hydro-distillation process. Reverchon *et al.* performed supercritical CO₂ extraction at a temperature of 40 °C and a pressure of 100 bar. They found out that percentages of many major components were nearly similar in both the processes. However, various other components such as oxygenated terpenes, sesqui and diterpenes which were not found in hydro distillate were identified in SFE extract. This can be attributed to their volatile nature [6].

Ibáñez *et al.* followed a two-step process to extract rosemary essential oils by super critical fluid followed by fractionation. They collected first fraction at 10MPa (100 bar) and 40 °C and the second fraction at 40MPa (400 bar). The extracts obtained at different conditions had different chemical profiles. Low temperature and pressure fractions evidently contained more volatile essential oil whereas high temperature and pressure fractions contained more antioxidants. They also found a direct correlation between drying methods and antioxidant properties [7]. In a similar study conducted by Carvalho Jr. *et al.* rosemary extracts were obtained by SFE process at 30-40 °C and 100-300 bar. The constituents were compared with the extracts obtained from hydrodistillation and various organic solvent extraction products. The antioxidant activity was higher for the extract obtained at 40 °C and 300 bar pressure though the yield was better at lower temperature and pressure conditions [8].

Comparison of antibacterial and antioxidant properties of rosemary extracts from SFE method and soxhlet extraction method was studied by Aziz K. Genena and his group. Total phenolic content was obtained over the temperature range 30-50 °C and pressure ranging from 100 to 300 bar indicating that extraction of total phenolic content is temperature and pressure independent [9]. Bensebia *et al.* studied the effect of extraction parameters on the yield of rosemary essential oil. The optimum pressure was 180 bar and optimum temperature was 40 °C. They found that addition of a polar co-solvent such as ethanol (3 wt.%) increased the yield [10]. Essential oils from Algerian rosemary were extracted using Supercritical CO₂ and the effects of different extraction parameters were studied by Ahmed Zermane *et al.* They observed optimal yield of 0.95g to 3.52g oil per 100 gram of dry rosemary at a temperature of 40 °C and pressure of 22 MPa (220 bar). The flow rate was 7g/min and particle size was 1mm [11]. Fornari *et al.* studied different extraction parameters for extraction of rosemary, thyme, sage and oregano. They also studied the effect of 5% ethanol as a co-solvent. For rosemary, addition of ethanol increased the yields of oxygenated compounds (Figure 1) [12].

Another comparative study of different extraction methods for rosemary essential oil extraction was reported by Conde-Hernández *et al.* They compared the impact of hydro-distillation, steam-distillation and supercritical CO₂ extraction process on the yield and composition of rosemary essential oil. They observed lower yield of the essential oils at higher temperature (50 °C) and lower pressure (10.34 MPa) whereas a higher yield at lower temperature (40 °C) and higher pressure (17.24 MPa). Interestingly, the yields from hydro-distillation, steam-distillation were found to be significantly higher with whole leaves (2.35%) rather than ground leaves (0.35%). Higher yield as well as





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antioxidant properties were observed with rosemary essential oils obtained from SCE process. The essential oil from SCE process showed nearly 14 times more antioxidant activity than the oils obtained from steam-distillation or hydro-distillation [13].

CONCLUSION

Rosemary and other herbs are source of various bioactive phytochemicals exhibiting antioxidant, antimicrobial, and antifungal properties. Rosemary essential oil can replace synthetic antioxidants and antimicrobial agents which are used in food and flavouring industries as a safer natural alternative. Supercritical CO₂ extraction is a highly efficient and cleaner technology to extract essential oils from various plant sources. As hydrodistillation and steam distillation methods tend to destroy many phytochemicals, supercritical CO₂ extraction process provides better yield, and retains most of the important compounds thus producing superior quality extracts with higher bioactivity. Also this process can be controlled by changing temperature and pressure and different fractions can be collected with different compositions and activities.

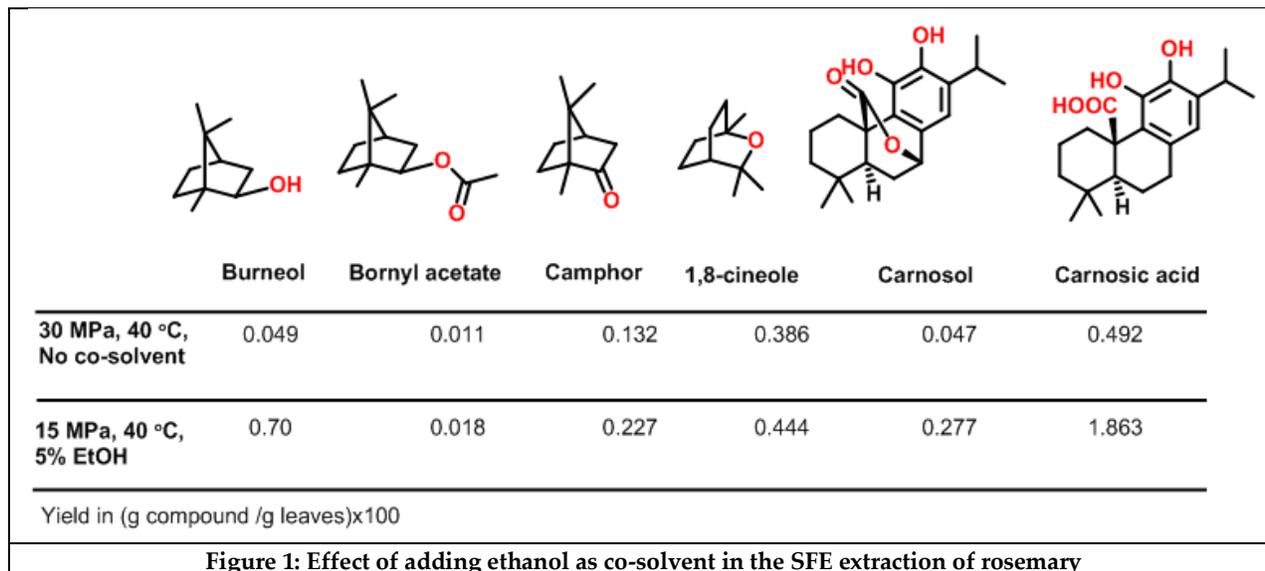
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Light Charge Particle Detection using Scintillators + SSB Detector

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ABSTRACT

In any nuclear reaction the excited compound nucleus (CN) decay via particle or gamma emission to reduce the excitation energy. It is important to detect those evaporated particles to know the dynamics of reaction. There are varieties of method to detect these particles starting from gas detector to solid state detectors. Here we have discussed about the detection of light charge particle using solid state (silicon) detector and scintillator detectors.

Keywords: CN, ER, SSB

INTRODUCTION

Detection of charge particle is an interesting topic since long time back for nuclear physics community. There are varieties of detectors has been devised for that, but each has its own advantage and limitation. Specially the detection for the evaporate particle which includes light to heavy is important to understand the reaction dynamics in any nuclear reaction. In the present paper we have discussed about the detection of light charge particle using SSB and scintillators.

METHODOLOGY AND RESULTS

To identify the charged ions that enter the telescopes (the mix of two detectors to detect the charge particles) two different techniques have been adopted $E - \Delta E$ and $\Delta E - t$. When the particles (protons, deuterons, tritons and α particles) have enough energy to go through the Si first stage of the telescope the identification is carried out with the $E - \Delta E$ technique. where ΔE represents the energy lost in the first detector of the telescope. From the Bethe-Block model is known that: $DE/Dx \propto MZ^2/E$ [1,2] where M, Z and E are mass, charge and initial energy of the particle,





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respectively. Δx is the detector thickness of the first stage of the telescope. From this it follows that in the matrix $E - \Delta E$, and equally, in the $E_R - \Delta E$ (E_R is the residual energy in the second stage of the telescope), the particles with different charge or different mass are placed in different hyperbola branches. A typical matrix collected with a telescope during an experiment has shown in Fig. 1. There are four regions which correspond to four different kinds of particles: protons, deuterons, tritons and alpha particles.

This method requires that the particle must have enough energy to pass through the ΔE stage of the telescope. The second identification technique concerns with the particles that stop in the ΔE stage (e.g. protons with energy $E_p < 6$ MeV or alpha particles with $E < 25$ MeV and fission fragments). A different method is used for the Wall and for the Ball telescopes of 8PLP. The Wall detectors are about 60 cm from the target. Consequently, the particles that stop in the ΔE stage can be identified by correlating the total energy lost (in this case ΔE is the total energy) and the TOF. The TOF is extracted directly by the time measured by the TDC. In fact, the start of the TDC is generated by the Si detector, and the stop by the RF signal. The technique of TOF [3,4] is based on the measurement of the time t , spent by the particle to span the distance L between the target and the detector, and its energy $E = \Delta E$. The connects between the TOF and the particle mass is the following relation: $t = L (M/2 \times \Delta E)^{0.5}$ In the matrix $\otimes Et$ the events concerning particles with different masses are clustered in different regions of the matrix and can be identified. In the case of the Ball telescopes, the flight path of only 15 cm is not enough to allow the separation between particles, due to the poor time resolution of the signal coming from the ΔE Si stages. To overcome this problem the pulse shape analysis of the signal [5] was chosen.

The PSA technique is based on the difference in the rise time for particles having different stopping power. In fact the total charge collection time reacts the rise time of the output signals. The discrimination is enhanced if particles are impinging on the ohmic side (generally named 'rear side'). This is due to the lower electric field in the entrance region and the lower velocity of the holes which are mainly responsible for the signal pulse formation. To perform PSA the Ball Si detectors are mounted with the rear side facing the target. From the electronics point of view, the PSA was obtained using the same electronics as the TOF, but changing the fraction and the internal delay of the CFD to have an output timing signal sensitive to the rise time of the input signal.

CONCLUSION

From the above discussion and picture it has clear that the detection of the LCP is very important for any nuclear reaction and special devices are their which uses special technique for this prpose. SSB and scintillators are one of the easy detectors, which one can use for this purpose specially the combination of $E - \Delta E$ gives the unique effect to detect these particles.

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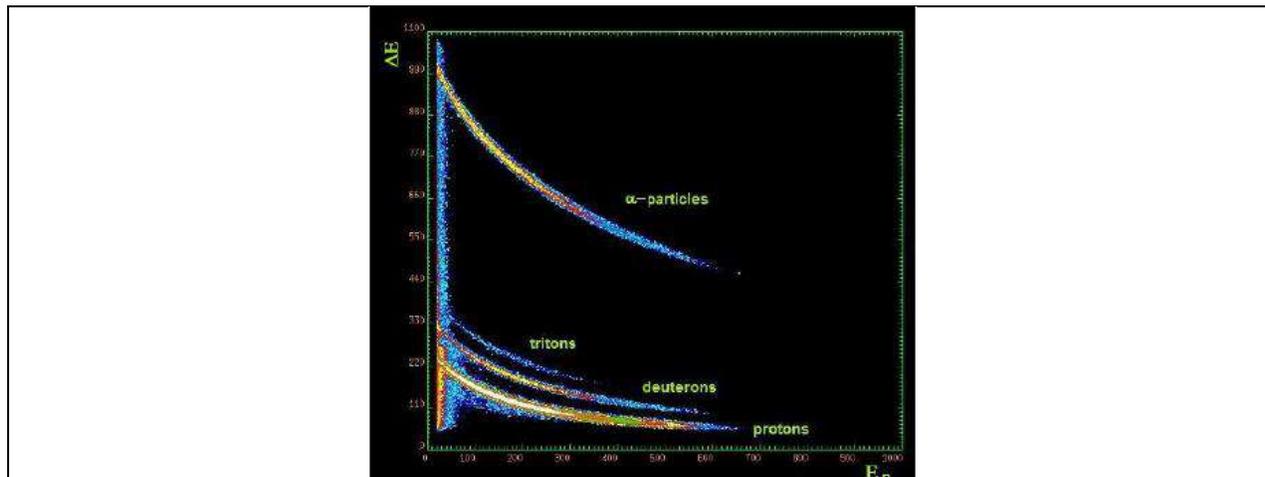


Fig.1 E- ΔE matrix used to identify light particles with higher energy using both the detector stages of the telescopes.





Embryotoxicity and Teratogenicity of *Taraxacum officinale* Leaf Extract on the Developing Embryos of the Zebrafish, *Danio rerio*

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ABSTRACT

The present study aimed to detect the existence of Phytochemicals and to assess the toxic and teratogenic activities of aqueous leaf extract of *Taraxacum officinale*. Qualitative analysis of *T. officinale* leaf extract revealed the presence of several medicinally important phytochemicals. Based on microscopic observation, the survival and sublethal endpoint were assessed and calculated. Assays for toxicity of *T.officinale* leaf extracts on *Danio rerio* embryos confirmed that mortality, hatchability, heartbeat rate, teratogenicity, and embryogenicity were concentration-dependent. Lethal effects were pronounced in treatment groups whereas sub-lethal and teratogenic toxicity effects like yolk deformities, scoliosis, edema, reduced mobility, stunted tail, and tail malformation were expressed in 32% of the exposed models. Tail malformations were the most marked teratogenic effect of the plant extract. On the whole, *T.officinale* leaves contain bioactive components that exhibit toxicity and teratogenicity in the zebrafish embryos. This study will help in the early prediction of the potential health risk of using this plant as conventional medicine.

Keywords: *Taraxacum officinale*, Zebrafish, toxicity, teratogenicity, LC50.





Jeba Preethi Jansi and Jeni Chandar Padua

INTRODUCTION

Plants are abundant in bioactive compounds and have been used as medicinal products [1] because of their richness of phytochemicals that can be used in the production and synthesis of drugs [2]. Additionally, due to their mild effects and very effective bioactivities, they are considered as an alternative medication [3]. Although plants possess enormous medicinal properties, it has been shown that a few of their chemical components are shown to possess mutagenic, carcinogenic, toxic and teratogenic effects. Hence forth, it is important to investigate the toxic and teratogenic effects of therapeutic plants.

Taraxacum officinale, commonly called dandelion is a globally distributed flowering herbaceous plant that has a position in the Asteraceae family. It is found to be a diuretic, cholorectic, and known to have anti-inflammatory, anti-oxidative, analgesic, anti-hyperglycemic, and anti-thrombotic activities, aside from the fact that dandelion is widely recognized as a weed [4,5]. Leaves are a rich source of an assortment of minerals and vitamins [6]. Leaves contain pharmacologically active compounds such as flavonoids (luteolin, apigenin, isoquercitrin, caffeic acid, and chlorogenic acid), terpenoids, and triterpenes [7]. The whole plant has been used to treat many diseases, including acne, eczema, high cholesterol, heartburn, gastrointestinal disorders, diabetes, and cancer. In Chinese, Arabian and Native American traditional medicine it is used to treat a variety of diseases related to liver, inflammation and cancer [8]. The aqueous leaf extract of this herb, however, has not yet been checked for its toxicity effects.

The capacity of substances to affect an organism is known as toxicity [9], while teratogenicity is known to be the malformation or unusual development of the shapes and forms caused by teratogens on the body of the developing embryo [10]. Therefore, toxicity testing is important to screen a sample's chemical compositions to ensure that they do not contain any life-threatening components. The embryonic and larval form of *Danio rerio* is increasingly used as a toxicological and teratological model due to the transparent larvae and embryo, fast development cycles, high fertility, ease of maintenance in the laboratory [11], high genetic homology to mammals [12], and its similarity to vertebrates of higher forms in embryonic development [13]. Zebrafishes can efficiently absorb small molecular compounds and thus serves as a promising model for drug screening and impact assessment of the teratogenic compounds [14,15]. Thus, this makes zebrafish embryo an advantageous assay in exploring many diversions of toxicology study yielding a prompt outcome [16]. The study was intended to evaluate the embryo-toxic and teratogenic effects of the *T.officinale* leaf extract using zebrafish embryos which could lead to the discovery of other potential bioactivities of this plant.

MATERIALS AND METHODS

Sample collection and preparation

The leaves of *T. officinale* were collected from CMST campus, Manonmaniam Sundaranar University. The leaves were washed with distilled water to expel any residue, chopped into pieces, and subjected to shade drying for 20 days. Dried leaves were made into a fine powder using a dry grinder. The powdered leaf material was processed for further extraction experiments in air-tight bags.

Extraction of *T. officinale*

The aqueous extract was prepared with the addition of 10g leaves powder of *T. officinale* in an Erlenmeyer flask containing 100 ml of distilled water in the extent of 1:10. The mixture was kept in a boiling water bath at 60°C for 1 hour. The extract was further filtered through Whatmann No.1 filter paper and put away in refrigerated condition until further assessment.



**Jeba Preethi Jansi and Jeni Chandar Padua****Preliminary Phytochemical screening of aqueous leaf extract of *T. officinale***

To detect the existence of active secondary metabolites, the aqueous extract of *T. officinale* was subjected to preliminary phytochemical examination. Standard techniques were followed to test each extract separately with particular chemical reagents [17]. The presence (+) or absence (–) of specific active ingredients was determined by visible colour change or precipitate formation.

In vivo* studies*Zebrafish maintenance and embryo selection**

Healthy adult Zebrafishes (*Danio rerio*) of wild-type (AB strain) were acquired from the Center for Marine Science and Technology, MS University, Tirunelveli. At a temperature of 27 ± 1 °C, pH 7 (± 0.5), conductivity 650 μ S/cm and a 12:12-hour day/night cycle, male/ female zebrafishes in the ratio of 2:1 ratio were kept in aquariums in a dark room. For the mating purpose, a cycle of 14 hr light/10 hr dark was adopted. After exposure to light in the morning, zebrafish start laying eggs within 30 minutes. The eggs were carefully collected after spawning and immediately rinsed with 2 mg/L methylene blue to prevent contamination. Viable embryos were used in the assay while those unfertilized eggs were discarded [18].

Experimental design and treatment

The viable embryos were treated with various concentrations of *T. officinale* leaf extract up to 72 hpf (hour post-fertilization). For each group, thirty embryos were treated. In triplicates, all the tests were completed.

***In vivo* toxicity**

According to OECD 2013 guidelines, toxicity assays were performed using zebrafish embryos [19]. By diluting stocks of the aqueous leaf extract of *T. officinale*, the test arrangements were made. Thirty viable eggs were chosen randomly and introduced into each of the 6 well plates with five distinct concentrations (10 μ l, 20 μ l, 40 μ l, 80 μ l, and 160 μ l) of *T. officinale* by dissolving in 3 ml embryo water. A compound microscope was used to visualize the development of embryos and larvae until 72 hours post-treatment. *D. rerio* embryos mortality was noted at 12h, 24h, 48h, and 72hpf. The mortality, hatchability, heartbeat rate of the embryos, and LC50 values were determined and photomicrographs were taken.

Teratogenicity assay

To assess if the compound has a teratogenic effect on the embryos, the teratogenicity assay was performed [20]. Thirty embryos were transferred to 6 well plate containing various concentrations of *T. officinale* (10 μ l, 20 μ l, 40 μ l, 80 μ l, and 160 μ l) was inspected for distortions every 24h under a compound microscope. Frequencies of malformations were recorded until 72 hpf. Zebrafish embryos were assessed for delayed development, tail malformation, head malformation, scoliosis, limited movement, edema, and so on. The normal development of the embryo was compared with previously described by Kimmel et al. [21].

Statistical analysis

The experiment was carried out in triplicates and the obtained data was provided employing Mean \pm Standard deviation (SD). The data thus obtained was analyzed statistically using SPSS version 22 software. The substantial differences between the experimental and control groups of embryos/larvae with a confidence interval of 95% were tested by one-way ANOVA with a Tukey comparison test.

RESULTS AND DISCUSSION**Phytochemical analysis**

Table 1 lists the phytochemical elements found in *T. officinale*, including tannins, saponins, phenols, alkaloids, flavonoids, steroids, carbohydrates, and terpenoids. The reported health benefits of *Taraxacum officinale* leaves could



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be attributed to these phytoconstituents (tannins, terpenoids, and phenolic compound) that may have medicinal value; tannins are plant metabolites well known for antimicrobial properties, and phenols retain antioxidant potential that could augment the body's resistance to pathology induced free radical generation [22]. Terpenoids, phenolics, and tannins have recently been discovered to be important components of several antibiotics used to treat prevalent pathogenic strains [23,24].

Mortality rate

Coagulation and the lack of heartbeat in zebrafish embryos are symptomatic of mortality [11]. The mortality of embryos was noted and the results were given in Mean \pm SD and stated in Table 2. Treatment with lower leaf extract concentrations (10 μ l and 20 μ l) resulted in mortality of $17.3 \pm 16.43\%$ and $30.6 \pm 14.91\%$ respectively, towards the end of the investigation at 72 hpf. Conversely, the embryos treated with a high dose of 160 μ l displayed a mortality rate of 100 % at 24hpf. Leaf extract concentration of 40 μ l showed $47.3 \pm 8.47\%$ at 48 hpf and the end of 24hpf, 50% mortality reached at the concentration of 80 μ l in *Danio rerio* embryos indicating $69.6 \pm 5.70\%$ mortality.

Lethal concentration dose (LC50) of the aqueous leaf extract

Probit analysis was used to measure the LC50 value for *T.officinale* leaf extract, which is shown by the statistical calculation of the amount of toxicant per body weight needed to regulate the 50 percent mortality rate of zebrafish embryos [25]. The logarithmic inference of the LC50 value using the embryo concentration and the mortality rate is shown in the figure 1, and the LC50 was determined to be 29.75 μ l.

Hatchability of zebrafish embryos

Embryo dechorionization is the major indicator of hatchability. Zebrafish embryos begin hatching at 48 hpf, and in normal conditions, finish hatching at 72 hpf [26]. In the treatment groups, however, the embryo-hatching rate was significantly reduced with hatching rates of $82.6 \pm 4.81\%$, $75.6 \pm 6.23\%$, and $67.6 \pm 6.76\%$ in the 10 μ l, 20 μ l, and 40 μ l respectively. Eggs hatchability was greatly reduced in the 80 μ l and 160 μ l showing $48 \pm 8.50\%$ and $11.3 \pm 51.24\%$ low hatching rates. These data showed a substantial dose-dependent decrease in the rate of hatching of treated groups of leaf extracts, which was significantly lower ($p < 0.05$) than that of the control groups. The percent hatchability for the control and leaf extract treated embryos at 72 hpf is shown in figure 2. A few embryos exhibited delayed hatching which could be because of formative anomalies in the developing embryos, bringing up the incompetence of the chorion to break; it also may well be explicated by the morphological anomalies detected in the embryos, which limit hatching [27].

Heartbeat rate

The normal heartbeat rate of zebrafish embryos ranges from 120 to 180 per min^[28,29]. The mean heartbeat rates per minute of zebrafish embryos exposed to various concentrations of leaf extract at 72 hpf revealed that the control embryos had the highest heartbeat rate of 157.3 per min, followed by the embryos treated with concentrations of 10 μ l, 20 μ l, 40 μ l, and 80 μ l, having heartbeats of $147 \pm 2.04\%$, $133 \pm 2.55\%$, $129 \pm 2.32\%$ and $115.3 \pm 3.97\%$ beats/min, respectively. No heartbeat was noticed in the *D. rerio* embryos as a result of early mortality at the concentration of 160 μ l. While looking at the outcomes, the heartbeat rate is decreased, when the treatment concentration increases.

Teratogenicity of Zebrafish embryos

The teratogenicity recorded in zebrafish is critical as it reflects the predictive intensity of the bioassay for assessing formative toxicity in vertebrates [30]. Teratogenic effects were observed in zebrafish embryos after exposure to leaf extract. The teratogenicity chart (Table 3) and morphological defects (Fig. 3) indicated that all the embryos treated with the least concentration (10 μ l) had fewer morphological defects, contrasted with the control. The treatment grouping 160 μ l was found to be very toxic to the embryos, with all the embryos dying within 36hpf. Pericardial edema, yolk sac edema, tail malformation, tail curvature, growth retardation, delayed hatching, and scoliosis were observed in embryos treated with leaf extract at the concentrations of 20 μ l, 40 μ l, and 80 μ l. A similar outcome is identified by Dulay et al. [31] in zebrafish embryos exposed to *G. Lucidum* extract showing tail malformation (bent tail and S-shaped tail) at 72 hpf as the most important morphological abnormality as seen in Fig 3f.



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The most conspicuous malformations were tail malformation, tail curvature, and yolk sac edema. The frequency of tail malformation may be attributed to warfarin, an anticoagulant coumarin derivative being the fingerprint morphological endpoint of toxicity [32]. Yolk sac edema and tail malformations were intermittently detected endpoints of zebrafish embryos after contact with the known embryo-toxic and teratogenic compounds such as valproic acid, retinoic acid, hydroxyurea, methoxyacetic acid, and boric acid^[33]. Thus, the yolk sac edema was likely due to the symptoms of hemostasis, hindered blood movement, or liquid incongruity. This hypothesis is upheld by a study suggesting that pericardial and yolk sac edema in zebrafish is associated with impaired blood flow and fluid imbalance [34]. The tail in Fig 3H showed a sharp kink in the center of the tail length coupled with a turn in the direction of the tail [35].

CONCLUSION

The study revealed that the toxic and teratogenic effects of *Taraxacum officinale* aqueous leaf extract triggered concentration dependent developmental toxicity in the embryos of zebrafish. Zebrafish embryos are intensely affected by high concentrations of treatment concentrations in terms of mortality, hatchability, and heartbeat rate. At 72hpf, the LC50 was found to be 29.75µl with teratogenic effects such as scoliosis, curved tail, twisted tail, and tail malformation. Advanced exploration is needed to define and elucidate the mechanisms acting specifically on particular teratogenicity in *D. rerio* embryos.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Table 1: Phytochemical Constituents of Aqueous Leaf Extract of *T. officinale*

Phytochemicals tested	Aqueous leaf extract of <i>T. officinale</i>
Tannins	+++
Saponins	++
Carbohydrates	++
Terpenoids	++
Steroids	+
Flavonoids	+++
Phenol	+
Alkaloids	++
Glycosides	+
Anthocyanin	-

Table 2: Mortality of *D. rerio* Embryos After 12, 24, 48, and 72 h of Exposure to Varying Concentrations of *T. officinale* Aqueous Leaf Extract (n=30)

Leaf extract Concentration	Mean mortality of the embryos (%)			
	12 hrs	24 hrs	48 hrs	72 hrs
Control	2.22	0.00	1.11	0.00
10 µl	5.55	7.77	2.22	0.00
20 µl	10.0	10.0	4.44	0.00
40 µl	22.22	8.88	4.44	2.22
80 µl	35.55	23.33	5.55	0.00
160 µl	55.55	37.77	8.88	0.00





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Table 3: Teratogenic Effects of *T. officinale* Leaf Extract in Zebrafish Embryos

Leaf extract concentration	Types of Toxicity						Teratogenicity Delayed hatch
	Lethal		Sublethal				
	Coagulation	No heartbeat	Pericardial edema	Yolk sac edema	Tail malformation	Scoliosis	
Control	-	-	-	-	-	-	-
10 µl	*	-	-	*	*	-	-
20 µl	*	*	*	-	*	*	*
40 µl	*	*	*	*	*	-	*
80 µl	*	*	*	*	nd	*	nd
160 µl	*	*	nd	nd	nd	nd	nd

- nil, * - present, nd- not detected

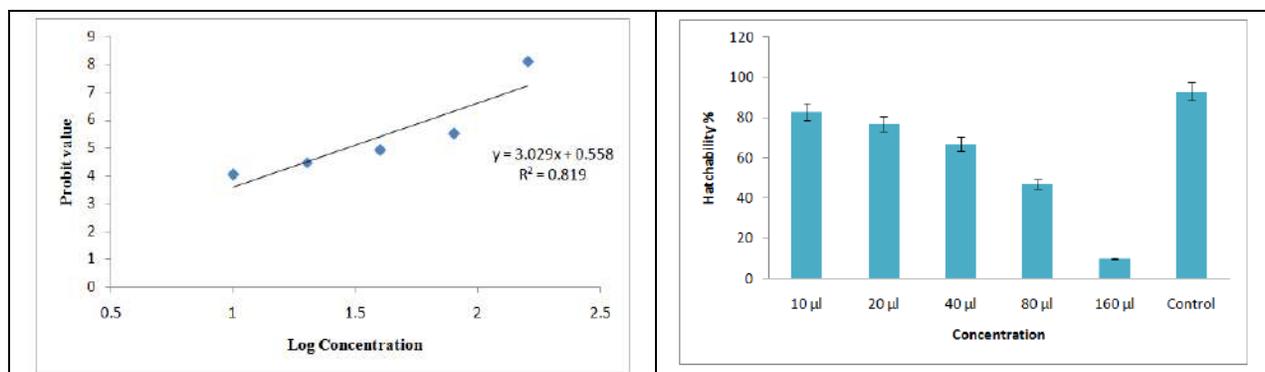


Figure 1: The linear regression curve of Log₁₀ Concentration versus probit of aqueous leaf extract of *T. officinale* on zebrafish embryos

Figure 2: Effect of various concentrations of *T. officinale* leaf extract on hatchability in zebrafish embryos; *p <0.05 when compared to corresponding control.

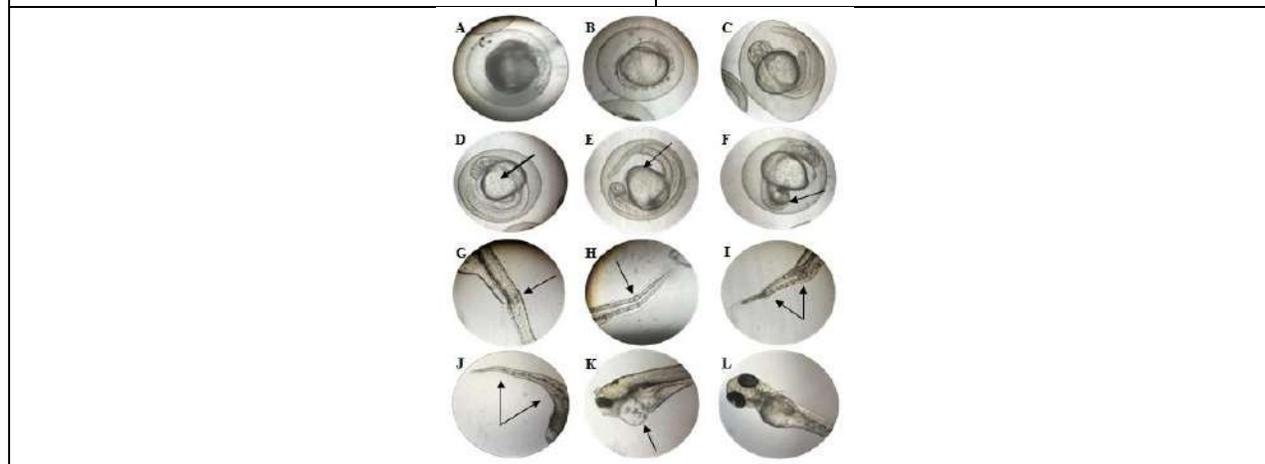


Figure 3: 10X magnification of Morphological malformations of zebrafish embryos exposed to *T. officinale* leaf extract from 6-72hpf: (A) Coagulated egg at 40µl concentration hpf (B) delayed developed egg at 160 µl concentration (C) Oval shaped egg at 80 µl (D,E) embryo showing yolk sac edema and abdominal edema at 36h and 42 hpf (F) embryo with deformed tail (G-I) embryos with tail malformations (J) embryo showing scoliosis at 72h (K) pericardial edema at 72h (L) Normal embryo at 72 hpf





Comparative Account of Density Functional Studies on the Electronic Structure and Spectroscopic Properties of Gallic Acid and Methyl Gallate Reveal their Immense Pharmacological and Biological Potential

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ABSTRACT

Several fruits and medicinal plants are known to harbor Gallic acid (GA) which is a natural phenolic compound having wide-spectrum therapeutic potential with many health-promoting pharmacological and biological effects. In the current study, Density functional theory (DFT) has been applied to compute bond length, Mullikan atomic charges, vibrational frequencies and electrostatic potential surfaces. The





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above calculations were carried out by using DFT (B3LYP)/6-311G,6-311G(d),6-311-G(d,p) geometries. Redistribution of charges on GA was reported to be over larger range while the Bond length of O-H in GA was found to be significantly higher in comparison to Methyl gallate (MG). This led us to conclude that GA molecule displays significant reactivity over MG. Comparative quantum chemical study of electronic structures, ground state properties and electrostatic potential surface studies are further suggestive of reactive sites in GA and MG.

Keywords: Density functional studies; Electronic structure; Electrostatic potential; GA; MG

INTRODUCTION

Gallic acid (GA) or 3,4,5-trihydroxybenzoic acid is a natural phenolic compound, which is a colorless or slightly yellow crystalline compound that is found in several fruits and medicinal plants, which has a number of health-promoting effects. GA has been isolated from different plant species such as *Quercus* spp. and *Punica* spp., via various chromatographical methods; however, from the industrial point of view, GA is produced through the hydrolytic breakdown of tannic acid using a glycoprotein esterase, namely tannase. GA and its derivatives such as MG have innumerable beneficial effects with reference to human health, which includes antioxidant nature of GA and MG along with other derived compounds such as lauryl gallate, propyl gallate, octyl gallate, tetradecyl gallate, and hexadecyl gallate [1]. Also, these molecules have anti-inflammatory, anti-cancer, and antineoplastic properties [2-3]. Moreover, these compounds also have been reported to have pharmacological and therapeutic activities in gastrointestinal, neuropsychological, metabolic, and cardiovascular disorders [4]. GA and its derivatives represent important additives in food technology, in reference to the thermodynamics, hydrogen atom transfer (HAT) which is governed by the O–H bond dissociation enthalpy (BDE) [5]. The characterization of Single Electron Transfer–Proton Transfer (SET-PT) could be carried out by the ionization potential and proton dissociation enthalpy (PDE) [4]. In the case of Sequential Proton-Loss Electron-Transfer (SPLET), deprotonation of an OH group is followed by the electron transfer from the formed phenoxide anion. The corresponding reaction enthalpies are proton affinity (PA) of the phenoxide anion and the electron transfer enthalpy, ETE [4]. The physical, chemical and biological properties have an association with the acidity of phenolic acids. In case of GA, the carboxylic group has higher acidity characteristics. Moreover, the acidities of three phenolic groups range from $pK_a = 8.8$ to $pK_a = 11.4$, the values obtained using Raman spectroscopy [5]. GA, a predominant polyphenol, has been shown to inhibit carcinogenesis in animal models and in vitro cancerous cell lines as well [5-6]. The inhibitory effect of GA on cancer cell growth is mediated via the modulation of genes which encodes for cell cycle, metastasis, angiogenesis and apoptosis. GA inhibits activation of NF- κ B and Akt signaling pathways along with the activity of COX, ribonucleotide reductase and GSH. Moreover, GA activates ATM kinase signaling pathways to prevent the processes of carcinogenesis [7]. The mapping of the anticancer efficacy of synthesized GA analogues can be done using modeling and artificial intelligence (AI) over a large range of concentrations. Pharmacodynamics modeling of anticancer potential of the synthesized compounds revealed that drug efficacy and response heterogeneity could be modulated by changing the exposure time to optimize therapeutic impact [8]. Ab-initio studies on bioactive molecules such as eugenol and carvacrol derived from *Ocimum species* have revealed the better reactive potential of Eugenol over Carvacrol in previous studies [9-10]. Similarly, the antimicrobial and other biological properties of GA and its derivatives seemed to be linked with the hydrolysis of ester linkage between GA and polyols like tannins hydrolyzed after ripening of many edible fruits. GA serves as a natural defense mechanism against microbial infections and modulation of immune responses [11]. Ab-Initio methods are being used for detailed studies of molecules having antimicrobial and therapeutic properties. Hartree fock and Density functional theory (DFT) methods with various basis sets are found to be very useful for determination of the molecular structures, intermolecular and intramolecular forces. Towards this goal, we carried out ab-initio studies on GA and MG to compare their reactive potential based on bond length, Mullikan atomic charges, vibrational frequencies and electrostatic potential surfaces.



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MATERIALS AND METHODS

The neutral gas phase of GA and MG were fully optimized by employing 6-311G,6-311G(d) and 6-311G(d,p) basis sets at HF and DFT level. Fig.1a and 1b are the optimized structures of GA and MG using the above 6-311G(d,p) basis set. Bond lengths, Mulliken atomic charges, vibrational frequencies and related modes of vibrations were studied by using these optimized structures of molecules. All calculations in the present work were carried out in the Department of Physics, MMEC, MM(DU), Mullana on i-5 Processor using G-03W and GAUSS VIEW 4.1 VERSION (4) of ab-initio quantum mechanical program[12].

RESULTS AND DISCUSSION

HF and DFT methods with 6-311G,6-311G(d) and 6-311G (d, p) basis sets have been employed to compute bond lengths, net atomic charges, vibrational frequencies and electrostatic potential surfaces of GA and MG which are derived from fruits and medicinal plants. The values of the bond lengths of GA and MG have been shown in Table 1 and Table 2. Upon comparisons of the bond lengths of GA and MG, it was observed that O11-H12, O13-H14, C15-O16 displayed higher bond length values for GA than MG indicative of the fact that GA is more reactive than MG which is consistent with the previous observation in literature [13]. This could be attributed to additional carboxylic group in GA molecule while carboxylic acid ester group is present in MG. The values of the net charges at various atomic sites in the units of 'e' have been shown in Table 3 and Table 4. These have been obtained through Mulliken population analysis using DFT and HF methods. The positive and negative values of the net charges at various atomic sites in a molecule are indicative of the fact that the total charges on the orbitals after the molecule is formed are lesser or more than the free atomic charges. Similarly, upon comparisons of atomic charges, it was observed that C1 and C3 of GA had more electropositive charge than MG while C4 and C5 of MG possessed more electronegative charge than C4 and C5 of GA. O9, O11 and O16 of GA were observed to be more electronegative than O9 of MG. H10, H12 and H14 of GA seem to be more electropositive than MG. Keeping in view of these observations, one may conclude that GA has a greater tendency to form ionic species as compared to MG.

IR spectra of GA and MG calculated at DFT(B3LYP)/6-311G(d,p) level, have been shown in Fig.2a and Fig. 2b. Computed frequencies and modes of vibrations at DFT(B3LYP)/6-311(d, p) are shown in Table 5 and Table 6. A typical infrared spectrum observed has been usually divided into two regions. The left half, below 1000 cm^{-1} to 400 cm^{-1} , is finger print region and the right half above 1000 to 4000 cm^{-1} , is known as the functional group region. The right half, above 2000 cm^{-1} although contains relatively few peaks, however, some diagnostic information could be found in this region of the spectrum. A very broad peak in this region between 3100 cm^{-1} and 3600 cm^{-1} indicates the presence of exchangeable protons, typically from alcohol, amine, amide or carboxylic acid groups [14]. The calculated IR frequencies at DFT(B3LYP)/6-311G(d,p) showed that O13-H14 stretching in GA is at 3849.39 cm^{-1} whereas in MG, it is at 3851.7 cm^{-1} . Similarly O11-H12, stretching is at 3795.41 cm^{-1} in GA and at 3837.04 cm^{-1} in MG. O9-H10 stretching is at 3789.97 cm^{-1} in GA and 3781.49 cm^{-1} in MG. These observations show that reactivity of GA is attributed to the presence of O-H groups. Stretching of C=O in GA is at 1801.4 cm^{-1} while in MG, it is at 1774.64 cm^{-1} which means that the reactivity of MG is due to ester group. O-H rocking and wagging are at higher frequencies in MG, that also supports the reactivity of GA which is attributed to O-H groups.

The electrostatic potential surfaces (ESP) of GA and MG are shown in Fig 3a and Fig. 3b. The potential increase has been reported to be in the order blue>green>yellow>orange>red. Blue colour represents the positive regions which is prone to nucleophilic attack and red colour represents the negative region that is more prone to electrophilic attack[15-16]. As can be seen from ESP surfaces of GA, that it has four possible sites for nucleophilic attack and one site for electrophilic attack. In case of MG, there are two sites for nucleophilic attack while two for electrophilic attack, again pointing towards GA having more reactive sites than MG.





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CONCLUSIONS

Density functional theory (DFT) and HF were employed to compute bond lengths, Mullikan atomic charges, vibrational frequencies and electrostatic potential surfaces which were carried out by using DFT(B3LYP)/6-311G(d),6-311G(d,p) geometries. Redistribution of charges on GA was reported to be over larger range while the Bond length of O-H in GA was found to be significantly higher in comparison to MG. Hence, A molecule displayed significant reactivity over MG. Comparative quantum chemical study of electronic structures, ground state properties and electrostatic potential surface studies are further suggestive of reactive sites in GA and MG. Future investigations are essential to further define the safety and therapeutic efficacy of GA and MG in humans given to their several beneficial effects including antioxidant, anti-cancer, anti-inflammatory, and antineoplastic properties.

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CONFLICT OF INTEREST

There exists no conflict of interest amongst authors regarding publication of this manuscript.

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Table -1 Bond lengths of Gallic acid computed with HF/6-311G,6-311G(d),6-311G(d,p) and DFT(B3lyp)/ 6-311G,6-311G(d),6-311G(d,p)

S.No.	Bond(s)	HF6-311G	HF6-311G(d)	HF6-311G(d,p)	DFT6-311G	DFT6-311G(d)	DFT6-311G(d,p)
1	C1-C2	1.3795	1.3833	1.3842	1.3958	1.3978	1.3986
2	C1-C6	1.3802	1.3821	1.3821	1.3897	1.3892	1.3895
3	C1-O9	1.3666	1.3438	1.344	1.3842	1.359	1.3593
4	C2-C3	1.3811	1.3844	1.3849	1.3944	1.3958	1.3963
5	C2-O11	1.3703	1.3519	1.3518	1.3883	1.3663	1.3662
6	C3-C4	1.3756	1.3768	1.3772	1.3871	1.3863	1.3865
7	C3-O13	1.3814	1.3586	1.358	1.4018	1.3761	1.3756
8	C4-C5	1.3933	1.3906	1.39	1.4078	1.4021	1.4018
9	C4-H7	1.0682	1.0733	1.0734	1.0798	1.0838	1.0827
10	C5-C6	1.3872	1.3834	1.3834	1.4027	1.3969	1.3969
11	C5-C15	1.4697	1.4861	1.4871	1.4699	1.4812	1.4823
12	C6-H8	1.0672	1.0718	1.0721	1.0786	1.0824	1.0815
13	O9-H10	0.9484	0.9417	0.9429	0.975	0.9666	0.9658
14	O11-H12	0.948	0.9416	0.943	0.974	0.9662	0.9658
15	O13-H14	0.9452	0.9395	0.9405	0.9696	0.9628	0.962
16	C15-O16	1.3514	1.3301	1.3302	1.3873	1.3605	1.3605
17	C15-O18	1.2116	1.1835	1.1833	1.2362	1.2082	1.208
18	O16-H17	0.9493	0.9438	0.9453	0.9757	0.9684	0.9678

Table -2 Bond lengths of Methyl gallate computed with HF/6-311G,6-311G(d),6-311G(d,p) and DFT(B3lyp)/ 6-311G,6-311G(d),6-311G(d,p)

S.No.	Bond(s)	HF6-311G	HF6-311G(d)	HF6-311G(d,p)	DFT6-311G	DFT6-311G(d)	DFT6-311G(d,p)
1	C1-C2	1.3849	1.3881	1.3887	1.4003	1.4014	1.402
2	C1-C6	1.3821	1.3828	1.3827	1.3932	1.3919	1.3919
3	C1-O11	1.3705	1.3473	1.3474	1.3886	1.3629	1.3632
4	C2-C3	1.3849	1.3869	1.3874	1.4006	1.401	1.4014
5	C2-O9	1.3597	1.3406	1.3411	1.3764	1.3539	1.3546
6	C3-C4	1.3752	1.3776	1.3776	1.385	1.3847	1.3848



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7	C3-O13	1.3819	1.359	1.3584	1.4022	1.3764	1.3759
8	C4-C5	1.3885	1.385	1.3849	1.404	1.398	1.398
9	C4-H7	1.0695	1.0742	1.0742	1.0813	1.085	1.0839
10	C5-C6	1.388	1.3863	1.3861	1.4031	1.3984	1.3982
11	C5-C15	1.4716	1.4879	1.4885	1.4718	1.4828	1.4831
12	C6-H8	1.0685	1.0737	1.0737	1.0804	1.0844	1.0832
13	O9-H10	0.9485	0.9416	0.9429	0.9761	0.9671	0.9664
14	O11-H12	0.9458	0.9394	0.9403	0.9716	0.9638	0.9625
15	O13-H14	0.945	0.9394	0.9405	0.9694	0.9627	0.9619
16	C15-O16	1.2154	1.1858	1.1858	1.2395	1.2105	1.2104
17	C15-O17	1.3414	1.3217	1.3216	1.3797	1.3539	1.3538
18	O17-C18	1.4437	1.4151	1.4165	1.4682	1.435	1.4364
19	C18-H19	1.0731	1.078	1.0793	1.0838	1.0877	1.088
20	C18-H20	1.0766	1.0801	1.0816	1.0879	1.0907	1.0912
21	C18-H21	1.0766	1.0801	1.0816	1.0879	1.0907	1.0912

Table -3 Net atomic charges of Gallic Acid computed with HF/6-311G,6-311G(d),6-311G(d,p) and DFT(B3lyp)/ 6-311G,6-311G(d),6-311G(d,p)

S.No.	Atoms	HF6-311G	HF6-311G(d)	HF6-311G(d,p)	DFT6-311G	DFT6-311G(d)	DFT6-311G(d,p)
1	C1	0.375216	0.387038	0.283268	0.262634	0.276774	0.171165
2	C2	0.289199	0.252591	0.194694	0.178619	0.169384	0.126043
3	C3	0.343679	0.328989	0.245227	0.24759	0.241573	0.145541
4	C4	-0.041648	-0.27618	-0.076058	-0.0071	-0.217903	-0.029509
5	C5	-0.218408	-0.222395	-0.293277	-0.183595	-0.161874	-0.25225
6	C6	-0.092606	-0.251297	-0.063738	-0.066629	-0.213349	-0.02436
7	H7	0.221158	0.25248	0.114515	0.193176	0.217507	0.103662
8	H8	0.233006	0.267526	0.134579	0.203314	0.229446	0.119143
9	O9	-0.768838	-0.68932	-0.454798	-0.619015	-0.562531	-0.356392
10	H10	0.437613	0.454843	0.278203	0.396418	0.411998	0.259312
11	O11	-0.793437	-0.723054	-0.495241	-0.646866	-0.596467	-0.395396
12	H12	0.453175	0.469016	0.293917	0.413357	0.427628	0.275836
13	O13	-0.804488	-0.726712	-0.499229	-0.669231	-0.613185	-0.412681
14	H14	0.42788	0.449202	0.279101	0.395955	0.414544	0.26839
15	C15	0.740002	0.682523	0.668892	0.467318	0.445875	0.431364
16	O16	-0.738949	-0.652489	-0.435997	-0.590124	-0.534835	-0.346377
17	H17	0.43955	0.446712	0.277737	0.39098	0.397212	0.252585
18	O18	-0.502102	-0.449471	-0.451795	-0.366801	-0.331797	-0.336076





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Table -4 Net atomic charges of Methyl Gallate computed with HF/6-311G,6-311G(d),6-311G(d,p) and DFT(B3lyp)/6-311G,6-311G(d),6-311G(d,p)

S.No.	Atom	HF6-311G	HF6-311G(d)	HF6-311G(d,p)	DFT6-311G	DFT6-311G(d)	DFT6-311G(d,p)
1	C1	0.324155	0.339517	0.253323	0.209326	0.230666	0.143808
2	C2	0.35951	0.322484	0.235487	0.237079	0.22586	0.164586
3	C3	0.310366	0.319988	0.244319	0.213508	0.229785	0.140071
4	C4	-0.071685	-0.277624	-0.080719	-0.032807	-0.222714	-0.037014
5	C5	-0.226259	-0.232672	-0.303612	-0.194116	-0.173647	-0.266074
6	C6	-0.068286	-0.286112	-0.083302	-0.021137	-0.22316	-0.031117
7	H7	0.221507	0.253489	0.117984	0.190948	0.215754	0.104287
8	H8	0.21457	0.24374	0.108883	0.185718	0.208271	0.097382
9	O9	-0.73706	-0.669374	-0.441251	-0.587869	-0.540747	-0.340677
10	H10	0.436889	0.453538	0.276401	0.397432	0.412777	0.259503
11	O11	-0.732823	-0.662145	-0.436868	-0.594984	-0.545986	-0.348984
12	H12	0.404623	0.428812	0.257788	0.374304	0.395295	0.24869
13	O13	-0.805019	-0.726815	-0.498648	-0.670425	-0.613594	-0.412353
14	H14	0.427955	0.449196	0.278858	0.396588	0.414994	0.268504
15	C15	0.743242	0.68994	0.701318	0.469272	0.454215	0.463487
16	O16	-0.531826	-0.474616	-0.475591	-0.394717	-0.357528	-0.358313
17	O17	-0.67259	-0.448787	-0.460152	-0.499965	-0.329497	-0.344412
18	C18	-0.171407	-0.388302	-0.003849	-0.269295	-0.439185	-0.111614
19	H19	0.187536	0.217666	0.100986	0.191771	0.215437	0.117102
20	H20	0.193313	0.22404	0.104324	0.199681	0.221502	0.121569
21	H21	0.19329	0.224036	0.104323	0.199689	0.221502	0.12157

Table - 5 Vibrational frequencies and modes of vibrations of Gallic Acid computed with DFT 6-311g(d,p).

S.NO.	DFT 6-311g(d,p)(cm-1)	New assignments	MODES
1	3849.39	O13-H14	STRETCHING
2	3795.41	O11-H12	STRETCHING
3	3789.97	O9-H10	STRETCHING
4	3775.48	O16-H17	STRETCHING
5	3218.2	C6-H8	STRETCHING
6	3195.84	C4-H7	STRETCHING
7	1801.4	C15=O18	STRETCHING
8	1659.85	C2-C3,C5-C6	STRETCHING
9	1654.22	C1-C2,C4-C5	STRETCHING
10	1563.05	C4-H7 and C6-H8	ROCKING
11	1505.8	O9-H10	ROCKING
12	1413.84	O11-H12	ROCKING
13	1398.51	O9-H10	ROCKING





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14	1362.86	O16-H17	ROCKING
15	1335.51	C4-H7	ROCKING
16	1296.68	O13-H14	ROCKING
17	1246.72	C4-H7 ,O11-H12	ROCKING
18	1204.49	O9-H10, C6-H8	ROCKING
19	1176.4	O13-H12	ROCKING
20	1173.05	O16-H17	ROCKING
21	1097.23	C6-H8	ROCKING
22	1042.08	O11-H12	ROCKING
23	953.64	C4-C5-C6	SCISSORING
24	891.79	C6-H8	WAGGING
25	837.36	C4-H7	WAGGING
26	787.65		DISTORTION
27	777.37	C4-H7,C6-H8	WAGGING
28	687.81	C1-C2-C3	WAGGING
29	672.03	O16-H17	ROCKING
30	639.8	O13-H14,O11-H12	ROCKING
31	616.66	O16-H17	WAGGING
32	579.1	C3-H4-H7	TWISTING
33	562.13		DISTORTION
34	536.49		DISTORTION
35	529.15	O16-H17	WAGGING
36	433.01	O9-H10	WAGGING
37	419.93	O16-C15-O18	ROCKING
38	378.11	O11-H12	WAGGING
39	349.19		DISTORTION
40	343.79	O11-H12	WAGGING
41	305.72	C3-O13,C1-O9	ROCKING
42	297.38	C2-O11	ROCKING
43	261.33	C6-H8	WAGGING
44	197.01	O13-H14	WAGGING
45	174.56	O16-C15=O18	ROCKING
46	168.84	O13-H14	WAGGING
47	111.36	O11-H12	WAGGING
48	66.34	18O=15C-16O	TWISTING





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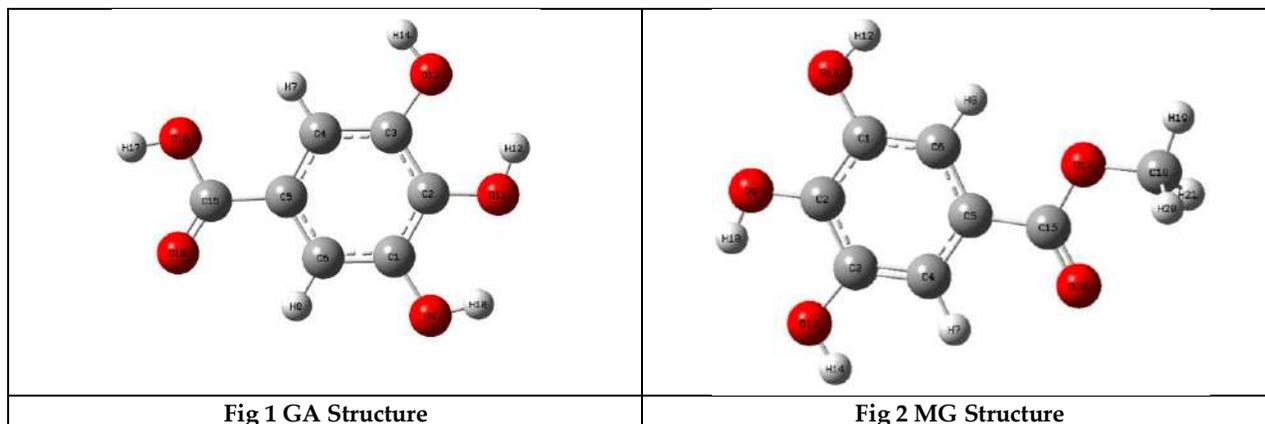
Table-6 Vibrational frequencies and modes of vibrations of Methyl Gallate computed with DFT 6-311g(d,p).

S.NO.	DFT 6-311g(d,p)(cm ⁻¹)	Bonds	MODE OF VIBRATIONS
1	3851.7	O13-H14	STRETCHING
2	3837.04	O11-H12	STRETCHING
3	3781.49	O9-H10	STRETCHING
4	3190.06	C6-H8	STRETCHING
5	3185.4	C4-H7	STRETCHING
6	3152.45	C18-H19	STRETCHING
7	3117.11	H20-C18-H21	ASSYMETRIC STRECHING
8	3046.9	C18-H19,C18-H20,C18-H21	SYMMETRIC STRETCHING
9	1774.64	C15=O16	STRETHCING
10	1661.28	C1-C2,C4-C5	STRETCHING
11	1644.56	C1-C6,C3=C4	STRETCHING
12	1574.28	C4-H7,C6-H8	ROCKING
13	1500.8	H20-C18-H21	SCISSORING
14	1481.92	H19-C18-H20	SCISSORING
15	1479.17	C1-C6,C3=C4	STRETCHING
16	1473.08	C18-H19	WAGGING
17	1419.22	O9-H10	ROCKING
18	1380.94	C2-C3,C4-C5	STRETCHING
19	1358.5	O11-H12	ROCKING
20	1318.7	O13-H14	ROCKING
21	1251.81	C6-H8	ROCKING
22	1238.89	C4-H7	ROCKING
23	1212.32	H19-C18-H20	ROCKING
24	1203.04	O11-H12	ROCKING
25	1176.76	O13-H14	ROCKING
26	1173.26	H20-C18-H21	ROCKING
27	1118.8	C4-H7,C6-H8	ROCKING
28	1034.26	O9-H10	ROCKING
29	1029.43	O17-C18	STRETCHING
30	928.5	C18-H19	ROCKING
31	858.95	C4-H7	WAGGING
32	838.52	C6-H8	WAGGING
33	810.23		DISTORTION
34	770.93	C4-H7,C6-H8	WAGGING
35	751.49		DISTORTION



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36	685.56	C1-C2-C3	WAGGING
37	633.16	C4-H7,C6-H8	ROCKING
38	581.51	C4-H7,C6-H8	WAGGING
39	562.09	C5-C4-H7	WAGGING
40	558.96		DISTORTION
41	534.89	C1-C2-C3	SCISSORING
42	453.74	O9-H10	WAGGING
43	418.86	C15=O16	ROCKING
44	364.5	O11-H12	WAGGING
45	336.88		DISTORTION
46	317.3	O11-H12	WAGGING
47	315.8	H19-C18-H20	ROCKING
48	303.53	C1-O11,C2-O9	ROCKING
49	293.72	O13-H14	ROCKING
50	258.22	C4-C5-C6	TWISTING
51	219.2	O13-H14	WAGGING
52	193.84	H20-C18-H21,O13-H14	ROCKING,WAGGING
53	146.78	H19-C18-H21	ROCKING
54	132.08		DISTORTION
55	124.96	H20-C18-H21,H19-C18-H20	ROCKING
56	87.55	H20-C18-H21	ROCKING
57	56.79	O16=C15-O17	TWISTING





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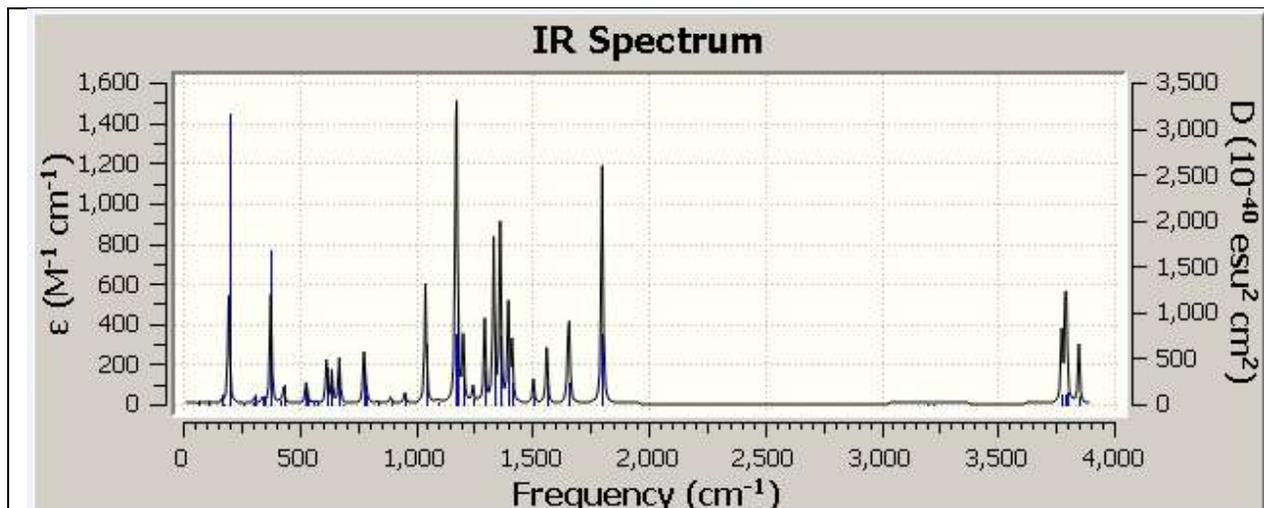


Fig 3 IR GA

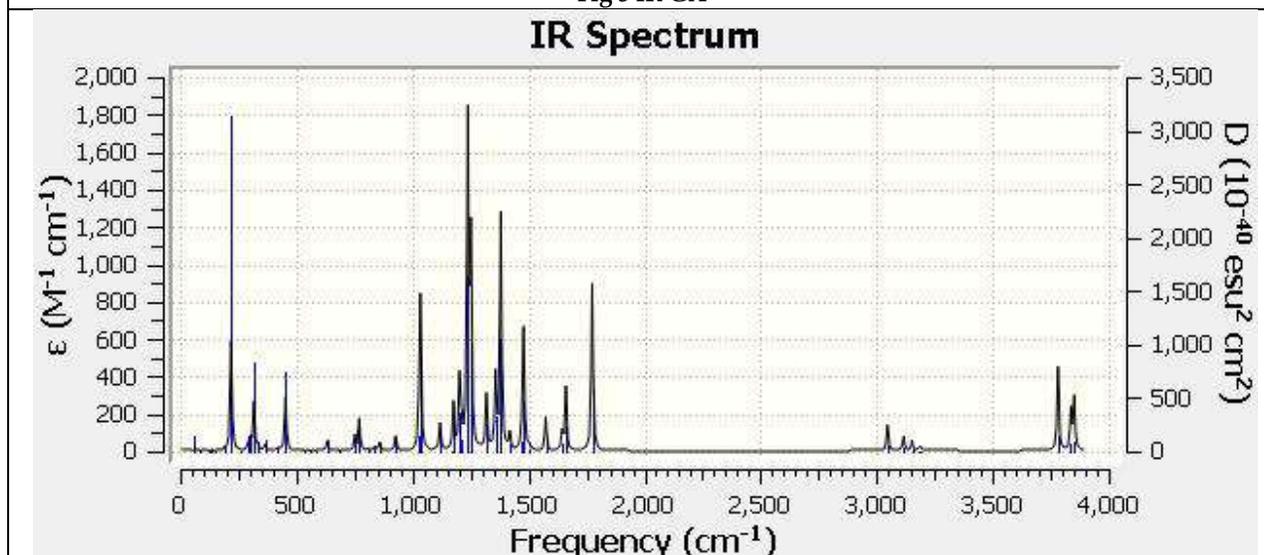


Fig 4 IR MG

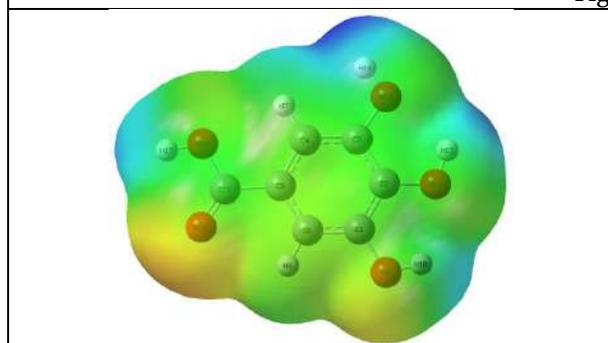


Fig. 5 Molecular electrostatic potential GA

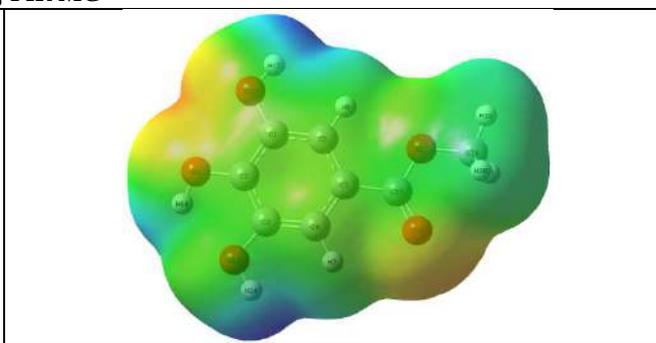


Fig. 6 Molecular electrostatic potential MG





A Study to Evaluate the Effectiveness of Comprehensive Oral Care on Knowledge and Practice Regarding Oral Health among School Children at Madurai

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ABSTRACT

The main objective was to evaluate the effectiveness of comprehensive oral care on knowledge and practice regarding oral health among selected school children. It was based on Wiedenbach's Helping Art of Clinical Nursing Theory. This study employed at Pre Experimental – One Group Pre Test & Post Test Design. Samples were selected using Purposive sampling method. This study was conducted at selected schools Madurai 14. Totally 120 students were included in the study. Comprehensive oral care was given to the participants for 12 weeks. Pre test oral health knowledge and practice mean were 25.3 and 31.1, standard deviations were 1.38 and 1.41 also mean % were 25 and 31 respectively. Post test oral health knowledge and practice mean were 83 and 87.3 and standard deviation was 1.07 and 1.15 respectively. (P value 0.000) school children who are taken comprehensive oral care by the trained health personal for 12 weeks had a statistically significant in improving knowledge and practice regarding oral health.

Keywords: oral health, oral care, school children, comprehensive care

INTRODUCTION

The mouth is one of the chief portals of entry of disease causing bacteria and is an ideal incubator for germs. It is an entrance to both respiratory and alimentary tract, a double gateway needing double guarding. Care of teeth and mouth helps to prevent tooth decay, gum disease, parotid gland infections and mouth sores. The survey, which was conducted by Indian Market Research Bureau across India with the aim of finding out about general standards of dental health and the nature of dental habits in the country. Amongst the most shocking of revelations is that nearly



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half of Indians do not use a toothbrush; only 51 percent of people surveyed said they regularly brushed their teeth using a toothbrush and toothpaste. Keeping this issue in mind the investigator aimed to conduct the effectiveness of comprehensive oral care on knowledge and practice regarding oral health among school children.

STATEMENT OF THE PROBLEM

A Study to evaluate the effectiveness of comprehensive oral care on knowledge and practice regarding oral health among school children At Madurai.

OBJECTIVES OF THE STUDY

1. To assess the pretest level of oral health knowledge and practice among selected school children
2. To evaluate the effectiveness of comprehensive oral care on knowledge and practice regarding oral health among selected school children
3. To correlate the relationship between oral health knowledge and practice among selected school children
4. To associate the pretest test level of oral health knowledge and practice with selected Demographic variables.

HYPOTHESES

1. **H1:** There is a significant difference between pretest and post test oral health knowledge and practice score among school children
2. **H2:** There is a significant association between pretest test level of oral health knowledge and practice and selected demographic variables.

MATERIALS AND METHODS

The research study was employed with quantitative research approach. The research design was pre experimental research design. The one group pretest and posttest design was adopted for this study. Totally 120 school students aged between 12-17 years were participated in this study. Purposive sampling technique was used and school children who satisfied the inclusion criteria were selected for this study.

SELECTION AND DEVELOPMENT OF STUDY INSTRUMENT

The tool used for data collection was an interview technique. It consists of three parts:

Section – A - Demographic Data

It included the student's Age in years, Gender, Religion, Educational status of parent, Occupation of parents, Income in rupees per month, Type of family, Area of living and food habits and type of drinking water.

Section – B

Questionnaire regarding Assessment of oral health Knowledge of school children

Section C

Questionnaire regarding assessment of oral health practice of school children

DATA COLLECTION PROCEDURE

Formal written permission for conducting the study was obtained from school administration. All participants were informed about the study. Informed consent in written form was received from participants and parents. 120 study participants who met the inclusion criteria were selected by using purposive sampling technique. Pre test assessment was done. The data regarding demographic variables, oral health knowledge and practice were assessed by self administered questionnaire. Comprehensive oral care was initiated to the study participants. Care of teeth with brushing by two times, flossing, mouth wash and regular oral checkup by health personnel were implemented and



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practiced by the participants for 12 weeks with constant supervision. Post test oral health status was assessed with same tool after 12 weeks. Data were analyzed for the findings.

DATA ANALYSIS AND INTERPRETATION**SECTION A- Distribution of students based on their demographic variables**

Table 1 denotes that most (82%) of participants were male and 18 % were female. Majority of the participants were belonged (79 %) to Hindu religion. 35 % of students parent were educated up to primary class only. 43 % of participants parent were daily wages. Most of the participants (71%) parent income was less than 5000 rupees per month. Majority (47 %) of the participants were belonged joint family. Vegetarian and non vegetarian were equally (32) participated. Most (63 %) of the participants were belonged village. Majority (73 %) of participants were taking drinking water from tap water source.

Section B**Evaluate the Effectiveness of Comprehensive oral Care by Comparison of Pretest and Post Test Oral Health Knowledge and Practice Scores among School Children**

Below table 2 reveals that pretest level of oral health knowledge and practice level of the participants. It was found that 53% were very poor and 40% were poor and 7% were average in knowledge and practice regarding oral health. No good or excellent. But in Post test the oral health knowledge and practice score showed that only 10% average and 40% good and 50 % were excellent. Below table 3 shows that pretest oral health knowledge mean 25.3 and standard deviation 1.38. Oral health practice mean 31.1 with standard deviation 1.41. Post test oral health knowledge mean 83 with standard deviation 1.07 and oral health practice mean 87.3 with standard deviation 1.15.

Below figure 1. showing pre test oral health knowledge and practice mean % were 25 and 31 respectively. It was found that Post test oral health knowledge and practice Mean % were 83 and 87 respectively. Below table 4. shows that 't' value of oral health knowledge was 31.22 and p value 0.000. 't' value of oral health practice were 34.43 and p value 0.000 Which indicates the study was highly significant.

Section C**Assess the Correlation between dental health knowledge and practice**

The above table 5 illustrates that there was a highly significant positive correlation had been found between oral health knowledge and practice among school children in pre test with 'r' value 0.19 and in post test 'r' value 0.695.

Section D**Distribution of demographic variables to find out association with Post test oral health knowledge and practice score**

Chi- square test results denote that there is a no significant association between post test oral health knowledge, practice and demographic variables.

Discussion Based On Objectives**The first objective was to assess the pre test level of knowledge and practice regarding oral health among school children**

The findings revealed that among the total number of 120 subjects. In Pretest 53% of the participant were with very poor oral health knowledge and practice and 40% were poor and 7% were average. No good or excellent.

The second objective was to evaluate the effectiveness of comprehensive oral care on knowledge and practice regarding oral health among selected school children

The post test findings of the study revealed that only 10% of the students were with average oral health knowledge and practice and 40% were with good and 50 % were having excellent oral health knowledge and practice. Pre test oral health knowledge and practice mean % were 25 and 31 respectively. It was found at Post test that oral health knowledge and practice Mean % were 83 and 87 respectively.





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Hence the hypothesis (H1) There will be significant difference between pre test and post test oral health knowledge and practice score was accepted.

- **The third objective was to correlate the relationship between oral health knowledge and practice among school children**

There was a highly significant positive correlation had been found between knowledge and practice in pre test with 'r' value 0.19 and in post test 'r' value 0.695.

- **The fourth objective was to associate the pretest test level of oral health knowledge and practice with selected Demographic variables.**

There was no association between pre test oral health knowledge, practice and demographic variables.

Hence the second Hypothesis

(H2) "There will be significant association between pre test oral health knowledge and practice scores and selected demographic variables" was rejected.

CONCLUSION

According to the results of this study, school children who are taken comprehensive oral care by the trained health personal for 12 weeks had a statistically significant in improving knowledge and practice regarding oral health. The comprehensive oral care is non invasive, free from side effects and highly feasible, the researcher concluded that it can be used as an effective intervention to improve knowledge and practice regarding oral health and prevention of oral related disease among school children.

Ethical clearance

Ethical clearance obtained from Institutional human ethical committee of Arupadai Veedu Medical College and Hospital, Puducherry.

Source of funding- Self

Conflict of Interest – I am doing my PhD in nursing at Vinayaka mission research foundation, Salem, India. As per curriculum I have to publish my original research at reputed journal. My specialty is M.Sc., Pediatric nursing. During my school health visit I have witnessing the majority of the school children were with poor oral health knowledge and practice. Keeping this mind the investigator conducted this research.

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Table 1 Frequency and percentage wise distribution of students according to demographic variables (n=120)

Topic	Demographic variables	Frequency	Percentage
Gender:	Male	98	82
	Female	22	18
Age(in years):	12	28	23
	13	26	22
	14	36	30
	15 and above	30	25
Religion:	Hindu	94	79
	Christian	12	10
	Muslim	4	3
	Others	10	8
Education of parents:	Non formal Education	26	22
	Primary	42	35
	Secondary	38	32
	Collegiate	14	11
Occupation of parents:	Unemployed	16	13
	Daily wages	32	43
	Private employee	38	32
	Govt. employee	14	12
Monthly income of parents :	Less than 5000	86	71
	Rs 5001-10000	18	15
	Rs10001-20000	14	12
	More than Rs.20000	2	2
Type of family:	Nuclear family	54	45
	Joint family	56	47
	Extended family	8	7
	Single parent family	2	2





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Topic	Demographic variables	Frequency	Percentage
Food habit:	Vegetarian	38	32
	Non Vegetarian	38	32
	Natural diet	36	29
	Fast food	8	7
Living place:	Village	76	63
	Town panchayath	8	7
	Town	18	15
	Corporation	18	15
Source of drinking water:	Well	6	5
	Lake	18	15
	Tap Water	88	73
	Osmosis Filter water	8	7

Table- 2 . Frequency and percentage wise distribution of pre test and post test Oral health knowledge and Practice among school children

Oral Health Knowledge and Practice	Pre test		Post test	
	f	%	f	%
Very poor	64	53	-	-
Poor	48	40	-	-
Average	8	7	12	10
Good	-	-	48	40
Excellent	-	-	60	50

Table-3. Comparison of Pre Test and Post Test Mean, SD and Mean% of Oral health knowledge and practice scores of school children N = 120

Overall	Max score	Pre test			Post test			Difference in mean%
		Mean	SD	Mean%	Mean	SD	Mean%	
Knowledge	100	25.3	1.38	25	83.0	1.07	83	58
Practice	100	31.1	1.41	31	87.3	1.15	87	56

Table- 4. Paired “t”-test assess the effectiveness of comprehensive oral care among school children N = 120

Overall	Post test		Pre test		‘t’-value	p-value
	Mean	SD	Mean	SD		
Knowledge	83	1.07	25.3	1.38	31.22	0.000***
Practice	87.3	1.14	31.1	1.41	34.43	0.000***

(*P<0.05 - significant and **P<0.01 & ***P<0.001 - Highly significant)

Table-5. Karl-Pearson test for the pre test and post test Oral health knowledge and practice N = 120

Overall (knowledge-practice)	‘r’-value	P-value
Pretest	0.19	0.14
post test	0.695	0.000***





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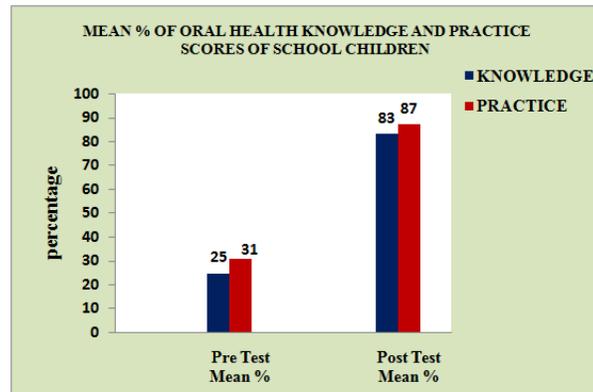


Figure 1. Pre and Post Test Mean % of oral health knowledge and practice among school children N = 120





Medical Device – A Regulatory View of Clinical Trials in EU

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ABSTRACT

Medical devices must be the machinery, tool, devices, implants, in-vitro calibrators or the software for the diagnosis, control, prevention or treatment of diseases for use in the healthcare sector. The safety of the public is a priority for the introduction of novel treatment devices for various diseases and their diagnosis. Industry and regulatory innovation collaborate to offer strategies used for the varied global marketplace also toward expand the value and protection of current strategies. Essential is to identify the identification of the real regulatory process that provides protection ethics besides additional governing conditions in particular country. Protection of the people is a top consideration when new medical gadgets are being launched to treat and diagnose a number of ailments. New industry and regulatory innovations work together to create devices for various international markets and enhance the quality and safety of outgoing devices on the market. Medical device studies differ considerably from clinical trials. The new EU legislation specifies that manufacturers must produce a complete summary for their evidence for any hazardous items. For maximal informed decision making to employ innovative medical technologies, complete openness is important. This report gives an overview on medical device testing, medical device categorization in Europe, the regulatory framework for medical device approval European Union.

Keywords: Medical devices, clinical trials, European union, regulatory framework of medical device, EU legislation.





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INTRODUCTION

Clinical trials, which strive to increase therapeutic information, enduring treatment, then bring confidence intended for groups to come, would be impossible to conduct without the participation of humans. [1]

As specified by the World Health Organization:

A medical experiment is an investigative learning in which social volunteers or sets of individuals are randomly assigned to one or more well-being-connected interferences in order to assess the effects on health outcomes. Medication, cells and other biological products, operations, radiological procedures, technology, behavioural therapy, care, etc, improvements, and prevention are all examples of interventions.

The following are some definitions of a clinical trial/investigation:

According to the 2017/745 Medical Device Regulation (EU) (MDR), a "clinical investigation" is "any systematic examination connecting one or more human subjects conducted to assess the device's safety or performance." The European Union Commission. The Medical Equipment Directive governs medical devices and Medical Device Regulation in Europe to protect patients and users (MDR). The MDR makes an explicit reference to ISO 14155:2011 in Article 64 with regard to clinical studies. (Clinical research of human medical devices - Good clinical practice) European Union Commission.

Definition of a Medical Device

The medical device (MD) is a type of health care invention that is becoming increasingly important enduring maintenance. The term "medical device" is moderately broad and encompasses a wide from basic bandages and thermometers to sophisticated equipment like computers, software, and implants. These products are used to diagnose, monitor, prevent, and help disabled individuals, as well as to treat acute and chronic diseases.

A medical device is defined by MDR (EU) 2017/745 in Europe as:

The MDR defines a medical device as "any instrument, equipment, appliance, software, implant, reagent, substance, or other object intended by the maker to be used, alone or in combination, for one or more of the following particular medical purposes:" - illness diagnosis, prevention, monitoring, prediction, prognosis, treatment or alleviation, injury or disability diagnosis, monitoring, treatment, relief, or compensation, investigation, replacement, or alteration of anatomy or a physiological or pathological process or condition, providing information through in vitro examination of specimens derived from the human body, including organ, blood, and tissue donations, and which does not achieve its primary intended action in or on the human body through pharmacological, immunological, or metabolic means, but which may be aided in its function by such means' European Commission.

Medical Device Classification

In Europe, the categorization of medical devices is divided into four degrees of safety.

- Class I - low risk
- Class IIa - medium risk
- Class IIb - medium risk - high
- Class III - high risk

The organization guidelines of the instruction examine the determination and the degree of risk to the patient or to the person who deals with the medical device. The group is designed to safeguard that acceptable administration and justification are in place. It's also looking into how long the medical device stays in the body and how invasive it is. [2] In order to demonstrate compliance with the new MDR the MD classification determines which conformity assessment route producers are required to do. The digression and control over the device by external parties is linked to the degree of risk. Defines formalized Emergo (2018).

DISCUSSION

Medical Devices Regulation

The European Commission, in near collaboration with Competent Authorities from Member States, is in charge of harmonizing regulations and regulating medical devices. Medical devices that are implantable, non-implantable or used in vitro diagnostics are all covered by legislation. The MDDs were phased out in favour of the MDR, which was



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approved on April 5, 2017. It is critical to understand the difference between a Directive and Regulation. The restrictions are quite specific and well-defined norms that are obligatory across all member countries, whereas Instructions have been adopted by the EU Legislature and implemented each member state must incorporate it into national legislation. Market of currently certified medical devices will have three years to meet the standards while IVDR manufacturers will have five years. [3] Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices, amending Directive 2001/83/EC, Regulation (EC) no. 178/2002, and Regulation (EC) no. 1223/2009, as well as recalling Council Directives 90/385/EEC and 93/42/EEC. The European Parliament and the Council adopted Regulation (EU) 2017/746 on in vitro diagnostic medical devices on 5 April 2017, repealing Directive 98/79/EC and Commission Decision 2010/227/EU. In Europe, the CE mark is necessary to allow free movement of items on the European market. Businesses must verify that their goods fulfil health, safety, and environmental protection criteria in order to receive the CE mark. [4]

The new guidelines feature a number of significant enhancements that will help to modernise the existing system. Stricter restrictions for high-risk gadgets are among them, thanks to a new pre-market review procedure comprising an EU-wide pool of specialists. Reinforcement of designation criteria and mechanisms for supervision by Notified Bodies. Certain cosmetic devices with the same features and risk profile as similar medical devices are included within the scope of these laws. Implementation of a new risk categorization system for in vitro diagnostic medical devices in compliance with international advice. Transparency was increased by developing a comprehensive EU database on medical devices and a device tracking system based on Unique Device Identification. The European Commission has proposed the use of an "implant card" that contains information on a patient's implanted medical equipment. Post-market surveillance obligations for manufacturers are being tightened. Improved EU-wide coordination tools for vigilance and market surveillance. The MDR intends to emphasise transparency and traceability more. The emphasis on clinical evidence and, in particular, the standardisation of European processes to ensure a more coordinated approach to clinical study approval. The focus on post-market surveillance ensures that the product is evaluated throughout its lifespan. [5]

Modification Reasons From Medical Devices To Medical Device Directives Regulation**Change from Directives to Regulation**

More specific criteria for clinical data and research are among the important changes introduced in the MDR. In the past, clinical rationales based on the equivalence of the device have been common practice but with the MDR equivalence it is less acceptable, particularly for higher risk gadgets. The producer is needed to show similarity by access to equivalent device data, and the MDR needs a contract between the manufacturer and the equivalent device maker to have access to technical documentation for that device. This implies that equivalence may only be requested for products for which a manufacturer has access to technical documentation from the European Commission. [6]

According to the MDR:

When clinical evidence is based largely or entirely on data from devices that are claimed to be similar to the device being assessed, the notified body should examine the appropriateness of the use of such data, taking into consideration variables such as new indications and innovation. The notified authority shall clearly record its conclusions about the claimed equivalency as well as the relevance and sufficiency of evidence to establish compliance. The notified body must assess the extent to which special claims are supported by particular pre-clinical and clinical data, as well as risk assessments, for any feature of the device claimed by the maker to be unique or for novel indications. The notified body checks the appropriateness of the clinical evidence and clinical evaluation, as well as the manufacturer's findings on conformity with the applicable general safety and performance standards. The verification should involve a review and appropriateness of the proposed PMCF plan, when applicable, to examine the sufficiency of the benefit-risk determination, risk management, instructions for use, user training, and the manufacturer's post-market monitoring strategy.



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The public will be allowed to access Eudamed, the European database on medical devices. Until the MDR, the Eudamed database functioned as an information tool, accessible to national competent authorities and the European Commission, and was used by European authorities for post-market monitoring. Despite the fact that clinical evidence is not a new need, the MDR has introduced a slew of additional clinical investigation criteria. Under the MDD, clinical evaluation reports (CERs) were required for lower risk devices, whereas clinical data was required for higher risk devices. CERs are still necessary, but the content and acceptability of CERs has altered. The first sentence of Annex XIV of the MDR says, "To organize, conduct, and document a clinical examination." The European Union Commission. A clinical development plan demonstrating the evolution from exploratory research, such as first-in-man studies, feasibility and pilot studies, through confirmatory studies, such as pivotal clinical investigations, and a PMCF as cited. The bulk of modifications to current European regulations are the result of popular desire for improved patient protection. 'In the past, high-risk technologies, such as implants, had considerably shorter approval processes on average. [7]

Clinical Evidence For Medical Devices

To comply with the MDR's essential requirements, known as general safety and performance requirements (GSPR) clinical data, as defined in the MDR, must be evaluated by a manufacturer. This clinical data could come from the device being evaluated or from a device that has been shown to be 'equivalent' to it. With the MDR, the clinical, technological, and biological elements that must be addressed in order to show equivalency were written into European legislation for the first time. The device under review and the claimed equivalent device must be similar to the degree that there is no clinically significant difference in the safety and clinical performance of the devices. [8]

Clinical Trial Designs

Clinical trials are classified into two types: observational studies and experimental trials.

Observational Studies

Nothing else or any treatments are required of participants in observational studies. Researchers simply measure specific aspects in groups of individuals, typically to learn about potential methods of prevention. Researchers' interests vary greatly from one study to another in measuring diverse aspects of general public health and welfare and evidence about themevery day dietary activities, for example and practise. Researchers may only need to measure them once or need to follow individuals done time to watch how things progress that are measured alter over time can vary between groups of individuals.

Interventional Trial

Interventional studies mean that the investigator intervenes at various stages throughout the study, as the name indicates. The most prevalent and powerful interventional studies were the randomised controlled trials, commonly referred to as experimental studies. Examples of pre-post study designs, non-randomized measured testing, and quasi-trials are examples of interventional studies. [9]

Clinical Trial Roles And Responsibilities

It is important that clinical trials include all relevant stakeholders. Depending on the trial, additional roles and duties may be necessary. The organisations and persons listed below are essential to clinical trials.

The subject or participant: The individual who participates in the experimental.

The sponsor: The organization or individual who is funding the experimental; it could be a company of pharma or equipment, theoretical, doctor, or clinic.

The principal investigator: The individual in charge of the investigatessite, who is usually a surgeon or a disease expert.

The study coordinator: The individual who assists the primarydetective, who is in responsible forthe trial daily source and organizing the various individuals or organizations complicated in the training.



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The Ethics Review Board/Investigational Review Board:The individual organization in charge of ensuring the truths, wellbeing, and security of those taking part in the experimental. Before the trial can begin, they must approve information about how it will be conducted.'A rare occurrence.

Clinical Trial Process For European Medical Devices

A clinical trial is always required when conducting research on new drugs. Small changes to a drug's composition may result in unexpected effects, necessitating the collection of clinical data before the changes can be implemented, according to the European Medicines Agency. Clinical trials of medical devices are not continuously necessary, and a hazard calculation determines whether or not one is performed.[10] Even though a bandage is a medical device, it presents no danger to people and hence does not require a clinical study. According to MDD, the trial of implantable and long-term invasive devices of class IIa or IIb can commence within 60 days of notice to the relevant authorities, except where a decision to the contrary based on the principle of public health has been notified. Commission of Europe. Under the MDR, Member States should, in the absence of any unfavourable judgments of the Ethics Committee, notify the patron of their decision within 45 days. The Member State may extend the term of 45 days by additional 20 days where consultations are necessary. European Union Commission.

Conducting The Clinical Trial

In conformity with the requirements established in the harmonised standards, EN ISO 14155, the scientific examination must be conducted in accordance with 2011 – Clinical study of human medical devices – Good clinical practise. The European Commission's website contains EN ISO 14155:2011 as a harmonized typical (2018g). The harmonized standard is a European standard established on request by the European Commission to generate a European Standard that offers solutions to fulfil the legislative need Cenelec recognised as a European Standards Organization (2017).[11] ISO 14155:2011 lays forth the design, conduct and reporting standards for human participant clinical studies. It was first released in 1996 and has received considerable review over the previous 20 years to address the unique research needs for medical equipment and better connect the criteria with the Good Clinical Practice (GCP) recommendations of the International Conference on Harmonization (ICH). The existing standard ISO 14155:2011, replacing parts 1 and 2, of ISO 14155:2003, is now closely matched to GCP recommendations.

Clinical Trial Guidance Documents For Medical Devices

A number of guidance publications are supported by the European Commission to help participants execute directives and rules on medical equipment. For producers and notified bodies participating in compliance assessment procedures, MEDDEVs encourage a consistent approach. MEDDEVs are established responsible for protection community strength in partnership with all members, including manufacturing organisations, well-being experts, Notified bodies and European Normalization Organizations. MEDDEVs are carefully drawn out and regularly revised following significant collaboration with all stakeholders. These materials are unique and approved by the Medical Devices Expert Group plenary meetings (MDEG). The strategies do not apply lawfully. The above investors and specialists from the competent establishments, however, should be involved in ensuring the uniform implementation of the applicable directive requirements, following the recommendations. They shall thus be followed 'European Union Commission.[12]

Informed Consent

All relevant information, including risks and benefits, must be given to every human subject that participates in a clinical trial. It is so crucial that all features of the clinical study be thoroughly understood and freely participated. 'Informed consent means a subject's free and voluntary expression of his or her willingness to participate in a particular clinical investigation, after having been informed of all aspects of the clinical investigation that are relevant to the subject's decision to participate or, in the case of minors and of incapacitated subjects, an authorisation or agreement from their legally designated representative to include them in the clinical investigation' European Commission.



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The MDR sets out certain conditions, such as documented, dated and signed consent of the interviewee as well as of the individual participating in the interview. If a person cannot agree, a legal demonstrative will sign on behalf of the participant. If a participant cannot write, an alternate technique may be used to provide the consent in the occurrence of at least one independent observer. [13] In this scenario, the observer should sign and date the notified consent. The full explanation of the nature, aims, advantages, implications, hazards of the clinical study should be communicated by all material presented to the participant or legal representative. It should also outline the rights and safeguards of the participants, in particular the ability to decline or reject the trial. In addition, the circumstances and period of the study, treatment options and any follow-up action should be addressed when the experiment has ended. Inclusive, succinct, clear, relevant and intelligible information should be supplied to the participant or the representative of the court. During a meeting with a participant of the investigative team of qualified individuals to domestic legislation, the information must be supported in advance.

Differences Between Medical Device And Drug Clinical Studies

An Ethics committee must approve both medical device and drug clinical studies, and medical investigations must undergo scientific and ethical scrutiny. According to the MDR: 'The ethical review shall be performed by an ethics committee in accordance with national law. Member States shall ensure that the procedures for review by ethics committees are compatible with the procedures set out in this Regulation for the assessment of the application for authorization of a clinical investigation. At least one lay person shall participate in the ethical review' European Commission.[14]

The necessary documents are comparable to those needed in the pharmaceutical research for an examination of the medical device. For a medical device clinical research, the phrase "clinical research plan" usually refers to the protocol of the study. The regulatory requirements for medical device clinical studies differ from the regulations for drug products, which have an effect on their clinical research design. The efficiency of the device is not legally necessary to receive a CE mark. The clinical trial attempts to show a medical device's safety and performance (conformity with claims). Drug research is to determine the safety and effectiveness of the medical product. Consequently, in medical device tests case numbers are generally lesser than in therapeutic tests. Therefore, the stage of a clinical trial which needs to be finished properly for CE marking is equivalent to Phase II when phase II drug development phase II requires confirmation of clinical activity of the medicine rather than phase III.

CONCLUSION

CE marketing or certification for every product to be marketed is obligatory in the European Union. All medical equipment is controlled by notified institutions. These notified authorities guarantee conformity with standards of quality and safety and approve CE marketing equipment. Directive on medical devices, minimise patient risk by reducing the marketing to doctors with required training & experience of innovative high-risk equipment with minimum clinical data. The new European laws should mandate the clinical efficiency and safety of pre-masks by means of a randomised controlled clinical study if possible. Every participant has to make efforts to improve the performance and security of new development devices to cure different types of ailments during clinical research such as researches, device production and government regulators.

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Table 1: Clinical Trial Classification of Drugs and Devices

Drugs	Devices
Phase 1 Safety and tolerance Healthy volunteers (20-100) subjects Major adverse effects	Pilot: Smaller population (10-30) subjects Preliminary safety and performance information
Phase 2 Safety and effectiveness Small population (50-200) subjects Approve dosing and major adverse effects	Pivotal: Larger population (150-300) subjects Control effectiveness and adverse effects
Phase 3 Safety and effectiveness Large population (100s to 1000s) subjects Control drug-drug interactions and minor adverse effects	Not Applicable
Phase 4 Post approval study Collect long-term data and adverse effects	Post-Approval Study: Collect long-term data and adverse effects





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Table 2: Clinical Trial Design of Drugs and Devices

Drugs	Devices
Randomisation is common Control group Large populations include placebos May compare to other approved therapies Ability to blind Difficult to visualise	Often no randomisation Control group Small population rarely uses placebos May compare to other approved therapies Difficult to blind Visualisation often included

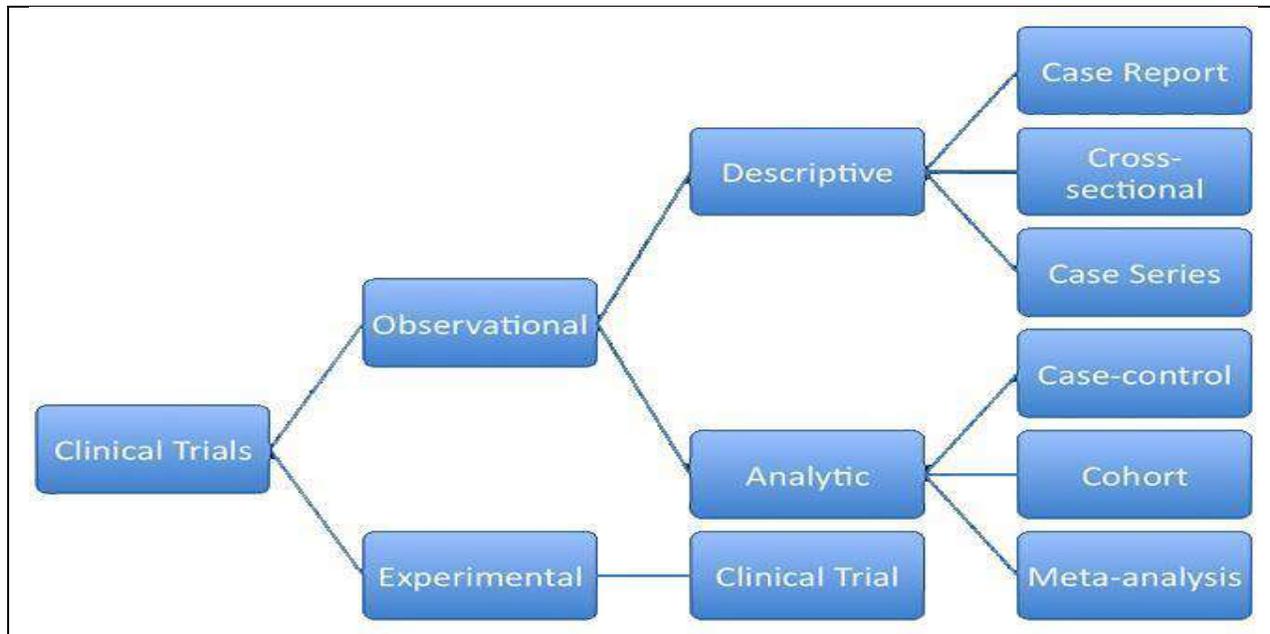


Figure 1: Clinical trial classification

Study design	Measures of disease	Measures of risk	Temporality
Ecological	Prevalence (rough estimate)	Prevalence ratio	Retrospective
Proportional mortality	Proportional mortality Standardized mortality	Proportional mortality ratio Standardized mortality ratio	Retrospective
Case-crossover	None	Odds ratio	Retrospective
Cross-sectional	Point prevalence Period prevalence	Odds ratio Prevalence odds ratio Prevalence ratio Prevalence difference	Retrospective
Case-control	None	Odds ratio	Retrospective
Retrospective and prospective cohort	Point prevalence Period prevalence Incidence	Odds ratio Prevalence odds ratio Prevalence ratio Prevalence difference Attributable risk Incidence rate ratio Relative risk Risk ratio Hazard ratio	Retrospective only Both retrospective and prospective Prospective only

Figure 2: Observational study design measures of disease, measures of risk, and temporality





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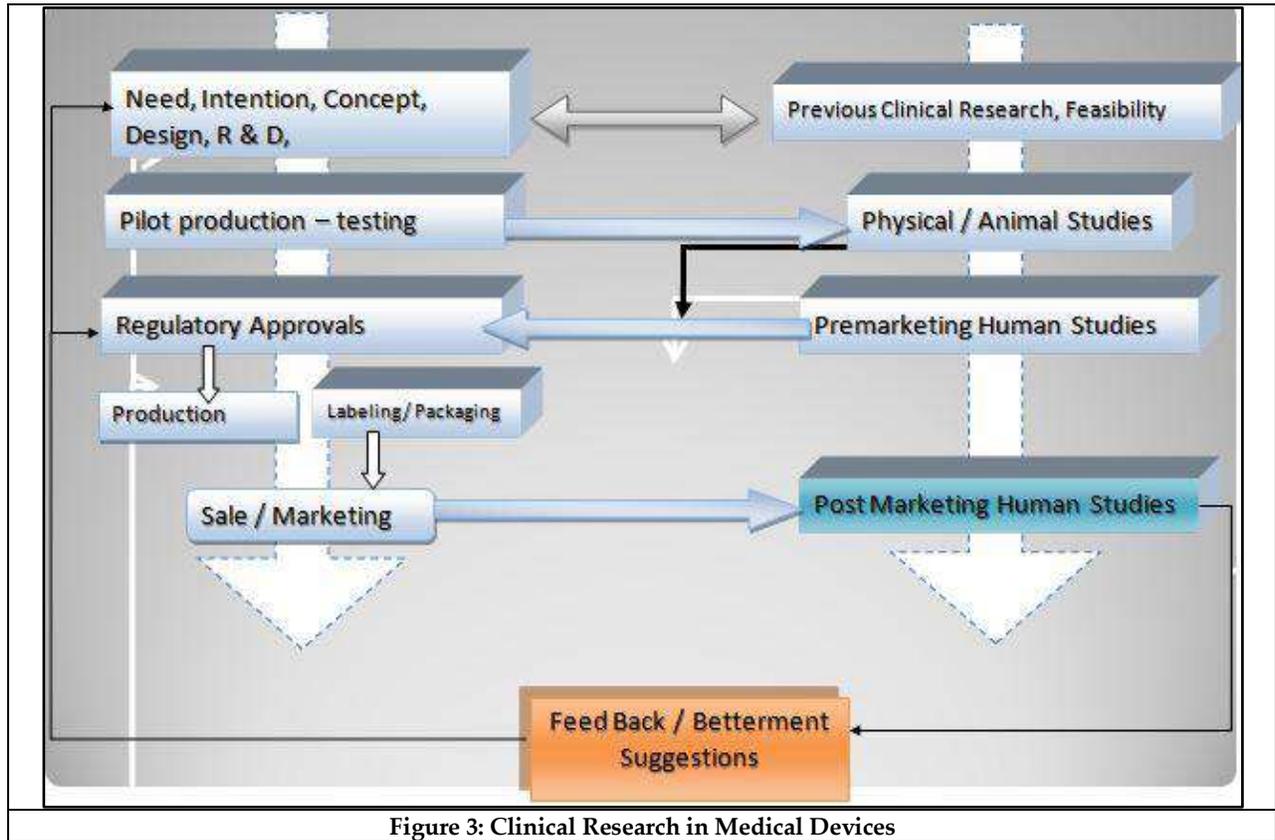


Figure 3: Clinical Research in Medical Devices





Role of *Ganoderma lucidum* (Curtis) P. Karst. as an Anti-Aging Medicine

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ABSTRACT

Several studies have demonstrated how to combat the negative effects of oxidative stress. In fact, biological age as we know is determined by a number of factors, including mental, hormonal, immune, and genetic factors, as well as lifestyle factors. When there is an imbalance between the production of oxidizing molecules (ROS) in the body and antioxidant defence, a condition known as "oxidative stress" develops. It is a key pathogenic mechanism of functional changes that can result in the most common degenerative pathology. Measuring "oxidative stress" is a great predictor of people's health and wellness, and it may be helpful to understand ageing processes as well. Anti-aging drug seeks to slow the process of aging and decrease the incidence of neurodegenerative disorders. *Ganoderma* harbors variety of supplements, vitamin complexes, nutrition as phytotherapeutic, nutritional supplements, homeopathic, and cosmetic products capable of activating and its rapid ability to heal skin problems. The goal of this article is to describe how the supernatural mushroom *Ganoderma lucidum* (Curtis) P. Karst. has the potential therapeutic value given to the presence of secondary metabolites.

Keywords: *Ganoderma lucidum*, Reishi Mushroom, Ageing, Reactive Oxygen Species, Anti-Oxidant

INTRODUCTION

Ageing is a stage of life that everyone goes through [1]. The global population is rapidly ageing, with the proportion of persons 60 and over expected to reach 22 percent by 2050, up from 11 percent in 2006 [2–3]. The world's elderly population is expected to outnumber children under the age of five by 2050, and the world's ageing population will





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attain two billion within the next 50 years.[3] With 140 million people aged 60 and up, India has the world's second largest ageing population (compared to 250 million people in China).[4] Furthermore, the elderly population's average annual growth rate is nearly three times that of India's overall population growth rate.[5] According to the underlying population dynamics, the proportion of India's population aged 60 and up would more than double, from 9% in 2015 to 19% in 2050. (Context 1). This would result in a population of over 320 million older people, roughly equal to the current population of the United States [4]. Globally, the proportion of older people is rapidly increasing as a result of increase in life expectancy, fertility rate, and large groups attempting to reach old age. Because of this demographic change, the World Health Organization and also the United Nations declared 2021–2030 the "Decade of Healthy Aging," a research, policy development, and outreach innovation designed to help communicate effectively the experience and needs of ageing. The public health ecosystem is being impacted by an ageing population [6].

Many issues arise as a result of the ageing population. While many people welcome the prospect of a longer life, it also brings with it a need for more healthy years instead of more years of disability & disease. Furthermore, health varies greatly among the elderly, with the some 80-year-olds having the same physical and mental ability as many 20-year-olds, whereas others experience a decrease in physical and/or mental ability [7]. There are several biological causes of ageing theories that contend that many different mechanisms contribute to the ageing process.[8] According to Kirkwood, the underlying cause is primarily due to the accumulation of random unrecovered molecular damage over time. This ultimately leads to cellular deformities and tissue dysfunction leading to increased inadequacy and age-related diseases. [9] Our cells have quality management systems in place such that molecular destruction can be recognized, repaired or removed. However, because of the energy demands of these systems, somatic maintenance is inefficient. All molecular components are vulnerable to damage, including DNA, proteins, fats, and organelles. Internal sources of damage include oxygen radicals and reactive nitrogen species (RNS), as well as external sources such as UV light, radiation, and toxins. In the context of ageing, exposure to sources of damage over a person's lifetime will vary between individuals, which may explain some of the variation in age [10].

Many studies on the molecular mechanisms of ageing have focused on specific principles like somatic mutation buildup, telomere shortening, protein damage, as well as mitochondrial dysfunction. However, it was realised in the late 1990s that individual pathways may not always be able to effectively describe the ageing process. And we must consider how these various mechanisms interact with one another. Damaged mitochondria, for example, produce more ROS, which results in increased impairment to all molecular components. The challenge of analyzing complex interactions inspired among the first unified mathematical methods of ageing, which led directly to a network theory of ageing [11]. Everything in nature follows a very precise logic that determines a perfect balance, and it is even feasible to differentiate biological phenomena in same living organism. They work together to allow normal functioning under usual circumstances and represent their adaptive capacity to modify the environmental conditions in which they live. O₂ and its interactions to biological systems have undoubtedly presented us with one of the most perplexing paradoxes. On the one hand, oxygen is required for vital events, but it also leading to the generation of so-called superoxide anion. In their electronic configuration, these radicals lack a paired electron, – i.e., an electron that occupies a specific orbital exclusively without sharing it with some other electron with the opposite charge. This is a state of high thermodynamic instability in which oxygen achieves a steady - state value by catching an electron from the nearby structures. It is obvious that this behaviour would result in a cascading mechanism because structures lacking electrons would be unstable, so they would tend to capture electrons from molecules, thereby broadening the phenomenon [12].

By raising lipid peroxidation and initiating disease processes, free radicals can cause severe and irreversible destruction to proteins, DNA, as well as biological membranes.[13–15] Under physiological conditions, the generation of oxygen free radicals does not increase 5% of the o₂ introduced with respiration; such concentration level do not constitute a specific pathogenic element. Whenever the production of these materials rises and exceeds the ability of our body's naturally found antioxidant defences, we experience oxidative stress. [16] The cell has a number of defences that defend it from oxidative damage caused by radicals and allow them to be eliminated or





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neutralized [17]. The antioxidative defense system is distributed throughout the body on a regular basis, both extracellular environment and intracellularly. Food can supply all proteins, including albumin, bilirubin, uric acid, and cholesterol, as well as various exogenous antioxidants, including ascorbate, tocopherols, and polyphenols, to plasma. The antioxidative defense system has its own concise compartment within the cell [18]. In actuality, the antioxidant system consists of a number of enzymes (superoxide dismutase, catalase, and peroxidase) as well as a variety of externally obtained chemicals (vitamins and substances similar in antioxidant activity, such as polyphenols, trace elements, etc.). A few of these compounds are liposoluble (e.g., tocopherol) and serve as first line defense against free radical attack once they enter the biomembrane structure. Others, such as ascorbate, are water soluble and primarily interfere with the cytoplasm and soluble matrix of cellular organelles. All of the components work together to maintain the equilibrium of antioxidant defences. In fact, a decline in antioxidant defense is mostly attributable to a loss of antioxidants, either absolute or relative [19].

Almost always, ageing is accompanied by a decrease in physiological function, which increases susceptibility to age-related diseases. It's an unavoidable physiological phenomenon, but the underlying mechanism has yet to be discovered, despite the passage of several decades. The oxidative stress and free radical buildup theories stand out among several theories associated with ageing. As people get older, their antioxidant system deteriorates, disrupting the delicate balance among radical oxygen species formation and removal, resulting in oxidative cellular damage [20]. Post-mitotic tissues, namely the brain, heart, as well as skeletal muscle, age more rapidly than other organs [21]. Cardiovascular contraction is highly dependant on oxidative phosphorylation and also the mitochondrial electron transport chain. Their dysfunction can cause an increase in oxygen radicals (ROS) production to an unhealthy level, resulting in structural and functional changes in the cardiac muscle, such as cardiac atrophy or compensatory hypertrophy, that also induce cardiac ageing [22]. The role of the novel natural substance *Ganoderma lucidum*, which can significantly avert oxidative stress, which has been reviewed in this study. Moreover, the article leads to gain insight into *Ganoderma lucidum*'s mechanism of action on anti-ageing issues as well.

Ganoderma lucidum

Ganoderma lucidum (Curtis) P. Karst. is a white rot fungus known in China as "ling zi," "ri shi" or Reishi in Japan, & "youngzi" in Korea. *Ganoderma lucidum*'s medicinal effects were first documented in the "Herbal Classic of Shen Nong" in 100 BCE, and had been noticed to improve health, increase vigour and vitality, and increase lifespan. [23] *Ganoderma lucidum* (Curtis) P. Karst. is a bitter-tasting mushroom with a woody consistency. This fungus is composed of approximately 90% water. The remaining 10% is made up of 10–40% protein, 2–8% lipids, 3–28 % carbohydrate, 3–32 percent fibre, 8–10percent ash, and vitamins & minerals such as selenium, potassium, calcium, phosphor, magnesium, iron, zinc, as well as copper. [24–25] Fruit bodies, mycelium, and spores contain the majority of the active components, which include polysaccharides, triterpenes, and peptidoglycans.[26] A number of preclinical and clinical studies on reishi have been conducted, recognising its numerous medicinal qualities, which are attributed to the variability of polysaccharides found in the fruit body and the spores.[27] Although *Ganoderma lucidum* (Curtis) P. Karst.had already been used as an elixir for ancient times, studies revealing anti-ageing and lifespan extension effects are just the tip of the iceberg. It is unknown whether *Ganoderma lucidum* (Curtis) P. Karst. has anti-ageing properties. As a result, the purpose of this review is to fully elucidate *Ganoderma lucidum*'s potential mechanism underlying anti-ageing effects in order to encourage its clinical utility as an anti-ageing herbal medicine [28].

Bioactive constituents in *Ganoderma lucidum*

Ganoderma lucidum (Curtis) P. Karst. contains a variety of active compounds, which include triterpenoids, polysaccharides, growth hormones (steroids), essential fats, amino acids, nucleosides, protein, as well as alkaloids. Because of their abundant supply in fungi, diverse structures, and significant bioactivities, triterpenes and polysaccharides have received a lot of attention (Table 1).

Polysaccharides

Polysaccharides are extracted from *Ganoderma lucidum* (Curtis) P. Karst. mycelium, fruit body, and fermentation liquid. Various components, structures, molecular mass, and consequences of *Ganoderma lucidum* (Curtis) P. Karst.





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polysaccharides distinguish the various developmental phases of *Ganoderma lucidum* (Curtis) P. Karst. (GLPs). The mycelium contains the most polysaccharides, while the fruit body contains the least. Fruit bodies contain mainly glucose and galactose, whilst the mycelium and spores contain primarily glucose [36]. *Ganoderma lucidum* (Curtis) P. Karst. Polysaccharides isolated from mushroom fruiting bodies may have anticancer effects via immunomodulation. *Ganoderma lucidum* (Curtis) P. Karst. polysaccharides extracted from fruiting bodies may have anticancer effects via immunomodulation. A group of polysaccharides with molecular weight range from 4×10^5 to 1×10^6 Daltons discovered in *Ganoderma lucidum* (Curtis) P. Karst. fruiting bodies and mycelia.[37] The basic structure of *Ganoderma lucidum* (Curtis) P. Karst. polysaccharide is a high-molecular-weight β -(1,3)-d-glucan (1,6)- β -d-glucosyl branching and the main polysaccharide constituents of *Ganoderma lucidum* (Curtis) P. Karst. Mannose, galactose, glucose, and rhamnose are among the sugars [38]. Polysaccharides in *Ganoderma lucidum* (Curtis) P. Karst. are most significant contributors to its bioactivity as well as medical uses. According to reports, these polysaccharides have antioxidant and immunomodulatory characteristic [39].

Triterpenoids

More than 200 triterpenoids have been isolated from *Ganoderma lucidum*(Curtis) P. Karst. fruit bodies, spores, and mycelia [40]. *Ganoderma lucidum*'s fruit body contains a high concentration and variety of Ganoderma triterpenoids, whereas the mycelium contains a few Ganoderma triterpenoids species. Ganoderma triterpenes have not been found in non-cracked *Ganoderma lucidum* (Curtis) P. Karst. spores [41]. There have been no reports directly linking *Ganoderma lucidum* (Curtis) P. Karst. triterpene's anti-aging effect, but its antioxidant effects indicates that *Ganoderma lucidum* (Curtis) P. Karst. triterpene may have a possible lifespan extension effect. Triterpenes from *Ganoderma lucidum* (Curtis) P. Karst. can prevent the body from oxidative stress-induced protein as well as lipid peroxidation [42]. Ganoderma triterpenoids are classified as ganoderic acid, ganoderiol, ganoderon, ganolactone, as well as ganoderyl based on their functional groups and side chains [43]. The very first triterpenoids extracted from *Ganoderma lucidum* (Curtis) P. Karst. were ganoderic acid A & B [44–45]. More than 100 triterpenoids with known chemical compositions and molecular arrangements have been found in *Ganoderma lucidum* (Curtis) P. Karst. since then. Over than 50 of these have been found in this mushroom. The large percentage are ganoderic and lucidenic acids, but other triterpenes like ganodelar, ganoderiol, and ganodermic acids were also discovered [46-47].

Proteins and polypeptide

Ganoderma lucidum(Curtis) P. Karst. has been linked to a number of bioactive proteins. Ling Zhi-8 is a polypeptide that contains 110 amino acid residues and an acetylated amino terminus [48]. The sequence & anticipated secondary structure of LZ-8 seem to be similar to those of the immunoglobulin heavy chain's variable region. LZ-8 was identified as first immunomodulatory protein derived from *G. lucidum* mycelial extracts using chromatographic and electrophoretic techniques [49]. Proteins and peptides are involved in a variety of important functions and systems in the body which are required for survival. Proteins and peptides also play important roles in diseases like cancer and diabetes. As a result, the use of peptides and proteins as medications is viewed as an appealing approach to combating a variety of diseases [50].

Vitamins and minerals

Several vitamins, including vitamins B1, B2, B6, beta-carotene, C, D, and E, have been reported from *Ganoderma lucidum* (Curtis) P. Karst. Calcium, sodium, potassium, phosphorus, iron, carbon, magnesium, as well as zinc are also present. *Ganoderma lucidum* (Curtis) P. Karst. contains chromium, arsenic, copper, manganese, silicon, aluminium, cobalt, molybdenum, nickel, and lead [51].

Enzymes

Laccase, β -1,3-glucanase, α -1,2-mannosidase, endo- β -1,3-glucanase, and glutamic protease were extracted from *Ganoderma lucidum*, with glutamic protease being most abundant protein.^[52]

Amino acids

Ganoderma lucidum (Curtis) P. Karst. contained eighteen different types of amino acids, the most abundant of which was leucine, which had powerful hypoglycemic and antioxidant properties [53].





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Nucleosides

Ganoderma lucidum (Curtis) P. Karst. contains nucleosides such as thymidine, cystidine, uridine, guanosine, inosine, and adenosine [54].

Steroids

More than 20 different kinds of sterols have been discovered in *Ganoderma lucidum* (Curtis) P. Karst., with their skeletons classified as ergosterol and cholesterol [55].

Effect on Skin

Because of its anti-aging, anti-melanogenesis, as well as skin barrier-enhancing properties, *Ganoderma lucidum*(Curtis) P. Karst. has been a main functional ingredient in several salve formulations.

Anti-aging and Antioxidant activity

UV light is a major environmental factor in skin ageing, causing rough wrinkles, dryness, as well as sagging [56]. UVB radiation stimulates MMP-1 secretion while decreasing collagen and elastin synthesis, which can hasten skin ageing [57]. By inhibiting ERK pathways, *Ganoderma lucidum* (Curtis) P. Karst. extract can inhibit UVB-induced MMP-1 expression & increase procollagen expression.[58] After UVB treatment, *Ganoderma lucidum*(Curtis) P. Karst. Polysaccharides could inhibit MMP-1 protein expression, encourage C-telopeptide of type I collagen protein, as well as inhibit ROS production in fibroblasts [59]. Long-term exposure to free radicals and reactive oxygen species (ROS) accelerates ageing and so many age-related diseases [60]. The antioxidant activities of protein content extracted from *Ganoderma lucidum*(Curtis) P. Karst. mycelium & fruiting bodies were investigated. Proteins from both mycelia & fruit bodies were discovered to have antioxidant properties [61]. The Comet assay was used to evaluate ROS generation and oxidative stress markers. Furthermore, when compared to positive control, an ethanolic extracts of *Ganoderma lucidum* (Curtis) P. Karst. can significantly decrease H₂O₂-induced ROS production [62].

Anti-melanogenesis effects

Skin pigmentation is caused by an abnormal buildup of melanin. Tyrosinase is an enzyme which controls the synthesis of melanin. *Ganoderma lucidum*(Curtis) P. Karst.could reduce the activity of tyrosinase as well as tyrosine-related proteins, both of which are known to inhibit hyperpigmentation. In contrast, the cAMP-dependent signaling cascade regulates melanogenesis by inhibiting cell phosphorylation. Reduced expression of the microphthalmia-associated transcription factor (MITF) results in decreased melanin production [63]. *Ganoderma lucidum* (Curtis) P. Karst. was used to produce the active substance, Ganoderma mannitol. Ganodermanondiol (10 M) significantly reduced melanin content in B16F10 melanoma cells when compared to arbutin (0.5 mM). Furthermore, the inhibitory effect of ganodermanondiol on CREB phosphorylation contributed to the reduction in microphthalmia-associated transcription factor expression and also melanin production. Phosphorylation of ERK and c-Jun N-terminal kinase (JNK) reported to inhibit melanin production, whilst p38 phosphorylation raised MITF expression & melanin production. Ganoderma nondiol induced ERK and JNK phosphorylation while suppressing p38 phosphorylation [64]. *Ganoderma lucidum* (Curtis) P. Karst. Polysaccharides is differed from Ganoderma Triterpenoids in that they have the ability to directly influence melanogenesis in melanocytes. GLP can reduce melanogenesis in melanocytes by suppressing the paracrine adverse effect of fibroblasts and keratinocytes through the fibroblast growth factor FGF-2/MAP kinase pathway [65]. *Ganoderma lucidum* (Curtis) P. Karst. can be used to treat pigmentary dermatoses like as solar lentigo, chloasma, wrinkles, and senile plaques.

Skin barrier-repairing activity

A wound compromises the skin's barrier, allowing bacteria to infiltrate and cause inflammation. *Ganoderma lucidum*(Curtis) P. Karst.can also be used as a wound-healing agent in vitro to successfully treat non-healing wounds [66]. Triterpenoids extracted and purified from *Ganoderma lucidum* (Curtis) P. Karst.are shown to improve frostbite healing through increasing the area of healing and declining the degree of clinical features in frostbitten mice skin tissue [67]. *Ganoderma lucidum* (Curtis) P. Karst. polysaccharide helps promote fibroblast migration, rises expression





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of a procollagen type I C-terminal peptide, and transforms growth factor-1 in fibroblasts, having allowed them to heal the wound [68]. As a result, *Ganoderma lucidum* (Curtis) P. Karst. could be used to enhance wound regeneration through barrier repair.

DISCUSSION

There has been a lot of amazing research done on the health benefits of mushrooms. Fungi and their metabolites were studied as essential nutraceuticals and cosmetic ingredients. Many biotechnological engineering researchers have discovered that various types of mushroom extracts & their bioactive metabolites have promising skin benefits. According to Traditional medicinal recommendations, the mushroom of "immortality," specifically *Ganoderma lucidum*, may be of primary importance in the fight against oxidative stress which can be used for their known anti-aging qualities with a combined action from inside and outside; nutraceuticals on the inside and cosmeceuticals on the outside. The process of creating cells in our bodies continues until we reach at a certain age. As we get older, this process weakens and waste cells begin to accumulate in the body. These cells eventually become the cause of old age. As a result of being severely ill or any ailment, old age comes early. The failure of the body's delicate organs, like heart, liver, & kidneys, causes the majority of people to die in older age. More time could be lived if such delicate organs remain healthy or whether healthy organs get transplanted if deteriorated.

However, due to the large number of old age people in the world, the above arrangement cannot be made for all. Aside from that, the donor & receiver being in the same condition is also a pre-requisite, which does not happen very often. Because ageing is a complex multifactorial process, it has always played an important role in defining the chronicity of cell overlap at various levels. The molecular changes can occur as we age, leading to cellular changes, which contribute to ageing. Reactive oxygen species, which have an unpaired electron in their outermost orbital, have high reactivity as well as chemical instability. They have the capacity to react with various molecules by which they come into contact and thus reduce or start releasing electron, in an attempt to manage them, resulting in the generation of other radicals based on the reactions, which are frequently spread out in a series. Cells physically produce oxygen free radicals (ROS) as a "waste product" of the mitochondrial electron transport chain's activity. Their accumulation is caused by "oxidative stress," which is defined as an imbalance of oxygen free radicals (ROS) and the antioxidant system's defense activity. In good health, our body is able to prevent free radical damage through natural defense mechanisms as antioxidants, which counteract the oxidizing action of reactive oxygen species. As a result, antioxidants are agents capable of neutralizing the damaging effects of free radicals.

Conflict of Interest

There exists no conflict of interest amongst authors regarding publication of this manuscript.

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Effectiveness of Incentive Spirometer and Diaphragmatic Breathing on Exertional Dyspnea in Individuals with Grade-I Chronic Obstructive Pulmonary Disease

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ABSTRACT

COPD is a disorder which affects 10% of people every year worldwide. The most common symptom experienced by patients with COPD is perceived respiratory discomfort (dyspnoea) during physical activity. Incentive Spirometry and Resistive Inspiratory Devices are widely used to improve inspiratory muscle strength and to reduce dyspnea. Total 120 patients were taken for this study. they were divided into 2 groups. That is 60 subjects in each group, Group A was given Incentive Spirometry and Group B was given Diaphragmatic Breathing Exercise. All subjects had performed 6 min walk test. At the end of 6 min walk test individuals were asked to tell his or her maximum perceived dyspnea during walking on Borg scale & other physiological parameters like: heart rate, systolic and diastolic blood pressure, Distance covered in 6 minute was measured. There was significant improvement in reducing dyspnoea in Group A compare to Group B where P value is <0.001. From the result of our study, it is concluded that both incentive spirometry and diaphragmatic breathing exercise reduce exertional dyspnea but incentive spirometry shows more significant difference compared to diaphragmatic breathing exercise in grade 1 COPD individuals.

Keywords: COPD, incentive spirometry, DBE, 6 min. walk test



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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a common and often devastating respiratory illness that afflicts ~10% of individuals over 40 years of age worldwide.[1,2] The most common symptom experienced by patients with COPD is perceived respiratory discomfort (dyspnoea) during physical activity. According to the 2012 American Thoracic Society statement, breathlessness (or dyspnoea) is “a subjective experience of breathing discomfort that consists of qualitatively distinct sensations that vary in intensity”[3]. Dyspnea is an important and debilitating symptom in patients with chronic obstructive pulmonary disease (COPD) [4]. Several pathophysiological factors are known to contribute to dyspnea. they include: 1) increased intrinsic mechanical loading of the inspiratory muscles; 2) increased mechanical restriction of the chest wall; 3) functional inspiratory muscle weakness; 4) increased Ventilatory demand related to capacity; 5) gas exchange abnormalities; 6) dynamic airway compression; or 7) cardiovascular effects [5].

In patients with COPD, Controlled breathing is used to relieve dyspnea by:1) Reducing dynamic hyperinflation of the rib cage and improving gas exchange, 2) Increasing strength and endurance of the respiratory muscles,3) Optimizing the pattern of thoraco-abdominal motion. In addition, psychological effects (such as controlling respiration) might also contribute to the effectiveness of controlled breathing [6]. Reduced endurance and strength of the inspiratory muscles are frequently observed in chronic lung disease and contribute to dyspnea sensation. Breathing techniques and body positions aim to improve the length- tension relationship or geometry of the respiratory muscles (in particular of the diaphragm) or increase strength and endurance of the inspiratory muscles. According to the length-tension relationship, the output of the muscle increases when operating at a greater length, for the same neural input. At the same time the efficacy of the contraction in moving the rib cage might improve. Also, the piston-like movement of the diaphragm increases and thus enhances lung volume changes. In contrast to what is often believed, diaphragm displacement and its contribution to tidal volume during resting breathing have not been shown to be different in COPD patients. During increased levels of ventilation, the contribution of the diaphragm is reduced in more severe COPD [7].

Incentive Spirometry and Resistive Inspiratory Devices are widely used to improve inspiratory muscle strength and to reduce dyspnea. These devices offer resistance while performing inspiration. Incentive Spirometer is a simple instrument which provides visual feed-back to the patient while performing inspiration, so that patient can achieve their present goals. It encourages deep breathing and a sustained inspiration[8]. Patients with COPD may reduce future COPD exacerbations by targeting risk factors that may cause readmission to the hospital, such as physical inactivity, reduced activity tolerance, impaired physical function, desensitization to shortness of breath, anxiety, and depression & also have beneficial effects on exercise capacity and exertional dyspnea in patients with COPD[9]. There is increase evidence that abnormal heart rate recovery (HRR) after the 6 min walking test (6MWT) is associated with morbidity and mortality in various respiratory diseases [10,11,12] However, the literature is scarce regarding the relationship between HRR after the 6MWT and prognosis in patients with chronic obstructive pulmonary disease (COPD) [13,14]. In chronic respiratory entities, abnormal autonomic cardiac response could be consequence of a lower parasympathetic activation and/or increased sympathetic tone, being widely accepted that parasympathetic activations play a protective role [15]. Consequently, it is possible that numerous factors present in COPD patients namely hypoxemia, dynamic hyperinflation, and medication could affect the autonomic cardiac response to exercise with adverse consequence in diseases prognosis. Deep breathing exercises and incentive spirometry help to increase the lung capacity and spirometry improves the lung functioning. So the proposed study was done to see the individual & comparative effect of deep breathing exercises and spirometry training.

METHODOLOGY

All the participants were assessed for different factors thoroughly with assessment format. Ethics committee approval was granted by Parul university institutional Ethics Committee for human research (PU-IECHR) with



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reference number PUIECHR/PIMSR/00/081734/2603. The participants were taken for the study by using Simple random sampling. Total number of participant was 120 of both the gender. With age group between 30 to 70 years. Patients were included if they were: 1) Individuals with class 1/ grade 1 gold classification of COPD. 2) Individual who were willing to participate in the study. 3) Individuals who were available during the period and data collection. 4) Both male and female individuals. Patients were excluded if they were: 1) Individuals with any musculoskeletal disorders, 2) neurological disorders, and any other cardio respiratory disorders. 3) Individuals who has critical condition. 4) Individuals who were previously trained.

Bases on Pulmonary Function Test subjects with grade 1 gold classification of COPD were selected for the study. Detailed cardio respiratory physiotherapy assessment was taken. Subjects who have fulfilled the inclusion criteria were included in the study and written consent was obtained from all the subjects. After the selection, the individual was divided in two groups with random sampling. Total 186 subjects were analyzed & out of 186 individuals total 120 individuals were selected for study. These subjects were randomly divided into two groups. Minimum 60 subjects were taken in each group: The Group A was given Incentive Spirometry and Group B was given Diaphragmatic Breathing exercise. Incentive Spirometry was given for 10 to 15 times in each session and Diaphragmatic Breathing exercise was given 10 to 15 time in each session. Both treatments was given 2 times per day for the period of 4 to 6 weeks. All subjects had performed 6 min walk test, at the end of 6 min walk test individuals were asked to tell his or her max perceived dyspnea during walking on Borg scale & other physiological parameters like: heart rate, systolic and diastolic blood pressure, Distance covered in 6 minute was measured

STATISTICAL ANALYSIS AND RESULTS

Data was checked for plausibility and cleaned. The kolmogorov-smirnov test was used to analyze normal distribution assumption of the quantitative outcomes. Descriptive and exploratory data analysis was performed on the total data set collected which may include parametric and non-parametric tests. For within group analysis, wilcoxon sign test was applied and for between group analysis, mann- whitney test will be applied. The p-values less than 0.001 will be considered statically significant. Statistical analyses will be performing using SPSS version 26.0 IBM Company. Within the group comparison where in group A distanced walked pre training mean value was 380.82 ± 19.96 and post training 451.88 ± 27.63 ($p < 0.001$) wherein Group B mean value was 388.47 ± 23.67 ($p < 0.001$). group A Heart Rate pre training mean value was 106.98 ± 3.14 and post training 99.35 ± 2.55 ($p < 0.001$) wherein Group B pre training mean value was 106.52 ± 3.71 and post training 102.08 ± 3.27 ($p < 0.001$). group A SBP pre training mean value was 135.33 ± 6.29 and post training 127.57 ± 5.82 ($p < 0.001$) where in Group B pre training mean value was 135.70 ± 8.56 and post training 131.73 ± 8.36 ($p < 0.001$). In group A DBP pre training mean value was 84.47 ± 3.50 and post training 79.87 ± 3.39 ($p < 0.001$) where in group B pre training mean value was 84.73 ± 6.12 and post training 81.70 ± 6.26 ($p < 0.001$). group A BORG'S Scale pre training mean value was 14.85 ± 1.16 and post training 9.87 ± 0.85 ($p < 0.001$) wherein group B BORG Scale pre training mean value was 14.08 ± 1.63 and post training 11.63 ± 1.48 ($p < 0.001$). group A SPO2 pre training mean value was 93.90 ± 0.82 and post training 97.30 ± 0.77 ($p < 0.001$) where in Group B SPO2 pre training mean value was 94.02 ± 0.91 and post training 95.27 ± 0.76 ($p < 0.001$).

Between the group comparison shows difference in distance walked in group A was 71.07 ± 22.20 and in group B 46.80 ± 16.64 shows significant difference in group A with p value < 0.001 . group A difference in Heart Rate was 7.63 ± 1.95 and in group B 4.43 ± 1.38 shows significant difference in group A with p value < 0.001 . group A difference in SBP was 7.77 ± 2.42 and in group B -1.03 ± 39.04 shows significant difference in group A with p value < 0.001 . group A difference in DBP was 4.60 ± 1.29 and in group B 3.03 ± 1.54 shows significant difference in group A with p value < 0.001 . shows significant difference in group A with p value < 0.001 . group A difference in BORG'S Scale was 4.98 ± 0.87 and in group B 4.5 ± 0.91 shows significant difference in group A with p value < 0.001 . group A difference in SPO2 was 3.40 ± 0.98 and in group B 1.25 ± 0.68 shows significant difference in group A with p value < 0.001 .





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DISCUSSION

In this study we have taken two groups. group A and group B contain 60-60 subjects. group A received Incentive Spirometry training and Group B received Diaphragmatic breathing training for 4 weeks. Before and after assessment was done on Borg scale & physiological parameters. In present study, both training groups when compared, results were found to be significant ($p < 0.001$). A recent systematic review completed in 2016 examined the evidence regarding patient compliance with incentive spirometry after cardiac, thoracic and abdominal surgery. They concluded that there is a scarcity and inconsistency of evidence regarding incentive spirometry compliance. They identified the importance of reporting this outcome measure to examine the effectiveness of this treatment adjunct. According to these results, Incentive Spirometry is highly effective in improving Inspiratory Capacity and reducing the dyspnea which could be due to improvement in ventilatory muscle strength which supports the results of Weiner *et al.*, they treated patients using Incentive Spirometry and other specific inspiratory muscle training and reported increased lung functions and inspiratory muscle strength. Any maneuver that emphasizes inflation will increase lung volume and maintain patency of the smaller airways. IS is the most widely prescribed technique for preoperative and postoperative lung expansion. In addition, IS is characterized by active recruitment of the diaphragm and other inspiratory muscles. It has been shown that the inspiratory muscles of patients with COPD are weaker than those of normal persons. It has been established that respiratory muscles can be trained like other skeletal muscles, and several reviews have been published that deal with ventilatory muscle training.

According to Mayuni *et al.*, repeated and routine diaphragmatic breathing helps with correctly using the diaphragm when breathing [16]. This technique is useful to strengthen the diaphragm and decrease respiratory work by reducing the rate of breathing, using less effort and energy to breathe. Diaphragmatic breathing increases tidal volume, decreases functional residual capacity, and enhances optimal oxygen uptake. DBEs are a combination of chest and abdominal breathing; they aim to relax the breathing muscles while performing deep inspiration. Patients concentrate on efforts to develop the diaphragm during controlled inspiration [17]. According to Windarti, diaphragmatic breathing exercises aim to improve breathing function, as well as train and regulate breathing by breathing properly. This technique is employed if an individual experiences sudden shortness of breath symptoms [16]. Additionally, DBEs aim to improve circulation and flex and strengthen the breathing muscles. According to Sudoyo, DBEs aim to reduce the feeling of shortness of breath with exercises that focus on abdominal breathing [7].

CONCLUSION

From the result of our study, it is concluded that both incentive spirometry exercise and diaphragmatic breathing exercise reduce exertional dyspnea but incentive spirometry exercise shows more significant difference compare to diaphragmatic breathing exercise in grade 1 COPD individuals.

Conflict of Interest: None

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Table 1.1 within group comparison

	GROUP A		Wilcoxon Test	P Value	GROUP B		Wilcoxon Test	P Value
	Mean	SD			Mean	SD		
Distance Walked Pre Training	380.82	19.96	6.736	<0.001	388.47	23.67	6.737	<0.01
Distance Walked Post Training	451.88	27.63			435.27	31.24		
Heart Rate Pre Training	106.98	3.14	6.761	<0.001	106.52	3.71	6.784	<0.001
Heart Rate Post Training	99.35	2.55			102.08	3.27		
SBP Pre Training	135.33	6.29	6.846	<0.001	135.70	8.56	6.193	<0.001
SBP Post Training	127.57	5.82			131.73	8.36		
DBP Pre Training	84.47	3.50			84.73	6.12		





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			6.915	<0.001			6.731	<0.001
DBP Post Training	79.87	3.39			6.12	6.26		
BORG'S Scale Pre Training	14.85	1.16	6.821	<0.001	14.08	1.63	6.819	<0.001
BORG'S Scale Post Training	9.87	0.85			11.63	1.48		
Spo2 Pre Training	93.90	0.82	6.826	<0.001	94.02	0.91	6.643	<0.001
Spo2 Post Training	97.30	0.77			95.27	0.76		

Table 1.2 Between The Group Comparisons

	GROUP A		GROUP B		Man Whitney Test	P Value
	Mean	SD	Mean	SD		
Difference Distance Walked	71.07	22.20	46.80	16.64	706.50	<0.001
Difference Heart Rate	7.63	1.95	4.43	1.38	298.00	<0.001
Difference SBP	7.77	2.42	-1.03	39.04	341.00	<0.001
Difference DBP	4.60	1.29	3.03	1.54	820.50	<0.001
Difference BORG'S Scale	4.98	0.87	2.45	0.91	94.00	<0.001
DifferenceSPO2	3.40	0.98	1.25	0.68	169.50	<0.001





Effectiveness of Neural Tissue Mobilisation Versus Deep Cervical Flexors Strengthening on Pain, Range of Motion and Quality of Life on Cervicogenic Headache among Female College Students- A Quasi Experimental Study

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ABSTRACT

Cervicogenic headache is a subgroup of secondary headache, stemming from a disorder of the cervical spine. The physiologic basis for this pain is convergence between trigeminal afferents and afferents from the upper three cervical spinal nerves. Approximately 47% of the global population suffers from a headache and 15-20% of those headaches are cervicogenic. This study aims to find out the effect of neural tissue mobilization versus deep cervical flexors strengthening on reducing pain and improving range of motion, quality of life among female college students over a period of 4 weeks. 30 subjects were selected and assigned into two groups by convenient sampling method. Group A received neural tissue mobilization and Group B received deep cervical flexors strengthening. VAS, HIT-6, goniometer were used as outcome measures to record pain, quality of life and ROM. The measurements were taken before and after the treatment over a period of 4 weeks. The results showed that both groups had improvement in their outcome measures. The group which received neural tissue mobilization technique showed more improvement than the group which received deep cervical flexors strengthening.

Keywords: Cervicogenic headache, Neural tissue mobilization, Deep cervical flexors strengthening, Visual AnalogueScale, Headache-impact test.





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INTRODUCTION

Siaastad et al, offered a hypothesis for “cervicogenic headache “in 1983. Cervicogenic headache is defined as “Chronic hemi cranial pain syndrome in which sensation of pain originates in cervical spine (C1-C3) or soft tissues of neck and is referred to the head” [1].Headache is affecting 66% of population globally and among this, tension type headache -38%, migraine – 10%, chronic daily headache -3%, cervicogenic headache- 2.5-4.1%. In case of Cervicogenic headache women are affected 4 times more than men [2].Nociceptive afferents from C1, C2, and C3 spinal nerves synapse in the dorsal horn at level of entry into the spinal cord with ascending and descending branches also synapsing in the adjacent one or more segments.Nociceptive afferents from the trigeminal nerve descend along the spinal tract from the pons into the upper two to four levels of the spinal cord to synapse here in the pars caudalis of the spinal nucleus of the trigeminal nerve.The trigeminal nerve is accompanied also by nociceptive afferents from the facial, hypoglossal, and vagus nerves which also synapse in the pars caudalis [3].Due to the caudal extent of the pars caudalis, it becomes overlapped both morphologically and functionally with the upper cervical segment of the dorsal horn, with the same cell types and lamination present in both.Through its multiple afferent fibres this area - now described as the trigeminocervical nucleus - effectively transmits primary pain perception for the entire head and upper cervical spine.A possible result of the overlap of central connections in the trigeminocervical nucleus is a phenomenon described as convergence.

The other study suggests that the second order neurons in the trigeminocervical nucleus misinterpret cervical afferent activity for that coming from several sources such as the trigeminal nerve and the cervical nerves. The brain therefore will perceive pain in the regions supplied by the trigeminal nerve as well as that supplied by the upper cervical nerves[4].

The concept of the trigeminocervical convergence was well demonstrated by showing that noxious stimulation of the greater occipital nerve increases central excitability of supratentorial afferents, and stimulation of the duramater increases trigeminocervical neuron responsiveness to cervical input[5].

Diagnostic criteria for cervicogenic headaches as described by the International Headache Society:

1. Pain localized in the neck and occiput, which can spread to other areas in the head, such as forehead, orbital region, temples, vertex, or ears, usually unilateral.
2. Pain is precipitated or aggravated by specific neck movements or sustained postures[6].
3. At least one of the following:
 - i. Resistance to or limitation of passive neck movements
 - ii. Changes in neck muscle contour, texture, tone, or response to active and passive stretching and contraction
 - iii. Abnormal tenderness of neck musculature
4. Radiological examination reveals at least one of the following:
 - i. Movement abnormalities in flexion/extension
 - ii. Abnormal posture
 - iii. Fractures, congenital abnormalities, bone tumors, rheumatoid arthritis, or other distinct pathology (not spondylosis or Osteochondrosis).

NEED FOR THE STUDY

Many people experience headaches, often due to problems related to their age, gender, health and overall lifestyle. College students are no different. Headache is among the commonest complaints in college students; it could be due to many physical or psychological stressors. Interestingly, cervicogenic headaches are one of the most common types of headaches in college going students. Students who have sustained forward head postures while reading and





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uncomfortable seating can develop headache co-exists with the neck pain. Females seem more predisposed to cervicogenic headaches affecting 4 times as many women as men [7,8]. There are some following causes that can develop cervicogenic headache:

Forward head posture:

In a study done by Watson and trot, they noted that forward head posture increases stress on the upper cervical segments which was also associated with weakness and decreased endurance of the deep neck flexors[9]. In contrast to that Zito et al, found no significant differences in the prevalence of forward head posture in CGH or migraine patients compared to control subjects[10].

Decreased strength of deep neck flexors:

Janda et al; noted that patients with cervical dysfunction often have weakness of their deep neck flexors. Several researchers have confirmed decreased strength and endurance of the deep neck flexors in CGH patients [11].

Abnormal Neural tissue mechanosensitivity:

According to Butler, 1991; Shacklock, 2005: Abnormal mechano sensitivity can potentially be addressed by the performance of neurodynamic sliders and neurodynamic tensioners which produce excursion and tension of the neural tissues respectively. There are various studies in the literature that have documented that pain in cervicogenic headache is due to musculoskeletal abnormalities like incorrect posture or any problem in muscular structures like tightness or weakness of muscles. Few studies stated there may be a problem present in the neural structures [12]. A case study has been done to illustrate that neural tissue mobilisation is an effective intervention which can be used in patients having cervicogenic headache [12]. It has been observed that no study has been done to compare the effects of neural tissue mobilisation and deep cervical flexors strengthening on Cervicogenic headache. So, this study intends to find the difference between neural tissue mobilisation and deep cervical flexors strengthening on cervicogenic headache among female college students. To study and find the effect of neural tissue mobilization versus deep cervical flexors strengthening in reducing pain and improving range of motion and quality of life on cervicogenic headache among female college students.

MATERIALS AND METHODS

Method of Collection of Data

Female college students of Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore were screened. After finding their suitability, subjects who had been diagnosed with cervicogenic headache were requested to participate in the study as per the inclusion criteria and exclusion criteria. Informed consent form was obtained from them. 30 Subjects with cervicogenic headache were placed into two groups Group A and Group B. Pretest and posttest was conducted on Group A and Group B by measuring pain through visual analog scale, active cervical range of motion through goniometer, quality of life through headache impact test-6.

Research Design: A Quasi- Experimental study design.

Criteria for Selection

Inclusion Criteria

- Age: 18-25 Years
- Subjects fulfilling the diagnostic criteria given by IHS (International Headache Society)
- Female college students.

Exclusion Criteria

- Dizziness or visual disturbance symptoms.
- Age group people of other than 18-25 years.
- Bilateral cervicogenic headache.
- Headache not of cervical origin.
- Known congenital, inflammatory and infectious condition of cervical spine.
- Patients on medication (Steroids, Pain Killers or Analgesics).

Measurement Tools

- Visual Analogue Scale



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- Universal Goniometer Of 0°-180°
- Headache Impact Test-6.

Treatment Techniques

Group A: Neural Mobilization Technique

Treatment duration and frequency: 2 set of 20 oscillations [6].

Treatment sessions: 12 sessions (3 sessions per week/ 4 weeks) [13].

Intervention duration: 4 weeks [13].

In this group subjects were treated with neural tissue mobilization technique for occipital nerve. It is a maneuver performed in order to produce a movement of neural structures relative to their adjacent tissues.

Patient position: Upright sitting position

Therapist position: on the affected side of the patient.

Hand placement: The therapist should place the right hand over the chin of the patient and left hand over the head.

The treatment consisted of upper cervical flexion followed by lateral flexion of neck away from the side of affection. 2 set of 20 oscillations was given for 3 sessions a week for a total of 12 sessions.

Group B: Deep cervical flexors strengthening technique

Treatment duration: 10 seconds hold and repeated them for 10 times [14].

Treatment session: 12 sessions (3 sessions per week/ 4 week) [15].

Intervention duration: 4 weeks [16].

Patient position: supine lying with the cervical spine in the neutral position and a stabilizer pressure biofeedback unit (chattanooga group, hixson, tn) should be placed under the cervical lordosis. The pressure sensor should be inflated to 20 mm hg. The patient should be then instructed to slowly nod the head. As muscular activation of the deep cervical flexors occurs, the cervical lordosis slightly flattens and registers as an increased in pressure on the pressure sensor. The activation score is the pressure that can be achieved and held steadily for 10 seconds. It was repeated for 10 times, 12 sessions (3 sessions per week) over a period of 4 weeks. The present study preferred deep cervical flexor strengthening technique using pressure biofeedback unit than traditional strength training, because the constant feedback encourages patients doing the exercise to perform it correctly and gets them more involved in the treatment. Feedback helps in motor learning which is a set of processes associated with practice or experience leading to permanent changes in the capability of responding. Positive reinforcement is the operative learning model [17].

DISCUSSION

This study was designed to determine the effects of neural tissue mobilization and deep cervical flexor strength training using pressure biofeedback unit in reducing pain, and improving ROM, quality of life in college going female students with cervicogenic headache. The study was carried out on 30 college female students, age group between 18-25 years. The subjects were divided into 2 groups Group A (Neural tissue mobilization), Group B (Deep cervical flexor strength training).

In Group A there were 15 subjects who received neural tissue mobilization. It is performed in order to produce a movement of neural structures relative to their adjacent tissues for 2 sets of 20 oscillations, 3 sessions per week. In Group B there were 15 subjects who received deep cervical flexors strengthening for 10 seconds hold and repeated them for 10 times, 3 sessions per week. It specifically addresses the impairments in deep neck flexor muscles found in cervicogenic headache patients. This is the first study in which both neural tissue mobilization and deep cervical flexor strengthening using pressure biofeedback was given to female college students with cervicogenic headache. Therefore, the lack of literature in this area limited the scope for direct comparison with other studies. The results of this study can be compared to other studies in a general way only due to differences in treatment protocols,



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subject population, measures taken, and duration of treatment. In this study both the groups showed a marked improvement in VAS, HIT-6, and goniometer scores, and there was a significant difference in the scores observed between the two groups.

It showed a statistically significant improvement in neural tissue mobilization group on reducing pain and improving quality of life when compared with the scores of deep cervical flexors strengthening group which gives an implication that the subjects in the neural tissue mobilization had improved better than the group which received deep cervical flexor strengthening. On improving the range of motion there is a statistically significant improvement in cervical extension alone of the neural tissue mobilization group when compared with the deep cervical flexor strengthening group. On contrary, there is no significant difference found between the groups to improve other range of motion measurements like flexion, right lateral flexion, left lateral flexion, right rotation, and left rotation.

In a study done by Oshin A Ferreira et al on the effectiveness of strain counter strain technique and neural tissue mobilization on cervicogenic headache, findings suggest that the SCS technique along with conventional treatment was more effective than neural mobilization to reduce pain, disability and to improve ROM [18]. An another case report done by Jaspreet Kaur et al, on the efficacy of neural mobilization and advice on postural correction in cervicogenic headache, a 42 year old female patient received neural mobilization and advice on postural correction. Outcome measures were Headache impact test, Visual analogue scale, and headache diary. This study concluded that neural mobilization is also an effective treatment in treating cervicogenic headache [12].

LIMITATIONS

- Future studies can be done for males.
- Further studies can be done with longer follow up.
- It can be further extended on to varying age group populations.
- It can be performed on bilateral cervicogenic headache.
- This study can be done for long duration.
- Different diagnostic criteria for cervicogenic headache like Sjaastad, Indian Association for the study of pain can be taken.
- Other outcome measuring reliability tests can be used.
- Immediate effects of these techniques can be analyzed.

CONCLUSION

On the basis of present study, both the techniques were able to reduce pain and to improve ROM, quality of life. Statistically, there is a significant difference between the effect of two techniques to reduce pain and to improve ROM, quality of life on cervicogenic headache. So, the conclusion is neural tissue mobilization is effective in reducing pain and to improve quality of life among female college students over a period of 4 weeks. Moreover, there is no significant difference of ROM values found between the two groups. But the reported range of cervical extension was greater in group A compared to the group B.

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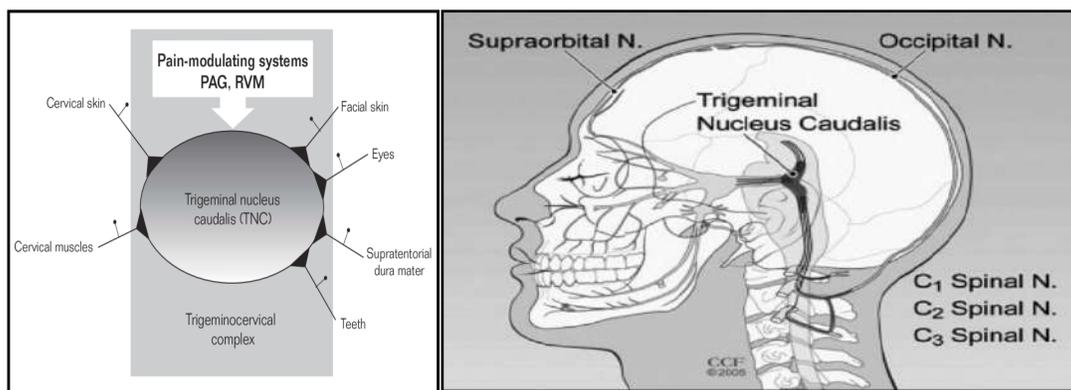


Figure 1: Convergence in the Trigemino Cervical Nucleus





Leading Learning: An Empirical Perspective Of Parental Involvement And Engagement At Secondary School Students

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ABSTRACT

Parents interactions with schools and with their children to promote academic success is known as parental involvement (Taylor&Hill, 2004). It is just an umbrella phrase that encompasses a wide range of parental actions and activities that are directly or indirectly connected to their children's education. Many studies have been undertaken throughout the years that show a link between parental participation and academic achievement, learning outcomes, and motivation in students. The purpose of this research was to investigate the impact of parental involvement on the academic achievement of senior secondary government and private school students, with special reference to gender. To perform the current study, 200 students were chosen at random from Gautam Buddha Nagar's senior secondary schools. Self-constructed questionnaire on parental involvement was used to collect data, and the CGPA scores of class 9 were used to assess academic progress. The current study found that senior secondary students from government and private school's boys and girls significantly different in terms of academic achievement and parental involvement, and there was no such difference between urban and rural senior secondary students in terms of academic achievement and parental involvement. The study's findings revealed that there was a strong positive link between parental involvement and academic achievement among Government school's senior secondary students as well as of Private senior secondary students.

Keywords: Parental Involvement, Academic Achievement, Senior Secondary Students, Government schools, Private schools

INTRODUCTION

Parental involvement is defined as any interaction between a parent and their children or school that benefits their child's development. According to Feuerstein (2000), parental involvement is an activity that involves a wide range



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of actions, such as discussing school with children and attending parent-teacher conferences. According to Harris and Goodall (2008), the phrases "parental engagement" and "involvement" are frequently used interchangeably, however they are not the same thing. They claim that parents might be "active" in school events without being "engaged" in their children's education. They said that parental involvement means parents went to school either formally like drop their children in school or informally like attend PTM whereas 'parental engagement' is when parents actively participate in their child's learning at home through extension or support activities, homework, and online activities. Holloway *et al.* (2008) describe parent participation as the beginning of both home-based and school-based behaviours such as supervising homework and interacting with educators. Parental involvement decreases as pupils become older, to the point that it is lower in secondary schools than in elementary schools (Stouffer, 1992). There are a variety of causes for the parent's and school's lack of participation. One of the causes for this is the education system's lack of awareness of unconventional households. The unconventional family is dealing with a number of issues that affect each family member. These factors can have a significant impact on the family's ability to participate in the student's education. There is almost certainly a time constraint. Simply said, there aren't enough hours in the day to get everything done. If a family member has died or divorced, the family's financial situation has most likely changed. The student/family may be ashamed if the school does not perceive the change. The entire essence of the family is changing, producing fear and confusion (Duncan, 1992; Lewis, 1992; Wanat, 1992). Schools must realize that parents' lack of involvement does not always indicate that they are ignoring their responsibility. They can only lack the time, money, or expertise to assist (Wanat, p. 47). Parents are frequently unwelcome in school. They believe that anything they have to contribute is insignificant and worthless. Furthermore, parents may not think that they have any information that the school would be curious to know about. This seems to be true especially if the parent has a limited educational background (Dixon, 1992; Vandergrift & Greene, 1992). It is quite conceivable that the parent is uninterested in his child's education or the school itself. It's possible that the parent does not value schooling (Vandergrift & Greene). Embarrassment is another factor that contributes to a lack of participation. It's possible that the parents are illiterate or don't know how to communicate in English. Communication might be difficult. Another source of embarrassment is memories of the parent's failure in school. The parent would not have much desire to return to a place that only served to remind him of his own failures (Brink & Chandler, 1993; Smith, 1991). Memories of a parent's failure in school are another cause of humiliation. The parents would not like to return to a location that simply served to remind him of his own mistakes (Brink & Chandler, 1993; Smith, 1991). Although there are several researches have been done in globally but some lacks of rest that's why researcher try to find out one more dimension of parental involvement is responsibility for learning outcomes. This study aims to assess the effect of parental involvement on the academic success of adolescent student's age 11 to 15 years old in India (Gautam Buddha Nagar District).

Need And Significance Of The Study

Adolescents' academic achievement is linked to parental involvement and their perceptions of them. Parents will benefit from understanding how their teenagers see them so that they may alter their child-rearing practices to ensure that their adolescents grow to their full potential. Adolescents must achieve great academic accomplishment in order to be healthy. Although there is a substantial body of work on adolescents' perceptions of parental involvement and academic performance, research has not concentrated on these issues from the perspective of one more dimension of parental involvement which is responsibility of learning outcome towards adolescents, particularly in India. However, very few knowledge is still available on how adolescents view parenting practices that their parents embrace, their consequences for their academic success. This work is especially significant in parenting research since it investigates the influence of parenting methods on good developmental outcomes in the Indian cultural context.

LITERATURE REVIEW

Singh M. and Mahajan P. (2021) designed to investigate the influence of parental participation on academic success of senior secondary students by gender and locality. To perform this study, there were 200 senior secondary school's





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students chosen at randomly from Jammu tehsil. The finding of the study indicated that there was significant difference between male and female senior secondary students in rural as well as urban areas in terms of their parental involvement & academic success, but no significant differences were observed between rural&urban senior secondary students in terms of parental involvement and academic achievement. According to the findings of the study, there was a strong positive link between parental involvement and academic success of both rural and urban students at secondary level. Azizi Yahaya et al., (2020) investigated that the academic success of students has been related to self-regulated learning, parental engagement, and homework. Moreover, with girls outperforming boys, gender also plays a part in academic success. Danbi Choe, (2020) concluded that the effects of encouragement from parents of young people and adults were different. The adolescent parental support study is more associated with higher adolescent academic achievement relative to the adult survey, but the adult academic support report is the strongest predictor of teen academic performance progress. Lara L. and Saracostti M. (2019) The current study sought to investigate the relationships between parental participation in school and children's academic progress. The results of a cluster analysis of 498 parents or guardians whose children attended second and third grades in 16 public elementary schools in Chile suggested the existence of three distinct profiles of parental involvement (high, medium, and low) when different types of parental involvement were considered (at home, at school and through the invitations made by the children, the teachers, and the school). The study found that there were disparities in children's academic accomplishment based on parental participation profiles, showing that children with less involvement reported poorer academic achievement. The result was consistent with worldwide research evidence, indicating the importance of focusing component in Latin American contexts. Boonk L. et al. (2018) The results first present how individual parental involvement variables correlate with academic achievement based on an age-related classification. Then we move to a more profound review of the literature to determine which variables are moderating or mediating the relationship between parental involvement and academic achievement. Finally, we describe the advancements that were made with studies from the last decade with special focus on the construct of parental involvement. S. Wilder (2014) This study combined the data of nine meta-analyses that investigated this influence, and it revealed conclusions that were generalizable across these investigations. The findings revealed a favorable association between parental participation and academic attainment. Furthermore, the results demonstrated that this association was highest when parental participation was characterized as parental expectations for their children's academic progress. However, when parental participation was defined as homework support, the influence on student academic progress was the smallest. Finally, it was discovered that the association between parental participation and academic success was stable across grade levels and ethnic groups. However, the strength of that association varied depending on the sort of assessment utilized to quantify student success.

RESEARCH METHODOLOGY

Objectives

- To identify the relationship between parental involvement and academic achievement of Government school senior secondary students and Private school senior secondary students of G.B. Nagar district.
- To find out the difference between academic achievement of senior secondary students of G.B. Nagar with special reference to gender.
- To explore the difference between parental involvement of senior secondary students of G.B. Nagar with special reference to gender.

Hypotheses Of The Study

There will be no significant relationship between parental involvement and academic achievement of Government senior secondary school students and Private senior secondary school students of G.B. Nagar. There will be no significant difference between Government school and Private School senior secondary Boys students and between Government school and Private School senior secondary Girls students of G.B. Nagar in terms of their parental involvement. There will be no significant difference between Government School senior secondary Boys and Girls students and between Private School senior secondary Boys and Girls students of G.B. Nagar in terms of their



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academic achievement. There will be no significant difference between Government school and Private School senior secondary Boys students and between Government school and Private Schools senior secondary Girls students of G.B. Nagar in terms of academic achievement. There will be no significant difference between Government Schools senior secondary Boys and Girls students and between Private Schools senior secondary Boys and Girls students of G.B. Nagar in terms of their parental involvement.

Procedure

The target population for this study was around 4080 senior secondary students enrolled in G.B. Nagar's senior secondary schools. A sample of 200 senior secondary students in 9th grade from government and private schools in G.B. Nagar was chosen with precision and care using a simple random technique, i.e. 5% of the target group from all senior secondary schools, which is representative of the total population from Government schools and Private schools in G.B. Nagar. The tool for collecting data was parental involvement scale self-constructed. This scale includes 35 items which measures three dimensions of Parental Involvement viz, Home-based involvement, School-based involvement, Responsibilities for learning outcomes. The scale has been evaluated in terms of content, construct, and discrimination validity. The discrimination validity was assessed using the 't' test, with the high group scoring 27 % and the low group scoring 27 %. All 't' values are significant at level 0.01 and the means of the higher group are likewise greater than the lower group, indicating the strong validity of parental involvement. The Cronbach Alpha Coefficient was used to assess the scale's reliability. All of the reliability coefficient values are more than 0.70. Thus, the Parental Involvement scale was a reliable scale with a reliability of 0.900, and the reliability for each dimension of Parental Involvement were 0.917, 0.900, and 0.923. The result of the CGPA score in class 9th was used to evaluate academic achievement. While administering parental involvement scale students were assured that their response would be kept confidential, so that they didn't hesitate to fill the required information asked in the scale.

Statistical Technique

Statistical techniques such as the mean, standard deviation, t-test, and coefficient of correlation were used to examine the data.

ANALYSIS AND DISCUSSION

The coefficient of correlation was derived in Table I to investigate the link between parental involvement and academic achievement of Government school and private school senior secondary pupils. The coefficient of correlation between parental involvement and academic achievement in terms of Government senior secondary students is 0.58 and the coefficient of correlation between parental involvement and academic achievement in terms of Private senior secondary students is 0.72. The coefficient of correlation is significant in terms of Government senior secondary students as well as Private senior secondary students. Hence the formulated hypothesis I is rejected. Therefore, from Table- I, it may be stated that there were a considerable association between parental involvement and academic achievement of Government as well as Private senior secondary students of G. B. Nagar. To point out current study, difference between Government and Private Boys senior secondary students and between Government and Private Girls senior secondary students of G.B. Nagar in terms of parental involvement, Mean, S.D. and t-value were computed in Table II

Table II shows that the computed t-value for Government and Private Boys senior secondary students is 0.062053, while the calculated t-value for Government and Private Girls senior secondary students is 0.006806. In the case of Government and Private Boys as well as Government and Private Girls senior secondary pupils, the t-value is minimal. As a result, hypotheses II is accepted. To point out current study, differences in parental involvement between Government Boys and Girls and Private Boys and Girls senior secondary students in G.B. Nagar, Mean, S.D., and t-value were calculated in Table III. It was evident from Table- III that the t-value calculated for Government Boys and Girls senior secondary students in terms of their parental involvement is 3.82658 which is



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significant at 0.01 level of significance. Hence the formulated hypothesis III is rejected. The calculated t- value for Private Boys and Girls senior secondary students in terms of their parental involvement is 0.392137 which is insignificant at 0.05 and 0.01 level of significance. Hence the formulated hypothesis III is accepted. Table III, it can be concluded that Government Boys and Girls senior secondary students differ significantly and Private Boys and Girls senior secondary students same insignificantly in terms of their parental involvement. To point out current study, difference between Government and Private Boys senior secondary students and between Government and Private Girls senior secondary students of G.B. Nagar in terms of their academic achievement, Mean, S.D and t-value were calculated in Table-IV.

Table-IV shows that the calculated t-value in the case of Government and Private Boys senior secondary students and between Government and Private Boys senior secondary students of G.B. Nagar in terms of academic achievement is 0.790422, which is insignificant, and the calculated t-value between Government and Private Girls senior secondary students is 5.86829, which is significant. Table IV shows that there is no significant difference in academic success between government and private Boys senior secondary students and a significant gap between government and private Girls senior secondary students in G.B. Nagar. As a result, in the case of Government and Private Boys senior secondary students, hypothesis IV is accepted, but in the case of Government and Private Girls senior secondary students, hypothesis IV is rejected. To point out current study, difference in academic achievement between Government Boys and Girls senior secondary students and Private Boys and Girls senior secondary students in G.B. Nagar, Mean, S.D., and t-value were computed in Table V. Table V shows that the t-values calculated for Government Boys and Girls senior secondary pupils are 0.119383 and 2.24337, respectively, in terms of their academic achievement, which are insignificant at the 0.05 and 0.01 levels respectively. As a result, the hypothesis V are accepted. As a result of Table V, it can be stated that the academic success of Government and Private Boys and Girls senior secondary students is not considerably different.

CONCLUSION

The accompanying data clearly show that increased academic attainment is linked to parents' active participation in their children's life. Parents of senior secondary students in either government or private schools may play an important role not only throughout their childhood but also during their key adolescence years, when they are being groomed to perform as responsible adults. Parents of Government's school senior secondary students are active in helping their children earn excellent marks, but not as involved as parents of Private's school senior secondary students in helping their children do better in the selected subjects of study, according to the study. On the basis of their differences in mean scores, it is also clear from the data that parents in both Government and private schools are more oriented toward boys' academic achievement than girls'. Many studies have shown that parents' involvement, direct attention, and freedom with their children can help their children attain academic success.

Recommendations

While there are several ways to improve parental participation at the secondary level, the success of any Programme is directly reliant on the principal's support and encouragement (Lewis, 1992). If the school is more aware of the conditions of nontraditional families, better communication may occur. One way the school may apply parenting tasks for the parents while the school takes care of educational matters. More communication between school and parents is desired, although other forms of communication are also necessary. Parent courses are a popular Programme that may help parents with parenting issues, homework/tutoring solutions, drug awareness, and communication skills. Parents, students, and the school may all benefit from these workshops. The usage of computers is a significant tool for parents to become engaged in their students' work. Many pupils, as well as their parents, are entering a new world. They may both learn about this fascinating world. One thing that must be done is to ensure that the programmes utilized are appropriate for the grade level and that there is a wide range of options (Rickelman & Henk, 1991).





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Table- I: In terms of Government school and private school senior secondary students in G.B. Nagar, the coefficient of correlation between parental involvement and academic achievement.

S.No.	Variables	N	Types of school	Coefficient of Correlation
1	Parental Involvement	100	Government secondary school	0.58
	Academic Achievement			
2	Parental Involvement	100	Private secondary school	0.72
	Academic Achievement			

Table- II: Mean, S.D. and t-value in terms of Parental Involvement.

S.No.	Category	N	Variable	Mean	S.D.	t-value	Level of Significance
1	Government Schools Boys	50	Parental Involvement	128.72	19.81	0.062053	insignificant
	Private Schools Boys	50		137.04	23.66		
2	Government Schools Girls	50	Parental Involvement	144.28	15.66	0.006806	insignificant
	Private Schools Girls	50		132.88	24.25		

Table- III: Mean, S.D and t-value in terms of Parental Involvement

S.No.	Category	N	Variable	Mean	S.D.	t-value	Level of Significance
1	Government Schools Boys	50	Parental Involvement	128.72	19.80710983	3.82658	significant
	Government Schools Girls	50		144.28	15.65508		
2	Private Schools Boys	50	Parental Involvement	137.04	23.65921	0.392137	Insignificant
	Private Schools Girls	50		132.88	24.24841		





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Table-IV: Mean, S.D, t-value in terms of Academic Achievement.

S.No.	Category	N	Variable	Mean	S.D.	t-value	Level of Significance
1	Government Schools Boys	50	Academic Achievement	63.02	16.26232	0.790422	Insignificance
	Private Schools Boys	50		124.76	6.575378		
2	Government Schools Girls	50	Academic Achievement	59.46	3.494052089	5.86829	significance
	Private Schools Girls	50		74.84	14.21458		

Table-V: Mean, S.D, and t- value in terms of Academic Achievement.

S.No.	Category	N	Variable	Mean	S.D.	t-value	Level of Significance
1	Government Schools Boys	50	Academic Achievement	63.02	16.26232	0.119383	Insignificance
	Government Schools Girls	50		59.46	3.494052089		
2	Private Schools Boys	50	Academic Achievement	124.76	6.575378	2.24337	Insignificance
	Private Schools Girls	50		74.84	14.21458		





Picture Comprehension with Neural Networks

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ABSTRACT

we are presenting a model architecture to describe an image and annotate it, with a human level description. Our model architecture is based on Convolutional Neural Networks for objects detection in image and provide a simple annotation at first, And then the results form Convolutional Neural Networks feed to a Recurrent Neural Network for the perception of the objects in the image. And then results feed into a pre-trained transformer model for human like language output. By stacking this neural network architectures we can enable a huge improvement in Picture comprehension (describing a picture). And also providing some negative rewards in case of wrong prediction and positive rewards if it handles the anomalies correctly. There are pre-existing Deep Learning models which uses Deep Visual-Semantic Alignments, but the architecture proposed by us, Can produce even more humanly description about the image.

Keywords: Convolutional Neural Networks, Recurrent Neural Networks, Deep Visual-Semantic Alignments, pre-trained transformer.

INTRODUCTION

Humans can annotate and describe about a picture or scenery with a very short observations. Our cerebral cortex and the neurons present are highly sensitive to perceptions like depth of the objects, spatial arrangement of them, and size of the objects. And also enables us to correlate facts about the specific objects and stitch them with the scene. This also enables us to detect any anomalies in the scene, For example, If there is car inside a lake, Humans would





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describe it as “This car have meet with an accident and should have fallen inside the lake”. But for neural networks it could be like “A car is parked inside water”. It is hard for neural network to understand that a car cannot be parked in a lake. And perceptions like depth is also hard for neural nets to understand. But humans can understand 3D perception in a photo which is a 2D representation of 3D scene. This depth perception is also very important to describe a scene. And also important attribute for image description is spatial understating of objects, which include where the objects is located in the image. Like Right, Left etc. Spatial understand also include size of the objects and orientation of the objects. With all this attributes we can provide a human level description of an image.

The model architecture proposed in this paper can locate and also can describe the annotations of the objects and their spatial features, like where the objects are located in the frame. And also features like position and orientation.

ARCHITECTURE BREAKDOWN

Convolutional Neural Network

The convolutional neural network is very fundamental for the proposed model. It is the base of the proposed architecture, rest of the components are built on top of convolutional layer. The convolutional layer localize the objects in the image and try to annotate them. The pre-trained model trained from CIFAR - 10 or Image Net will provide us with all the class labels we need. Convolutional Neural Networks have a convolution layer followed by a neural network layer for probabilistic predictions. And to rank the output class possibilities. Generally convolutional neural have, input layer where the image is feed into the network as a column vector and then it is followed by a convolutional layer and then by an activation function (ReLU or Sigmoid) and then by a pooling layer. This is layer are connected for multiple times and then followed by a fully connected layer.

Recurrent Neural Network

The Recurrent Neural Networks works very well with spatial and orientations features. It consist of a fully connected input layer and followed with a various hidden layers. More the hidden layers, more parameters are available to adjust. Recurrent neural networks differs from rest of the neural networks, because the output is feedback into the hidden layer, for dynamic temporal analysis. We use it for prediction of spatial and orientation of objects in image.

Generative Pre-Trained Transformer

The Generative Pre-Trained Transformer is also known as GPT - 3 is a third generation language model. With the inputs from the previous layers. And this model produces a clean and human like language description of the input image. With raw inputs like, “Black Dogs” or “Blue Car parked” the GPT can produce outputs like “There is a black dog in the image” or “Parked image of a Blue car”. This model can produce rich output texts. This model can also perform text completion tasks. The GPT-3 is pre trained model with 175 billion parameters.

STACKING OF INDIVIDUAL COMPONENTS

Our Model architecture consist of various components stacked on each other creating a single large model. The breakdown of the model is as follows.

DYNAMIC LOCALISATION OF OBJECTS IN IMAGE

With our true image we impose a template for localizing the annotated objects on the image. By imposing this template to our input image, the recurrent neural network can go ahead and map the spatial feature with minimum error. As you can tell from the figure 5. Player 1 is kicking the ball from bottom left corner. And the ball is at the centre of the image and the goal post is at the top of the image and the goal keeper is trying to defend the goal from top right. The template provides localised zones for the neural network to annotate it effectively. In case of any mis predications can also cross verified using this sample template imposing method. The annotated image may look as shown below were the objects in the image and even colours are annotated. With the deep annotated image and imposable reference template, the neural network can produce sentences like, “Bottom Left Yellow shirt man kicking”, “Ball center”, “Red shirt Goal Keeper top right” and “Goal post top”. This generated text may be apt description of the object and space, but it is not a rich text. And it not like human like. These sentences are feed into





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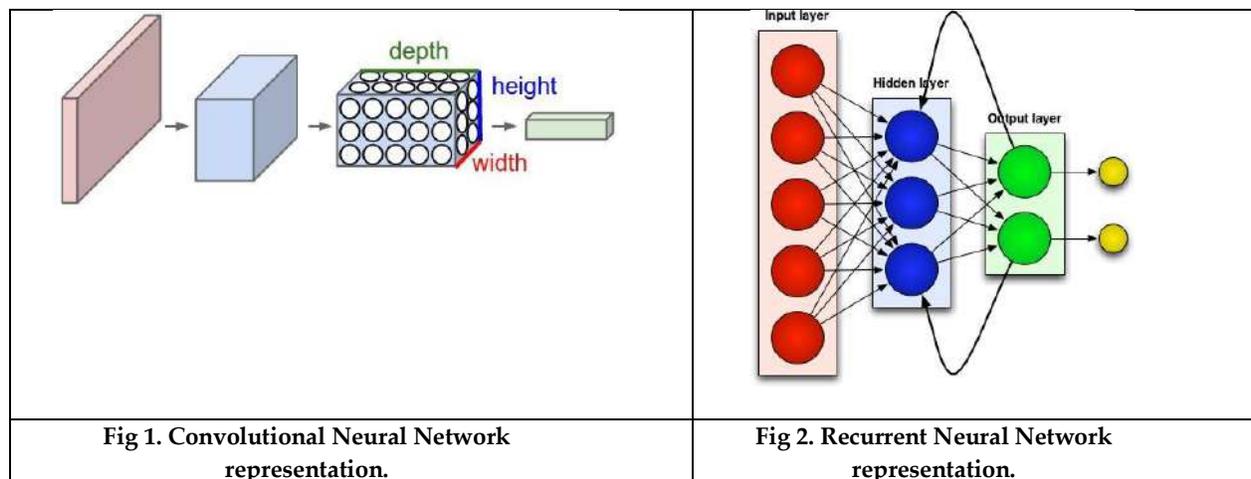
GPT-3 model and the result will be like. “In bottom right man wearing yellow shirt kicking, the ball is in the center, in top right Goal keeper is wearing red shirt standing before the goal, which is on top”

CONCLUSION

By this Stacking architecture we can achieve human like description of the given image. This architecture has a very small latency during test time, because all the stacked neural networks are pre trained. The prediction is even boosted by the GPT-3.

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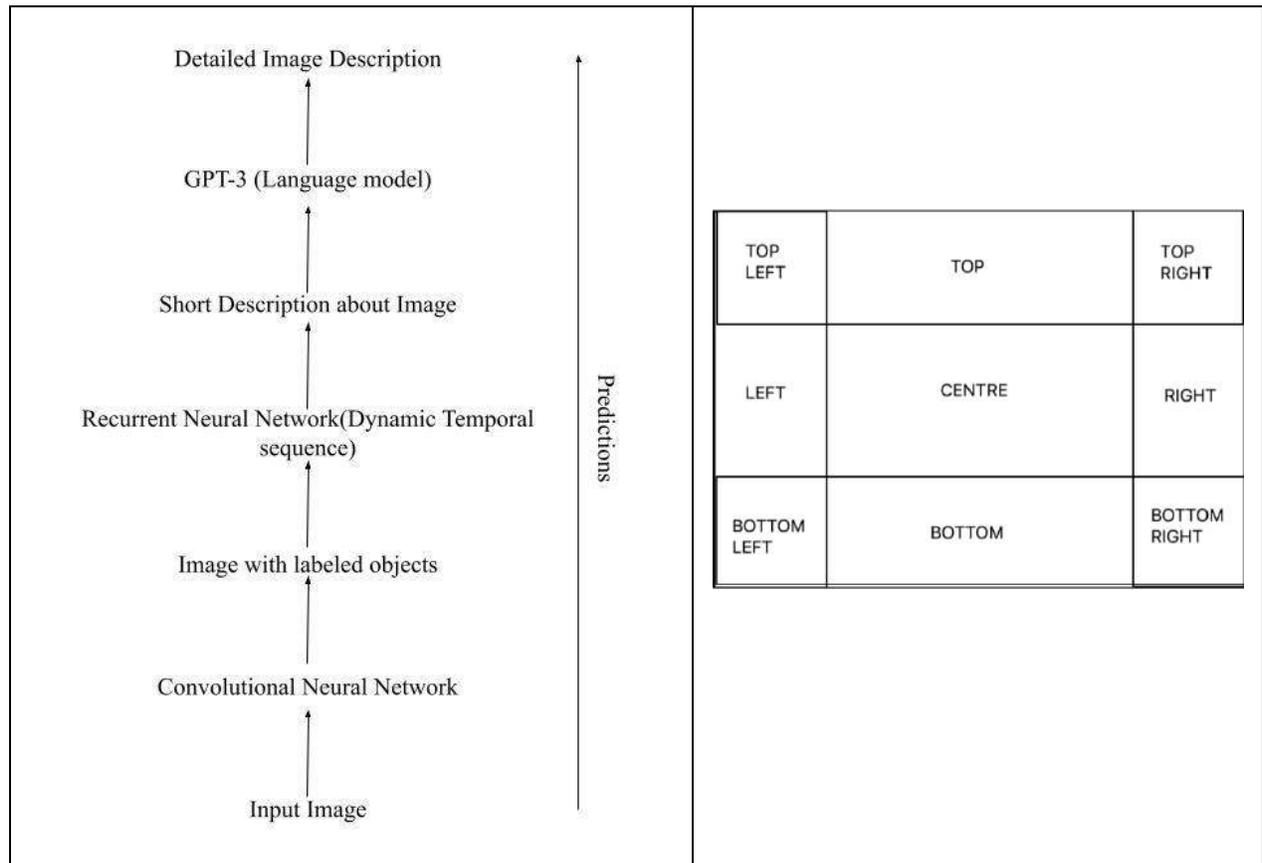


Fig 3. Model Stack

Fig 4. Imposable template

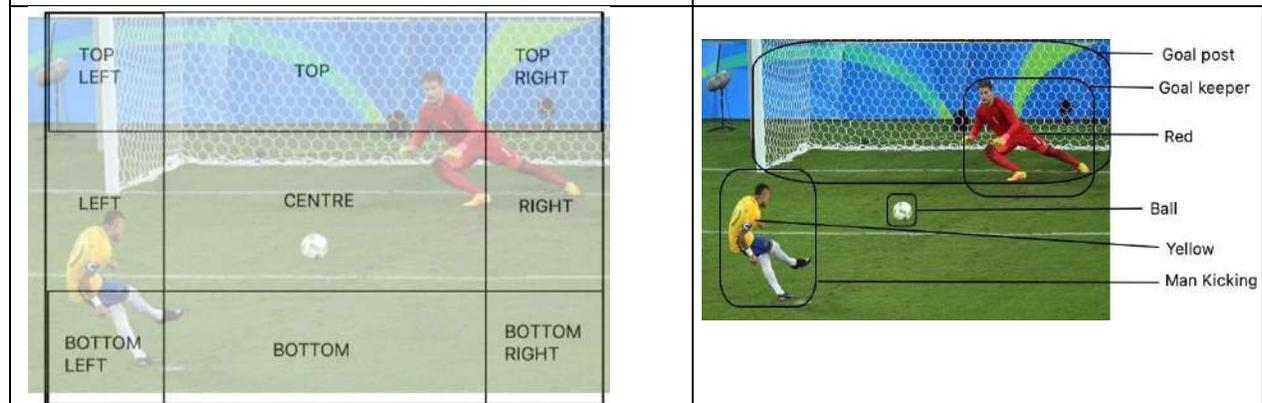


Fig 5. Imposed Image with template

Fig 6. Deep annotated image





Plant Leaf Disease Detection using Convolutional Neural Networks

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ABSTRACT

Plant diseases are the most prevalent cause of poor yields and therefore lower agricultural incomes for farmers. Currently, experts are putting out their best efforts to develop a system that can detect plant illnesses on its own. Accurate diagnosis of plant diseases may aid in the discovery of a treatment as soon as possible in order to control the loss. This research seeks to establish a unique strategy for predicting plant diseases by using machine learning techniques in conjunction with other data sources. The Pre trained Convolutional Neural Networks Models such as AlexNet, VGG16 & ResNet are used in this paper for extracting features from plant leaf for detection and classification of diseases in plant leaves. Based on the experimental results it is concluded that pre-trained CNN Architecture ResNet performs better for plant leaf diseases detection system with the F1-Score is 99%. The findings of the experiments demonstrate that plant diseases may be reliably diagnosed.

Keywords: Plant diseases, CNN, Machine Learning

INTRODUCTION

Farming is one of the most common forms of employment in India. Every country's economy is dependent on agriculture in some way or another. Advancement in the field of agriculture is mainly intended to meet the growing



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demand of population. A modernization of the agricultural sector is required to ensure its survival in the current climate. Crops are impacted by bacterial and fungal diseases, which are both contagious. This has a significant negative impact on the productivity of farmers. The crop must be in good condition in order to provide an ideal yield. The detection of illnesses with our naked eyes will always be a time-consuming and difficult procedure. It is vital to monitor the farm on a continuous basis in order to do this. This is a time-consuming procedure. The expense of doing so might be prohibitively expensive when the farm is large in size. Even agricultural professionals are unable to detect illnesses and come up with a solution to the issue as a result of this obstacle. Farmers would benefit greatly from the development of an automated system that can detect and diagnose plant illnesses.

This approach may serve as a tool to alert farmers at the appropriate time so that they may take the required precautions. Various plant diseases may cause damage to plant components such as leaves, fruits, seeds, and other reproductive organs. These diseases affect distinct regions of the plant's body in a unique way. The leaves of a plant are the most vital element of the plant. It is directly detrimental to the plant's life cycle if the leaf of the plant is infected with a pathogen. There are many types of illnesses that typically affect leaves, such as bacterial disease, fungal disease, and so on. As a result, it is critical to diagnose plant diseases as early as possible.

Machine Learning Based Approach

Machine learning seems to be a more promising approach to addressing this problem. Based on digital plant pictures, many machine learning (ML) methods are presented for the automated identification and classification of plant diseases. Plants are afflicted by a variety of illnesses that manifest themselves in many sections of the plant's body, including the leaf, stem, seed, and fruit. Various areas of the plant's body are susceptible to different diseases. The leaves are widely regarded as the most important element of the plant, and it is only with the assistance of the leaves that the process of photosynthesis can be carried out. If a plant's leaf is susceptible to disease, it will have an immediate impact on the plant's life cycle. Setting up a system that automatically identifies and categorizes illnesses is critical to dealing with them fearlessly and effectively. For resolving this problem, machine learning is likely to be a viable alternative. Several machine learning algorithms have recently been presented for the diagnosis and classification of plant diseases based on photographs of plants. Although such automated technologies have provided solutions to the difficulties, the greater difficulty comes in ensuring that the findings of the tests are consistent and robust. In this research, machine learning methods are used to identify plant illness.

LITERATURE REVIEW

Several researchers, including Revathi et al. [1] have developed a novel technique for the identification of visual disorders in plants. It is necessary to create and pre-process digital photographs of the plant. After that, the extraction methods, such as edge detection, colour space, and textural elements, are carried out on the data. The characteristics that have been retrieved are handed on to the classifiers for classification. The goal of this study is to use image processing to locate the diseased portion of a cotton leaf.

The authors of [2] presented a technique in which a colour picture is entered and then colour altered. Using the threshold setting, the green pixels in the picture are concealed and removed from the final image. Following that, the segmentation procedure is carried out. The texture statistics are produced for each and every credible segment in the dataset. When it comes to illness classification, the next classifier is applied. In their study, the authors [3] examine and contrast the categorization approaches that are utilised in image processing. In this work [4] authors presented segmentation-based approaches as an alternative to segmentation-based techniques. Kumar et al [5] employed a procedure that included a sorting process followed by grading of the citrus fruits to get their results. It is able to identify the kind of fruit based on the combination of photos of fruits. Kulkarni and colleagues [6] have made use of artificial neural networks (ANN). The classification problem is solved using an ANN classifier, while the feature extraction problem is solved using a Gabor filter. It made advantage of both texture and colour characteristics.



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The authors in [7] utilised a Gaussian filter to reduce noise, and a threshold was applied to produce the green component of the final product. It is proposed in [8] that an image processing technique be used for the detection of jute plant disease. It is capable of detecting stem infections in the jute plant with high accuracy. Camargo and colleagues [9] created a system that, with the use of colour picture analysis, can prolong the visual symptoms of plant illnesses. A Gray scale picture of an infected plant is transformed into colour transformations such as I3a, I3b, and H using the algorithm that has been built in the first step. First, the translated picture is segmented based on the intensity distribution in the histogram, which is used to segment the original image. This approach is particularly useful when attempting to target a single picture with a wide range of brightness. Once the segmentation procedure is complete, the area that has been collected is optimised in order to remove pixel portions that are not inside the targeted area.

There was complete agreement on the outcomes, which demonstrated that they were successful in detecting the sick areas of the plant. Camargo et al [10] have developed an algorithm for the identification of sick spots in plants that is based on image processing techniques. The image is taken in its initial state, and then the colour change is applied. The use of the Gaussian filter can improve the quality of transformed images by a significant amount. After that, segmentation is carried out in order to determine the region of interest. In this case, the location of the optimal threshold distinguishes the segments. In the next step, the segmented areas are classified and labelled as unhealthy or healthy using the SVM classifier. It is suggested in [11] to conduct an overview of several detection strategies that make use of image processing. The authors of [12] employed a genetic approach to identify plant illnesses, which they described in detail. For disease diagnosis, the only approach now available is on-site leaf analysis by specialists, from which plant disease identification and detection may be achieved. This activity requires the participation of many professionals and the frequent monitoring of the plant, both of which are prohibitively costly when performed on larger farms.

Mohanty et al. [13] used GoogleNet and AlexNet to identify 54,306 plant leaf photos as healthy or sick in the Plant Village dataset, and they discovered that GoogleNet had a somewhat greater average classification effect than AlexNet on the dataset. With respect to the test set, the trained deep convolutional neural network model achieves a 99.35 percent accuracy. A straightforward method of using intelligent mobile phones to diagnose plant illnesses in horticultural crops involves building a deep learning model on a growing and publicly accessible picture dataset, which is expanding all the time. A deep residual neural network-based updated technique developed by Picon et al. [14] was used to diagnose a wide range of plant ailments under real-world acquisition situations.

For the purpose of early sickness detection, numerous enhancements have been suggested. According to the statistics, all of the diseases assessed had an AuC score more than 0.80, which indicates that they were all severe. Selvaraj et al. [15] used the transfer learning technique to retrain three CNN architectures, and the results were promising. For accurate predictions, deep transfer learning was used to construct networks utilizing pre-trained illness detection models, which were then used to form networks. Deep learning was suggested by Fuentes et al. [16, 17] for recognizing diseases and pests in tomato plant pictures taken at different camera resolutions, and it has been used in many studies. Multiple CNN object detectors were used in conjunction with deep learning meta-architectures to detect objects. Data expansion, as well as local and global class annotation, were used to improve training accuracy while simultaneously reducing false positives. Training and testing were carried out using a large-scale tomato disease dataset, which was employed throughout the process. A total of nine distinct pests and illnesses were successfully identified by the algorithm from the complex circumstances.

In a few countries, suitable agricultural facilities are not accessible due to lack of resources. As a result, they believe that employing professionals is essential. However, this results in actions that are both costly and time demanding. During such circumstances, the recommended technique has been shown to be useful in assessing large crop yields. The non-manual diagnosis of plant disease by examining the state of the leaves reduces the cost and makes it simpler to identify the illness. This aids machine vision in the provision of robot guiding, inspection, and image-based autonomous process control, among other functions. More time and effort are required for the visual method of leaf





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disease detection. However, this work is less precise and can only be completed in certain regions. Non-manual, or automated, plant disease detection, on the other hand, easier, more accurate, and takes up less time than manual plant disease detection.

MATERIALS AND METHODS

Our major goal is to construct a model that can identify input plant leaf images as healthy or sick based on their appearance as shown in the figure 2. Detection of a disease on a plant leaf also allows for the identification of the disease kind. In this work, we evaluate the pre trained CNN architecture for the detection and classification of leaves suffering from common ailments.

Dataset

In order to train sophisticated visualization and classification algorithms, a large amount of data must be collected. In general, when training with huge volumes of data, machine learning and deep learning systems perform better than when training with little amounts. In this work the Plant Village database [18, 19] is used for analysis. Each of the 14 plants included in this "dataset" has a total of 54,323 images, which are separated into 38 groups of healthy leaves and plants suffering from various ailments. 11654 photos of three different plant kinds were utilized in this study: apple (7771 images), potato (3763 images), and rice (120 images). For the purpose of training and evaluating deep learning systems, the data volume should be separated into two types of sets: research sets and evaluation sets. The dataset developed as a result of this research is unique in that it comprises photos of varying sizes and has more sensor power than any other dataset available. Preprocessing, feature extraction, feature selection, and classification of plant leaf images were performed using images acquired from the PlantVillage dataset [19]. A selection of few images of leaf diseases are shown in Figure 1 from the dataset.

Convolutional Neural Networks

Convolutional Neural Networks (CNNs) have a different architecture than traditional neural networks. CNN layers contain neurons that are organized in three dimensions, namely, width, height, and depth, and each layer in a CNN converts a three-dimensional input volume into a three-dimensional output volume of neuron activations, resulting in a three-dimensional output volume of neuron activations. In a typical CNN design, there are three sorts of layers: convolutional, pooling, and fully associated layers, to name a few examples. It is not necessary for all neurons in one layer to be coupled to all neurons in the next layer for the network to function properly. A feature map is created by performing a series of convolutions and pooling operations on the input data with the help of a filter to generate a feature map. The result of the convolution layer is a combination of the feature maps that have been merged.

The convolution layer is regarded to be the most important building component of a CNN, and as a result, training a CNN takes a significant amount of time. A technique called convolution is used to the input in order to calculate the outputs of neuronal networks in each of these layers. The parameters of convolution layers are shared sets of weights (also known as kernels or filters), which have extremely narrow receptive fields [20], which are used to create the convolution layers. Figure 3 depicts a typical CNN design for three-variate time series categorization using convolutional neural networks.

Input layer: A total of $N \times K$ neurons are included in the input layer, where k signifies the variate number of input time series to be processed and N specifies the duration of each univariate series to be processed

Convolution layer: Perform convolution operations on the time series of the preceding layer with convolution filters. Here are various filter parameters that should be established beforehand based on domain knowledge or simply based on tests, such as filter numbers m , convolution strides s , and the size of the convolutional filter $K \times I$, where k





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represents the varying value of the time - series data in the previous layer and I specifies the length of the filter in the next layer. In this layer, it is also necessary to define a nonlinear transformation function, denoted by the letter f. For instance, if the preceding layer contains k-variate time series and the length of each univariate is N, after the convolution operation, we get m-variate time series and the length of each univariate is $\lfloor (N-1)/8+1 \rfloor$, where $\lfloor \cdot \rfloor$ denotes rounding down.

Pooling layer: When a feature map is partitioned into N equal-length segments, the average or maximum value of each segment is used to represent the segment as a whole in the pooling layer. Downsampling convolution output bands reduces the variability in the hidden activations, which is a benefit of the pooling process.

Feature layer: In the end, a set of feature maps represents the original time series after it has been subjected to various convolution and pooling processes. Create a new long time series by connecting all of the feature maps together, which will serve as the final representation of what was originally entered into the feature layer.

Output layer: The output layer comprises n neurons, which correspond to the number of classes of time series in the input layer. The feature layer is completely integrated with this layer. In the classification job, the most often used technique is to use the maximum output neuron as the class label for the input time series, which is called the maximum output neuron method.

Training of CNN

The CNN is trained via a sequence of training examples $((x_1, y_1), (x_2, y_2), \dots, (x_{Nsample}, y_{Nsample}))$ with $x_t \in R^{N \times k}, y_t \in R^n$ for $1 \leq t \leq Nsample$. The input to the network is represented by the multidimensional or unilateral time series x_t , while the goal output is represented by the vector y_t . The network is trained according to the following several steps

Step 1 Initialize the network. Determine the CNN architecture composes convolution layer and pooling layer, as shown in Figure 3. Make sure the number of neurons in the input and output layers are set to match the classification task's specifications. Set all the CNN parameters. Use a modest random integer to set the weights and bias. A popular example of an activation function is the sigmoid function and a learning rate.

$$f(x) = Sigmoid(x) = \frac{1}{1+e^{-x}} \tag{1}$$

Step 2 Using a random number generator, choose a sample from the practise data.

Step 3 Calculate the output of each layer.

(i) The convolution layer's output may be expressed as follows:

$$P_r(t) = g \left(C_r((t-1)l+1), C_r((t-1)l+2), \dots, C_r(tl) \right) \tag{2}$$

where $\omega_r \in R^{N \times k}$ denotes the input time series or the output of the preceding layer, s denotes the convolution stride, $C_r(t)$ refers to the t^{th} component of the r^{th} feature map, $\omega_r \in R^{l \times k}$ and $b(r)$ refer to the weights and bias of the r^{th} convolution filter

(ii) The pooling layer's output may be expressed as

$$P_r(t) = g \left(C_r((t-1)l+1), C_r((t-1)l+2), \dots, C_r(tl) \right) \tag{3}$$





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The pooling strategy is represented by the function g , with the average or maximum pooling strategies being the most often utilised. It goes without saying that the pooling procedure has the effect of lowering the amount of point data while maintaining the same feature map.

(iii) It's possible to express the result of the output layer in terms of

$$O(j) = f(\sum_{i=1}^M z(i) \omega_f(i, j) + b_f(j)), j = 1, 2, \dots, n \quad (4)$$

The weights of the link between the feature layer and the output layer $\omega_f \in R^{M \times n}$ denoted by z , the final feature map in the feature layer, and b_f is the output layer bias. As a result, the mean-square error may be expressed as

$$E = \frac{1}{2} \sum_{k=1}^n e(k)^2 = \frac{1}{2} \sum_{k=1}^n (O(k) - y(k))^2 \quad (5)$$

Step 4 Gradient descent is used to adjust the weights and bias.

$$p = p - \eta \frac{\partial E}{\partial p} \quad (6)$$

where p is the worth of the constraint, and p denotes to ω_r , ω_f , b , or b_f in this CNN.

Step 5 Choose another training sample and go to **Step 3** until all the samples in the training set have been trained.

Step 6 Increase the iteration number. If the iteration number is equal to the maximum value which is set previously, stop the algorithm. Otherwise, go to **Step 2**.

AlexNet: The fundamental advance in CNN performance was made possible in large part because of this concept. Image identification and categorization issues are among the most difficult to solve it was topped the ILSVRS 2012 contest. It was developed at the University of Toronto by Krizhevsky et al. [21]. It comprises of 5 pairs of Conv layers preceded by ReLU, 3 max-pooling layers, and 3 fully connected layers as shown in figure 4.

VGG16: Developing on the success of CNNs in image identification, Zisserman & Simonyan [22] of the Oxford University introduced an architecture which is effective & simple for CNNs. Visual Geometry Group was the name given to this innovative architecture (VGG). There are 5 Pool layers and 13 Conv levels in VGG16 Net, with all hidden layers packed with ReLU and just one completely linked layer as shown in figure 5.

ResNet: In the ILSVRC-2015 competition, a Microsoft algorithm which was presented by He et al. [23] was awarded first place. The ResNet architecture is composed of 5 stages, with the last 4 stages including a residual slab (for example, Conv2-4 (56x256x56) in the stage 2. Each residual block is adjacent to 3 layers with convolutions of 1×1 and 3×3 . 2 Pool layers, 17 Conv layers, and 1 fully linked layer comprise ResNet networks as shown in figure 6.

Performance Metrics

Performance is evaluated using industry-standard classification methods: Precision, Recall, & F1-Score. A "true positive" (TP) occurs when we expect the answer to be "yes." The authentic output remained "yes". A false positive (FP) occurs when we expect a yes answer but get a no. True Negative (TN) is a term that refers to situations in which we expected a negative outcome. Actually, the answer was NO, while A false negative (FN) is one in which we incorrectly assumed that the answer was "no." YES, that was the final outcome are the 4 tags were created from the confusion matrix castoff to compare the efficiency of various deep learning techniques.





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Precision is defined as the ratio of exact positive outcomes to positive outcomes predicted by the classification model.

$$\text{Precision} = \frac{TP}{TP+FP} \quad (7)$$

Recall [39,40] is defined as the proportion of accurate positive findings to the total no. of related samples (There were no false negatives in any of the samples).

$$\text{Recall} = \frac{TP}{TP+FN} \quad (8)$$

The F1 Score, is used to measure a system accuracy, is calculated as a weighted harmonic average of precision & recall. It has a range of values (0,1)

$$F1 = 2 \frac{\text{Precision} \cdot \text{Recall}}{\text{Precision} + \text{Recall}} \quad (9)$$

RESULTS AND DISCUSSION

In this work MATLAB2021a and the WEKA tool for feature extraction and training. The experimental findings of the suggested framework are presented and discussed in this section. Based on the analysis it is observed that the pre-trained CNN Architecture ResNet achieves 98 % of precision and 97% of recall are observed from the Table I and the model's cumulative performance is calculated using the F1-Score is 99%. Based on the Figure 7 it is observed that the pre-trained CNN Architecture ResNet performs better for plant leaf diseases detection system.

CONCLUSION

In this paper the Pre trained Convolutional Neural Networks Models such as AlexNet, VGG16 & ResNet are used in this paper for extracting features from plant leaf for detection and classification of diseases in plant leaves. Based on the experimental results it is concluded that pre-trained CNN Architecture ResNet performs better for plant leaf diseases detection system with the F1-Score is 99%. The findings of the experiments demonstrate that plant diseases may be reliably diagnosed.

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Table 1. Analysis of Pre trained CNN Architecture

CNN	Precision	Recall	F1 Score
AlexNet	0.952	0.954	0.966
VGG16	0.974	0.972	0.985
ResNet	0.982	0.97	0.992





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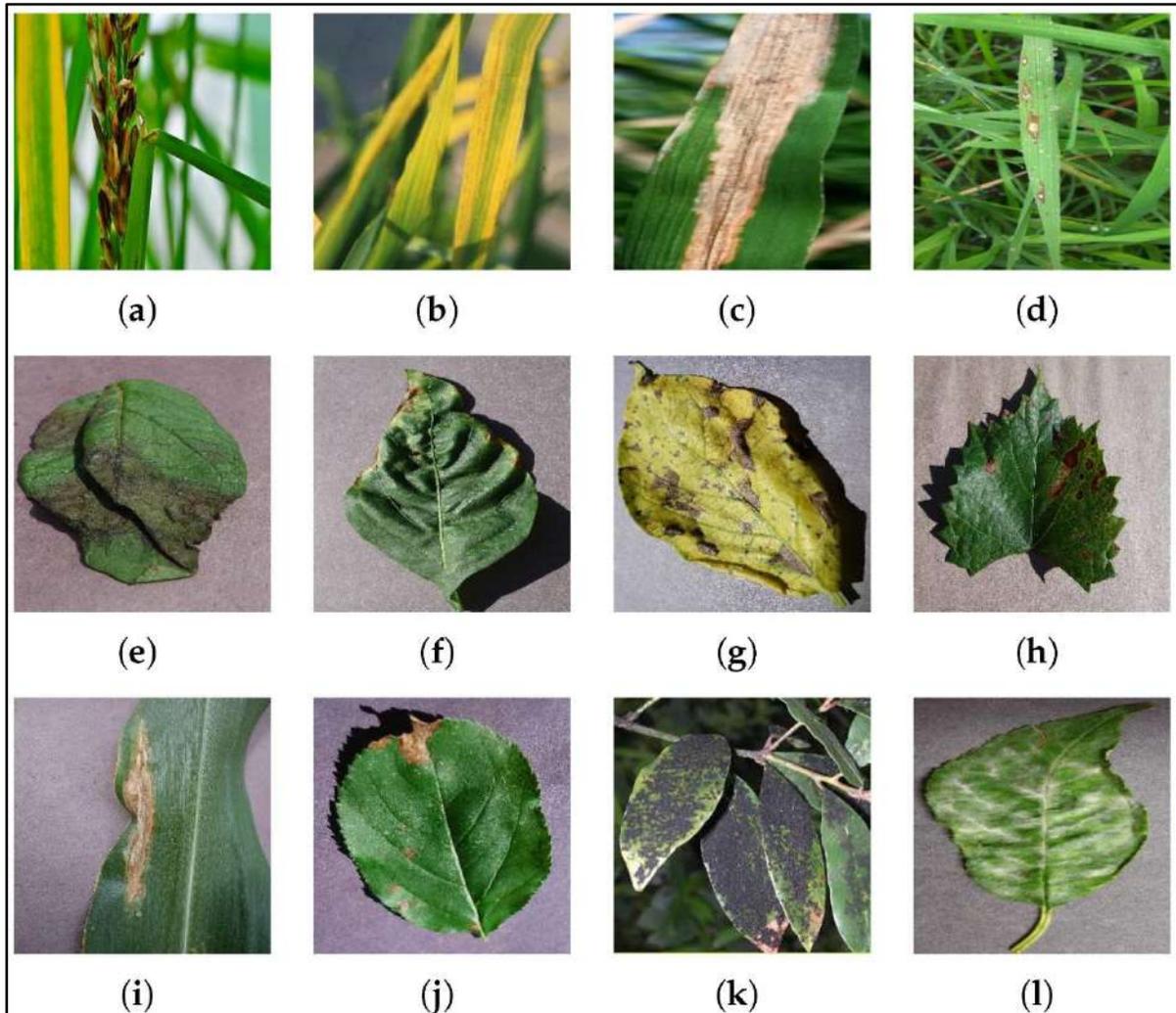


Fig.1. Samples of plant leaf disease images under numerous health conditions in various backgrounds and having different symptoms: (a) Rice Sheath-rot, (b) Rice Tungro, (c) Rice Bacterial leaf-blight, (d) Rice Blast, (e) Potato Late-blight, (f) Pepper Bacterial-spot, (g) Potato Early-blight Pepper Bacterial-spot, (h) Grape Black-measles, (i) Corn Northern Leaf-blight, (j) Apple Black-rot, (k) Mango Sooty-mold, and (l) Cherry Powdery-mildew [19]



Fig.2. Block diagram for proposed approach of plant leaf disease detection





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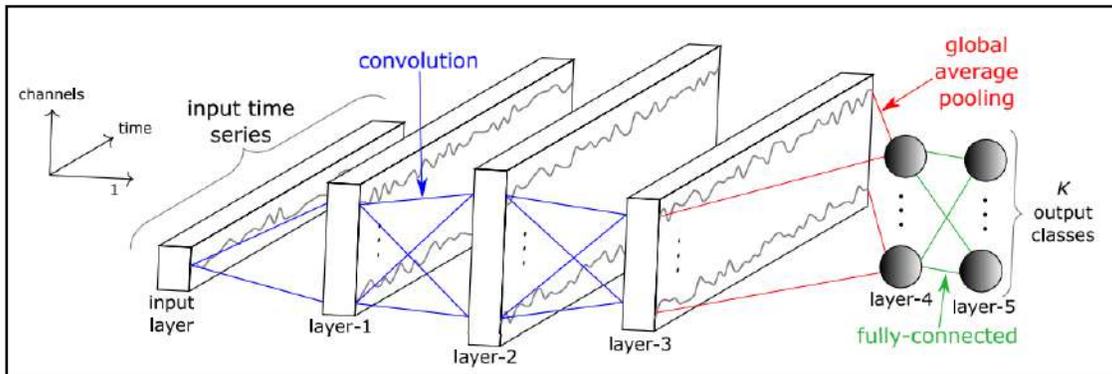


Fig. 3. Fully Convolutional Neural Network Architecture

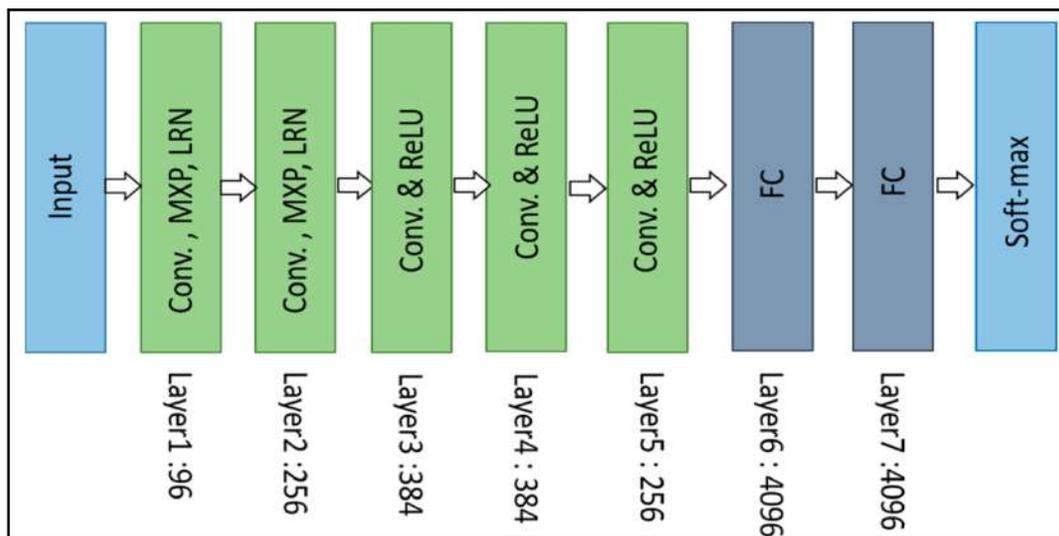


Fig.4. Architecture of AlexNet.



Fig.5. The Standard VGG-16 Network Architecture.





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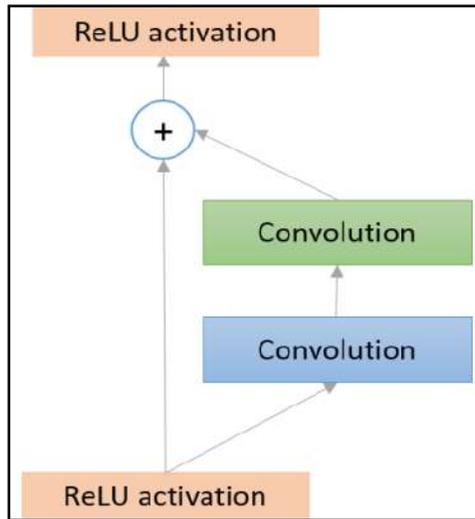


Fig.6.Basic diagram of Residual block.

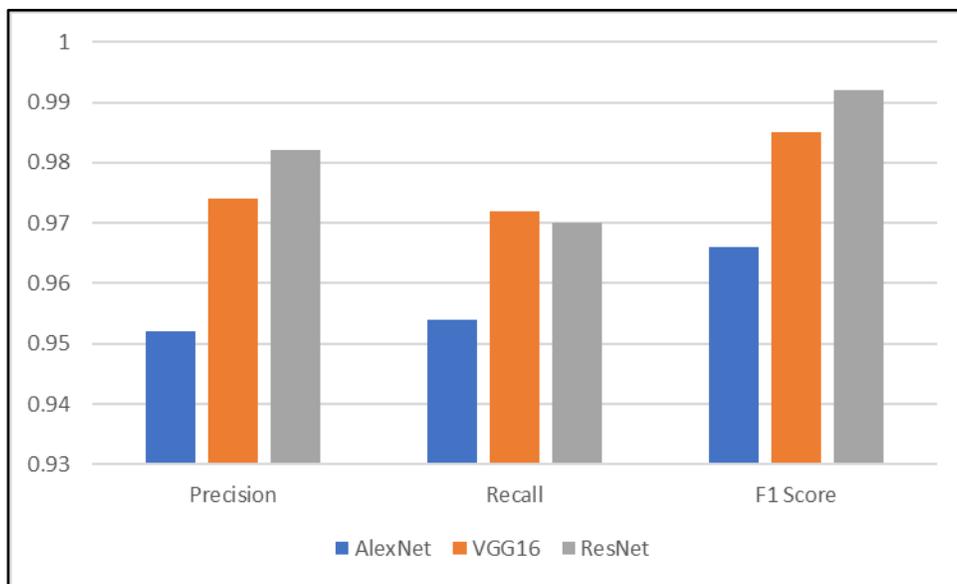


Fig.7.Analysis Plot for Pre trained CNN Architecture





Relative EP Matrices in Minkowski Space

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ABSTRACT

In this paper, we have introduced the concept of relative EP matrix of a rectangular matrix relative to a partial isometry in Minkowski space \mathcal{M} . We established the relationship between T-symmetric, T-normal, T-EP matrices and their properties in Minkowski space \mathcal{M} .

Keywords: Moore-Penrose inverse, Partial isometry, EP matrix, T-EP matrix, Minkowski adjoint, Minkowski inverse, Minkowski space.

MSC Subject Classification: 15A09, 15A27, 15B57.

INTRODUCTION

The classical Minkowski space is a fictitious four-dimensions space-time, which is named for the German mathematician Hermann Minkowski. The symbols H^* , H^- , H^m , H^\dagger , $R(H)$ and $N(H)$ denote the conjugate transpose, Minkowski adjoint, Minkowski inverse, Moore-Penrose inverse, range space and null space of a matrix H respectively. Formally, it is a four dimensional real vector space equipped with non-degenerate, symmetric bilinear form with signature $(+, -, -, -)$. Then it is often denoted by $\mathbb{R}^{1,3}$, in which the metric matrix is $G = \text{Diag}(1, -I_3)$. In this paper, we denote the Minkowski space by \mathcal{M} , which is an n dimensional complex vector space with the metric matrix $G = \text{Diag}(1, -I_{n-1})$, where I_{n-1} denotes the identity matrix of order $n - 1$. It is easy to check that $G^* = G$ and $G^2 = I_n$. Let $\mathbb{C}_{m,n}$ be the set of $m \times n$ complex matrices. The Minkowski adjoint of a matrix $A \in \mathbb{C}_{n,n}$ is denoted by A^\sim , and is defined as $A^\sim = GA^*G$, where A^* denotes the conjugate transpose of A .





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Renardy [10] gets some equivalent conditions for a matrix to have singular value decomposition in \mathcal{M} . The Minkowski inverse of a matrix $A \in \mathbb{C}_{n,n}$ in \mathcal{M} is defined as the unique matrix $X \in \mathbb{C}_{n,n}$ satisfying the following four conditions:

$$AXA = A, XAX = X, (AX)^\sim = AX, (XA)^\sim = XA$$

However, the Minkowski inverse of a matrix does not exist always as in the case of Moore-Penrose inverse of a matrix. It is proved that the Minkowski inverse of a matrix $A \in \mathbb{C}^{m \times n}$ exists if and only if $rk(AA^\sim) = rk(A^\sim A) = rk(A)$. A matrix $A \in \mathbb{C}^n$ is said to be m -symmetric if $A = A^\sim$. Also, presented the notion of range symmetric matrices in Minkowski space. Further developed the concept of Minkowski inverse of the range symmetric matrices and its equivalent conditions. The EP matrices have been generalized in many ways. The first such generalization known as bi-EP matrices is given by Hartwig and Spindelbock in [3]. Since then on several of their generalizations have appeared some of them being conjugate EP matrix [6], k-EP [7], weighted EP matrices [11], m-EP [5], k-core EP [2], and k-DMP [2]. All these extensions have been defined on the set of complex square matrices. Our aim here in this work is to extend the class of EP matrices to a class of relative EP matrices when matrices are rectangular matrices.

T-EP Matrices in Minkowski Space \mathcal{M}

In this section we introduce the concept of relative EP matrix for a matrix $H \in \mathbb{C}^{m \times n}$ with respect to a partial isometry $T \in \mathbb{C}^{m \times n}$ in Minkowski space. We present various equivalent characterizations of this new class of matrices and we show that this class of matrices contains the following two classes of matrices in Minkowski space.

Definition 2.1. Let $H, T \in \mathbb{C}^{m \times n}$ in the Minkowski space \mathcal{M} . Then H is called relative symmetric to T (or, in short, T -symmetric) if $H = T(GHG)^\sim T$.

Definition 2.2. Let $H, T \in \mathbb{C}^{m \times n}$ in the Minkowski space \mathcal{M} . We say that H is relative normal to T (or, in short, T -normal) if $H = T(GTG)^\sim H = H(GTG)^\sim T$ and $H(GHG)^\sim T = T(GHG)^\sim H$.

Lemma 2.1. Let $H \in M_n(\mathbb{C})$ in the Minkowski space \mathcal{M} . Then, $N(H) = R((GHG)^\sim)^\perp$ and $N((GHG)^\sim) = R(H)^\perp$.

Lemma 2.2. If T is a partial isometry in the Minkowski space \mathcal{M} if and only if $T^m = (GTG)^\sim$.

Theorem 2.1. Let $H, T \in \mathbb{C}^{m \times n}$ in the Minkowski space \mathcal{M} . If H is T -symmetric in \mathcal{M} then H is T -normal in \mathcal{M} .

Proof. Since H is T -symmetric in \mathcal{M} , $H = T(GHG)^\sim T$.

We prove H is T -normal in \mathcal{M} .

Now,

$$\begin{aligned} H(GTG)^\sim T &= T(GHG)^\sim T(GTG)^\sim T \\ &= T(GHG)^\sim (T(GTG)^\sim T) \text{ (Using } T(GTG)^\sim T = T) \\ &= T(GHG)^\sim T \\ &= H. \end{aligned}$$

Similarly,

$$\begin{aligned} T(GTG)^\sim H &= T(GTG)^\sim T(GHG)^\sim T \\ &= (T(GTG)^\sim T)(GHG)^\sim T \text{ (Using } T(GTG)^\sim T = T) \\ &= T(GHG)^\sim T \\ &= H. \end{aligned}$$

Also,

$$\begin{aligned} H(GHG)^\sim T &= (T(GHG)^\sim T)(GHG)^\sim T \\ &= T(GHG)^\sim (T(GHG)^\sim T) \\ &= T(GHG)^\sim H. \end{aligned}$$

Hence H is T -normal in \mathcal{M} .





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We now define the concept of relative EP matrix respect to a partial isometry T in Minkowski space as follows:

Definition 2.3. Let $H, T \in \mathbb{C}^{m \times n}$ in the Minkowski space \mathcal{M} . Then we say H is relative EP to T (or, in short, T -EP) if $\mathcal{R}(H) = \mathcal{R}(T(GHG)^{\sim}T)$ and $H = H(GTG)^{\sim}T$.

Remark 2.1. In particular, when the partial isometry T is the identity matrix above definition reduces to $\mathcal{R}(H) = \mathcal{R}((GHG)^{\sim})$, which implies that H is an EP matrix in Minkowski space \mathcal{M} . We next show that a T -normal matrix is a T – EP matrix in Minkowski space.

Theorem 2.2. Let $H, T \in \mathbb{C}^{m \times n}$ in the Minkowski space \mathcal{M} . If H is T -normal in \mathcal{M} then H is T – EP in \mathcal{M} .

Proof. Let H be T -normal in \mathcal{M} .

Then $H = H(GTG)^{\sim}T = T(GTG)^{\sim}H$.

and $H(GHG)^{\sim}T = T(GHG)^{\sim}H$

(or equivalently $(GTG)^{\sim}H(GHG)^{\sim} = (GHG)^{\sim}H(GTG)^{\sim}$).

Clearly, $H = H(GTG)^{\sim}T$.

It remains to show that $\mathcal{R}(H) = \mathcal{R}(T(GHG)^{\sim}T)$.

In fact,

$$\begin{aligned} \mathcal{R}(H) &= \mathcal{R}(H(GHG)^{\sim}) \\ &= \mathcal{R}(T(GTG)^{\sim}H(GHG)^{\sim}) \text{ (Using } (GTG)^{\sim}H(GHG)^{\sim} = (GHG)^{\sim}H(GTG)^{\sim}) \\ &= \mathcal{R}(T(GHG)^{\sim}H(GTG)^{\sim}) \text{ (Using } H = T(GTG)^{\sim}H) \\ &= \mathcal{R}(T(GHG)^{\sim}T(GTG)^{\sim}H(GTG)^{\sim}) \\ &= \mathcal{R}(T(GHG)^{\sim}T(T(GHG)^{\sim}T)^{\sim}) \\ &= \mathcal{R}(T(GHG)^{\sim}T). \end{aligned}$$

Hence H is T -EP in \mathcal{M} .

The following two lemmas are the characterizations of relative EP matrices in Minkowski space.

Lemma 2.3. Let $H, T \in \mathbb{C}^{m \times n}$ in the Minkowski space \mathcal{M} . Then the following are equivalent:

- (i) $H = T(GTG)^{\sim}H$;
- (ii) $\mathcal{R}(H) \subseteq \mathcal{R}(T)$;
- (iii) $\mathcal{N}((GTG)^{\sim}) \subseteq \mathcal{N}((GHG)^{\sim})$;
- (iv) $H^m = H^m T(GTG)^{\sim}$.

Proof. (i) \Rightarrow (ii).

Let us assume that $H = T(GTG)^{\sim}H$.

To prove that $\mathcal{R}(H) \subseteq \mathcal{R}(T)$.

$$\begin{aligned} \mathcal{R}(H) &= \mathcal{R}(H(GHG)^{\sim}) \\ &= \mathcal{R}(T(GTG)^{\sim}H(GHG)^{\sim}) \text{ (Using } H = T(GTG)^{\sim}H) \\ &= \mathcal{R}(T(GHG)^{\sim}H(GTG)^{\sim}) \text{ (Using } (GTG)^{\sim}H(GHG)^{\sim} = (GHG)^{\sim}H(GTG)^{\sim}) \\ &= \mathcal{R}(T(GHG)^{\sim}T(GTG)^{\sim}H(GTG)^{\sim}) \text{ (Using } H = T(GTG)^{\sim}H) \\ &= \mathcal{R}(T(GHG)^{\sim}T(T(GHG)^{\sim}T)^{\sim}) \\ &= \mathcal{R}(T(GHG)^{\sim}T) \end{aligned}$$

implies $\mathcal{R}(H) \subseteq \mathcal{R}(T)$.

(ii) \Rightarrow (iii).

Let us assume that $\mathcal{R}(H) \subseteq \mathcal{R}(T)$.

To prove that $\mathcal{N}((GTG)^{\sim}) \subseteq \mathcal{N}((GHG)^{\sim})$.

Using Lemma 2.1, we have $\mathcal{R}(H) \subseteq \mathcal{R}(T)$

$$\Rightarrow \mathcal{N}((GHG)^{\sim})^{\perp} \subseteq \mathcal{N}((GTG)^{\sim})^{\perp}$$

$$\Rightarrow \mathcal{N}((GTG)^{\sim}) \subseteq \mathcal{N}((GHG)^{\sim}).$$

(iii) \Rightarrow (iv).

Let us assume that $\mathcal{N}((GTG)^{\sim}) \subseteq \mathcal{N}((GHG)^{\sim})$.





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To prove that $H^m = H^m T(GTG)^\sim$.

Since $\mathcal{N}((GHG)^\sim) = \mathcal{N}(H^m)$, we have

$$\mathcal{N}((GTG)^\sim) \subseteq \mathcal{N}(H^m) \Rightarrow H^m = H^m ((GTG)^\sim)^m (GTG)^\sim.$$

Since $T^m = (GTG)^\sim$, we have

$$\begin{aligned} H^m &= H^m ((GTG)^\sim)^\sim (GTG)^\sim \\ &= H^m T(GTG)^\sim. \end{aligned}$$

(iv) \Rightarrow (i).

Let us assume that $H^m = H^m T(GTG)^\sim$.

To prove that $H = T(GTG)^\sim H$.

Since $H^m = H^m T(GTG)^\sim$

Premultiplying by $(GHG)^\sim H$, we obtain

$$\begin{aligned} (GHG)^\sim H H^m &= (GHG)^\sim H H^m T(GTG)^\sim \\ (GHG)^\sim &= (GHG)^\sim T(GTG)^\sim. \end{aligned}$$

Taking Minkowski adjoint on both sides, we have

$$\begin{aligned} ((GHG)^\sim)^\sim &= ((GHG)^\sim T(GTG)^\sim)^\sim \\ GHG &= ((GTG)^\sim)^\sim T^\sim ((GHG)^\sim)^\sim \\ GHG &= GTGT^\sim GHG \end{aligned}$$

Pre and post multiplying by G , we have

$$\begin{aligned} GGHHG &= GGTGT^\sim GHGG \\ H &= TGT^\sim GH \\ H &= T(GTG)^\sim H. \end{aligned}$$

Hence the proof.

Similarly one can show the following:

Lemma 2.4. Let $H, T \in \mathbb{C}^{m \times n}$ in the Minkowski space \mathcal{M} . Then the following are equivalent:

- (i) $H = H(GTG)^\sim T$;
- (ii) $\mathcal{N}(T) \subseteq \mathcal{N}(H)$;
- (iii) $\mathcal{R}((GHG)^\sim) \subseteq \mathcal{R}((GTG)^\sim)$;
- (iv) $H^m = (GTG)^\sim T H^m$.

Proof. (i) \Rightarrow (ii).

Let us assume that $H = H(GTG)^\sim T$.

To prove that $\mathcal{N}(T) \subseteq \mathcal{N}(H)$.

$$\begin{aligned} \mathcal{N}(H) &= \mathcal{N}((GHG)^\sim H) \text{ (Using } H = H(GTG)^\sim T) \\ &= \mathcal{N}((GHG)^\sim H(GTG)^\sim T) \text{ (Using } (GHG)^\sim H(GTG)^\sim = (GTG)^\sim H(GHG)^\sim) \\ &= \mathcal{N}((GTG)^\sim H(GHG)^\sim T) \text{ (Using } H = H(GTG)^\sim T) \\ &= \mathcal{N}((GTG)^\sim H(GTG)^\sim T(GHG)^\sim T) \\ &= \mathcal{N}((T(GHG)^\sim T)^\sim T(GHG)^\sim T) \\ &= \mathcal{N}(T(GHG)^\sim T) \\ \mathcal{N}(H) &\supseteq \mathcal{N}(T). \end{aligned}$$

(ii) \Rightarrow (iii).

Let us assume that $\mathcal{N}(T) \subseteq \mathcal{N}(H)$.

To prove that $\mathcal{R}((GHG)^\sim) \subseteq \mathcal{R}((GTG)^\sim)$.

Using Lemma 2.1, we have $\mathcal{N}(T) \subseteq \mathcal{N}(H)$

$$\begin{aligned} \Rightarrow \mathcal{R}((GTG)^\sim)^\perp &\subseteq \mathcal{R}((GHG)^\sim)^\perp \\ \Rightarrow \mathcal{R}((GHG)^\sim) &\subseteq \mathcal{R}((GTG)^\sim). \end{aligned}$$

(iii) \Rightarrow (iv).

Let us assume that $\mathcal{R}((GHG)^\sim) \subseteq \mathcal{R}((GTG)^\sim)$.

To prove that $H^m = (GTG)^\sim T H^m$.





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Since $\mathcal{R}((GHG)^\sim) = \mathcal{R}(H^m)$, we have
 $\mathcal{R}(H^m) \subseteq \mathcal{R}((GTG)^\sim) \Rightarrow H^m = (GTG)^\sim((GTG)^\sim)^m H^m$.
 Since $T^m = (GTG)^\sim$, we have
 $H^m = (GTG)^\sim((GTG)^\sim)^\sim H^m$
 $= (GTG)^\sim T H^m$.

(iv) \Rightarrow (i).

Let us assume that $H^m = (GTG)^\sim T H^m$.

To prove that $H = H(GTG)^\sim T$.

Since $H^m = (GTG)^\sim T H^m$

Post-multiplying by $H(GHG)^\sim$, we obtain

$$H^m H(GHG)^\sim = (GTG)^\sim T H^m H(GHG)^\sim$$

$$(GHG)^\sim = (GTG)^\sim T (GHG)^\sim.$$

Taking Minkowski adjoint on both sides, we have

$$((GHG)^\sim)^\sim = ((GTG)^\sim T (GHG)^\sim)^\sim$$

$$GHG = ((GHG)^\sim)^\sim T^\sim ((GTG)^\sim)^\sim$$

$$GHG = GHGT^\sim GTG$$

Pre and post multiplying by G , we have

$$GGHGG = GGHT^\sim GTGG$$

$$H = H(GTG)^\sim T.$$

Hence the proof.

We now use these lemmas to obtain some characterizations and properties of $T - EP$ matrices in Minkowski space.

Theorem 2.3. Let $H, T \in \mathbb{C}^{m \times n}$ in the Minkowski space \mathcal{M} . Then the following are equivalent:

- (i) H is $T - EP$ in \mathcal{M} (i.e., $\mathcal{R}(H) = \mathcal{R}(T(GHG)^\sim T)$) and $H = H(GTG)^\sim T$;
- (ii) $\mathcal{R}(H) = \mathcal{R}(T(GHG)^\sim T)$ and $\mathcal{N}(T) \subseteq \mathcal{N}(H)$;
- (iii) $\mathcal{N}((GHG)^\sim) = \mathcal{N}((GTG)^\sim H(GTG)^\sim)$ and $\mathcal{R}((GHG)^\sim) \subseteq \mathcal{R}((GTG)^\sim)$;
- (iv) $\mathcal{R}(H) = \mathcal{R}(T(GHG)^\sim)$ and $\mathcal{N}(T) \subseteq \mathcal{N}(H)$;
- (v) $\mathcal{R}(H) = \mathcal{R}(T(GHG)^\sim)$ and $H = H(GTG)^\sim T$;
- (vi) $\mathcal{N}((GHG)^\sim) = \mathcal{N}(H(GTG)^\sim)$ and $\mathcal{R}((GHG)^\sim) \subseteq \mathcal{R}((GTG)^\sim)$.

Proof. (i) \Leftrightarrow (ii).

H is $T-EP$ in $\mathcal{M} \Leftrightarrow \mathcal{R}(H) = \mathcal{R}(T(GHG)^\sim T)$ and $H = H(GTG)^\sim T$.

$$\Leftrightarrow \mathcal{N}(H) = \mathcal{N}((GHG)^\sim H) \text{ (Using } H = H(GTG)^\sim T)$$

$$\Leftrightarrow \mathcal{N}(H) = \mathcal{N}((GHG)^\sim H(GTG)^\sim T) \text{ (Using } (GHG)^\sim H(GTG)^\sim = (GTG)^\sim H(GHG)^\sim)$$

$$\Leftrightarrow \mathcal{N}(H) = \mathcal{N}((GTG)^\sim H(GHG)^\sim T) \text{ (Using } H = T(GTG)^\sim H)$$

$$\Leftrightarrow \mathcal{N}(H) = \mathcal{N}((GTG)^\sim H(GTG)^\sim T(GHG)^\sim T)$$

$$\Leftrightarrow \mathcal{N}(H) = \mathcal{N}((T(GHG)^\sim T)^\sim T(GHG)^\sim T)$$

$$\Leftrightarrow \mathcal{N}(H) = \mathcal{N}(T(GHG)^\sim T)$$

$$\Leftrightarrow \mathcal{N}(H) \supseteq \mathcal{N}(T).$$

(ii) \Leftrightarrow (iii).

$$\text{If } \mathcal{R}(H) = \mathcal{R}(T(GHG)^\sim) \text{ and } \mathcal{N}(T) \subseteq \mathcal{N}(H). \Leftrightarrow \mathcal{N}((GHG)^\sim)^\perp = \mathcal{N}((T(GHG)^\sim T)^\sim)^\perp \text{ and } \mathcal{R}((GTG)^\sim)^\perp \subseteq \mathcal{R}((GHG)^\sim)^\perp$$

$$\Leftrightarrow \mathcal{N}((GHG)^\sim)^\perp = \mathcal{N}((GTG)^\sim H(GTG)^\sim)^\perp \text{ and } \mathcal{R}((GHG)^\sim) \subseteq \mathcal{R}((GTG)^\sim)$$

$$\Leftrightarrow \mathcal{N}((GHG)^\sim) = \mathcal{N}((GTG)^\sim H(GTG)^\sim) \text{ and } \mathcal{R}((GHG)^\sim) \subseteq \mathcal{R}((GTG)^\sim).$$

(ii) \Rightarrow (iv).

Let us assume that $\mathcal{R}(H) = \mathcal{R}(T(GHG)^\sim T)$ and $\mathcal{N}(T) \subseteq \mathcal{N}(H)$.

To show that $\mathcal{R}(H) = \mathcal{R}(T(GHG)^\sim)$.

Since $\mathcal{R}(H) = \mathcal{R}(T(GHG)^\sim T)$, we obtain

$$\mathcal{R}(T(GHG)^\sim T) \subseteq \mathcal{R}(T(GHG)^\sim) \text{ and } \text{rank}(H) = \text{rank}(T(GHG)^\sim T).$$





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Moreover, $rank(T(GHG)^\sim) \leq rank((GHG)^\sim) = rank(H) = rank(T(GHG)^\sim T)$.

Therefore, $\mathcal{R}(H) = \mathcal{R}(T(GHG)^\sim)$.

(iv) \Rightarrow (ii).

Suppose $\mathcal{R}(H) = \mathcal{R}(T(GHG)^\sim)$ and $\mathcal{N}(T) \subseteq \mathcal{N}(H)$.

To prove that $\mathcal{R}(H) = \mathcal{R}(T(GHG)^\sim T)$.

In fact, clearly $\mathcal{R}(T(GHG)^\sim T) \subseteq \mathcal{R}(H)$.

Also, as $\mathcal{R}(H) \subseteq \mathcal{R}(T)$ implies $T(GTG)^\sim H = H$ by Lemma 2.3 (i).

$$\begin{aligned} \text{So, } rank(H) &= rank(T(GHG)^\sim) \\ &= rank(T(GHG)^\sim T(GTG)^\sim) \end{aligned}$$

$$\leq rank(T(GHG)^\sim T)$$

Therefore $\mathcal{R}(H) = \mathcal{R}(T(GHG)^\sim T)$.

(iv) \Leftrightarrow (v).

If $\mathcal{R}(H) = \mathcal{R}(T(GHG)^\sim)$ and $\mathcal{N}(T) \subseteq \mathcal{N}(H)$.

$$\Leftrightarrow \mathcal{R}(H) = \mathcal{R}(T(GHG)^\sim) \text{ and } \mathcal{N}(T(GHG)^\sim T) = \mathcal{N}(H)$$

$$\Leftrightarrow \mathcal{N}((T(GHG)^\sim T)^\sim T(GHG)^\sim T) = \mathcal{N}(H)$$

$$\Leftrightarrow \mathcal{N}((GTG)^\sim H(GTG)^\sim T(GHG)^\sim T) = \mathcal{N}(H) \quad (\text{Using } H(GTG)^\sim T = H)$$

$$\Leftrightarrow \mathcal{N}((GTG)^\sim H(GHG)^\sim T) = \mathcal{N}(H) \quad (\text{Using } (GTG)^\sim H(GHG)^\sim = (GHG)^\sim H(GTG)^\sim)$$

$$\Leftrightarrow \mathcal{N}((GHG)^\sim H(GTG)^\sim T) = \mathcal{N}(H)$$

$$\Leftrightarrow H(GTG)^\sim T = H.$$

(v) \Leftrightarrow (vi).

If $\mathcal{R}(H) = \mathcal{R}(T(GHG)^\sim)$ and $H = H(GTG)^\sim T$

$$\Leftrightarrow \mathcal{N}((GHG)^\sim) = \mathcal{N}((T(GHG)^\sim)^\sim) \text{ and } \mathcal{N}(H) = \mathcal{N}((GHG)^\sim H)$$

$$\Leftrightarrow \mathcal{N}((GHG)^\sim) = \mathcal{N}(H(GTG)^\sim) \text{ and } \mathcal{N}(H) = \mathcal{N}((GHG)^\sim H(GTG)^\sim T)$$

$$\Leftrightarrow \mathcal{N}((GHG)^\sim) = \mathcal{N}(H(GTG)^\sim) \text{ and } \mathcal{N}(H) = \mathcal{N}((GTG)^\sim H(GHG)^\sim T)$$

$$\Leftrightarrow \mathcal{N}((GHG)^\sim) = \mathcal{N}(H(GTG)^\sim) \text{ and } \mathcal{N}(H) = \mathcal{N}((GTG)^\sim H(GTG)^\sim T(GHG)^\sim T)$$

$$\Leftrightarrow \mathcal{N}((GHG)^\sim) = \mathcal{N}(H(GTG)^\sim) \text{ and } \mathcal{N}(H) = \mathcal{N}((T(GHG)^\sim T)^\sim T(GHG)^\sim T)$$

$$\Leftrightarrow \mathcal{N}((GHG)^\sim) = \mathcal{N}(H(GTG)^\sim) \text{ and } \mathcal{N}(H) = \mathcal{N}(T(GHG)^\sim T)$$

$$\Leftrightarrow \mathcal{N}((GHG)^\sim) = \mathcal{N}(H(GTG)^\sim) \text{ and } \mathcal{N}(H) \supseteq \mathcal{N}(T)$$

$$\Leftrightarrow \mathcal{N}((GHG)^\sim) = \mathcal{N}(H(GTG)^\sim) \text{ and } \mathcal{R}((GHG)^\sim)^\perp \supseteq \mathcal{R}((GTG)^\sim)^\perp$$

$$\Leftrightarrow \mathcal{N}((GHG)^\sim) = \mathcal{N}(H(GTG)^\sim) \text{ and } \mathcal{R}((GTG)^\sim) \supseteq \mathcal{R}((GHG)^\sim).$$

Hence the proof.

Theorem 2.4. Let $H \in \mathbb{C}^{m \times n}$ be a T – EP matrix in the Minkowski space \mathcal{M} , then the following hold:

(i) $H = T(GTG)^\sim H$.

(ii) $rank(H) = rank(T(GHG)^\sim T) = rank(T(GHG)^\sim) = rank((GHG)^\sim T)$;

(iii) $((GTG)^\sim H)^m = H^m T$;

(iv) $(H(GTG)^\sim)^m = TH^m$;

(v) $((GTG)^\sim H(GTG)^\sim)^m = TH^m T$.

Proof. (i) Since H is T-EP in \mathcal{M} , we have

$$\mathcal{R}(H) = \mathcal{R}(T(GHG)^\sim T), \text{ and so } \mathcal{R}(H) \subseteq \mathcal{R}(T).$$

Now, Lemma 2.3 implies $H = T(GTG)^\sim H$.

(ii) By definition of T-EP matrix in \mathcal{M} it is clear that

$$rank(H) = rank(T(GHG)^\sim T).$$

$$\text{Thus, } rank(H) = rank(T(GHG)^\sim T)$$

$$\leq rank(T(GHG)^\sim)$$

$$\leq rank(H)$$

$$= rank(T(GHG)^\sim T)$$

$$\leq rank((GHG)^\sim T)$$

$$\leq rank(H),$$





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Therefore $rank(H) = rank(T(GHG)^\sim T)$
 $= rank(T(GHG)^\sim)$
 $= rank((GHG)^\sim T)$.

(iii) We check this by direct verification.

Let $X = H^m T$. By definition of T-EP matrix in \mathcal{M} and part (i), we have $H = H(GTG)^\sim T$ and $H = T(GTG)^\sim H$, respectively.

Thus, $(GTG)^\sim HX(GTG)^\sim H = (GTG)^\sim HH^m T(GTG)^\sim H$
 $= (GTG)^\sim HH^m H$

$$\begin{aligned} &= (GTG)^\sim H, \\ X(GTG)^\sim HX &= H^m T(GTG)^\sim HH^m T \\ &= H^m HH^m (GTG)^\sim \\ &= H^m (GTG)^\sim \\ &= X, \end{aligned}$$

$$\begin{aligned} ((GTG)^\sim HX)^\sim &= ((GTG)^\sim HH^m T)^\sim \\ &= (GTG)^\sim (HH^m)^\sim T \\ &= (GTG)^\sim HH^m T \\ &= (GTG)^\sim HX, \end{aligned}$$

$$\begin{aligned} (X(GTG)^\sim H)^\sim &= (H^m T(GTG)^\sim H)^\sim \\ &= (H^m H)^\sim \\ &= H^m H \\ &= H^m T(GTG)^\sim H \\ &= X(GTG)^\sim H. \end{aligned}$$

By uniqueness of the Minkowski inverse we have $X = ((GTG)^\sim H)^m$.

Conditions (iv) and (v) can also be proved in the same manner.

Properties of square T-EP Matrices in Minkowski space \mathcal{M}

In this section, we have obtained the properties of square Relative EP matrices in Minkowski space.

Theorem 3.1. Let $H, T \in \mathbb{C}^{n \times n}$ in the Minkowski space \mathcal{M} . If H is EP in \mathcal{M} and $H(GHG)^\sim$ is T-EP in \mathcal{M} . Then H is T-EP in \mathcal{M} .

Proof. Since $H(GHG)^\sim$ is T-EP in \mathcal{M} , by definition we have

$$\begin{aligned} \mathcal{R}(H(GHG)^\sim) &= \mathcal{R}(T(H(GHG)^\sim)^\sim T) \\ &= \mathcal{R}(TGHGH^\sim T) \end{aligned}$$

and $\mathcal{N}(T) \subseteq \mathcal{N}(H(GHG)^\sim)$.

$$\text{Thus } \mathcal{N}(T) \subseteq \mathcal{N}(H(GHG)^\sim) = \mathcal{N}((GHG)^\sim) = \mathcal{N}(H) \tag{1}$$

where the last equality is due the fact that H is EP in \mathcal{M} .

$$\begin{aligned} \text{Also, } \mathcal{R}(H) &= \mathcal{R}(H(GHG)^\sim) \\ &= \mathcal{R}(TH(GHG)^\sim T) \\ &\subseteq \mathcal{R}(T(GHG)^\sim) \end{aligned}$$

and $rank(T(GHG)^\sim) \leq rank((GHG)^\sim) = rank(H)$.

Therefore, $\mathcal{R}(H) = \mathcal{R}(T(GHG)^\sim)$.

In consequence, from equation (1) and Theorem 2.3 (iv), we have H is T-EP in \mathcal{M} .

Theorem 3.2. Let $H, T \in \mathbb{C}^{n \times n}$ in the Minkowski space \mathcal{M} . Let T be a normal partial isometry in \mathcal{M} . Then the following hold.

(i) If $H = T(GTG)^\sim H$ then $(TH)^m = H^m (GTG)^\sim$.

In particular, $H^m = (TH)^m T$.

(ii) If $H = H(GTG)^\sim T$ then $(HT)^m = (GTG)^\sim H^m$.

In particular, $H^m = T(HT)^m$.

Proof. (i) we check this by direct verification.

Let $X = H^m (GTG)^\sim$.

Since $H = T(GTG)^\sim H$ and T is normal $(T(GTG)^\sim)^\sim = (GTG)^\sim T$, we have





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$$\begin{aligned}
 THXTH &= THH^m(GTG)\sim TH \\
 &= THH^mT(GTG)\sim H \\
 &= THH^mH \\
 &= TH, \\
 XTHX &= H^m(GTG)\sim THH^m(GTG)\sim \\
 &= H^mT(GTG)\sim HH^m(GTG)\sim \\
 &= H^mHH^m(GTG)\sim \\
 &= H^m(GTG)\sim \\
 &= X, \\
 (THX)\sim &= (THH^m(GTG)\sim)\sim \\
 &= T(HH^m)\sim(GTG)\sim \\
 &= THH^m(GTG)\sim \\
 &= THX, \\
 (XTH)\sim &= (H^m(GTG)\sim TH)\sim \\
 &= (H^mT(GTG)\sim H)\sim \\
 &= (H^mH)\sim \\
 &= H^mH \\
 &= H^mT(GTG)\sim H \\
 &= H^m(GTG)\sim TH \\
 &= XTH.
 \end{aligned}$$

By uniqueness of Minkowski inverse, we have $X = (TH)^m$.
 It remains to show that the last affirmation of part (i), In fact, from Lemma 2.3 (i) and the fact that T is normal in \mathcal{M} , we obtain

$$\begin{aligned}
 H^m &= H^mT(GTG)\sim && \text{(Using } T(GTG)\sim = (GTG)\sim T) \\
 &= H^m(GTG)\sim T \\
 &= (TH)^mT.
 \end{aligned}$$

(ii) Can also be proved in the same manner.

Theorem 3.3. Let $H, T \in \mathbb{C}^{n \times n}$ in the Minkowski space \mathcal{M} , and T a partial isometry such that H and $(GTG)\sim$ commute. Then H is T -EP in $\mathcal{M} \Leftrightarrow H$ is EP in \mathcal{M} and $H = H(GTG)\sim T$ in \mathcal{M} .

Proof. Let us assume that H is T -EP in \mathcal{M} .
 To prove that H is EP in \mathcal{M} .
 suppose H and $(GTG)\sim$ commute and H is T -EP in \mathcal{M} .
 By definition, $\mathcal{R}(H) = \mathcal{R}(T(GHG)\sim T)$.
 Since H and $(GTG)\sim$ commute, $(GHG)\sim$ and T commute.
 Therefore, $\mathcal{R}(H) = \mathcal{R}((GHG)\sim T^2) \subseteq \mathcal{R}((GHG)\sim)$.
 However, $rank(H) = rank((GHG)\sim)$ implies $\mathcal{R}(H) = \mathcal{R}((GHG)\sim)$.
 Thus H is EP in \mathcal{M} .
 Conversely, Let H be EP in \mathcal{M} and $H = H(GTG)\sim T$.
 Since H is EP in \mathcal{M} , $\mathcal{R}(H) = \mathcal{R}((GHG)\sim)$.
 Thus $\mathcal{R}(T(GHG)\sim) = \mathcal{R}(H(GTG)\sim) \subseteq \mathcal{R}((GHG)\sim) = \mathcal{R}(H)$.
 Since $H = H(GTG)\sim T$, we obtain
 $rank(H) = rank(H(GTG)\sim T) \leq rank(H(GTG)\sim) = rank(T(GHG)\sim)$.
 So, $\mathcal{R}(T(GHG)\sim) = \mathcal{R}(H)$.
 Now, by Theorem 2.3 (v), H is T -EP in \mathcal{M} .

Theorem 3.4. Let $H, I \in \mathbb{C}^{m \times n}$ be T -EP in \mathcal{M} such that $rank(T(G(H + I)G)\sim T) = rank(H + I)$ and $\mathcal{R}(H + I) = \mathcal{R}(H) + \mathcal{R}(I)$. Then $H + I$ is T -EP in \mathcal{M} .

Proof. Since H and I are T -EP in \mathcal{M} , by definition we have





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$\mathcal{R}(H) = \mathcal{R}(T(GHG)^{\sim}T)$ and $H = H(GTG)^{\sim}T$ and also,

$\mathcal{R}(I) = \mathcal{R}(T(GIG)^{\sim}T)$ and $I = I(GTG)^{\sim}T$.

In consequence, from $\mathcal{R}(H + I) = \mathcal{R}(H) + \mathcal{R}(I)$, we obtain

$$\begin{aligned} \mathcal{R}(T(G(H + I)G)^{\sim}T) &\subseteq \mathcal{R}(T(GHG)^{\sim}T) + \mathcal{R}(T(GIG)^{\sim}T) \\ &= \mathcal{R}(H) + \mathcal{R}(I) \\ &= \mathcal{R}(H + I). \end{aligned}$$

Thus, $\text{rank}(T(G(H + I)G)^{\sim}T) = \text{rank}(H + I)$.

Implies $\mathcal{R}(T(G(H + I)G)^{\sim}T) = \mathcal{R}(H + I)$.

Moreover, it is easy to see that $(H + I) = (H + I)(GTG)^{\sim}T$

Hence $H + I$ is T-EP in \mathcal{M} .

CONCLUSION

In this paper, we have concluded the algebraic structure of the Relative EP matrices in Minkowski space.

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Long Short Term Memory based Plant Leaf Disease Detection

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ABSTRACT

In agriculture, poor yields are sometimes caused by plant diseases, which diminish farmers' profits. A device that can identify plant ailments on its own is currently being developed by specialists. The faster a cure can be discovered, the more controllable the loss will be. Accurate identification of plant diseases. The goal of this study is to use deep learning methods in concert with other data sources to develop a unique approach for forecasting plant diseases. To analysis and classify the plant leaf disease Recurrent Neural Network (RNN) & Long Short-Term Memory (LSTM) architectures are used to extract disease-related properties from plant leaves. The F1-Score of 99.4 %, based on the results of the experiments, shows that LSTM is superior at detecting plant leaf diseases. It is clear from the results of the trials that plant diseases may be accurately identified.

Keywords: Plant diseases, RNN, LSTM, Deep Learning

INTRODUCTION

The majority of Indians earn their living via farming. Agriculture has a significant role in the economies of all countries. Progress in agriculture is primarily aimed at meeting the rising population's desire for food. The present environment necessitates the upgrading of the agriculture industry. Bacterial and fungal infections, both of which are infectious, affect crops. Farmers' production suffers greatly as a result of this. In order to have the best possible yield, the crop must be in excellent shape. It will always take a long time and be difficult to diagnose diseases with our naked sight. To achieve this, it is essential to keep an eye on the farm at all times. It takes a long time to do this task.



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It may be very costly to do so if the farm is really vast. As a consequence of this problem, even agricultural experts are unable to diagnose infections and provide a cure. Automated systems that can identify and diagnose plant diseases would be very useful to farmers. Using this method, farmers may be alerted at the right moment so that they can take the necessary safeguards. Leaf, fruit, and seed damage may be caused by a variety of plant diseases. Other reproductive organs might also be affected. Different parts of the plant are affected by these diseases in a unique manner. A plant's leaf is its most critical component. Having a pathogen in the leaf of a plant may have a direct impact on its ability to reproduce. Leaves may be infected by a variety of ailments, including fungal disease, bacterial disease, and so on. As a consequence, early detection of plant diseases is crucial.

Deep Learning Based Approach

To solve the plant leaf detection issue, it seems that deep learning has greater promise. Many Deep learning (DL) algorithms are described for the automated detection and categorization of plant diseases based on digital plant images. The leaf, stem, seed, and fruit of a plant may all be affected by a wide range of ailments, which present themselves in various ways. There are several illnesses that affect different parts of the plant's body. Photosynthesis can only occur with the help of the plant's leaves, which are often considered as the plant's most critical component. As soon as a plant's leaf becomes infected, the plant's life cycle will be affected. Illnesses may be dealt with courageously and successfully if they are identified and categorised automatically. Machine learning is likely to be a feasible approach to fixing this issue. The detection and categorization of plant diseases based on pictures has recently been given by many machine learning methods. Because of this, it is difficult to ensure that the results are consistent and robust, even if automated technologies have solved the problem of difficulty. Machine learning techniques are used in this study to detect plant disease.

LITERATURE REVIEW

It has been created by many researchers like Revathi et al. [1] to identify visual abnormalities in plants. In order to properly document the plant, it is important to take and process digital images. After that, the data is subjected to several extraction techniques, including edge detection, colour space, and textural aspects. The classifiers receive the retrieved attributes and use them to make a classification decision. This research aims to identify the sick area of a cotton leaf using image processing.

Arivazhagan et al. [2] demonstrated a method for modifying the colour of a colour image. The green pixels in the photo are hidden and deleted from the final image by using the threshold setting. On the next step, we'll do the segmentation. For each and every believable section, texturing statistics are generated. The next classifier is used for sickness categorization.

Singh, V., & Misra, A [3] compare and analyse several image processing classification algorithms. As an alternative to segmentation-based strategies, the authors offered segmentation-based approaches in this paper. To reach their findings, Kumar et al [5] used a system that includes sorting and grading of citrus fruits. Based on a mixture of fruit images, it can figure out what sort of fruit it is. Artificial neural networks (ANNs) have been used by Kulkarni and others [6]. (ANN). An ANN classifier is used to handle the classification issue, while a Gabor filter is used to tackle the feature extraction challenge. It used both texture and color features to its advantage.

Pranjali, B., & Anjil, A.[7] utilized a Gaussian filter to reduce noise, and a threshold was applied to produce the green component of the final product. Reza et al. [8] proposed that an image processing technique be used for the detection of jute plant disease. It is capable of detecting stem infections in the jute plant with high accuracy. Camargo et al.[9] created a system that, with the use of color picture analysis, can prolong the visual symptoms of plant illnesses. A Gray scale picture of an infected plant is transformed into color transformations such as I3a, I3b, and H using the algorithm that has been built in the first step. First, the translated picture is segmented based on the intensity



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distribution in the histogram, which is used to segment the original image. This approach is particularly useful when attempting to target a single picture with a wide range of brightness. Once the segmentation procedure is complete, the area that has been collected is optimized in order to remove pixel portions that are not inside the targeted area.

The results were unanimous, indicating that they were effective in identifying the plant's infected parts. Image processing methods have been used by Camargo et al. [10] to design an algorithm for the detection of diseased patches in plants. Before applying the colour modification, the photograph is captured in its original form. The quality of converted photographs may be greatly enhanced by using the Gaussian filter. After that, the area of interest is identified via segmentation. In this situation, the segmentation is based on where the ideal threshold is located.

The SVM classifier is then used to categories and identify the separated regions as healthy or unhealthy. Arti et al. [11] compiled a list of several detection methods that involve image processing for their study. Using a genetic method, Vijay et al. [12] were able to detect plant ailments in great detail. Only on-site leaf analysis by professionals is now available for disease diagnosis, allowing specialists to identify and diagnose plant diseases. It is excessively expensive to undertake this activity on a big farm since it need several specialists and constant monitoring of the plant.

Mohanty et al. [13] used GoogleNet and AlexNet to identify 54,306 plant leaf photos as healthy or sick in the Plant Village dataset, and they discovered that GoogleNet had a somewhat greater average classification effect than AlexNet on the dataset. With respect to the test set, the trained deep convolutional neural network model achieves a 99.35 percent accuracy. A straightforward method of using intelligent mobile phones to diagnose plant illnesses in horticultural crops involves building a deep learning model on a growing and publicly accessible picture dataset, which is expanding all the time.

A deep residual neural network-based updated technique developed by Picon et al. [14] was used to diagnose a wide range of plant ailments under real-world acquisition situations. For the purpose of early sickness detection, numerous enhancements have been suggested. According to the statistics, all of the diseases assessed had an AuC score more than 0.80, which indicates that they were all severe. Transfer learning was utilised by Selvaraj et al. [15] to retrain three CNN architectures, with encouraging results. With the use of pre-trained sickness detection models, which were subsequently utilised to create networks, deep transfer learning was employed to develop networks capable of making accurate predictions.

Deep learning was suggested by Fuentes et al. [16, 17] for recognizing diseases and pests in tomato plant pictures taken at different camera resolutions, and it has been used in many studies. Multiple CNN object detectors were used in conjunction with deep learning meta-architectures to detect objects. Data expansion, as well as local and global class annotation, were used to improve training accuracy while simultaneously reducing false positives. Training and testing were carried out using a large-scale tomato disease dataset, which was employed throughout the process. A total of nine distinct pests and illnesses were successfully identified by the algorithm from the complex circumstances.

Due to a lack of resources, agricultural infrastructure that may be useful are out of reach in certain nations. As a consequence, they see the need for hiring experts. This, however, leads to activities that are time consuming and expensive. The proposed method has been shown to be beneficial in appraising big crop yields in certain situations. Using leaves as a non-invasive diagnostic tool decreases labour costs while also making it easier to spot disease in plants. Other applications include robot guidance, inspection, and image-based autonomous process control using machine vision. Visual leaf disease identification requires more time and effort. As a result, it can only be done in certain areas. In contrast, non-manual plant disease detection, or automated detection, is more accurate and less time-consuming than manual detection of plant diseases.





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MATERIALS AND METHODS

Our major goal is to construct a model that can identify input plant leaf images as healthy or sick based on their appearance as shown in the figure 2. Detection of a disease on a plant leaf also allows for the identification of the disease kind. The LSTM network is used as a classifier in this article. Traditional models of deep learning, such as the feed-forward neural network (FNN), do not include the LSTM model. In the suggested technique, LSTM is used as the classifier since it is capable of spotting and processing these variances for pattern recognition. To analysis and classify RNN&LSTM architectures are used for the detection and classification of leaves suffering from common ailments.

Dataset

There must be a vast quantity of data gathered in order to train advanced visual and classification algorithms. In general, machine learning and deep learning systems perform better when trained with large quantities of data than when trained with small amounts. The Plant Village database [18, 19] is utilised in this study for research purposes.

Each of the 14 plants included in this "dataset" has a total of 54,323 images, which are separated into 38 groups of healthy leaves and plants suffering from various ailments. 11654 photos of three different plant kinds were utilized in this study: apple (7771 images), potato (3763 images), and rice (120 images).

It is important to divide the data volume into research and evaluation sets for the purposes of training and testing deep learning systems. There are no other datasets like this one since it contains photographs of varied sizes and greater sensor power than any other.

It is important to divide the data volume into research and evaluation sets for the purposes of training and testing deep learning systems. There are no other datasets like this one since it contains photographs of varied sizes and greater sensor power than any other.

Recurrent Neural Networks

The RNN is experiential as a group of Feedforward Neural Network (FNN), where the hidden neurons of the preceding time step are connected to the hidden neurons of the subsequent time step. Hidden neurons H_t are acquired by combining the weight of the preceding repetition cycle W_h with the weight of the current input information W_x . And so on, this procedure will prolong the next time repetition cycle. In this way, the RNN can take advance of chronological information but not consider the signal as a combination of secluded points. The output of the existing repetition epoch is based not only on the existing input, but also on the information of the preceding repetition epoch. In Figure 3 the configuration of the many-to-one RNN model is exposed.

X is the input and H is the vector of hidden layer:

$$H_t = \tanh(W_h H_t - 1 + W_x X_t) \quad (1)$$

According to the chain rule, the network loss gradient is

$$\frac{\partial E_k}{\partial W} = \frac{\partial E_k}{\partial H_k} \frac{\partial E_k}{\partial H_{k-1}} \dots \frac{\partial H_2}{\partial H_1} \frac{\partial H_1}{\partial W} = \frac{\partial E_k}{\partial H_k} \left(\prod_{t=2}^k \frac{\partial H_t}{\partial H_{t-1}} \right) \frac{\partial H_1}{\partial W} \quad (2)$$

and derivative is





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$$\begin{aligned}\frac{\partial H_t}{\partial H_{t-1}} &= \tanh'(W_h H_{t-1} + W_x X_t) \cdot \frac{d}{dH_{t-1}} [W_h H_{t-1} + W_x X_t] \\ &= \tanh'(W_h H_{t-1} + W_x X_t) \cdot W_h\end{aligned}\quad (3)$$

Combining (2) and (3)

$$\frac{\partial E_k}{\partial W} = \frac{\partial E_k}{\partial H_k} \left(\prod_{t=2}^k \tanh'(W_h H_{t-1} + W_x X_t) \cdot W_h \right) \frac{\partial H_1}{\partial W} \quad (4)$$

Concurring to W_h and $\tanh < 1$, $(\tanh'(W_h H_{t-1} + W_x X_t) \cdot W_h)$ may be less than 1 or greater than 1, which will cause the gradient to vanish or detonate [20]. Even if K is not large the concluding gradient may rapidly get misshapen due to the multiplication effect, depending on the random weight initialization.

This is the reason RNN can be flimsy and vulnerable to initialization. Also, a much smaller or larger gradient can extensively change the weight update of the network, making it hard to unite this problem is called vanishing and exploding gradient.

Long Short-Term Memory

Recurrent neural networks, of which the Long Short-Term Memory is a subset. Recurrent neural networks often have a 'short term memory' in that they employ information from the previous neural network to build the current one. In essence, the knowledge presented above is put to use in the current activity. RNN is unable to adequately express long-term interdependence in a problem. The long short-term memory (LSTM) was specifically designed for this purpose. It has a long-term memory for information. While LSTMs were first examined in a variety of challenging situations, they are now being used in a wide range of learning scenarios that differ much from those in which these advancements were first tested.

Memory cells that can retain their state over time and non-linear gating units that normalize the information flow into and out of the cell are the underlying concepts of the LSTM architecture. Many improvements to the LSTM design have been achieved since its inception in the early 1990s [22]. It was Graves and Schmidhuber [22, 23] who first outlined the LSTM configuration that is often utilized in prior publications.

The structure of a LSTM unit is shown in Figure 4. LSTM, on the other hand, substitutes the \tanh activation with erudite gate control in the incline flow and hence can realize better steadiness and recital. LSTM network was proposed to improve the RNN structure [22]. Compared to traditional RNN, LSTM introduces a specially designed LSTM unit to stylishly control the flow of hidden state information from a one-time step to the next.

In LSTM, X_t and H_t are the input vector and the network is hidden state vector at the time repetition epoch t , correspondingly. C_t is a vector which is accumulated in the external memory unit. The communication among the unit state vector, the input vector, and the hidden state vector is attained through the forgetting gate (f_t), input gate (i_t) and output gate (o_t). The calculation of forgetting a gate vector is:

$$f_t = \sigma(W_f \cdot [H_{t-1}, X_t] + b_f) \quad (5)$$

where, $[H_{t-1}, X_t]$ is the concatenated vector of the other hidden state vector H_{t-1} and the existing input vector X_t , W_f and b_f are the weight and bias of f_t which are determined by network training, σ is the sigmoid activation function.





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The flow of information in the vector C_t is controlled by dot multiplication of elements. The provisional state vector \tilde{C}_t is computed by:

$$\tilde{C}_t = \tanh(W_c \cdot [H_{t-1}, X_t] + b_c) \quad (6)$$

where W_c and b_c are the weight and unconventionality of f_c , \tanh is the \tanh stimulation function.

The calculation of input gate vector is

$$i_t = \sigma(W_i \cdot (H_{t-1}, X_t) + b_i) \quad (7)$$

where W_i and b_i are the weight and eccentricity of I_t . They are determined by network training.

The state of new cell state C_t in the time step t is updated by both forget gate and input gate via pointwise multiplication,

$$C_t = f_t * C_{t-1} + i_t * \tilde{C}_t \quad (8)$$

The current hidden state is gritty by the new state and the write gate O_t . similar to f_t and i_t , O_t can be written as

$$O_t = \sigma(W_o[H_{t-1}, X_t] + b_o) \quad (9)$$

Then, hidden state H_t at the current time step t is computed by pointwise multiplying by O_t by $\tanh(C_t)$:

$$H_t = O_t * \tanh(C_t) \quad (10)$$

The value hidden state H_t is computed with the use of C_{t-1} and H_{t-1} from the preceding time step as well as the existing input X_t . H_t is then used by the neural network to compute the output at the existing time step. LSTM neural network inherits the advantages of RNN in dealing with sequential forecast problems and solves the vanishing gradient problem. Therefore, it is chosen as the ideal mathematical model to analysis and classify the plant leaf disease.

Performance Metrics

Performance is evaluated using industry-standard classification methods: Precision, Recall, & F1-Score. A "true positive" (TP) occurs when we expect the answer to be "yes." The authentic output remained "yes". A false positive (FP) occurs when we expect a yes answer but get a no. True Negative (TN) is a term that refers to situations in which we expected a negative outcome. Actually, the answer was NO, while A false negative (FN) is one in which we incorrectly assumed that the answer was "no." YES, that was the final outcome are the 4 tags were created from the confusion matrix castoff to compare the efficiency of various deep learning techniques.

Precision is defined as the ratio of exact positive outcomes to positive outcomes predicted by the classification model.

$$Precision = \frac{TP}{TP+FP} \quad (11)$$

Recall [39,40] is defined as the proportion of accurate positive findings to the total no. of related samples (There were no false negatives in any of the samples).





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$$\text{Recall} = \frac{TP}{TP+FN} \quad (12)$$

The F1 Score, is used to measure a system accuracy, is calculated as a weighted harmonic average of precision & recall. It has a range of values (0,1)

$$F1 = 2 \frac{\text{Precision} \cdot \text{Recall}}{\text{Precision} + \text{Recall}} \quad (13)$$

RESULTS AND DISCUSSION

In this work MATLAB2021a and the WEKA tool for feature extraction and training. The experimental findings of the suggested framework are presented and discussed in this section. Based on the analysis it is observed that the LSTM Architecture achieves 98.4 % of precision and 97.5% of recall are observed from the Table I and the model's cumulative performance is calculated using the F1-Score is 99.4%. Based on the Figure 5 it is observed that the LSTM architecture performs better for plant leaf diseases detection system.

CONCLUSION

In this paper the Recurrent Neural Network (RNN) & Long Short-Term Memory (LSTM) architectures are used for extracting features from plant leaf for detection and classification of diseases in plant leaves. Based on the experimental results it is concluded that LSTM Architecture performs better for plant leaf diseases detection system with the F1-Score is 99.4%. The findings of the experiments demonstrate that plant diseases may be reliably diagnosed.

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Table 1. Analysis of Pre trained CNN Architecture

Architectures	Precision	Recall	F1 Score
RNN	0.966	0.962	0.985
LSTM	0.984	0.975	0.994



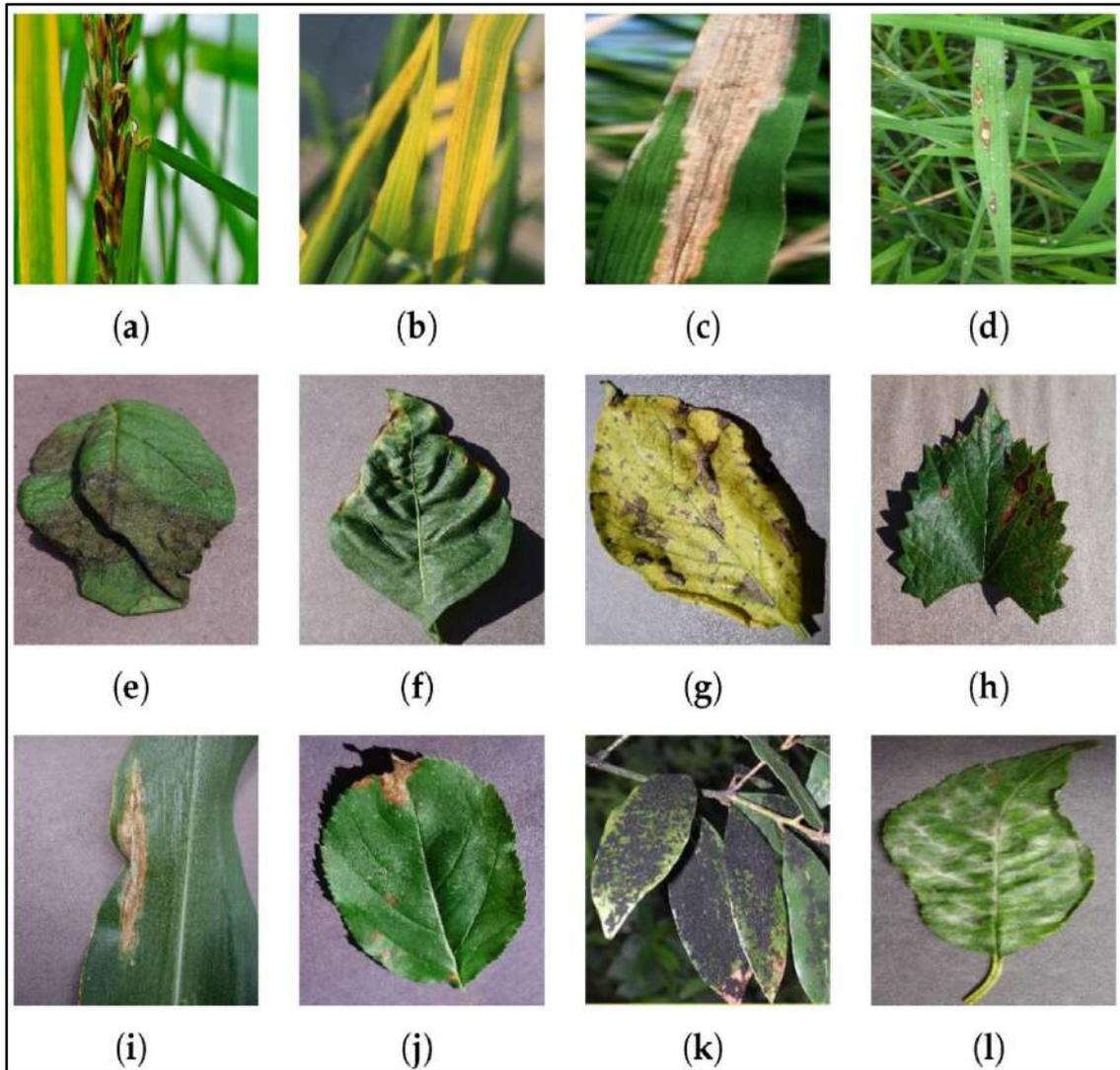


Fig.1. Samples of plant leaf disease images under numerous health conditions in various backgrounds and having different symptoms: (a) Rice Sheath-rot, (b) Rice Tungro, (c) Rice Bacterial leaf-blight, (d) Rice Blast, (e) Potato Late-blight, (f) Pepper Bacterial-spot, (g) Potato Early-blight Pepper Bacterial-spot, (h) Grape Black-measles, (i) Corn Northern Leaf-blight, (j) Apple Black-rot, (k) Mango Sooty-mold, and (l) Cherry Powdery-mildew [19]

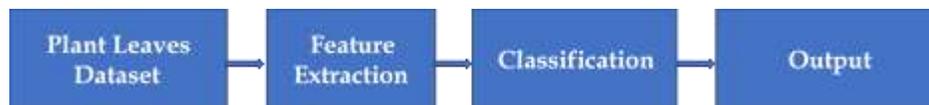


Fig. 2. Block diagram for proposed approach of plant leaf disease detection





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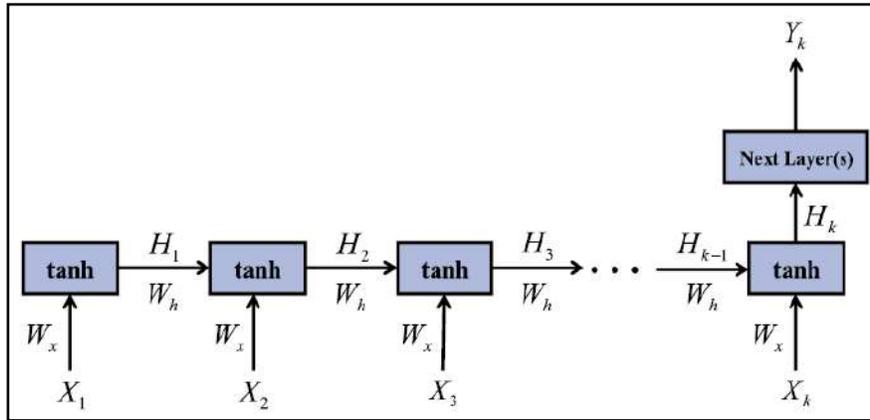


Fig. 3.The configuration of the many-to-one RNN model.

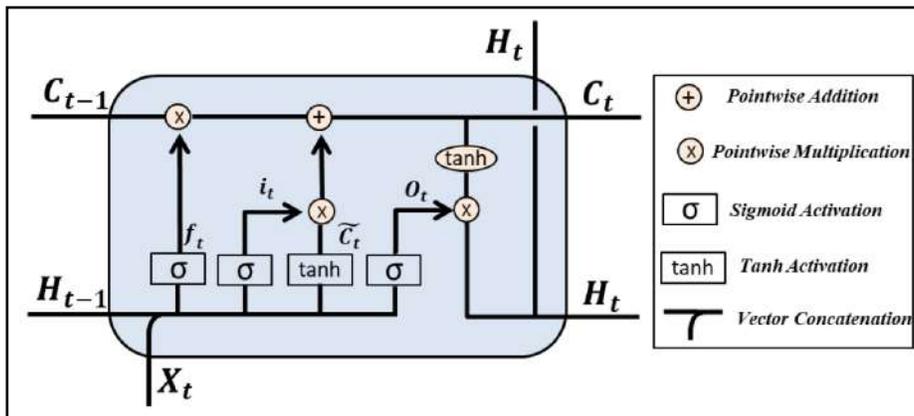


Fig.4.The structure of the LSTM.

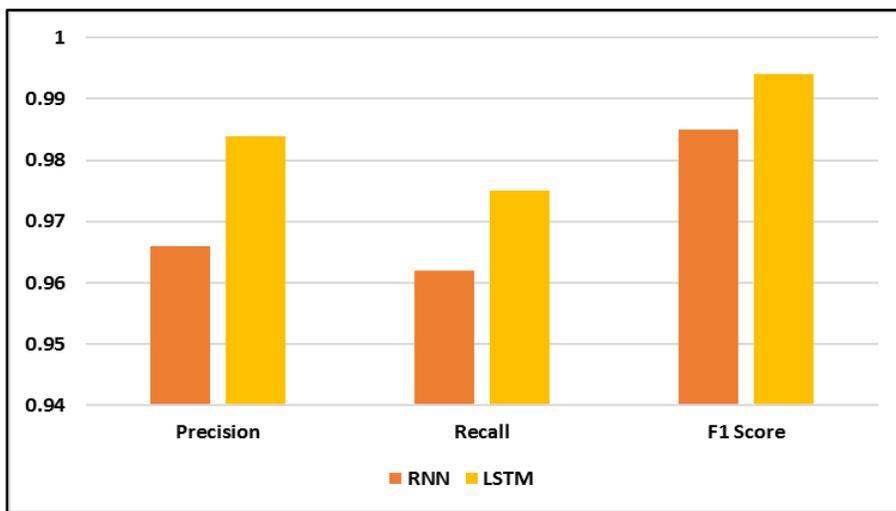


Fig. 5.Analysis Plot for RNN& LSTM Architecture





Association of Arbuscular Mycorrhizal Fungi in *Casuarina equisetifolia* L. Growing in Shoreline Ecosystem

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ABSTRACT

Casuarina trees are planted in the coastal areas with high soil salinity to act as a bio shield against wind and tsunami. Studies on arbuscular mycorrhizal fungi associated with casuarina in saline conditions are fewer. In the present study the community composition of AMF in *Casuarina equisetifolia* L. growing in the coastline of Karaikal, India with high soil salinity (3.15-6.15 mS) is investigated. Monthly AMF colonization in Casuarinaroots ranged from 14.08(±1.05) - 20.57(±1.10) % with no significant variation, however spore density ranged from 95.2(±2.28) -180.2(±5.32)/100g soil with significant monthly variation (P<0.05) Electrical conductivity exhibited positive significant correlation with hyphae (P<0.01). Total Phosphorus exhibited a significant negative correlation with vesicles (P<0.01) and AMF colonization (P<0.05) and positive correlation with spore density (P<0.05). Further, a significant negative correlation was observed between AMF colonization and spore density (P<0.05). A total of 21 AMF species belonging to two orders (Glomerales and Diversisporales), four families (Glomaraceae, Acaulosporaceae, Gigasporaceae, Diversisporaceae) and eight genera (*Funneliformis*, *Glomus*, *Rhizophagus*, *Septoglomus*, *Acaulospora*, *Dentiscutata*, *Scutellospora*, *Diversispora*) within the phylum Glomeromycota were isolated from the rhizosphere of Casuarinaseedlings. The present study indicated the ability of arbuscular mycorrhizal fungi to colonize saline environment with dominance of *Glomus fasciculatum*, *G. lamellosum*, *G. macrocarpum* and *Dentiscutata reticulata*.

Key words: Arbuscular mycorrhizal fungi, *Casuarina equisetifolia* L., Diversity and Rhizosphere





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INTRODUCTION

In coastal habitat plants have to endure extreme conditions due to high temperature, high soil salinity, water stress and low nutrients [1]. To cope with such situations and ensure successful establishment, plants have developed a strategy in which they form mutualistic relationship with arbuscular mycorrhizal fungi (AMF). This interaction benefits the plants not only in acquiring mineral nutrition but also facilitates increased adaptability to drought, salinity, soil densification, pathogens and heavy metals [2,3,4,5] present in the soil. Mycorrhizal symbiosis is the most ancient and widespread form of fungal symbiosis with almost 90% of terrestrial plants [6,7]. This non host specific symbiosis suggests that mycorrhiza exhibits profuse ecological differentiation and specialization, resulting in fungal types which can establish itself in nonconforming environment [8,9,10]. Soil salinity in the coastal region poses serious challenge for plant growth. *Casuarina* sp. can enter a symbiotic relationship with ectomycorrhizal fungi (EMF) and/or arbuscular mycorrhizal fungi (AMF) [11] which benefits the plants to cope with various abiotic stresses e.g., salinity and drought [12] by increased uptake of water and nutrients [13,14]. Considering the practical importance of mycorrhizal fungi for woody plants [15] and their ability to alleviate salt stress [12, 16], study on mycorrhizal diversity associated with *Casuarina equisetifolia* growing in the shoreline with high salt concentration is crucial. Thus, the present study was aimed at analyzing the diversity and colonization by AM fungal associated with *Casuarina equisetifolia* L. growing in saline soil of Eastern Coastal region of India.

MATERIALS AND METHODS

Study sites and Sampling

Sampling of roots and rhizosphere soil was done from January to May, 2019 from *Casuarina* plantation in the eastern coastline (latitude 10.9254° N, longitude 79.8380° E) of Karaikal, Pondicherry, India. Sampling was done twice monthly in an area of 100 x 50²m. Three random *Casuarina* seedlings for root and 250g rhizosphere soil were sampled. A composite sample was prepared by mixing all the three samples. The collected roots were fixed in FAA (Johansen 1940) for colonization study while the collected rhizosphere soils were sieved (2mm) to remove debris for AMF spore isolation and physicochemical analysis.

Estimation of AMF Colonization:

The fixed roots upon washing with tap water were cut into 1cm segments. The root segments were macerated using 10% KOH at 90°C for 1h following washing with tap water and staining with Trypan blue [17]. Lactoglycerol was used to mount the stained root samples on glass slides to examine fungal structures. Magnified intersection method was followed to identify arbuscules, vesicles and hyphae for estimation of fungal colonization [18].

Spore Isolation, Identification, and Enumeration

Wet sieving and sucrose centrifugation method was used for isolation of AMF spores [16]. Soil water suspension of twenty-five grams was filtered through a series of 710 to 37µm sieve. The residues obtained were transferred to beaker and filtered through gridded filter papers. The filter papers were spread on Petri dish and spores were counted using a light microscope at 40x magnification. The spores were pulled out from the filter paper, stained and mounted on glass slide using Melzer's reagent and polyvinyl-lacto-glycerol. Morphological identification of the spore was done on the basis of colour, ornamentation and wall characteristics using identification keys provided by International Culture Collection of Vesicular and Arbuscular Mycorrhizal Fungi (<http://www.invam.caf.wvu.edu>), AMF phylogeny (www.amf-phylogeny.com) and Schüßler's Glomeromycota Species List. Relative abundance was calculated following Zhao and Zhao [19].

$$\text{Relative abundance} = \frac{\text{Number of spores of a species or genus}}{\text{Total number of spores isolated}} \times 100$$





Soil Physico-Chemical Analysis

Soil temperature was noted using soil thermometer at the time of sampling. The soil moisture content (%) was determined by drying 10g of the sample in hot air oven 105°C for 24h. Soil pH and electrical conductivity was determined using a digital pH electrode [20] and conductivity meter [21]. Total phosphate and soil organic carbon content was determined using the ignition method [22] and wet oxidation method [23] respectively.

Statistical Analysis

One-way analysis of variance (ANOVA) and Tukey's test was performed to compare the means of colonization variables. Also, Pearson's correlation was performed between soil physicochemical properties, AMF colonization and spore density using Origin 7.0 software.

RESULTS AND DISCUSSION

Soil pH varies between 6.9-8.4. Soil temperature ranged from 26.9-31.4°C, Electrical conductivity ranged from 3.15-6.15mS, Soil moisture content ranged from 11.1-24.8%, Total phosphate and Soil Organic Carbon ranged from 0.11-0.09% and 0.05-5.0 % respectively. The mean values of soil physicochemical properties showed significant variation ($P < 0.05$) during the sampling periods (Table 1). Arbuscules, vesicles, and hyphae were observed in all root samples (Fig 1). The total AMF colonization ranged from 14.08-21.57% with no significant monthly variation at $p < 0.05$. (Table 2). However, a significant variation in fungal structure viz., arbuscules, vesicles and hyphae were observed during the sampling period ($p < 0.05$). Spore density ranged from 95.2-180.2 per 100g soil (Table 2) with a significant variation at $p < 0.05$ between sampling periods. The roots of Casuarina showed low root fungal colonization (17.93 %) in contrast to high spore density in the rhizosphere soil under salinization as supported by other workers [24,25,26]. AMF colonization was negatively correlated with spore density which may be due to stimulated sporulation at low root-colonization levels as supported by Aliasgharzadeh [27]. Low colonization by AMF in casuarina trees may be due to poor spore germination and hyphal formation further salinization may alter enzymatic activity leading to inhibition of cellular expression thus, decreasing mycorrhizal colonization of the roots [26,31]. Also, dry and harsh soil of saline environment hinders colonisation process due to lack of proper hydration resulting in long term dormancy stage facilitating spore accumulation as evident with positive correlation between moisture content, AMF colonisation and spore density (Table 3).

Electrical conductivity exhibited negative significant correlation with hyphae. Moisture content exhibited positive significant correlation with AMF colonization and spore density. Total Phosphorus exhibited a significant negative correlation with vesicles and AMF colonization and positive correlation with spore density. Further, a significant negative correlation was observed between AMF colonization and spore density in the rhizosphere soil of Casuarina. Negative significant correlation between electrical conductivity and hyphae suggests lower hyphal length with increased salinity as supported by Juniper and Abbott [30] who, reported that under high NaCl concentration germination rate and hyphal length development from the germinated spores decreases. Our result indicate that total phosphorus is inversely related to AMF colonization suggesting the increase availability of phosphorus lead to lower root infection which is in compliance with reported of Deng *et al.* [32]. Positive correlation of total phosphorus with spore density was observed which is in contrast to the finding of Liuet *al.* [33], who observed no significant influence of phosphorus on AMF community composition. However, Kahiluoto *et al.* [34] illustrated that phosphorus can change species composition and increase AMF diversity.

A total of 21 morphotypes were isolated from the rhizosphere soil of Casuarina belonging to two orders, four families, and eight genera within the phylum Glomeromycota. The dominant AMF species isolated were *Glomus fasciculatum* Gerdemann & Trappe (9.0%) followed by *G. lamellosum* Dalpé, Koske & Tews and *Dentiscutata reticulata* (Koske, Mill. & Walker) Sieverd., F.A. Souza & Oehl (6.0%) and *Glomus macrocarpum* (Tul. & Tul) Berch & Fortin (5.4%). The relative abundance of AMF species from the rhizosphere soil followed the trend *Glomus* > *Acaulospora* > *Dentiscutata* > *Rhizophagus* > *Scutellospora* > *Septoglomus* > *Diversispora* > *Funneliformis* with 43.7, 10.7,



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9.1, 8.7, 8.1, 4.9, 2.9 and 2.8% respectively. The highest isolation frequency was recorded for *G. etunicatum* (Becker & Gerd.) Walker & Schüßler and *D. reticulata* (Koske, Mill. & Walker) Sieverd., F.A. Souza & Oehl (100%) and the lowest was recorded for *Funneliformisgeosporum* (Nicolson & Gerd.) Walker & Schüßler and *Rhizophagus aggregatus* Schenck & Sm (50%). Species richness of AMF was recorded to be highest for *Glomus* sp. (10) and the lowest was recorded for *Diversispora* sp., *Funneliformis* sp. and *Septoglomus* sp. (1) (Table 4). High species diversity of AMF in saline soil indicates that mycorrhizal fungi maintain a diverse community by competing for resources using various strategies as indicated by Estrada *et al.*, and Koske [35,36]. In the present investigation, *Dentiscutata reticulata* and *Glomus* sp. were the most frequent AMF species isolated, suggesting their competitive nature and ability to survive in salt stress conditions. The high species diversity found in the rhizosphere of *Casuarina equisetifolia* L. as indicated by high spore density from the study site points towards the survival strategies implemented by AMF species through sporulation which helps to survive unfavorable condition. McMillen [37] demonstrated that NaCl inhibited spore germination and hyphal growth but resulted in increased spore population in saline soils. Of the 21 species isolated most belong to Glomerales, which are known as dominant communities in various ecosystems [35,38,39,40]. The order Glomerales was represented by the genera *Glomus* being the highest (10 species). The abundance of *Glomus* species has repeatedly been reported from most disturbed ecosystems such as agricultural system [41,42] and coastal areas [43] due to its ability of sporulate, colonise from fragments and form anastomoses [41,44,45]

The present study indicated high AMF diversity in the saline soil of *Casuarina equisetifolia* L. with highest abundance of *Glomus* sp. and *Dentiscutata reticulata*. A comprehensive study is required, including *Glomus* and *Dentiscutata* sp., to examine role played by mycorrhizal fungi in alleviation of salt stress in *Casuarina equisetifolia* L. therefore, appropriate in-vitro studies involving inoculation of resident AMF species to *Casuarina* seedling as well as other crop species under high salt concentration will be carried out in further studies. Also, the lower rate of mycorrhizal colonization in the roots needs to be addressed by emphasizing on the factors responsible for lower colonization. Furthermore, the present study highlighted that despite high salt concentration AMF can survive and sporulate and provide a rich reservoir of potentially beneficial consortium which could be used to evaluate the role of AMF species in mitigation of salt stress in other economically important plants.

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Table 1: Physicochemical Properties of Rhizosphere Soil.

Soil properties	Mean values ± SE
pH	7.71±0.18
Temp (°C)	28.33±0.41
EC (mS)	4.57±0.25
MC (%)	16.70±1.30
TP (%)	0.027±0.008
SOC (%)	1.9451±0.58

Note: EC = Electrical conductivity, MC = Moisture content, TP = Total Phosphorus, SOC = Soil Organic Carbon





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Table 2: AMF Colonization and Monthly Spore Density.

Months	Arbuscules	Vesicles	Hyphae	Total AMF	Spore density/100g
January	5.43±0.16 ^a	10.28±0.52 ^a	1.82±0.44 ^a	17.53±2.45 ^a	105.4±4.19 ^a
February	5.91±0.12 ^a	9.39±0.12 ^b	6.27±0.32 ^b	20.57±1.10 ^a	95.2±2.28 ^a
March	6.22±0.13 ^a	5.18±0.40 ^c	2.68±0.20 ^a	14.08±1.05 ^a	180.2±5.32 ^b
April	7.34±0.20 ^b	9.82±0.27 ^{ab}	2.22±0.14 ^a	19.38±2.23 ^a	166.0±4.12 ^b
May	7.44±0.20 ^b	9.75±0.37 ^a	0.92±0.24 ^a	18.11±2.64 ^a	120.6±4.08 ^b

Note: Mean±SE with the same letter on top does not differ significantly according to Tukey's HSD test (P=0.05).

Table 3: Correlation Coefficients between AMF Colonization, Spore Density and Soil Physicochemical Properties.

Factors	pH	T	EC	MC	TP	SOC	Arb	Ves	Hyp	AMF	SD
pH	1	-	-	-	-	-	0.84*	-	-	-	-
Temperature (T)°C		1	-	-0.8	-	-	-0.82	-	-	-	-0.74
Electrical conductivity (EC) mS			1	-	-	-	-	-	-0.96**	-	-
Moisture content (MC) %				1	-	-	-	-	-	0.87*	0.88*
Total Phosphorus (TP)%					1	-	-	-0.98**	-	-0.85*	0.89*
Soil Organic Carbon (SOC)%						1	-	-	-	-	-
Arbuscules (Arb)							1	-	-	-	-
Vesicles (Ves)								1	-	0.8	-
Hyphae (Hyp)									1	-	-
AMF colonization (AMF)										1	-0.81
Spore density (SD)											1

Note *Significant at p < 0.05. **Significant at p < 0.01.

Table 4: AMF Species Isolated from Rhizosphere Soils of *casuarina equisetifolia* l. with their Relative Abundance (RA) and Isolation Frequency (IF).

	AMF Species	RA%	IF%
	Glomerales		
	Glomaraceae		
1	<i>Funneliformis geosporum</i> (Nicolson & Gerd.) Walker & Schüßler	2.8	50
2	<i>Glomus caledonius</i> Gerdemann & Trappe	3.3	70
3	<i>G. clarum</i> Nicolson & Schenck	2.9	70
4	<i>G. etunicatum</i> (Becker & Gerd.) Walker & Schüßler	2.4	100
5	<i>G. fasciculatum</i> Gerdemann & Trappe	9.0	80





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6	<i>G. lacteum</i> Rose & Trappe	2.9	70
7	<i>G. lamellosum</i> Dalpé, Koske& Tews	6.0	90
8	<i>G. macrocarpum</i> (Tul. &Tul) Berch& Fortin	5.4	80
9	<i>G. mosseae</i> (Nicolson & Gerd.) Gerd. & Trappe	3.3	60
10	<i>G. multicaule</i> Gerd. &Bakshi	3.3	70
11	<i>G. versiforme</i> (Karst.) Berch& Fortin	5.2	80
12	<i>Rhizophagus aggregatus</i> Schenck & Sm	5.4	50
13	<i>R. fasciculatus</i> (Thaxt.) Walker &Schüßler	3.3	70
14	<i>Septoglo mus constrictum</i> (Trappe) Sieverd., Silva & Oehl	4.9	80
Diversisporales			
Acaulosporaceae			
15	<i>Acaulosporadelicata</i> Morton	4.9	60
16	<i>A. kentinensis</i> Kaonongbua, Morton & Bever	5.8	60
Gigasporaceae			
17	<i>Dentiscutata heterogama</i> (Nicolson & Gerd.) Sieverd, Souza &Oehl	3.1	70
18	<i>D. reticulata</i> (Koske, Mill. & Walker) Sieverd., F.A. Souza &Oehl	6.0	100
19	<i>Scutellospo rabiornata</i> Spain, Sieverd. &Toro	3.8	80
20	<i>S. calospora</i> (Nicolson & Gerd.) Walker & Sanders	4.3	70
Diversisporaceae			
21	<i>Diversispo ratrimurales</i> (Koske& Halvorson) Walker &Schüßler	2.9	60

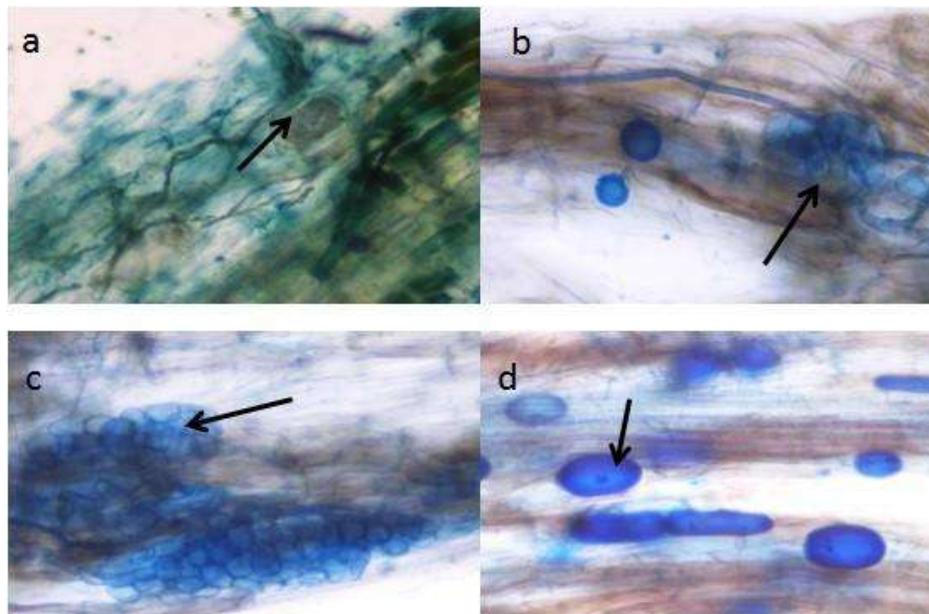
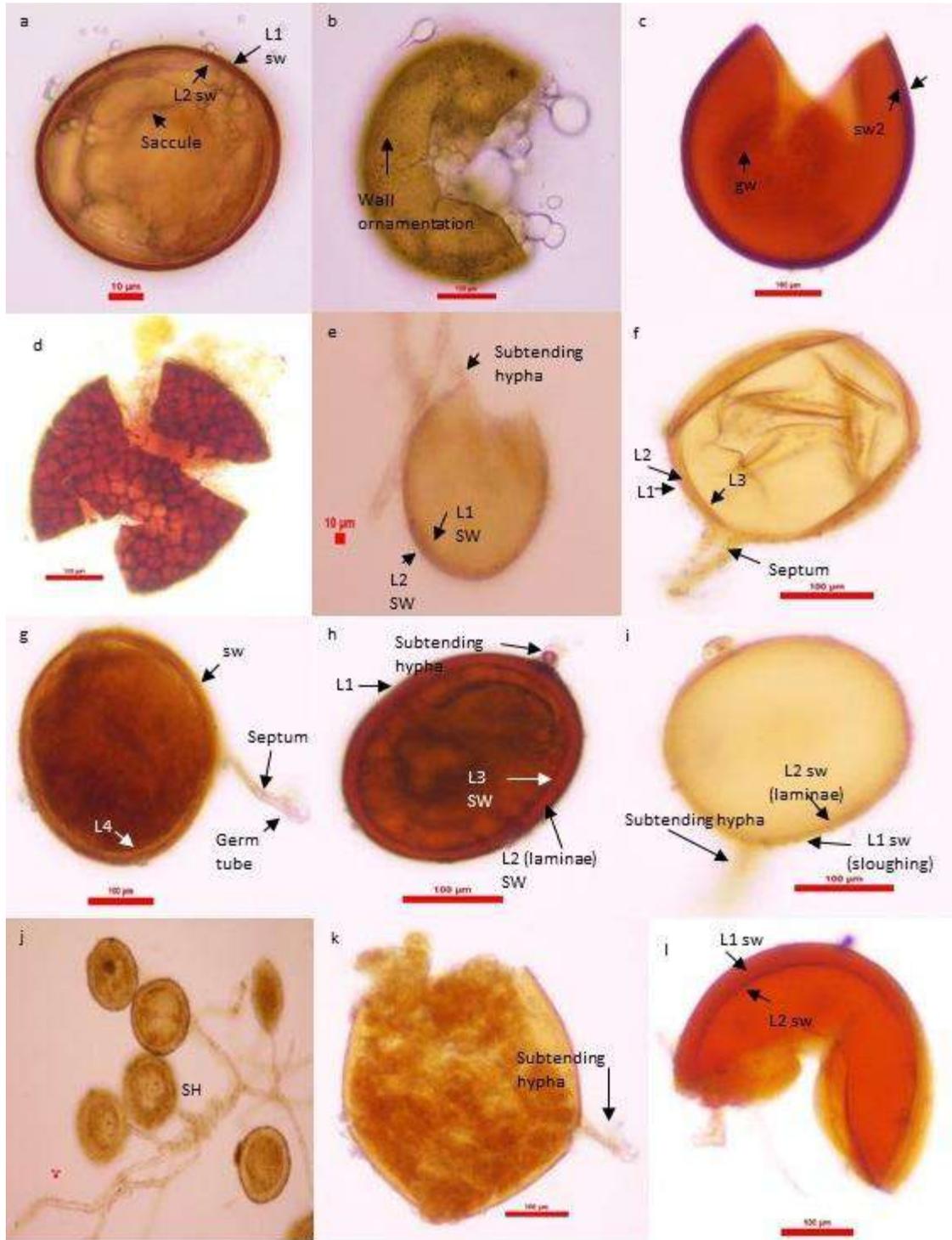


Figure 1: Mycorrhizal colonization in *Casuarina equisetifolia* L. showing Arbuscules (a), hyphal coil (b), sclerotia (c) and vesicles (d)





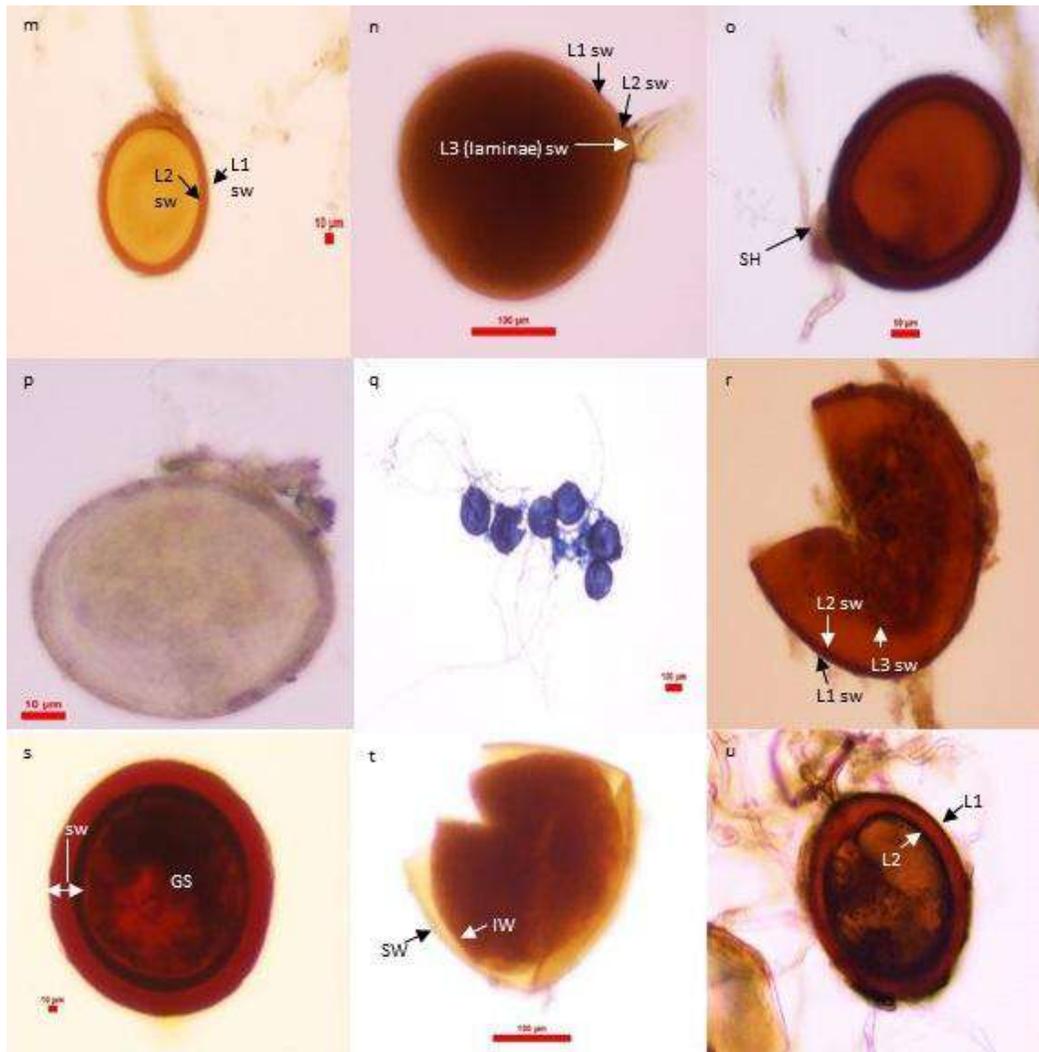


Figure 2: Arbuscular mycorrhizal fungi isolated from the rhizosphere soil of *Casuarina equisetifolia*. (a) Presence of sporiferous saccule in *Acaulospora delicata*. Bar=10 μ m; (b) ornamentation on the wall of *A. kentinensis*. Bar=100 μ m; (c) *Dentiscutata heterogama*. Bar=100 μ m. germinal wall closely paired; (d) outer wall ornamented in *D. reticulata*. Bar=100 μ m; (e) spores foremed singly in soil with subtending hypha in *Diversispora trimurales*. Bar=10 μ m; (f) sloughing of spore wall of *Funneliformis geosporum*. Bar=100 μ m; (g) germ tube emerging from the lumen of subtending hypha with septum of *Glomus caledonius*. Bar=100 μ m; (h) insertion of L3 into subtending hypha of *G. clarum*. Bar=100 μ m; (i) presence of inner laminae and sloughing of outer wall of *G. etunicatum*. Bar=100 μ m; (j) subtending hypha of *G. fasciculatum*. Bar=10 μ m; (k) single layered spore wall of *G. lacteum*. Bar=100 μ m; (l) *G. lamellosum*. Bar=100 μ m; (m) bilayered with thickening of spore wall of *G. macrocarpum*. Bar=10 μ m; (n) layer 3 laminae of *G. mosseae*. Bar=100 μ m; (o) multiple attached hyphae and thick spore wall of *G. multicaule*. Bar=10 μ m; (p) laminate innermost layer of *G. versiforme*. Bar=10 μ m; (q) loose cluster without a peridium of *Rhizophagus aggregatus*. Bar=100 μ m; (r) three layered spore wall of *R. fasciculatus*. Bar=10 μ m; (s) *Scutellospora biornata*. Bar=10 μ m; (t) two layered juvenile spore of *S. calospora*. Bar=100 μ m; (u) bilayered spore wall with subtending hypha of *Septoglomus constrictum*. Bar=100 μ m. where, L=spore wall layer, sw=spore wall, gs=germination shield, iw=inner wall.





Outlier Detection using Fuzzy Approach

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ABSTRACT

Through the wisdom of free open text data, Data Analytics helps businesses grow and make decisions by revealing some fascinating patterns and trends in knowledge discovery. Customers' perspectives express sensible opinions that are not always true. We present a strong fuzzy based circle clustering strategy to find positive-definite outliers in this work. Text data from free handlers gives prior knowledge about the product opinion, which can be used to find knowledge discovery and pattern trends. Positive-definite reviews and comments on the internet encourage consumers and sellers to work together. Negative consequences may yield valuable insights for business, individuals, and government development. There are two parts to this paper: Data preprocessing, a circle-generating technique for clusters, and outlier detection using fuzzy logic. The engaging and fascinating experimental examination of the positive-definite outlier opinion yields accurate and appropriate conclusions with a degree of ambiguity in the mathematical model.

Keywords: fuzzy, outliers, opinion, clusters, free text handlers.

INTRODUCTION

Data mining, also known as non-trivial extraction of implicit data, is a collection of techniques that includes classification, clustering, data summarization, change analysis, and anomaly detection. It uncovers hidden patterns and combines machine learning, deep learning, and statistical methodologies. It also looks for global patterns, decision support, estimating, forecasting, and novel correlation patterns, among other things. However, Data Mining



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must meet certain criteria, such as innovation, repository trends, understandability, veracity, and interest. Estimation, prediction, classification, association, and grouping are just some of the jobs and procedures it may perform. To create comprehensive records of the target variable and categorical predictor variable, estimation delivers the approximate value of a numeric variable. Point estimation, confidence interval estimation, simple linear regression and correlation, and multiple regression are some of the estimate methods available. The future holds predictions that are similar to classification and estimation. For example, using KNN, you can forecast the price of a stock for the next three months and the winners of a cricket team for the next ten years. The target variable is categorical rather than numeric in classification, which is similar to estimation. It is based on a predetermined set of predictor and target variables. Affinity analysis (or market basket analysis) uses association rules to determine which traits "go together." It also aims to discover the principles of association between two or more traits with a degree of confidence and support. Clustering is a grouping of records that are similar but have no target variable. It may not attempt to categorise, estimate, or forecast the value of a target variable. Within the cluster, record similarity is maximised, while outside the cluster, it is minimised. Clustering is a useful exploratory data analysis technique for detecting outliers. Clustering is an unsupervised learning technique that uses unlabeled training data to uncover classes within the data. A cluster are similar that contains collection of data objects. It's organised based on how similar it is. Clustering is widely employed in real-world issues for a variety of purposes. It is used in applications like as market research, pattern identification, data analysis, image processing, and the medical industry to simplify enormous datasets in order to uncover something new. The homogeneous 'n' data points correspond to the 'c' clusters of the partitioning matrix 'U'. Cluster Analysis uses class labels to help create taxonomies without analysing data items. The data objects are grouped using the maximization of intraclass similarity and minimization of interclass similarity principles. Section 2 describes outlier analysis, Section 3 describes related work, Section 4 includes various aspects of outlier detection, Section 5 includes applications of outlier detection , Section 6 includes clustering, Section 7 includes fuzzy clustering and Section 8 describes experimental results and discussion.

OUTLIER ANALYSIS

Outlier analysis is a methodology for detecting uncommon patterns that arise as a result of computational error, wrong input, sampling error, exceptional of true mistake, and native data modifiability. In a nutshell, it's the process of identifying patterns in a dataset that don't follow the predicted pattern. Anomalies, oddities, aberrations, discordant item, an exception, a surprise, and aberrant behaviour are all terms used to describe outliers. Both phrases are used interchangeably in the context of outlier discovery to transfer significant positive-definite data to other domains. The data analyst is fascinated by the common characteristics that exist across all application domains. Outlier detection relies heavily on the "interestingness" of real-world relevance. Data analysts are uninterested in noise and regard it as a barrier to outlier detection. Global, contextual, and collective outliers are the three sorts of outliers. Fraud detection, intrusion detection, image processing, health care informatics, surveillance, medical diagnosis, predictive maintenance, cyber-intrusion, and other applications are among its capabilities. It is necessary to recognise outliers in order to accept the uncertain and imprecise. To detect it, first cluster the data, then compute the centroid and distance using the Euclidean distance approach, then compute OF (objective function) and proclaim it as an outlier if it falls below a certain threshold.

Several variables make detecting outliers difficult in general. a)It's not always easy to distinguish between normal and abnormal behaviour. The anomalous point is close to the normal zone, which operates normally and appears to be more difficult to solve. It may lead to the induction of factors based on labelled data, data kind, anomaly type, and so on. b)In the future, the current concept of actual behaviour may not be well reflected. For each domain, the definition of abnormal behaviour may be different. For example, in medical diagnostics, changes in body temperature may be close to normal when evaluating the patient's therapy, and malevolent enemies may be considered typical behaviour. In the stock market, oscillations in the rise or fall in the value of a stock may be close to the normal zone, with outlier points being considered typical. c)Training labels and validating critical models is a difficult task. d)Noise is difficult to partition and eliminate since it is identical to true outliers. Data points that deviate from the norm are known as outliers. Some statistical tests, such as density-based and distance-based approaches, can be used to detect it. Data preprocessing, fuzzy clustering, and outlier analysis are the three sections



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of this research. The capacity to deal with noise and high dimensionality are the obstacles. Here are some questions to consider when we try to spot outliers.

- a) "Are all of the patterns interesting?"
- b) "How does the pattern appeal to you?"
- c) "Can a data mining system come up with the most interesting ones?"
- d) "It's finally novel?"

RELATED WORK

Outliers have been examined in the statistical field since the 19th century[21]. Anomaly observations were used to test a statistical model for noise accommodation through immunization [33]. For several research commodities, several application fields for detecting outliers have been researched [10]. In [6][3][43], a survey of statistics on outlier detection was conducted. For anomaly identification, [44] looked at a complete survey of statistics research. Noise accommodation is a structured robust analysis on linear regression for identifying outliers, noise removal is removing unwanted objects before analysis, Teng et al[68], identifying theft in credit card fraud detection and novelty detection is detecting novel patterns and outliers [48][49]. The study of sensitive data being sent to an unauthorised machine [41]. [25] investigates surprising observations in spacecraft sensors. The study looks at a survey of cyber-intrusion detection [51] as well as a study on intrusion detection [18]. [9] and [31] both conducted surveys on anomaly detection. [6] conducted a review on identifying anomalies in numeric and symbolic data. The investigation of outliers detection in various strategies is shown in Table 1.

In general, different aspects such as the nature of data input, label availability, types of anomalies, and the output of the application domain driven by requirements and limitations establish a distinct formulation of the problem. We evaluated the size of the structure and its worth as an extra element.

The type of data input is classified according to the type of data instance. Sequence data (time series, protein sequences, genome sequences, data sequences), geographical data (ecological data, vehicular traffic data), and graph data are all examples of data types (connect with edges to other vertices). It is dependent on the type of data, which can be univariate or multivariate. Different statistical models were employed to analyse data instances that were categorised as binary, categorical, and continuous. In [66]; Point, contextual, and collective anomalies are the three categories of anomalies. "Point anomalies" are defined as "data points that are aberrant in relation to other data points." Point anomalies are the simplest, and the outlier point is located outside the normal region's boundaries. For example, in credit card fraud detection, the anomaly is defined as a large amount spent in comparison to usual spending. ii) "Contextual anomalies" are defined as "data objects that stray significantly from a certain context." Contextual is also known as conditional because it is reliant on a choice. [63] It has two types of attributes: environmental and behavioural. The neighbourhood of a data instance is determined by contextual attributes, which are based on geographic and time series data. Non-contextual data characteristics are determined by behavioural attributes. The context is regarded as an oddity, while the behaviour is regarded as usual. As a result, the data instance's key property is categorised as anomalous in context and normal in behaviour attributes. iii) "Collective anomalies" are defined as "a data object that is abnormal in relation to the complete data set." Sequence data, graph data, and spatial data are all used. The types of anomaly detection are summarised in Table 2.

VARIOUS ASPECTS OF OUTLIER DETECTION

In general, different aspects such as the nature of data input, label availability, types of anomalies, and the output of the application domain driven by requirements and limitations establish a distinct formulation of the problem. We evaluated the size of the structure and its worth as an extra element. The type of data input is classified according to the type of data instance. Sequence data (time series, protein sequences, genome sequences, data sequences), geographical data (ecological data, vehicular traffic data), and graph data are all examples of data types (connect with edges to other vertices). It is dependent on the type of data, which can be univariate or multivariate. Different statistical models were employed to analyse data instances that were categorised as binary, categorical, and



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To evaluate if a data instance is anomalous or typical, a data label that encompasses all sorts of behaviour is typically costly and correct. Through the labelling mechanism, a significant amount of human work is required to obtain labelled training data sets. It records a labelled set of unusual data instances that are more challenging to understand than regular behaviour. Airline safety mechanism, for example, where unusual behaviour leads to rare events. When it comes to spotting outliers, there are three types of data labels: supervised, unsupervised, and semi-supervised. Two fundamental challenges in supervised anomaly detection are rather difficult. First, when compared to typical class instances, the anomalous class instance is extremely rare. As a result, an uneven distribution of class instances is discovered when compared to the predictive model. Second, to represent the label training dataset, several false anomalous data points are created. Unsupervised anomaly detection does not require training data labels, so an implicit assumption is made for anomalous instances that are close by and normal instances that are far away. Only the normal class has training data of labelled cases in semisupervised anomaly detection. Labeled instances are not required for the Anomaly class. Fault detection in spacecraft, for example. It's tough to spot outliers in test data if the engine is malfunctioning. As a result, the anomalous model is constructed in the same way as normal behaviour. Table 3 describes the various approaches of outlier detection.

Outliers in score and labels must be reported as part of the detection process. Using scoring algorithms, an Outlier Score is assigned to each instance that is dependent on degree. Using a threshold value, the data analyst selects the top outliers. They assign labels to each instance's control parameters for each approach.

APPLICATIONS OF OUTLIER DETECTION

Many applications detect outliers, including intrusion detection, fraud detection, medical health analysis detection, quality control, financial applications, web log-based analytics, Earth science applications, satellite image analysis, anti-terrorism, pharmaceutical research, data leakage prevention, time series monitoring, and more. Image processing detection [61], Text data outlier detection [64], Industrial damage detection [39], Novelty detection in robots [15], Sensor networks detection, [12], criminal activities detection [46], video monitoring detection, astronomical data detection [20], Management of customer complaints[29] are discussed.

CLUSTERING

" Clustering is an unsupervised learning technique that involves collecting similar objects and analysing them." There are several types of clustering methods, including partitioning methods (k-means, k-medoids), grid-based methods (Wavecluster, clique, sting), hierarchical methods (divisive, dendrogram, agglomerative), model-based methods, and density-based methods (denclue, optics, dbscan). It is widely used in many fields such as data mining, digital image processing, statistics, biology, deep learning, and machine learning, and is widely used in many applications such as data analysis, pattern recognition, market research, and image processing using software packages such as R programming, Python, and SPSS. Clustering aids the business sector in grouping customers based on common patterns. It also aids in economic market research, insurance forecasting, home planning, earthquake analysis, document classification, and geographic data analysis. It works with a wide range of properties,





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as well as arbitrary forms, noise, outliers, data object scalability, constraint-based grouping, and interpretability. Clustering tendency, non-uniform distribution solved by Hopkins test as null hypothesis (H0), and alternate hypothesis (H1) are used to evaluate clusters (Ha) ii) Clustering quality, which is determined by metrics like the intrinsic measure-silhouette, Fig(14)(15), and the extrinsic measure. iii) Number of optimal clusters (k), as determined by i)empirical and elbow methods, and ii)domain knowledge approach, as shown in granularity Fig(11). iii)statistical approach: the value of the gap statistic corresponds to clusters. Figure 1 depicts many cluster types.

Cluster validity indices are used to compare clustering algorithms or two cluster sets in terms of compactness and connectivity. It determines whether or not there is a cluster owing to noise. Internal cluster validation, external cluster validation, and relative cluster validation are all methods for determining cluster validity. The inter-cluster distance $d(a, b)$ can be one of the following: a) single linkage distance, b) complete linkage distance, c) average linkage distance, or d) centroid linkage distance. Complete diameter linkage distance, average diameter linkage distance, and centroid diameter linkage distance are all examples of intra-cluster distance $D(a)$. Figure 2 depicts both well-separated and poorly-separated clustering.

J. C. Dunn proposed the Dunn index in 1974 as an internal metric for evaluating clustering techniques. It's used to distinguish compact, well-separated clusters within cluster variance. When the Dunn index is high, clustering is good, and the DI maximisation is used to determine the optimal number of clusters. In equation(1), the dunn index is defined as

$$\text{Dunn index}(U) = \min_{1 \leq i \leq c} \left\{ \min_{1 \leq j \leq c, j \neq i} \left\{ \frac{\delta(X_i, X_j)}{\max_{1 \leq k \leq c} \Delta(X_k)} \right\} \right\} \tag{1}$$

Where X_i and X_j are the cluster's intercluster distances. X_k is the cluster's intracluster distance.

The Davies–Bouldin index was created in 1979 by David L. Davies and Donald W. Bouldin to evaluate clustering techniques internally. The dataset's size and characteristics are assessed. Clustering is beneficial if the DB index value is low. In equation(2), the DB index is defined as follows:

$$\text{DB index}(U) = 1/k \sum_{i=1}^k \max_{i \neq j} \left\{ \frac{\Delta(X_i) + \Delta(X_j)}{\delta(X_i, X_j)} \right\} \tag{2}$$

Where, X_i and X_j denote the cluster's intercluster distance. X_k represents the cluster's intracluster distance.

Measuring the distances between two clusters' similarity is significant and vital. Euclidean and Manhattan distances are the two methodologies used. B. The equation for calculating Euclidean distance is as follows: (3)

$$J(U, V) = \sum_{i=1}^n \sum_{j=1}^c (\mu_{ij})^m \| x_i - v_j \|^2 \tag{3}$$

The pearson correlation distance, Eisen cosine correlation distance, and Spearman correlation distance are all correlation-based distances that may be calculated using equation(4) (5). The average dissimilarity between clusters is measured as average distance. The average silhouette of all rows is called Average Silhouette. The number of clusters that maximises this value is used.

$$J(U, c_1, \dots, c_c) = \sum_{i=1}^c J_i = \sum_j^c \sum_j^n u_{ij}^m d_{ij}^2 \tag{4}$$





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$$c_i = \sum_{j=1}^n u_{ij}^m x_j / \sum_{j=1}^n u_{ij}^m \tag{5}$$

Summary of clustering types:

J.A. Hartigan and M.A. Wong created the k-means algorithm to create a small number of clusters from a large number of data with no outliers. Discrete data, as an example. The fundamental goal is to partition n observations with p dimensions into k clusters with the lowest possible sum of squares. This approach locates a "local" optimal solution. Each row of the kth cluster has p variables and contains n and k observations. The jth row of the kth group provides a missing value for ith variable. It is normalised by subtracting the mean from the total and dividing by the standard deviation, which is indicated by zij.

Algorithm 1: K-means clustering

1. Decide on the number of clusters you want to use.
2. Choose k cluster centres.
3. Assign all data objects to the cluster centre that is the closest to you.
4. In each cluster, calculate new cluster centres.
5. Repeat the procedure until the cluster centre calculation remains unchanged.

Algorithm 2: Hierarchical clustering

1. Determine the smallest element dij in each of the n clusters.
2. Combine clusters i and j into a single new cluster, k (nk=ni+nj).
3. Use the distance formula to calculate distances.
4. Go through the instructions again. N-1 iterations are required for 1 to 3 objects.

Agglomerative hierarchical clustering algorithms are represented by a dendrogram, which is a tree diagram. The objects are never separated as the two clusters are combined into a single new cluster. The distance between clusters is defined by the horizontal axis, while the cluster items are represented by the vertical axis. The dij of n items represents the distance between I and j clusters. Figure 3 depicts an example dendrogram as well as the procedures involved in hierarchical clustering.

FUZZY CLUSTERING

Jim Bezdek invented soft clustering in 1981. It is the data object that contains members of all clusters with membership degrees ranging from 0 to 1. It has a high degree of membership that is close knit and a low degree of membership that is dispersed. Cluster prototype and membership degree are used to build the matrix. where 'n' denotes the number of data points 'vj' denotes circle centre cluster, 'm' denotes index of fuzziness index, $m \in [1, \infty]$, 'c' denotes cluster centre, 'ij' denotes membership degree, and 'dij' denotes Euclidean distance in equation(1)(2)(3). Figure (4) depicts the fuzzy membership function, which is given by equation (6)(7)(8).

$$\sum_{i=1}^c u_{ij} = 1, \forall j = 1, \dots, n \tag{6}$$

$$\mu_{ij} = 1 / \sum_{k=1}^c (d_{ij} / d_{ik})^{(2/m - 1)} \tag{7}$$

$$v_j = (\sum_{i=1}^n \mu_{ij}^m x_i) / (\sum_{i=1}^n (\mu_{ij}^m)), \forall j = 1, 2, \dots, c \tag{8}$$

Membership degree, =0,.....,1, is the fuzzy indication of similarity in fuzzy sets. If the membership degree is equal to





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1, the object belongs to the set completely, and if it is equal to 0, the object does not belong to the set at all. Clusters with comparable values have a higher possibility of belonging to a set. The following equation (9) can be used to calculate fuzzy clustering, goal function, cluster centre, and membership degree (10)

$$u_{ij} = 1 / \sum_{k=1}^c (d_{ij} / d_{kj})^{2/(m-1)} \tag{9}$$

Where,

$$d_{ij} = ||c_i - x_j|| \tag{10}$$

clustering that is a little fuzzy Partially categorising clusters is possible using partition clustering algorithms. Each individual is a member of only one cluster in regular clustering.

ALGORITHM OVERVIEW

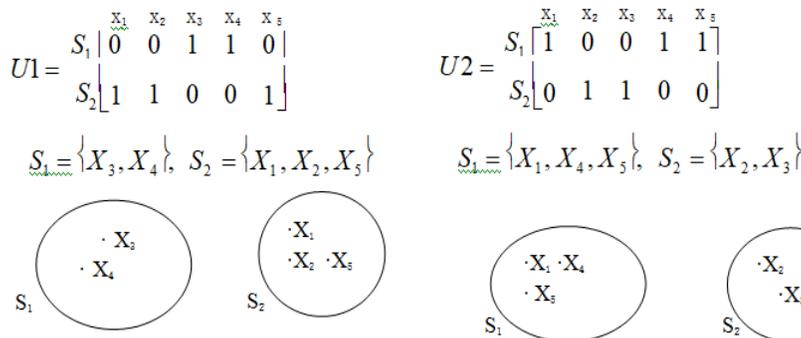
The algorithm's major criteria include efficiency, scalability, performance, and optimization. There are a variety of algorithms for reducing (or eliminating) outliers. However, it is possible that some critical buried data will be lost. The approach is first preprocessed to decrease noise and then partitioned using fuzzy set theory, a linear model, and the mean of clusters. Outliers are recognised using an objective function once the next circle generation process has been overrun. Outliers are often dismissed as exceptions (or) noise by many data mining tools.

FUZZY CIRCLE BASED – (CENTROID) CLUSTERING PROPOSED

Free text handlers opinions are clustered into two categories as positive definite and negative opinions. Fig (5) shows how blobby clusters are based on degree of fluidity and defined as "blobbiness clusters" which are proportional to the corresponding degree. The following equation (11) follows how to structure the clusters. The opinions of free text handlers are divided into two groups: positive definite opinions and negative definite opinions. Blobby clusters are characterised as "blobbiness clusters" that are proportionate to the relevant degree of fluidity, as seen in Fig (5). The structure of the clusters is determined by the following equation (11):

$$f(x, y, z) = \sum_{k=0}^{-n} b_k \cdot e^{-ak^{r^2}k} - T \tag{11}$$

Where x,y,z are the parameters, T is the threshold, a and b are the blobby adjustments, and e and k are negative values. Let X= X1, X2, X3, X4, X5 be separated into two subsets, each of which is a non-empty matrices with each element belonging to a single Clustering





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Algorithm 3: Circle generating algorithm

1. $r, (x_c, y_c)$
2. $p_0 = 1 - r$
3. $p_k < 0, p_{k+1} = 2x_{k+1} + 1$
4. $p_k > 0, p_{k+1} = 2x_{k+1} - 2y_k + 1$
5. $2x_{k+1} = 2x_k + 2$
6. $2y_{k+1} = 2y_k + 2$

Condition:

$$\text{fuzzycircle}(x, y) = x^2 + y^2 + 1$$

If $\text{fcircle}(x, y) < 0 \rightarrow (x, y)$, inside circle cluster

If $\text{fcircle}(x, y) = 0 \rightarrow (x, y)$, on circle cluster

If $\text{fcircle}(x, y) > 0 \rightarrow (x, y)$, outside circle cluster

Using the aforesaid approach, we have created new cluster centroids. Using the Euclidean distance between data points and every circle centroid, a new centroid value is produced until the threshold value is less than 0.

Pseudocode

1. Initialize membership matrix U
2. Calculate fuzzy cluster circle center C
3. Find the distance between different circle centers using Euclidean distance.
4. Calculate OF until the value is below threshold.
5. Otherwise, Fix a random point 'p', to get optimum value—repeat from step 2

Objective

The goal of employing a fuzzy based circle generating clustering algorithm to find outliers and identify influential factors in opinion mining.

Points to Remember

1. IQR is calculated when data is normalised with a Z-score (-3 to +3). (Inter Quartile Range)
2. Preparation of Data is done by a) Graphical Method b) Numerical Method
3. Research Methodology a) High Average Point b) Influential Observations c) Simple Linear Regression
4. Methods of Assessment - Algorithms for fuzzy clustering and generating circles
5. Outliers: LOF 1, (No consistent data point)
No Outliers: LOF > 1 (consistent data point)

Idea

To discover top dissimilar clusters, choose n data points and k clusters.

1. Identify data that is incongruent (cluster the data)
2. Look for outliers in your data (calculate the centroid of each cluster)
3. Methodology: A distance-based strategy
4. Compare the distance around the outlier object assumption to the distance around the non-outlier object assumption (finding the distance)
5. Using the circle-generating technique, make the circle significantly equivalent to the distance between neighbours (To decide anomaly).

Note: Before detecting outliers, noise must be eliminated.





DISCUSSION OF EXPERIMENT RESULTS

By using R programming version 3.6.1 and installing the basic package “ppclust” (23rd July 2019). Our technique has been tested on two datasets: Advertising and stock data. We are confident that our system will find internal coincidence quality outliers in a high proportion of cases. When the aforementioned two datasets are compared using the proposed method, the stock data dataset has a higher quality % than the Advertising dataset. Fig (6) shows the boxplot of outliers. Fig (7) shows the membership degree of stock_data dataset. Fig (8)(9)(10)(11)(12) shows the fuzzy membership calculations. Fig (13) displays the optimal number of clusters. Fig (14) displays rows and columns of Advertising dataset. Fig (15)(16) shows the silhouette with 3 clusters and 2 clusters.

CONCLUSION

By 2025, our corporate economy will have matured due to customer-specific requirements. The competition between various pressures will be eliminated, and a successful outcome will lead to customer satisfaction. The suggested technique addresses the uncertainty, noise, and incompleteness of data in a networked context, enhancing the power of discovery patterns. The product's interestingness measurements are determined by pattern constraint and pattern evaluation in opinion mining based on user beliefs (or) expectations. The views of free text handlers (customers) are useful in determining the style of marketing that will result in an increasing profit for a specific product. We can better comprehend the targeted strategy. Fuzzy clustering is a great way to boost business growth. Our method appears to be effective based on the outcomes of our tests. When applied to the stock data dataset, the revised suggested technique produces positive results. In the future, the research will concentrate on fuzzy-genetic outlier detection.

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Table 1: A study on Outlier detection Techniques.

Techniques	References
Clustering based	[35][29][30]
Classification based	[66][19]
Information Theoretic	[45]
Statistical based	[4]
Spectral based	[36]
Hierarchical clustering	[11]
DB-SCAN	
Density based	
Fuzzy Clustering	

Table 2: Types of Anomaly Detection

Types of anomaly		Example	References
Point		Credit card – Fraud detection	[9]
Contextual	i)Contextual attributes Spatial data	Latitude and Longitude of a location- rainfall	[40] [60]
	ii)Behavioral attributes Time series data	Weather forecasting, Medical diagnosis	[71][58]
Collective	Sequence data	Event log management system	[23][24]
	Graph data	Rainfall in a month	[50]
	Spatial data	Temperature in a particular region	[60]

Note: “By incorporating the context information, both the types-point and collective-are transformed to contextual.”

Table 3: Modes of Outlier Detection

Modes of Data Label	References	Description
Supervised	[72] [37][38] [70] [69] [13] [65] [1]	Training data is required
Unsupervised	[9]	No training data label is required
Semi-supervised	[24] [17] [16] [25]	Combination of supervised and unsupervised technique

Note: The Semi supervised model is an unsupervised model with low outliers in test data and high robustness in training data.





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Table 4: Applications of Outlier Detection

Applications	Key Challenge	Techniques Studied	References
Host based Intrusion Detection System	<ul style="list-style-type: none"> ✓ Large amount of data, results in false alarm rate. ✓ Collective anomaly. ✓ No point anomaly. ✓ Semi-supervised and Unsupervised 	<ul style="list-style-type: none"> ✓ Neural Networks ✓ Rule-based systems ✓ Statistical Profiling – Histograms ✓ Mixture of models 	[52] [18] [32] [42][62] [24] [16]
Network based Intrusion Detection System	<ul style="list-style-type: none"> ✓ Anomalies change over time ✓ To evade real time intrusion by outside hackers ✓ Point anomaly 	<ul style="list-style-type: none"> ✓ Parametric and Non-Parametric modelling ✓ Neural networks ✓ Clustering based system ✓ Support vector machine ✓ Rule-based systems ✓ Bayesian networks ✓ Nearest Neighbor based ✓ Statistical profiling- Histograms 	[27][28]
Credit card Fraud Detection	<ul style="list-style-type: none"> ✓ Immediate Online detection to track the credit card fraudulent transaction ✓ Only Profiling and clustering techniques are used ✓ Very critical to handle the geometric position at the earliest to track. 	<ul style="list-style-type: none"> ✓ Statistical profiling- Histograms ✓ Information-Theoretic 	[26] [8] [7]
Mobile Phone Fraud Detection	<ul style="list-style-type: none"> ✓ Requires large accounts of calling activity ✓ May be continuous or discrete ✓ Unfavour destinations 	<ul style="list-style-type: none"> ✓ Parametric statistical modelling ✓ Rule-based ✓ Neural networks ✓ Statistical profiling- Histograms 	[2][59][53][67][5][22][14].

Table 5: Comparative Result analysis of dataset

Algorithm	Dataset	No of records	No of clusters	Result percentage
FCM	Advertising	200	3	44.06%
	Stock_data	3000	3	52.52%
Fuzzy circle based clustering approach	Advertising	200	3	61.92%
	Stock_data	3000	3	69.48%





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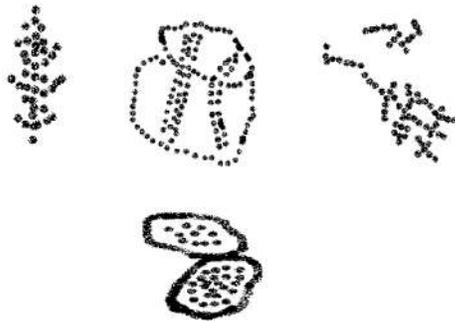


Figure 1: Different forms of clusters

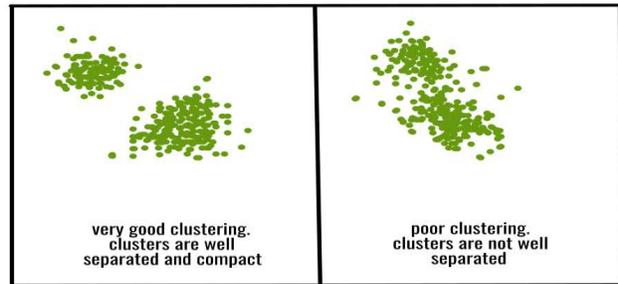


Figure 2: Good Clustering and Bad Clustering

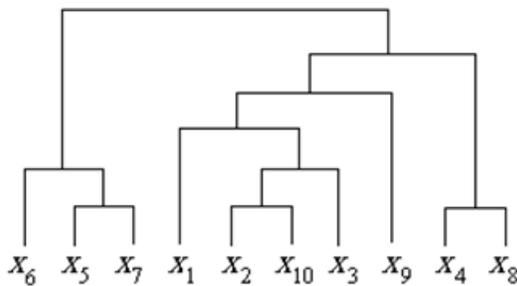


Figure 3: A Sample Dendrogram

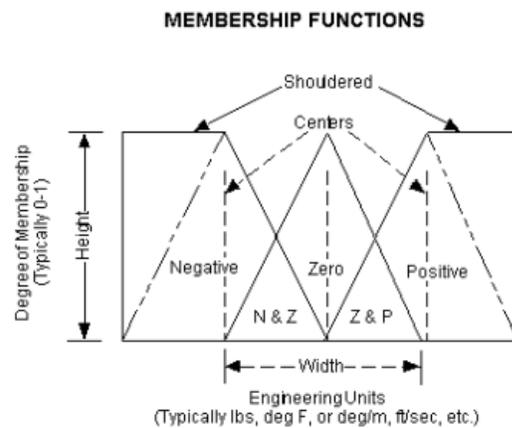


Figure 4: Fuzzy Membership function

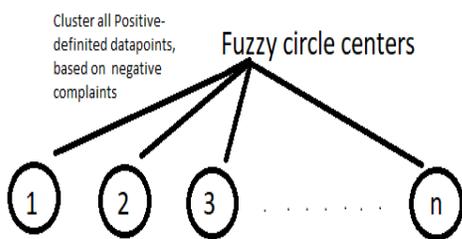


Figure 5: Bloby clusters are identified and circled

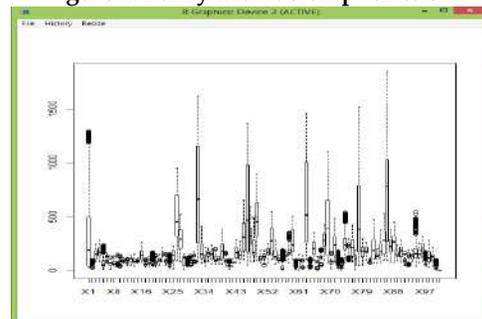


Figure 6: Boxplot of stock_data dataset

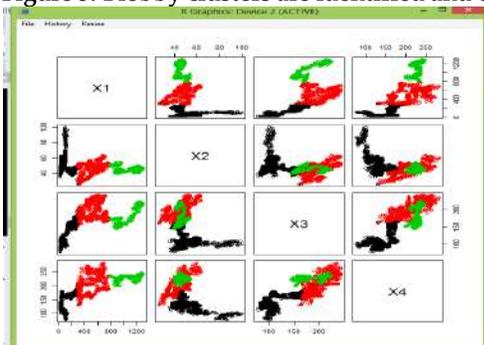


Figure 7: Crisp fuzzy membership degree

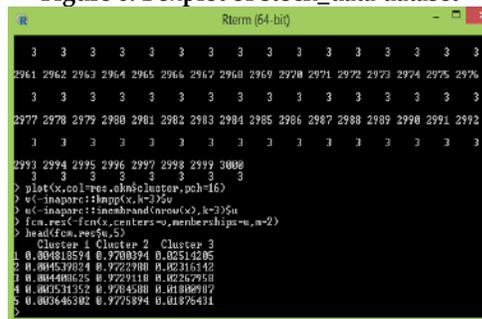


Figure 8: Fuzzy membership degree -Advertising





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```

Rterm (64-bit)
Cluster 1 Cluster 2 Cluster 3
1 0.004918594 0.9700394 0.02514205
2 0.004539824 0.9722988 0.02361442
3 0.004400625 0.9729118 0.02267958
4 0.003531352 0.9794580 0.01808987
5 0.003646382 0.9725894 0.01876431
> fcm.res<-fcm(x,centers=u,memberships=u,n=2,eta=2)
> head(fcm.res$u,5)
Cluster 1 Cluster 2 Cluster 3
1 0.004918597 0.9700393 0.02514207
2 0.004539827 0.9722987 0.02361444
3 0.004400627 0.9729118 0.02267957
4 0.003531354 0.9794580 0.01808988
5 0.003646383 0.9725894 0.01876432
> head(fcm.res$ft,5)
      [ ,1]      [ ,2]      [ ,3]
[1,] 1.075341e-05 0.0002903240 3.633804e-05
[2,] 1.056617e-05 0.0003034911 3.491231e-05
[3,] 1.060636e-05 0.0003139095 3.533728e-05
[4,] 1.046348e-05 0.0003088159 3.456043e-05
[5,] 1.054681e-05 0.0003792214 3.515007e-05

```

Figure 9: Fuzzy membership degree -- stock_data

```

Rterm (64-bit)
2990 4.209094e-03 0.0119465543 0.984043900
2991 2.548029e-03 0.0072510399 0.994923315
2992 6.292362e-03 0.0179767990 0.9765100586
2993 5.136367e-03 0.0141944669 0.9806691659
2994 9.046303e-03 0.0248093262 0.9568643707
2995 6.521011e-03 0.0170505585 0.9752926309
2996 9.435390e-03 0.0250370329 0.9655267771
2997 1.123395e-02 0.0274657672 0.9592942920
2998 1.063836e-02 0.0270911395 0.9515200886
2999 1.155982e-02 0.0102763948 0.95301645865
3000 1.605452e-02 0.0400609435 0.9430045379
> res.fcm$u
      X1      X2      X3      X4
Cluster 1 81.0985 49.76000 114.3760 142.3949
Cluster 2 504.5254 46.45044 200.2567 209.1970
Cluster 3 1070.3011 45.23375 104.4706 226.7274
> summary(res.fcm$clusters)
      Min. 1st Qu. Median Mean 3rd Qu.  Max.
res.fcm$u 1.000 1.000 1.523 2.000 3.000
> summary(res.fcm)
Number of data objects: 3000
Number of clusters: 3

```

Figure 10: clusters of stock_data dataset

```

Rterm (64-bit)
2990 0.010505402 0.02709412 0.9615209
2999 0.01155902 0.030027640 0.9516146
3000 0.01616452 0.04006094 0.9430045
Descriptive statistics for the membership degrees by clusters
      Size      Min      Q1      Mean      Median      Max
Cluster 1 1775 0.4053531 0.9606304 0.9303910 0.9053685 0.9806370 0.9904530
Cluster 2  881 0.4821615 0.6693426 0.8058412 0.8289596 0.9475412 0.9965757
Cluster 3  344 0.4755421 0.8421680 0.8878001 0.9331659 0.9699499 0.9996957
Dunn's Ruziness Coefficients:
dunn_coef normalized
0.8407674 0.7611540
Within cluster sum of squares by cluster:
      1      2      3
12124911 19628933 7963354
(between_SS / total_SS = 89.48%)
Available components:
[[1]] "u"
[[1]] "cluster" "csize" "sumsqrs" "k" "n"
[[1]] "iter" "best.start" "func.val" "comp.time" "inpargs"
[[1]] "algorithm" "call"
      Min. 1st Qu. Median Mean 3rd Qu.  Max.
res.fcm$comp.time [1] 44.06
res.fcm$func.val [1] 29823503
res.fcm$iter [1] 88
res.fcm$comp.time [1] 44.06
res.fcm$best.start [1] 1

```

Figure 11: coefficients of stock_data dataset

```

Rterm (64-bit)
Within cluster sum of squares by cluster:
      1      2      3
12124911 19628933 7963354
(between_SS / total_SS = 89.48%)
Available components:
[[1]] "u"
[[1]] "cluster" "csize" "sumsqrs" "k" "n"
[[1]] "iter" "best.start" "func.val" "comp.time" "inpargs"
[[1]] "algorithm" "call"
      Min. 1st Qu. Median Mean 3rd Qu.  Max.
res.fcm$comp.time [1] 44.06
res.fcm$func.val [1] 29823503
res.fcm$iter [1] 88
res.fcm$comp.time [1] 44.06
res.fcm$best.start [1] 1

```

Figure 12: computation time of stock_data dataset

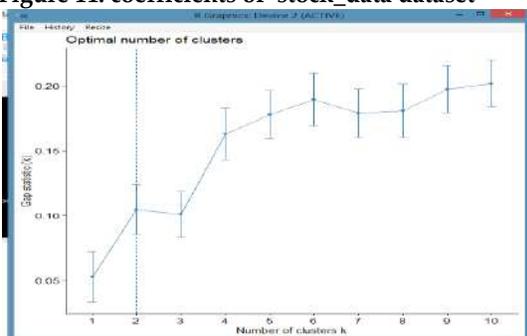


Figure 13: Displaying Optimal number of clusters

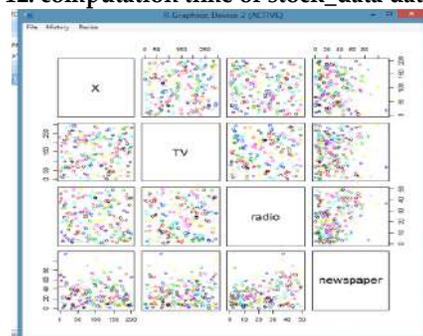


Figure 14: Displaying –Advertising dataset

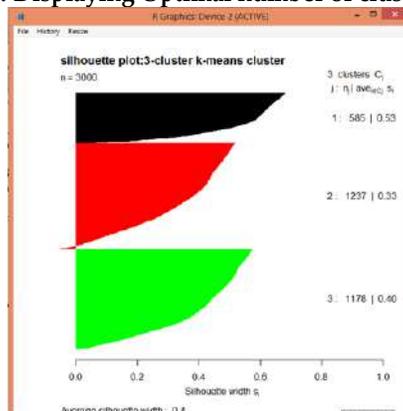


Figure 15: Silhouette with 3 clusters

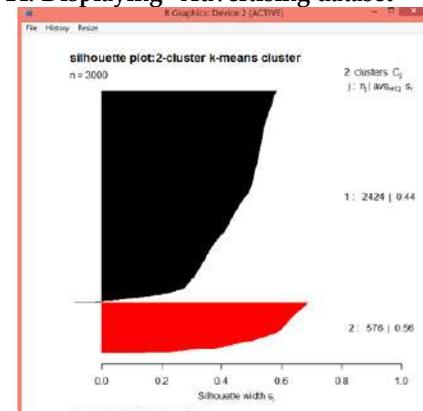


Figure 16: Silhouette with 2 clusters





Nonlinearity based Harmonic Controlled Inverter for Electric Vehicle By Using IGBT Heat Reduction Module

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ABSTRACT

Electric vehicle (EV) developments have made a major breakthrough over the past years, largely driven by advancements in motor drives, power converters, batteries, and system design. Generally, EVs will be categorized as hybrid electric vehicles, plug-in electric hybrid vehicles, and more electric vehicles, depending on the vehicle electrification. EVs serve a requirement that's cost-sensitive and innovations are being introduced to spice up their system performance while driving down costs, additionally to initial costs, key component reliability is a very important performance indicator that affects operational costs and will make the difference between marketplace success and failure of a product. The electrical vehicle is seen as a possible replacement for current-generation automobiles and to handle the rising pollution. In electrical drives, Insulated Gate Bipolar Transistor (IGBT) modules deliver power to the motor and generate lots of warmth during switching. However, heat generation in IGBT hinders the performance of the electrical vehicle. The foremost existing techniques analyze the warmth however doesn't control the heat production within the IGBT module, and also high heat density leads to failing components. Thus to reduce the heat generated in the IGBT module, the work proposed an amalgam illogical controller that handles nonlinearity and provides a fast response. This controller is further tuned by Amended Moth System (AMS) to scale back heat consumption within the IGBT module with electrical parameters. Furthermore, the IGBT is complex to excessive voltage and excessive temperature therefore this current and voltage harmonics within the inverter are reduced by a seamless genuine adaptive filter



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that detects and suppresses the desired harmonics within the estimated back-EMF, consequently reducing the harmonic position error within the estimated rotor position. Even though there is a problem with a wide range of frequency within the motor. To diminish the switching frequency supremacy harm checker is incorporated which is forced to decide on active and zero voltage vectors alternatively, to decrease the variation within the switching frequency. Thus the proposed outcomes efficiently tackle the problem in EV and greatly reduce heat consumption in the IGBT module.

Keywords: Electric Vehicle, Insulated Gate Bipolar Transistor, Amalgam Illogical Controller, Amended Moth System (AMS), Seamless Genuine Adaptive Filter, Supremacy Harm Checker

INTRODUCTION

Electric vehicle (EV) developments have made a big revolution over the past, largely driven by advancements in electric motor drives, power converters, batteries and system design. Generally, EVs will be categorized as hybrid electric vehicles, plug-in electric hybrid vehicles, more electric vehicles, and all-electric vehicles, depending on the vehicle electrification [1]. EVs serve a requirement that's cost-sensitive and new innovations are being introduced to spice up their system performance while driving down costs [2]. Additionally to initial costs, key component reliability is a vital performance indicator that affects operational costs and will make the difference between marketplace success and failure of a product. Power electronic converters are frequently utilized in electrical traction drive applications, while multichip insulated -gate bipolar transistor (IGBT) power modules are the foremost commonly used control switches for onboard power converters of the EVs [3]. Yet IGBTs are considered to be at risk to failure, and the situation would only deteriorate when working under an EV's bonnet within the harsh environment. Insulated gate bipolar transistor (IGBT) may be a core device for power transmission and transformation, and encompasses a wide selection of applications in areas like smart grids, rail transit and distributed renewable energy generation [4].

The main failure parts include bonding wires, solder layers, and chips. Consequently, it is important to ensure the reliability of the IGBT module during the operations [5]. IGBT module's internal structures slowly age and fail when the module is subjected to long-term thermal and mechanical stresses Obviously, IGBT modules' efficiency and reliability are limited by the stringent conditions that result in exposure to high temperatures, high humidity levels, extreme cyclic loading and mechanical stress [6]. The isolated gate bipolar transistor (IGBT) modules were widely used among various control electronic devices in the EV drive systems due to their high power and high switching speed. The cost of IGBT modules in the drive systems accounts for about 10 percent of the vehicle's total cost; therefore, further work on the IGBT module's reliability is necessary [7]. Insulated gate bipolar transistors (IGBTs) are highly demanded in the generation of wind energy, high-speed rails, and hybrid vehicles that require power Semiconductor devices with a large voltage range from 300 V to 6.5 kV and higher current handling capacity than MOSFETs. [8]. Although the exact percentage of IGBT failure was not known, the survey found that IGBTs were the most commonly used devices (42%) among power semiconductor devices followed by MOSFETs (27%), thyristors (14%), PiN diodes (10%), etc. IGBT module failures are motor drive applications and high-speed railway applications, respectively. There is a strong demand for further improvement in the reliability of the IGBT modules for safety- critical and mission-critical applications. Redundancy may not be the best option in those applications [13]. EVs are the ultimate eco-cars that emit no CO₂. Nearly all manufacturers of automobiles are actively developing EVs and some of them have been sold out. Also, the power of the EV motors is currently over a 100 kW. The inverters' output power which controls these motors also exceeds a 100 kW [14]. We have a tendency to develop a high- power inverter for HEVs and EVs for these requirements, using direct water and double-sided cooled module technology [15].





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Power Electronics Converter for Electric Vehicle

An Electric Vehicle is a vehicle that uses a mix of various energy sources, Fuel Cells (FCs), Batteries and Super capacitors (SCs) to power an electrical drive system. The vehicle uses an oversized traction battery pack to power the electrical motor. In Electric Vehicle the most energy source is assisted by one or more energy storage devices, which is employed energy storage devices are batteries and super capacitors. The impedance depends on many parameters, e.g. temperature, state-of-charge, health, and point of operation. Each device might therefore be operated at an inappropriate condition, e.g. health and efficiency. Power Electronics Controller accustomed manages the flow of electricity delivered by the traction battery, controlling the speed of the electrical traction motor. By introducing DC/DC converters one can chose the voltage variation of the devices and therefore the power of every device may be controlled. The IGBTs are a high-voltage, high-current switch connected directly to the traction motor within the Electric vehicles. The battery pack is managed and monitored by a battery management system (BMS) and charged via an on-board AC/DC converter module.

Heat Reduction on IGBT Module

Generation of heat in IGBT during the switching process and to reduce the heat generated in the IGBT module in the EV inverter. The foremost existing techniques analyze the heat but do not control the heat production in the IGBT module, also high heat density failing components. Furthermore, the IGBT is complex to excessive voltage and excessive temperature therefore the current and voltage harmonics in the inverter is needed to be reduced for the productive working of the motor. Existing methods use filters that shrink the harmonics only to a particular level. Besides, an individual Petrol Particulate Filter (PPF) is suitable only to reduce pollution, where the harmonics are high enough to be eliminated. Finally, the switching frequency has a direct impact on the generation of heat in IGBT. Power from the battery is boosted up using the Buck-Boost Converter which is a DC- DC Converter. The output from the Buck-Boost converter is injected into an inverter where IGBT is a very popular semiconductor that combines the voltage characteristics of bipolar junction transistors such as low conduction losses, and high input impedance with the Insulate gate of a MOSFET. Due to the overload of work in the inverter the Insulated Gate Bipolar Transistor (IGBT) module, produce large consumption of heat. Amalgam logic controller is introduced to remove the little number of harmonics and heat reduction from the system to make the high accurate efficiency. Amended Moth System is used to suppress by a seamless real adaptive filter technique utilizing the back-EMF method, which also minimizes position error. Finally, the switching frequency in the motor drive has been reduced by integrating the supremacy harm checker. Finally, the reduced switching frequency helps to decrease the heat production in IGBT.

Amalgam Illogical Controller

Amalgam Illogical Controller is used in the IGBT module to analyze and monitor heat production. PD component is used to mitigate the steady-state error, and to maintain the system's damping and speed characteristics. Nevertheless, the steady-state error cannot be eliminated; hence, the second stage uses a PI controller and the overall controller is known as the illogical Amalgam controller. This controller retains the functionality of both a Fuzzy Logic Controller (FLC) and a PID by eliminating the error on the steady-state. The controller will be able to handle nonlinearity and provide a quick response due to PD control and adequate stable PI output.

$$TF_{AIC} = X_{OUTPUT} * \left\{ (K_{P1} + SK_D) + \left(K_{P2} + \frac{K1}{S} \right) \right\} \quad (1)$$

It analyzes the steady-state error and the nonlinearity in the Insulated gate bipolar transistor (IGBT) module with the controller.





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Amended Moth System

The proposed amended moth system is used to fine-tune the Amalgam Illogical Controller for IGBT module heat reduction in electric vehicles. This controller is used in all subsystems that are present in the power system. It results in a simple system, minimal maintenance demand, with less controllable variables. The schematic diagram of the proposed work is shown in Fig.1, where an SG adaptive filter position observer is used to suppress the harmonics found in the back-EMF. The position of the rotor is calculated using the traditional extended PMSM back-EMF model as described below.

Harmonic Reduction on Inverter by Using SGAF

A seamless Genuine Adaptive Filter(SGAF) is employed to detect and suppress the harmonics in the estimated back-EMF, consequently reducing the harmonic position error in the estimated rotor position. Figure 3 provides the BRLS adaptive filter's design block diagram for the detection and suppression of harmonics. $D(k)$ refers to the original signal, consisting of the simple signal $f(k)$ and the harmonic signal $h(k)$. $X(k)$ refers to the input signal associated with the harmonic high-order components. The output signal $Y(k)$ of the filter can be obtained by iterative computation employing the input signal $X(k)$ interpreted by the BRLS adaptive algorithm. The output signal $Y(k)$ can control the real, high-order harmonic components $h(k)$ after the adaptive filter converges. These signals will be used in the irrelevant (k) vector of harmonic information. While to improve the filtering efficiency of the Seamless Genuine adaptive filter, the output signal $Y(k)$ is also introduced into the $e(k)$. You can obtain the harmonic information vector $\phi(k)$ by:

$$\phi(k1) = [X(k1) Y(k-1) X(k) Y(k-1)] \quad (2)$$

The desired fundamental component in the estimated back-EMF can be derived directly from the error signal $e(k)$, which is obtained by subtracting the output E_{of} from the original signal $E_{original}$ is expressed as follows:

$$e(k) = E_{of} - Y(k) \quad (3)$$

Thus the harmonics are detected and suppressed then the harmonics removed voltage and current helps to efficient motor performance.

Control the Power Frequency By SHC

Supremacy Harm Checker (SHC) helps to cut the Permanent Magnet Synchronous Motor (PMSM) switching frequency. To rising the switching frequency using one active voltage vector normally selected from the cost function, and to force the engine to pick the zero voltage vectors in the next switching series. Through this process, the motor is forced to respectively choose active and zero voltage vectors. So the switching frequency variance will diminish. Each switching series predicts all possible states under certain conditions in the conventional method. Therefore, it is not certain that the switching state shifts in every series of switches, and therefore the switching frequency is not constant. This means one voltage vector may be chosen for two or more switching sequences, or it may adjust at each control interval. It can be inferred that the explanation for variable switching frequency is the evaluation at each switching interval of all possible candidates.

Simulation Blocks

Amalgam illogical controller to reduce large consumption of heat production in IGBT using Amended Moth System are shown below figures and also the simulated representation of each parameter in the simulation has been deliberated following, Figure 6 states the overall Simulink design of the proposed heat-reduced insulated gate bipolar transmission (IGBT) module in the electric vehicle inverters. This provide effectively describes the efficiency of our proposed work by analyzing the reduction of heat in IGBT and the output current and voltage for the IGBT



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and the filter. Also comparing the obtained experimental results with the conventional Approaches for harmonics and switching frequency comparison. The proposed system has been implemented in MATLAB/SIMULINK to demonstrate competent power utility.

RESULTS

Comparison of the Proposed Controller

The suggested work has been compared with previously existing approaches for harmonics such as Active stunt power filter, LCL filter, and single tuned filter. And for switching frequency such as model predictive control method to reduce the common-mode voltage (MPC-RCMV), Variable Switching frequency pulse width modulation (VSF-PWM), and Random Pulse width modulation (PWM). Amalgam illogical controller which optimized amended moth system heat reduction in the IGBT module here the heat 170 reduced in the range of 20 (W/ (m² K)) in terms of time per second in Fig. 10. The three-phase output current of IGBT after the reduction of heat using an amended moth system empowered amalgam illogical controller. The current calculated the minimum average output current of the driver output within 0.5 seconds in figure 11. Then the output voltage of IGBT after the reduction of heat using an amended moth system empowered amalgam illogical controller is in figure 12. Where the figure 13 gives the experimental results of the approximate EMF by a seamless genuine adaptive filter technique which results at a frequency of 10 H. The root-mean-square (RMS) voltage of a sinusoidal source of electromotive force (V_{rms}) is used to characterize the source.

Speed of the Permanent Magnet Synchronous Motor (PMSM) in the electric drive utilizing time per second. The proposed insulated gate bipolar transistor in an electric vehicle is carried out with the 700 m/s speed within the time interval of 0 to 1 sec in figure 14. Supremacy harm checker reduced switching frequency in permanent magnet Synchronous motor and it highly reduced from 400 Hz to 5 Hz in terms of allowable ripple is in figure 15. The output of harmonics reduced output voltage within the time from 0.05 to 0.45 sec. Harmonic is the voltage or current that is at all of the system's specific frequencies or intervals shown in figure 16. The proposed solution could control the load transition effectively and minimize dramatically the harmonic ripples with a seamless genuine adapter filter. Then speed of the Permanent Magnet Synchronous Motor (PMSM) in the electric drive utilizing time per second. The proposed insulated gate bipolar transistor in an electric vehicle is carried out with the 700 m/s speed within the time interval of 0 to 1 sec which is shown in figure 17. Fig. 18 states the comparison for exiting filter in terms of total harmonic distortion in current and voltage the existing approaches such as Active stunt power filter (ASPF), LCL filter, and single tuned filter (STF). From the above observation, it is depicted that the total harmonic distortion for the single tuned filter (STF) [22] is 3.3%, compared with this the LCL methodology is slightly reduced by 2.5%, then the technique of active stunt power filter (ASPF) the obtained value is 0.5% and finally the proposed filter which overpowers all the above filter with reduced harmonic distortion of 0.3%. Thus the proposed filter achieves highly reduced total harmonic distortions with the existing techniques. Fig. 19 shows the relationship between the number of switching cycles in a motor and the switching frequency in an existing method like model predictive control (MPC) [19], variable switching frequency (VSF), and Random pulse width modulation (PWM). From the graph, it is noted that the high harmonic distortion is determined from the VSC-PWM -700, MPC-RCMV-555, and random PWM-500 with a different number of cycles. By comparing other techniques, our proposed system converts the low voltage input into high voltage output as 400hz with high accuracy in performance. Then the production of heat would be reduced in IGBT switches with fewer harmonic disturbances in current and voltage. Reduction of switching frequency leads to a slight decrease in heat production of IGBT. Then the proposed system generates a better output under various switching frequencies with high efficiency.





CONCLUSION

Power electronics is one of the current regions of electrical engineering which has seen a lot of advancements in recent times and has impacted human life in almost every sphere. Due to the overload of work in the inverter, the Insulated Gate Bipolar Transistor (IGBT) module produces a large consumption of heat. To reduce enormous heat generation in IGBT an amalgam illogical controller is proposed to reduce steady-state and nonlinearity problems and enhance the parameters using an amended moth system to control the heat production in IGBT. Then harmonics generated in current and voltage gets reduced by a seamless genuine adaptive filter for efficient motor performance. Finally, voltage and current with reduced harmonics of 0.27% support the efficient working of the motor in which the switching frequency has reduced by supremacy harm Checker. Voltage with reduced switching frequency will minimize the heat generated in the IGBT module. Hence it has effective outcomes when compared to the previous methods and reduces the heat in the IGBT module to drive an electric vehicle. The proposed approach significantly reduced heat generation in electric drives of the isolated gate bipolar transistor (IGBT) with the controller, which further reduced switching frequencies and harmonic distortions substantially to assist electric vehicles to function effectively.

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Table1.Comparison of switching frequency produced by the proposed Filter

S.No	Methods	Comparison of Total Harmonic Distortion
1	Active Stunt Power Filter	0.5%
2	Single Tuned Filter	3.3%
3	LCL Filter	2.5%
4	Proposed Filter	0.3%

Table 2.Comparison of switching frequency produced by the proposed Controller

S.No	Methods	Comparison of Switching Frequency
1	Model Predictive Control	500Hz
2	Variable Switching Frequency	700Hz
3	Random PWM	500Hz
4	Proposed PWM	400Hz





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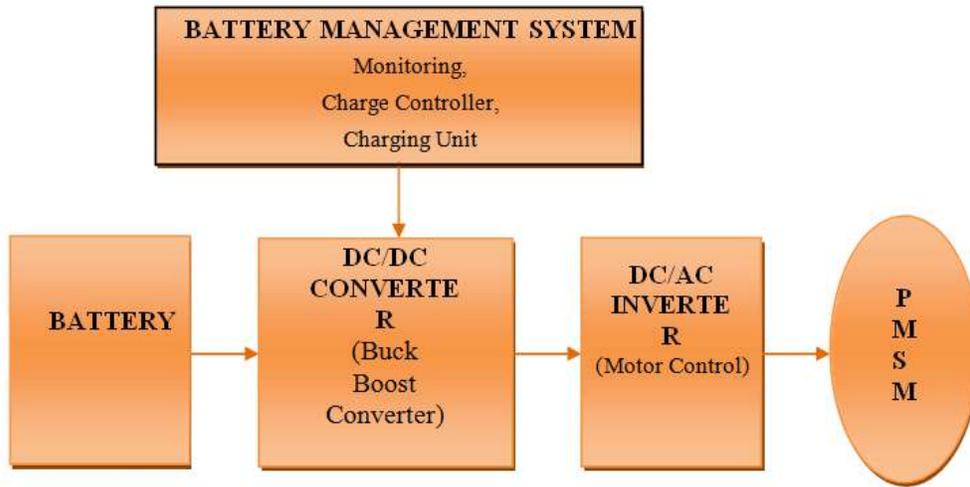


Figure 1. Basic electrical architecture of the Electric Vehicle

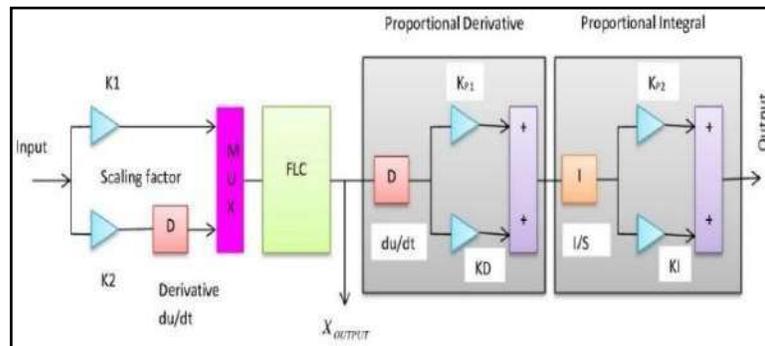


Figure 2. Configuration of the Amalgam Illogical Controller

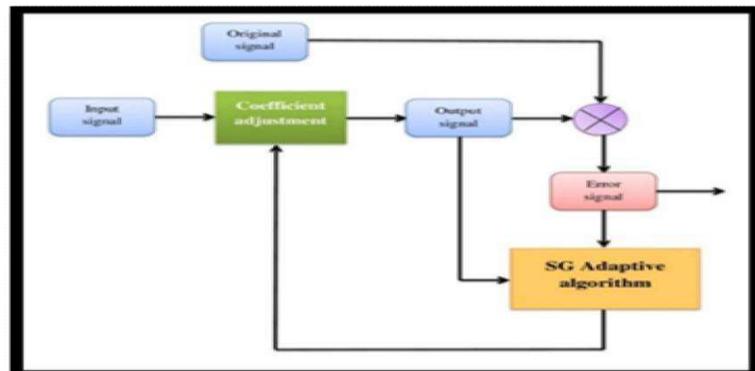


Figure 3. Structure of harmonic detection and suppression by using an adaptive filter based on the SG adaptive algorithm.





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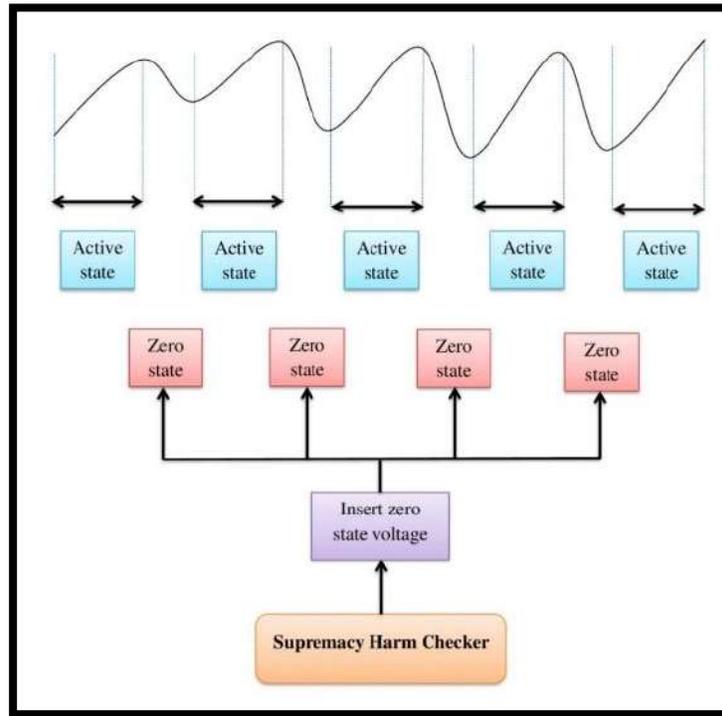


Figure 4.Reduce Switching Frequency using Supremacy Harm Checker

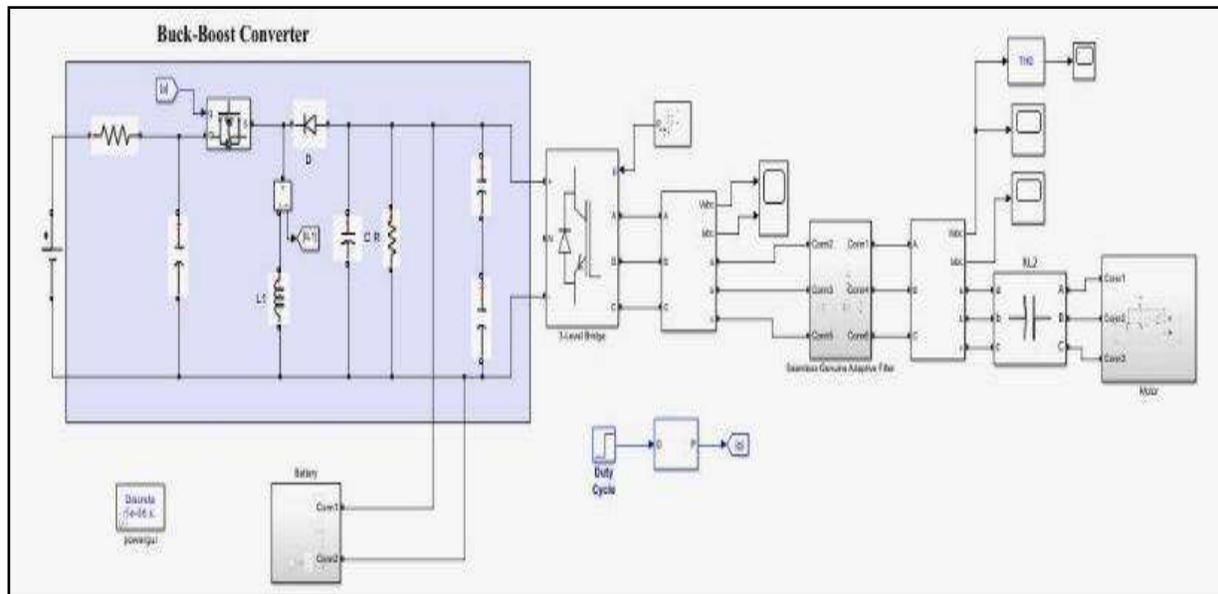


Figure 5.Simulink Diagram





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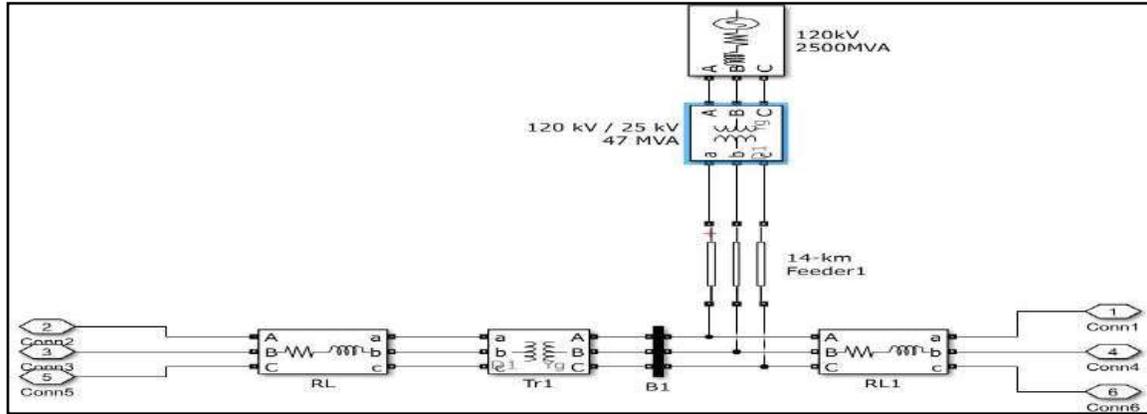


Figure 6.Simulink Diagram – IGBT

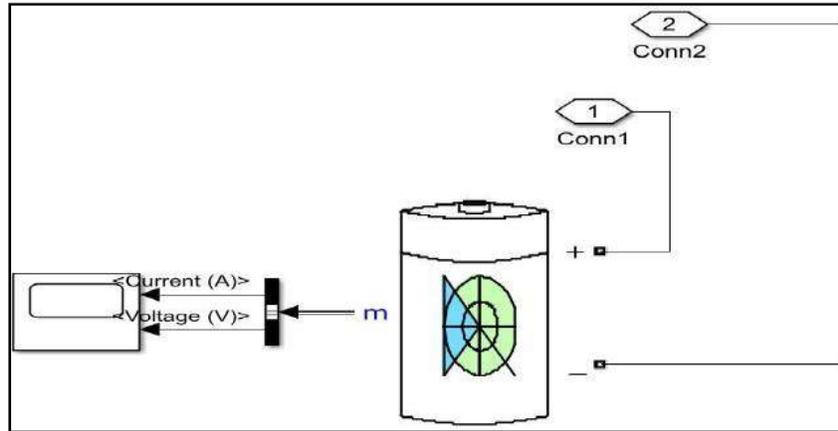


Figure 7.Simulink Diagram – Battery

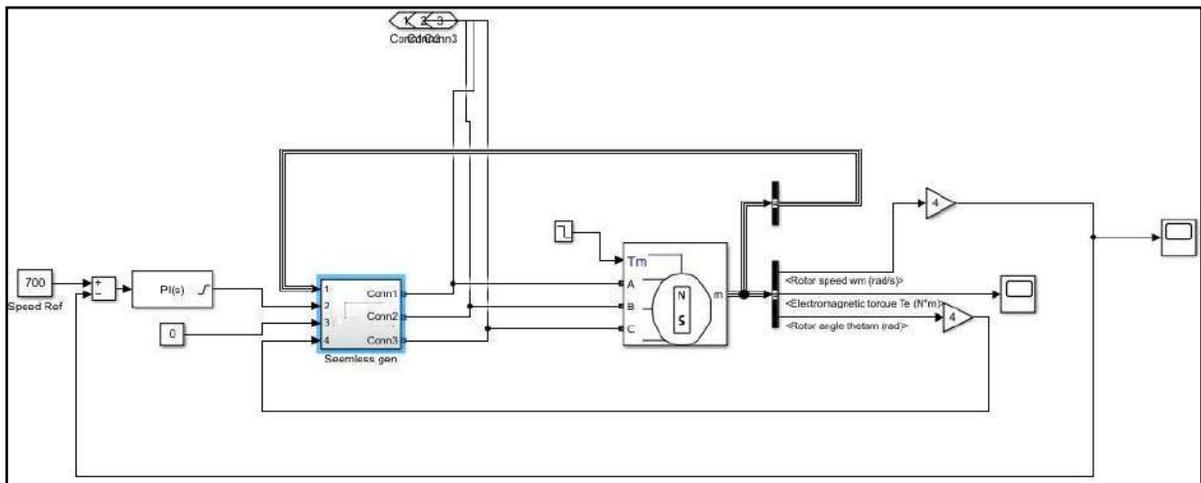


Figure 8.Simulink Diagram – Proposed Motor Unit



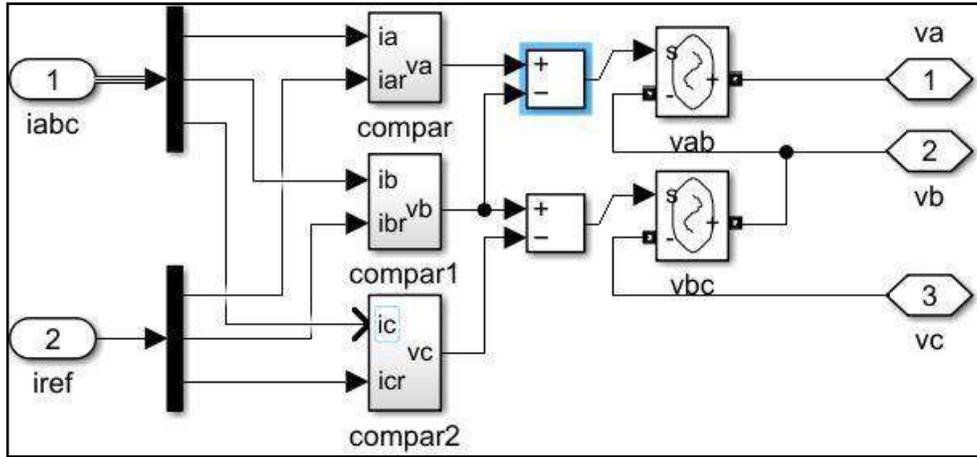


Figure 9.Simulink Diagram – PWM Inverter

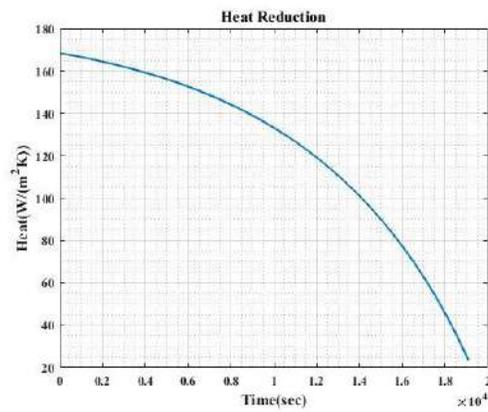


Figure 10.Heat reduction in IGBT

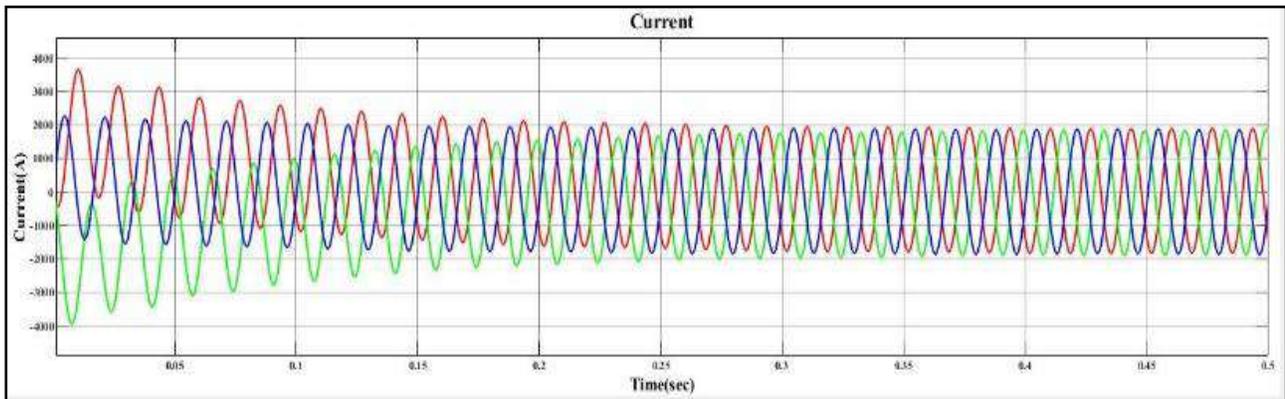


Figure 11.Output current of IGBT





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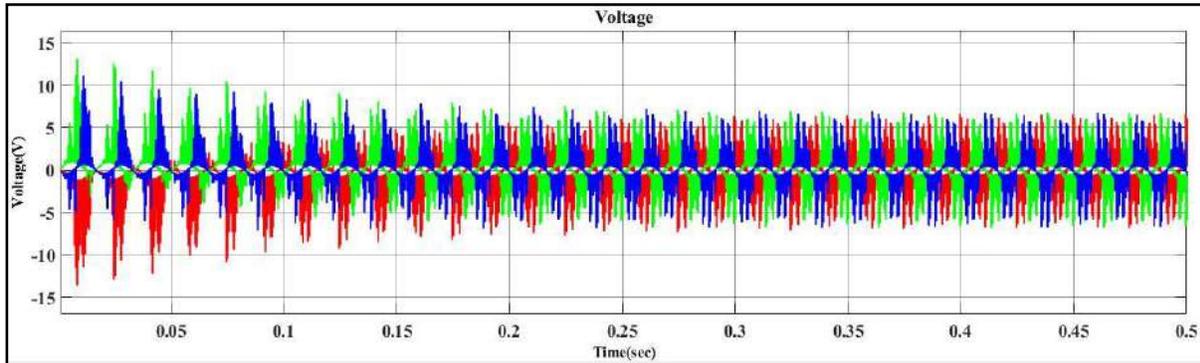


Figure 12. Output Voltage of IGBT

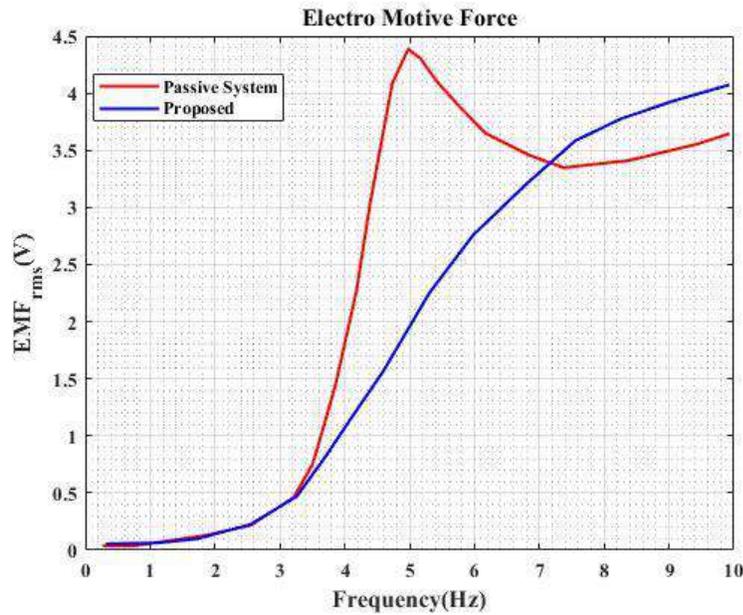


Figure 13. Electromotive Force

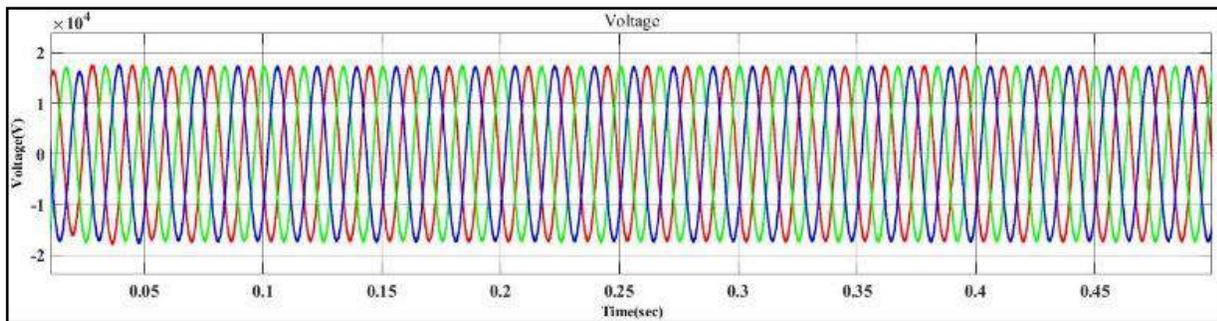


Figure 14. Harmonics reduced current from the filter





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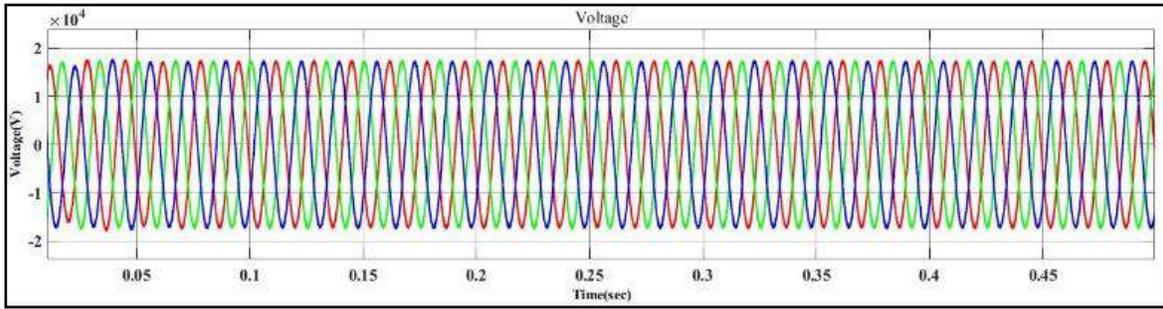


Figure 15. Harmonics reduced voltage from the filter

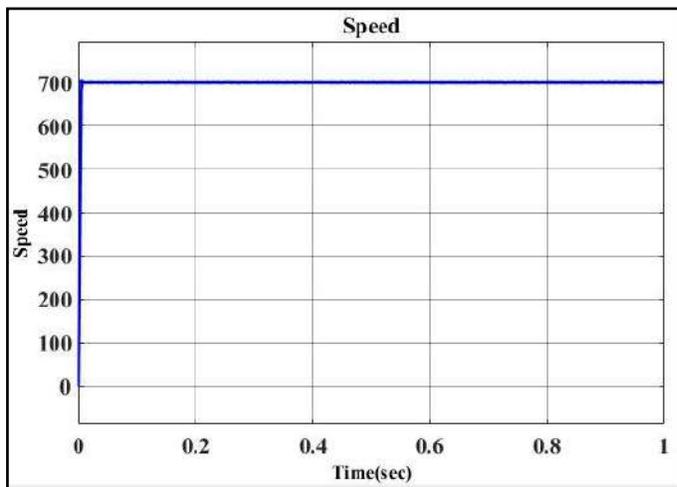


Figure 16. Speed of PMSM motor

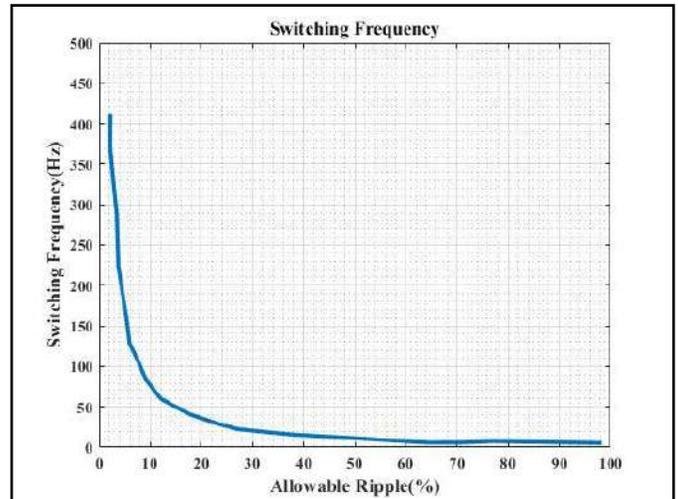


Figure 17. Reduced Switching Frequency

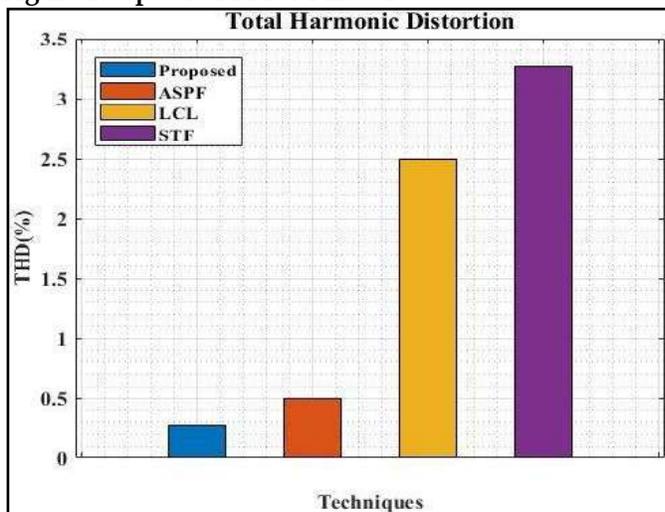


Figure 18. Total Harmonic Distortion Comparison

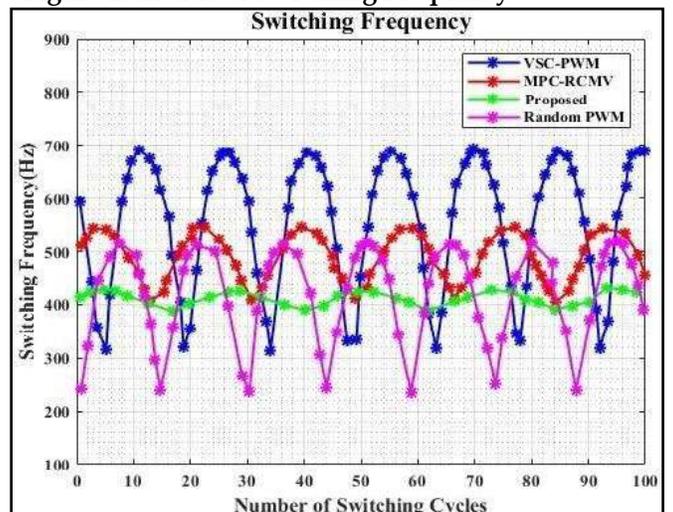


Figure 19. Comparison of switching frequency





***In vitro* Antioxidant and Antimicrobial Activity of *Allium odorum* Leaves Extracted with Different Solvents and their Phytochemical Screening - A Comparative Study**

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ABSTRACT

North-east India, the reservoir of plant diversity is rich in medicinal and aromatic plants. A huge population here is still dependent on these medicinal plants for curing various ailments. Various diseases that require pharmaceutical medications are also associated with many risk factors. Extracts from such medicinal plants are capable of minimizing side effects caused by chemically synthesized drugs. The present study was conducted to detect the presence of various potent phytochemicals, evaluation of *in vitro* antimicrobial and anti-oxidant activity from the leaf extracts of *Allium odorum*. Extraction was carried out using solvents with varied polarity. Various phytochemicals such as saponins, phenols, tannins, flavonoids etc were found to be present. The extract showed anti-microbial activity with a clear zone of inhibition of 2.5mm and 2.8mm against *E. coli* (gram negative) and *S. aureus* (gram positive) respectively for hexane; 5mm (*E. coli*) and 5.2mm (*S. aureus*) for acetone and 5.7mm (*E. coli*) and 6mm (*S. aureus*) for distilled water extracts. The antioxidant properties of the extracts were evaluated where 200µg/ml of hexane extract showed a maximum value of 68.20%. The above results conclude that leaves of *Allium odorum* possesses various pharmacologically active molecules which can be further





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characterized by molecular techniques. Further prospects include validating the use of these herbal drugs through animal model study.

Keywords: *Allium odorum*, North-east, Phytochemicals, Antimicrobial, Hexane, Acetone, Pharmacologically active.

INTRODUCTION

Allium odorum commonly known as Chinese Chives is a member of *Allium*, the second largest genus of monocotyledons, belongs to the family *Liliaceae* [1]. Among all the species of *Allium* found in India, onion and garlic are commonly used as vegetable and medicine. The warm tropical climate of the Northeast region provides appropriate habitat for a wide variety of cultivated and wild edible species of *Allium* [2]. It is thought to originate from China, spread to Japan, Korea, India, Nepal, Thailand and Philippines [3]. Chinese Chives leaves are considered to be harvested 3-4 times per year without damaging the plant [4], with leaves growing about 15 to 25cm in height, leaves are green, narrowly linear, flattish, 3 to 6mm wide, looks a lot like a grass [5]. The leaf base is more fleshy and scaly. Leaves are mostly consumed by the native people as raw, cooked or boiled besides consuming as salads and soups [6]. The bulbs and leaves are collected throughout the year while autumn and winter being the best harvesting time. These leaves as well as the bulb are known to act as moths repellants [7].

There are studies reporting immune system stimulation, anti cancer, anti fungal and antioxidant activities of this species [8]. In vitro studies confirms that some plants of *Allium* species have the ability to reduce the parameters which are taken as a risk factors in cardiovascular diseases such as raised serum total cholesterol, increase low density lipoprotein, increased platelet aggregation, hypertension and smoking[9,10,11]. Due to increasing demand for natural ingredient, the use of plant extract is a viable alternative product for healthy lifestyle. This work aims to monitor the in vitro the antibacterial and antioxidant activity of the leaf extracts prepared using solvents of varied polarity. The extracts were also tested for the presence of potent phytochemical constituents.

MATERIALS AND METHODS

Collection and preparation of sample

Chinese chives (*Allium odorum*) were obtained from Silchar district of Assam, India. It was collected, washed and cleaned to remove the dirt and dust using distilled water and was transferred to departmental laboratory, Assam down town University. They were identified based on their vernacular name. The plant samples were then cut into small pieces and were spread all over the water bath tray and were kept over the hot water bath to provide steam so as to remove excess chlorophyll at a temperature of 60°C. After the excess chlorophyll has been removed, the sample was kept on the filter paper at room temperature for shade drying. Later they were grounded into fine powder using an electrical grinder and stored in an air tight container.

Solvent extraction

The extraction was carried out using distilled water, Hexane and Acetone. The plant samples were mixed with the solvent using 1:10 ratio (g/ml) [12]. They were later filtered using Whatman no 1 filter paper followed by centrifuging at 3000 rpm for 5 minutes. The filtrates were stored in three different Falcon tubes for future use.

Phytochemical Screening

Phytochemicals are the chemical constituents produced by plants, through primary or secondary metabolism. They play a great role in plant growth or defense against pathogens. Various phytochemicals test were carried out which



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include tests for Alkaloids, Carbohydrates, Proteins, Flavonoid, Phenol, Saponin, Fixed oils, Terpenes, Glycoside, Tannin etc.[13,14,16]

Test for Terpenes - 5ml of the plant extract taken in a test tube was added to 2ml of chloroform and few drops of conc. Sulfuric acid is added to the walls of the test tube.

Tests for Carbohydrate (Benedict's test) - 1ml of the plant extract are added to 1ml of the Benedict's reagent. It is heated in a water bath for 2-3 minutes.

Tests for Protein (Ninhydrin test) - 2 drops of the Ninhydrin solution was added to 2ml of aqueous filtrate. Water bath the sample for 20 minutes

Test for Tannin - 2ml of plant extract is mixed with a few drops of 5% ferric chloride.

Test for Flavonoid (Lead Acetate Tests) - 2ml of the plant extract is mixed with a few drops of 10% Lead acetate.

Tests for Steroids (Salkowski Test) - 2ml of plant extract is taken in a test tube and added to 1ml of chloroform and a few drops of Sulphuric acid is added to it. It is shaken well and allowed to stand.

Test for Saponin (Foam test) - 5ml of the plant extract is taken in a test tube and shaken vigorously for 5 minutes.

Test for Phenolic compounds - 2ml of the plant extract is added to 1ml of ferric chloric and modified by adding 1ml of potassium ferricyanide.

Test for Fixed oils (Oil Stain Test) - One drop of the sample was put and pressed on a filter paper.

Antimicrobial Activity

The modified disc diffusion method [14] was used in this study. Bacterial Cultures were ordered from HiMedia. The three solvent extracts were used to test for antimicrobial activity against two bacteria namely *Escherichia coli* (ATCC® 25922), and *Staphylococcus aureus* (ATCC® 25923). The zone of inhibition was then measured with a scale to determine extract against bacterium [15, 16].

Antioxidant activity

The free radical scavenging capacity of hexane extract was determined by using DPPH assay according to the previously described method with slight modification. The stock solution of 1M DPPH was prepared in methanol and kept at 20°C until analysis. Fresh 0.1 mM DPPH working solution was prepared by diluting 10 mL stock solution with 90 mL methanol and added to the different concentration of the plant extracts (100µg/ml, 150µg/ml and 200µg/ml). The DPPH free radical scavenging was determined in a UV Vis spectrophotometer (Systronic UV-Vis Spectrophotometre-117) by measuring absorbance at 517 nm against a blank solution by taking ascorbic acid as standard [16, 17].

$$\% \text{ Inhibition} = \frac{\text{Absorbance (Control)} - \text{Absorbance (Sample)}}{\text{Absorbance (Control)}} \times 100$$





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RESULTS AND DISCUSSION

Phytochemical screening

The medicinal value of plants mainly depends on different bioactive compounds present on it which may lead to different pharmacological impact on human. The present study reveals the presence of different phyto-constituents like terpenes, flavonoid, steroids, saponin, carbohydrates, proteins, phenol in *Allium odorum* which is supported by various previous studies and are shown in table 1. The hexane extract gave maximum presence of phytochemical in the study. A recent study have revealed the presence of different phytochemicals like sterol, tannin, flavonoid, saponin, phenol, amino acid, sugar and diterpenes with ethanolic extraction [17]. Another study have shown the presence of different phytochemical like flavonoids, steroids, polyphenols, quinones, and monoterpenoid /sesquiterpenoid in ethanolic extract of *Allium odorum* [18].

Antibacterial activity

Antibacterial activity of *Allium odorum* showed high resistance against the tested bacteria. Distilled water extracts showed higher inhibition activity against *Staphylococcus aureus* followed by *E coli* which are depicted in table 2. It is reported that plants of *Allium* genus are widely used as antibacterial in traditional knowledge in NE India as well as other part of the world [15].

Antioxidant activity

Since, among all the three extracts (hexane, acetone and distilled water), hexane extracts showed the presence of maximum phytochemicals, therefore antioxidant activity was evaluated only for the hexane extracts of Chinese chives leaves. Screening of antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl(DPPH) method showed a significant percentage of inhibition based on the different concentration [14]. Ascorbic acid was used as a standard and a positive control (OD 0.738) was maintained.

The antioxidant activity by DPPH method revealed that hexane extracts of *Allium odorum* exhibited high antioxidant activity of 68.20% with a concentration of 200 µg/ml. A recent study has reported the antioxidant activity of aqueous and ethanolic extract of *Allium odorum* where they found that ethanolic extract has shown more inhibition than the aqueous extract with an inhibition rate of 84.85% and 65.88% respectively [17]. It is also reported that *Allium* derived polyphenols attributed to their antioxidant activity which are having other functional properties like antimicrobial, anti-inflammatory, anticancer effects etc [9, 19, 20]. Hence, it can be highlighted that, *Allium odorum* provides nutritional properties which will be of huge significance in improving human health and medicinal world.

CONCLUSION

The Chinese chives or *Allium odorum* leaves are used by different tribes of the region where they eat them fresh or cook them to cure many diseases. Not much work has been reported in this plant from North East region of India to prove their efficacy scientifically. The finding of this study may help in future large scale experiments.

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Conflict Of Interest

The authors declare no conflict of interest.





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Table 1: Phytochemical screening of different extracts

Sl. No	Phytochemicals	Hexane Extract	Acetone extract	Distilled water extract
1	Terpenes	+	+	-
2	Carbohydrate	+	+	+
3	Protein	+	+	+
4	Tannin	-	-	-
5	Flavonoid	+	-	+
6	Steroid	+	-	+
7	Saponin	+	-	+
8	Phenol	+	+	+
9	Fixed oils	+	-	+

Table 2: Antimicrobial activity of different extracts against selected bacterial species

Sl No.	Species	Hexane extract	Acetone extract	Distilled water extract
1	<i>E. coli</i>	0.25cm	0.5cm	0.57cm
2	<i>S. aureus</i>	0.28cm	0.52cm	0.6cm

Table 3: Antioxidant activity of hexane extract

Sl No.	Concentration of sample ($\mu\text{g/ml}$)	Absorbance (Values represent mean \pm SD, n=3)	% of inhibition	IC50 value ($\mu\text{g/ml}$)
1	100	0.433 \pm 0.35	41.32%	81.82
2	150	0.362 \pm 0.23	50.87%	
3	200	0.235 \pm 0.18	68.20%	





Effect of Diaphragmatic Breathing Exercise with Inspiratory Muscle Training to Improve Peak Expiratory Flow Rate and Exercise Tolerance in Asthma Patients

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ABSTRACT

The study is to find the effect of Diaphragmatic breathing exercise with Inspiratory muscle training to improve peak expiratory flow rate and exercise tolerance in asthma patients. 20 asthma patients between 35-55 years of age were selected randomly using simple random sampling method. The pretest measurement of peak expiratory flow rate and exercise tolerance was measured using the peak flow meter and six minutes walk test for all the subjects. After the pretest assessment the subjects received diaphragmatic breathing exercise with inspiratory muscle training for a period of 8 weeks and on the end of 8th week post test measurement of peak expiratory flow rate and exercise tolerance was done using the peak flow meter and six minutes walk test for the group in a similar fashion as that of pretest measurement. The results of the study concluded that Diaphragmatic breathing exercise with Inspiratory muscle training improved peak expiratory flow rate and exercise tolerance in asthma patients.

Keywords: Asthma, Inspiratory muscle training, Peak expiratory flow rate, Exercise tolerance test, Diaphragmatic breathing exercise

INTRODUCTION

Asthma was a unique disease with variable progression and severity of presentation over different time. The prognosis of the patients depends on the severity of the disease, but very importantly the degree of control with treatment. Majority of the patients may be symptom free for long periods, but few of them may be patients with severe persistent asthma, who may develop progressive loss of lung function. Asthma was an inflammatory disease in the airway, thus resulting in increased airway responsiveness, obstruction, mucus hyper-production and involvement in the airway wall repairing. Asthma was an obstructive lung disease. Asthma was common among developing countries with poor ecological conditions and pollution. Asthma is a reversible airflow obstruction



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associated with the increased airway responsiveness and airway swelling [1]. The breathing retraining programme aims at helping individuals with asthma in their everyday life. Medications to treat the asthmatic condition can be widely classified into long-term controllers and short-term relievers [2]. Non-pharmacological involvement has obtained attention in the treatment of asthma. Those involvements are breathing exercises, yogic practices, inspiratory muscle training, physical activity, and the other strategies such as stoppage of smoking, prevention of occupational exposure, the indoor allergens, and even weight reduction, among others [3]. Non-conventional therapies such as homeopathy, acupuncture, aromatherapy, massage, dietary supplements, physiotherapy [4]. The Diaphragmatic Breathing exercises have been used by physiotherapists and also by the other professionals in order to control the symptoms of asthma. The convention for training the breathing generally pays attention to tidal and minute volume and to encourage relaxation, regular exercise at home, the moderation of breathing pattern minutes, nasal breathing, holding of breath, lower rib cage and abdominal breathing [5]. In the last twenty years the corticosteroid inhalers has become the great therapy agent for asthma, the death rate of asthma has decreased [6]. Meantime allergic diseases, such as asthma, have increased in the past fifty years associated with urbanization [7]. Cases will get increased by more than hundred million by 2025 [8]. Recently the asthma has not been acknowledged as a simple Th2 disease, which is featured by IgE upgrading and somewhat eosinophilia. Th17 and Th9 cell subtypes are well known to contribute the swelling or increasing the smooth muscles contraction or stimulating the mast cells. Asthma is a chronic airway swelling disorder of the lungs that leads to structural and functional changes, thus resulting in increased bronchial responsiveness and the airflow stoppage. Exacerbations might be fatal and these are more repeated and more serious in high-risk clients or clients with uncontrolled asthma. Factors involves viral infections, allergens, tobacco smoke, physical exercise, stress, some medications like non-steroidal anti-inflammatory drugs and beta-blockers might trigger or even worsen asthma symptoms. Some of the phenotypes are identified, such as allergic asthma, non-allergic asthma, and late arising asthma. During quiet breathing, the primary muscle responsible for ventilation is the diaphragm. Inspiratory muscle training showed a significant improvement in inspiratory muscle strength and an increased exercise tolerance [9]. Broncho dilatation, gaseous exchange improves peak expiratory flow rate and exercise tolerance. The aim of the study is to find the effect of diaphragmatic breathing exercise with inspiratory muscle training to improve pulmonary status in asthma patients. The study is essential as most of the general population suffers from this problem and also to make physiotherapists to realize the benefits received from the alternative approaches like diaphragmatic breathing exercise with inspiratory muscle training to improve peak expiratory flow rate and exercise tolerance in asthma patients [10].

MATERIALS AND METHODOLOGY

Twenty acute asthma patients of Vinayaka Missions Kirupananda Variyar Medical College and Hospital, Salem between 35 to 55 years of age were selected randomly using simple random sampling method are included for the study. Patients having cardiac and psychiatric problems are excluded from study. The group underwent a pretest assessment of exercise tolerance and peak expiratory flow rate with the help of six minutes walk test and peak flow meter. Six minutes walk test was performed by using materials like chalk powder, stop watch, whistle, meter tape were used to collect the data for this test. A hard and flat 200 meters walkway was marked for six minute walk test. Subjects were asked to stand in the start line and were instructed to walk in their self pace and rest as needed back and forth along the marked walkway for a period of six minutes following a whistle blow. Stop watch & whistle were used to start and stop the test. The distance walked was measured by multiplying the number of times of full completion of marked walkway with 200 meters and the excess using a meter tape. The distance walked in six minutes was recorded with a help of a meter tape in meters.

Peak expiratory flow rate was measured using peak flow meter. The sliding pointer was set to zero before the start of the procedure. The subjects were asked to stand straight and hold the handle of the peak flow meter. They were further asked to take a deep breath and put the mouth piece in their mouth and seal their lips and teeth tightly around the mouthpiece, following which they were asked to blow out as hard and as fast as they can. The number on



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the scale corresponding to the sliding pointer was noted. The pointer was rest to zero every time and the best of three recording was taken into account. The numerical value corresponding to the slide pointer was taken as the score. After the pre test measurement was over, the subjects received diaphragmatic breathing exercise with inspiratory muscle training for 8 weeks and then posttest measurement was taken. Diaphragmatic breathing exercise was done by placing hands on diaphragm and the patient was asked to breath out through mouth and breath in through nose and then diaphragm was palpated by its movement. Inspiratory muscle training was done for a period of eight weeks twice a day The training was given for six days in a week. Inspiratory muscle trainer is a device which consist of mouth piece and with adjustable resistance. Inspiratory muscle trainer resistance is altered according to patient condition from acute to severe. Place nose clips to close the nose. Inspiratory muscle trainer is placed in mouth started with minimal resistance and gradually resistance increased. Treatment time was 10 to 15 minutes. Diaphragmatic breathing exercise with Inspiratory muscle training has been shown to improve inspiratory muscle strength, improve exercise tolerance and peak expiratory flow rate in asthma patients.. Reviews of work done on training the respiratory muscles using inspiratory muscle training which reduce bronchospasm and improves exercise tolerance (9,10). Non-pharmacological treatment methods are as important as pharmacological treatment in asthma patients in order to take symptoms under control and to prevent the frequency of exacerbation. . After treatment with diaphragmatic breathing exercise and inspiratory muscle training the posttest collected data was subjected to statistical analysis using paired 't' test .

RESULTS AND DISCUSSION

The results of the study were derived from the statistical analysis using the paired t-test . The results using a paired t-test revealed that there was a significant improvement in peak expiratory flow rate and exercise tolerance in asthma patients. The results of the study showed that difference between the pretest and post test mean values were statistically significant thereby indicating that peak expiratory flow rate and exercise tolerance had significantly improved following diaphragmatic breathing exercise with inspiratory muscle training for a period of eight weeks .The improvement in peak expiratory flow rate and exercise tolerance may be because obstruction in air passage was reduced, broncho spasm was reduced and utilisation of primary muscles of respiration was increased so the peak expiratory flow rate was increased. Diaphragmatic breathing exercise with Inspiratory muscle training using inspiratory muscle training device improves strength of the inspiratory muscles to improve ventilation perfusion ratio of the lungs and increases the metabolic needs of the tissues and oxygen supply which increases the muscle contraction to meet the exercise tolerance of the individuals to reach the task.

CONCLUSION

The results of the study make us to conclude that Diaphragmatic breathing exercise with Inspiratory muscle training improved Peak expiratory flow rate and Exercise tolerance in asthma patients.

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Table 1. The collected data were analyzed using paired 't' test

Variables	Pre test Mean	Post test Mean	Mean difference	Standard deviation	't' calculated value	't' tab value
Peak expiratory flow rate scores (liters /minute)	375.5	393.5	18	6.95	11.61	2.09
Exercise tolerance test scores (meters)	373.3	395.85	22.55	5.826	17.346	2.09

t calculated value > t table value.





Production and Characterisation of Bioplastic from Vegetable Peels an Ideal Strategy for Food Waste Disposal

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ABSTRACT

Industries, trash disposal, and other factors contributed to environmental contamination. Because plastics are non-biodegradable, they pose the greatest harm to the environment. Based on the foregoing, a sustainable material that is also biodegradable is required. Bioplastics are a type of material that falls within this category. As a result, research was conducted to manufacture bioplastic from vegetable peels and to characterize it using FTIR (Fourier Transform Infrared Spectroscopy) analysis. FTIR analysis was used to characterize the product. The FTIR spectrum was acquired at wavelengths ranging from 500 to 4000cm⁻¹.

Keywords: bioplastic, vegetable peels, waste, food, biodegradable

INTRODUCTION

As a result, food waste management has become more important, because food waste contains valuable biomass that might be used to make environmentally friendly industrial products. Biotransformation of vegetable and fruit wastes could thus contribute to the generation of beneficial industrial goods. Converting food waste into biodegradable, environmentally friendly polymers could be a viable alternative to synthetic plastics (Jasmine, et al., 2021). Bioplastics are plastics made from renewable biomass sources such vegetable fats and oils, maize starch, straw, wood chips, food waste, agricultural by-products, and microorganisms, as well as used plastic bottles and other containers. Bioplastic is transparent, flexible, long-lasting, effective as a barrier, and heat resistant. Bioplastics are divided into



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three categories: 1. Bioplastics made of starch, 2. bioplastics made of cellulose, and 3. bioplastics made of protein (Jerez et al, 2007) . Disposable items such as packaging, crockery, cutlery, pots, bowls, and straws are made of bioplastics (Beevi, et al., 2020). Based on the foregoing considerations, an attempt was made to synthesis biodegradable plastic material from banana peels and potato starch, and to describe the resulting bioplastic material using FTIR analysis.

MATERIAL AND METHODS**Sample collection**

For this investigation, bacteria were isolated from soil samples and cow dung, as well as cow milk samples and effluent, in order to screen for the best PHA-producing bacteria.

Isolation of Polyhydroxyalkanoic Acids (PHAs) Producing Bacteria.

To extract bacteria from the sample, the materials were processed in the lab using standard techniques of serial dilution up to 10^{-7} and spread plating on Nutrient Agar. At 37 degrees Celsius, the plates were incubated for 24 hours. By streaking colonies on Nutrient agar plates numerous times, colonies with specific features were selected and purified.

Screening for PHA producing bacteria

For screening, all of the isolated bacterial cultures were streaked on Soil Minimal Salt Medium with Bagasse from Product. The Soil Minimal Salt Medium with Bagasse is prepared as follows: 500 mL Minimal Salt Medium, 1 mL Micronutrient solution, 1 mL Nile Red, and 2 gram Bagasse Following autoclaving, the sterile media was transferred to Petri plates under aseptic conditions.

Gram's Staining

Gram staining was performed to identify the unknown bacteria.

Identification of PHA producing isolates

Isolated colonies from Nutrient agar were streaked on the sterilised Soil Minimal Salt Medium containing Bagasse by Product. Eight isolated colonies were streaked on a single plate and incubated at 37°C for 96 hours. Incubated plates were examined for PHA synthesis under UV light.

Extraction of PHA from bacterial cells:

Mass culturing was used to extract the PHA synthesized by the bacterium cells. 16 hours of fresh cultivation of the selected bacterial strain of potential bacterium Isolate was inoculated in different medium composition, including 1liter of Nutrient broth with vegetable peels extract, water with vegetable peels extract, and incubated in a rotatory shaker at 37°C for 96 hours. During the media preparation, potato and pumpkin peels were weighed and crushed in a mixer before being mixed with the media. Following growth, the biomass collected in the culture flask was subjected to PHB extraction using boiling chloroform as the solvent because it is known to be effective in extracting polymer.

Disruption of Cells by Chemical Methods and PHB Estimation

In test tubes, nutrient broth was prepared and colonies were inoculated. The medium was incubated for 24-96 hours at room temperature. Every 24 hours, the amount of PHB was estimated. A total of 5 mL of culture was centrifuged for 10 minutes at 10,000 rpm. The supernatant was decanted, and the pellet was suspended in 2.5 mL sodium hypochlorite and 2.5 mL chloroform for 1 hour at 30°C. At room temperature, the above content was centrifuged for 10 minutes at 1500 rpm. The upper hypochlorite phase, the middle chloroform phase with undisturbed cells, and the bottom chloroform phase with PHB were obtained. The contents were centrifuged at 1500 rpm for 10 minutes at room temperature to separate the top and middle phases, and the phase other than chloroform with PHB was



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carefully removed. The chloroform phase containing PHB was treated with concentrated sulphuric acid. It was then cooked for 10 minutes in a water bath at 100°C.

FT-IR

The functional groups included in the bioplastic samples were identified using the FT-IR approach, which used a wavelength range of 4000–500 cm⁻¹ and a resolution of 2 cm⁻¹. Middle IR beam splitter: Germanium-coated KBr (Standard). In the end, the spectrum data was plotted graphically. Shimadzu Corporation's IRTracer-100 Fourier Transform Infrared Spectrophotometer (FT-IR).

RESULT AND DISCUSSION

Environmental pollution has existed for millennia, but it became more prevalent in the 19th century as a result of the development of new industries. Industries, radioactive compounds, and trash dumping in oceans and on land pose a severe hazard to the environment and surrounds since they can be eradicated or reused (Jayachandra et al, 2016). Plastic is made from organic chemicals such as crude oil, natural gas, and other similar substances. Some harmful chemicals, such as acetone, methylene, chloride, styrene, benzene, sulfuroxides, nitrogen oxides, methanol, and other volatile organic compounds, are routinely emitted during the manufacture of plastics. These hazardous acids pollute the ecosystem significantly (Pintu and Hossain, 2016). Plastic is the most serious environmental threat since it is not biodegradable. Every environmentalist and wildlife conservationist are concerned about them. We are throwing plastics into the ocean, which has resulted in a disaster for the organisms who dwell there. The plastic's main component is a polymer (polypropylene/polystyrene), which can leach into water and enhance water toxicity. Aquatic species mistake the plastic objects that float on the water's surface for food and feed on them, eventually leading to death (Ginting et al, 2015). Plastic is a huge contaminant on the planet. As a result, bioplastic is being developed to replace synthetic plastic and reduce pollution. Bioplastics are plastics made from renewable biomass sources such as vegetable fats and oils, corn starch, and can degrade in both anaerobic and aerobic settings. The agents that predominantly decomposed organic waste to generate bioplastic were microorganisms and algae (Pintu and Hossain, 2016). As a result, research was conducted to obtain bioplastic film from pumpkin and potato peels, as well as to investigate the properties of synthesized bioplastic using FTIR analysis.

In this study, possible PHB-accumulating bacteria were extracted from a soil sample, cow dung, cow milk samples, and effluent, and potential strains were chosen for further study. Serial dilution and spread plating on Nutrient Agar were conducted for each diluted sample. Colonies with unique morphologies were chosen after incubation (Figure:1), sub-cultured, and kept at 4°C for future use. For screening, all of the isolated bacterial cultures were streaked on Soil Minimal Salt Medium with Bagasse from Product. For additional testing, nine separate bacterial cultures were chosen. Pre-treated sugarcane bagasse (56 %) was the most cost-effective carbon source, followed by corn cobs (52 % PHB). Yu et al. (Yu et al., 1998) obtained 54 percent PHB using bagasse hydrolytes from *Cupriavidus necator* and reported comparable results. Paramjeet et al. (Paramjeet et al., 2012) employed *Pseudomonas aeruginosa* to extract 60% PHB from sugarcane bagasse. On the other hand, teff straw and banana peel produced less biomass and PHB (Getachew & Woldesenbet, 2016).

For screening, all eight isolated bacterial cultures were streaked on Soil Minimal Salt Medium containing 2% Bagasse by Product (Figure.2). All of the cultures were gram positive Bacilli, according to the Gram staining results. Incubated plates were examined for PHA formation under UV light. Only two of the eight cultures showed Promising results. They showed high fluorescence under UV light when grown on 2 percent bagasse plates containing Nile red, as shown in Figure 3.



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The interactions between different components and changes in chemical compositions of the mixtures were investigated using FTIR spectroscopy (Figure 4). To identify the probable biomolecules included in the produced bioplastic film, FTIR measurements were performed. Figure 4 depicts the results of FTIR analysis of produced bioplastic. The sample's FTIR spectrum was acquired at a wavelength in the range of 500-4000 cm^{-1} , according to the results of the FTIR analysis. Peaks corresponding to the O-H functional group were also found in plasticized materials, ranging between 3435 and 2922 cm^{-1} . This is due to the fact that both of the plasticized materials utilized, glycerol and sorbitol, are polyols (Ano et al., 2017), which include a large number of O-H groups, resulting in broad peaks between 3600 and 3200 cm^{-1} (Chen et al., 2015a, 2015b). Cao et al. observed similar peaks for O-H spanning between 3200 and 3600 cm^{-1} (Cao et al., 2008). In all unplasticized and plasticized samples of COM bioplastics, there were a few peaks between 979 and 1058 cm^{-1} , indicating the C-O-H group, and 1058–1189 cm^{-1} , indicating the C-O-C group. Peaks at 1381.71 cm^{-1} were caused by primary amine, which produces two N-H stretching absorptions, whereas the peak at 1647 cm^{-1} was caused by alkane C-H bonds.

CONCLUSION

The goal of this research was to create an eco-friendly and cost-effective bioplastic using plant resources. One of the key issues in bioplastic material synthesizing is the bioplastic made from banana peels and potato starch. The current report focuses on the synthesis and characterization of natural polymeric materials of this sort. Certainly, there is a long way to go in terms of research for both cost-effective and environmentally beneficial bioplastic or biopolymer goods. However, synthesis of bioplastic from fruit waste is a more dependable process since it is more cost-effective and makes better use of trash. However, this research will be a centralized project that can be used on a wide scale to generate enormous quantities of plastic to meet the needs of any business.

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Soil Sample



Bagasse by Product

Fig. 1: Bacterial colony Growth on Nutrient Agar Media after Spread Plate



Fig.2: PHA Producing Bacterial Growth on 2% bagasse Plates Containing Nile Red

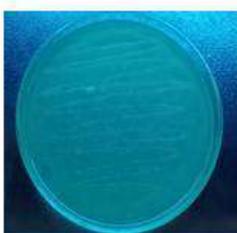


Fig.3: PHA producing bacterial growth on 2% bagasse plates containing Nile red under UV Light

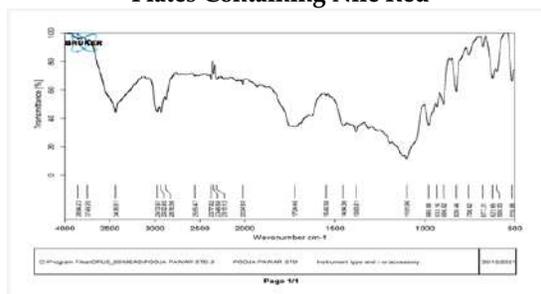


Fig. 4: FTIR Analysis





Energy-Efficient and High-performance FIR Adaptive Filter Architecture of Wireless Sensor Networks for IoT applications

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ABSTRACT

Adaptive filters are used to adapt to signal-changing settings, spectral overlapping among noise and signal, and unpredictable or time-varying noise. The adaptive FIR filter is extensively employed as a signal preprocessing stage in wireless sensor networks (WSN) and the internet of things (IoT). Signal preprocessing could assist in minimizing the amount of energy used in communication between nodes while also improving the efficiency of data transmission. The most common adaptive filtering technique utilizes the least mean squares (LMS) algorithms. The multiply and accumulate (MAC) process is the spine of LMS adaptive filters. The usage of multiple MAC units can boost the system's speed, but the cost of the system rises as the multipliers take up a lot of space and consume more energy. When LMS is implemented as a completely reliable architecture on a hardware platform, the multiplier evolves a bottleneck for higher-order filters, resulting in significant size, expenditure, and energy needs, rendering the design unsuitable for practical implementation. FIR filters are frequently realized using distributed arithmetic (DA) based scheme. DA-based designs replace multipliers with look-up tables (LUT), where the precomputed partial product (PPs) is saved. LUTs are required for filtering and weight updating processes. The size of the LUT grows exponentially with the increasing order of filters. To minimize the size of a LUT, offset binary coding (OBC) is utilized, resulting in a smaller memory size. The suggested method stores OBC input sample and filter weight combinations in two independent LUTs. Compared to prior techniques, the proposed architecture provides significant area and power reductions without

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breaking the LUT into two smaller LUTs. The OBC-DA-based architecture outperforms the traditional MAC-based architecture in terms of throughput. Also, it saves a large amount of sliced LUT and flip-flops compared to conventional systems.

Keywords: Wireless Sensor Networks (WSN), Internet of Things (IoT), Adaptive filter; FIR filter; distributed arithmetic (DA); look up tables (LUT); least mean square (LMS); offset binary coding (OBC); chip area; power consumption; throughput.

INTRODUCTION

A wireless sensor network (WSN) groups spatially separated sensor nodes that communicate wirelessly to form a network [1]. A sensor node measures the fluctuation of current events in its surroundings utilizing its sensor(s). The ADC unit transforms these measures into comparable electric signals, which the node's processor processes. The node can wirelessly transfer the information recorded by its processor to other nodes or the base station using its transceiver. WSNs are utilized in various sectors, including health, transportation, monitoring of the environment, power grids, and defense [2-11]. The most important aspect of data transit from the sensor to the sink is noise, significantly impacting WSN's performance. Noise cancellation in WSN has received a lot of interest as a technique for removing noise from signals and improving signal quality [12]. The use of traditional filters would cause the desired signal to be distorted. The adaptive filter is a crucial component of noise cancellation since it reduces noise without a prior understanding of the random noise and input signal [13]. FIR filters provide huge benefits over IIR filters, such as stability and linear phase, and are the most widely used in adaptive processing. Pre-processing is needed for efficient data collection performance since it decreases the quantity of data transmitted across the communication channels. FIR filter is often utilized as a signal pre-processing method in WSN. In this perspective, it is required to devote more effort to developing an efficient architecture for digital signal processing (DSP) operations like FIR filters, which are widely utilized in video and audio signal processing in WSN [14]. Because higher-order filters' complexity and circuit size are too enormous, so real-time processing of these filters with the desired area and power metrics is difficult [15].

Traditional n-tap FIR filter necessitates n- multiply-and-accumulation (MAC) blocks. Adaptive filters are divided into two portions, one for updating the filter's coefficients and the other for calculating the outcome. The least mean square (LMS) algorithm updates the weights (or coefficients). LMS possesses a higher rate of convergence, greater resilience, and greater simplicity [16]. Due to the logic complexities and resource utilization, n-MAC blocks are costly to implement in FPGA and raise the computational difficulty. The hardware implementation is challenging due to the closed-loop adaptive procedure and corresponding LMS algorithm. Multipliers in MAC-based FIR filters take up a lot of space on the device and utilize much power. Multiple MAC units can boost system speed, but the expense of the system rises due to the multipliers' occupying a large area. The MAC-based LMS adaptive filter architecture necessitates more space, money, and power, greatly restricting the improvements in throughput, motivating looking for new designs for LMS adaptable filters. Multipliers consume far more resources on hardware than adders, motivating to look for architecture that does not need multipliers. A multiplier-less design based on distributed arithmetic (DA) is developed to tackle this problem [17].

The basic idea of the DA method is to use the shift and addition operations instead of multiplication, which decreases the area greatly. The convolution procedure is performed using the DA approach, replacing multipliers with look-up tables (LUT), resulting in minimal resource usage and increased throughput. MAC units are replaced with LUTs to save space and hardware resources. The DA technique can be utilized with higher-order FIR filters, and it is an effective approach for computing PP's coefficient values and storing the PPs. This technique executes the filtering by shifting and accumulating operations on these PPs. On the other hand, the LUT's size will grow



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exponentially as the coefficient increases. It is quite simple to realize if the coefficient is little. While the coefficient is large, it will consume FPGA storage resources and slow down calculations. The content of LUTs in adaptive filters is a function of tap weights, which must be updated for every input. As a result, the recently estimated PPs will substitute the contents of LUTs. The filters' PPs are stored in LUT at several address locations and demand B clock-cycles (B is the word length) to obtain the filter outcome for the input word width of B. Filter must fetch the LUT sequentially for B bit positions followed by shift-accumulated (SA) for B bits to obtain the filter outcome. With increasing filter order, the size of the LUT increases exponentially. Because each address location must be updated regularly, the LUT complexity increases when the filter is made adaptive. However, as the size of the LUT increases with larger N, this implementation becomes ineffective for more extensive values of N [18].

Several architectures for implementing the adaptive FIR filter utilizing DA have been presented. Allred et al. [19] presented an architecture that uses two distinct LUTs for weight updating and filtering operations. For implementation, this technique has two LUT intricacies. Even though the memory needs decreasing, the size of the LUT climbed exponentially when the order of the filter grew. As an outcome, these designs are incompatible with adaptive filters of higher order. Rui Guo et al. [20] presented an architecture to solve this problem by employing only one LUT, which performs weight updating and filtering operations. Higher sampling rates are not achieved because, for each new sample, the design requires multiple cycles to update LUT. M.S. Prakash et al. [21] developed an offset binary coding (OBC) technique in which the size of the memory-based LUT is reduced in half. Combinational circuits and adders are used in this design. The throughput rate has greatly increased while consuming less power and less space. However, large LUT decomposes into two small LUTs to attain higher throughput. Because two multiplexed LUTs are used simultaneously, the power usage of such a device increases. Furthermore, the additional hardware required for LUT decomposition resulted in a further increase in size and energy consumption. S.Y. Park et al. [22] proposed an increased speed with minimal adaptation delay architecture, significantly reducing energy consumption. In concurrently, the contents of the LUTs are updated. It entails operating at an adjustable frequency. Yet, the smaller the frequency, the smaller the area used, and the greater the frequency, the greater the area used. It has a greater hardware complexity, particularly for an FIR filter with higher orders. B. K. Mohanty et al. [23] proposed a block-LMS (BLMS) technique to implement an FIR filter architecture in which LUT decomposition is performed. In this architecture, hardware resources and energy usage are reduced. Atul A. Chandekar et al. [24] proposed BLMS based architecture which uses the same LUT to perform correlation and convolution functions. Speed and throughput are increased using parallel processing. The proposed system requires fewer LUTs and areas, and the number of flip flops is independent of block size.

As inspired by earlier work, to minimize the size of a LUT, delay offset binary coding (OBC) is utilized, resulting in a smaller memory size. OBC algorithm employed to optimize the structure of PPs, resulting in a smaller memory size. The suggested method stores OBC input sample and filter weight combinations in two independent LUTs. Compared to prior techniques, the proposed architecture provides significant area and power reductions without breaking the LUT into two smaller LUTs. The OBC-DA-based architecture outperforms the traditional MAC-based architecture in terms of throughput. Also, it saves a large amount of sliced LUT and flip-flops compared to conventional systems. The article is systematized as follows: Section-2 and Section-3 go over the background information needed, Section-4 goes over the proposed design, Section 5 goes over the simulation findings, and finally, Section-6 concludes the proposed work.

MAC-based LMS adaptive filter

Figure 1 depicts a traditional architecture of the LMS adaptive filter. The adaptive FIR filter consists of an FIR filter for output computation and a weight update module for weight adaptation. Furthermore, it consists of a smaller unit for error calculation. The filter module processes the input signal, and the filter coefficient weights are updated utilizing the LMS algorithm.





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Figure 2 depicts the output unit of the FIR filter with a 4-tap containing specific multipliers, adders, and registers to store previous inputs, following LMS equations. The error calculation unit, shown in Figure 3, is the same independent of the order of the filter. The basic structure of the weight updating module consists of adders and multipliers, which are depicted in Figure 4. Furthermore, the design is clear and simple to implement. However, if the filter's order rises, processing unit requirements also increase, increasing the area and energy consumption. Furthermore, there will be low multipliers and adders because a DSP/FPGA system has finite resources. Another disadvantage of implementation is that the system's critical path delay increases as the filter order increases. As a result, designing a large-order LMS adaptive FIR filter for real-time is difficult. Equations (1), (2), and (3) govern the LMS algorithm. The Adaptive filter output is represented as "y" in equation (1), filter weight "h" in equation (2), and the mean square error "e" in equation (3).

$$y[n] = \sum_{k=0}^{N-1} h_k[n].x[n - k] \tag{1}$$

$$e[n] = d[n] - y[n] \tag{2}$$

$$h_k[n + 1] = h_k[n] + (\mu \cdot e[n].x[n - k]) \tag{3}$$

The input signal is represented by x, while "N" stands for the filter order, and n is between 0 and N-1. D represents delay flip flop. The desired or target output is denoted by d, while the step-size or convergence coefficient of the filter is denoted by μ . Convergence coefficient regulates the adaptive filter's mean steady-state error and convergence. Normally, μ is chosen in the negative exponent of 2, and as a result, the right shift function can be employed to multiply μ and e[n] in equation (3). Equation (1) governs the filter's output by accumulating filter weights' products with related filter inputs. As per equation (2), the difference between current and target output determines the error. According to equation (3), updating the filter weights is performed using the error value, previous inputs, current inputs, step size, and previous weights.

LMS adaptive FIR filter using OBC-DA

Figure 5 depicts LMS adaptive FIR filter using DA. It consists of W-LUT for filtering operation and X-LUT for updating input samples. It also has a shift accumulating unit (SA) and error calculation unit. The input signal x(n) is processed to obtain the y(n) output signal. The following are the mathematical equations required to design a fixed coefficient DA-based FIR filter. Each of the input samples x (n-k) is represented in the form of a 2s complement shown in equation (4). The word length of the input samples is denoted by B.

$$x_k = x(n - k) = -x_{k, B - 1} + \sum_{j=1}^{B-1} x_{k, B - 1 - j} 2^{-j} \tag{4}$$

It is possible to write from equation (4) as $x_k = \frac{1}{2} [x_k - (-x_k)] = \frac{1}{2} [x_k - (x_k ')]$ where $x_k '$ is the 2s complement of x_k . As a result, the equation (4) becomes:

$$x_k = \frac{1}{2} [-(x_{k, B - 1} - x'_{k, B - 1}) + \sum_{j=1}^{B-1} (x_{k, B - 1 - j} - x'_{k, B - 1 - j}) 2^{-j} - 2^{-(B-1)}] \tag{5}$$

The OBC scheme is represented in equation (5). Now select,

$$d_{k, j} = \begin{cases} -(x_{k, j} - x'_{k, j}), & j \neq B - 1 \\ -(x_{k, B - 1} - x'_{k, B - 1}), & j = B - 1 \end{cases} \tag{6}$$





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We derive equations (7) by replacing equations (5) and (6) in equation (1) and reordering them.

$$y(n) = \sum_{j=0}^{B-1} \left(\frac{1}{2} \sum_{k=0}^{N-1} h_k d_{k,j} \right) 2^{-j} - \frac{1}{2} \left(\sum_{k=0}^{N-1} h_k \right) 2^{-(B-1)} \tag{7}$$

Describe,

$$b_{B-1-j} = \frac{1}{2} \sum_{k=0}^{N-1} h_k d_{k,j} \quad 0 \leq j \leq B-1 \tag{8}$$

$$P_{initial} = -\frac{1}{2} \sum_{k=0}^{N-1} h_k \tag{9}$$

$$y(n) = \sum_{j=0}^{B-1} (b_{B-1-j}) 2^{-j} + (P_{initial}) 2^{-(B-1)} \tag{10}$$

If the filter order is N, it can be seen from equation (8) that b_{B-1-j} can have 2^N different filter weight combinations. Nevertheless, because OBC combinations are symmetric, " 2^{N-1} " half terms must be stored in LUT. XOR gates can be utilized to acquire the remaining half of the combination of OBC which is shown in Figure 6. Minimal area, lower power consumption, and fast LUT access are all advantages of this method and it stores OBC input sample and filter weight combinations in two independent LUTs. The LSBs form the address lines to the W-LUT, holding the PPs from each register. The output will be produced by successive content readings from W-LUT following the SA operation. From the LUT, which is then subjected to SA, any combination of PPs of filter weights can be retrieved. The number of combinations stored in LUT grows exponentially as the number of filter taps increases, i.e., 2^N . LUT can be cut in half, and the input sample is represented as $x_k = \frac{1}{2} [x_k - (-x_k)] = \frac{1}{2} [x_k - (x_k)']$, generally known as OBC.

Because OBC combinations are symmetric, just 2^{N-1} half terms must be stored in LUT. Figure 7 shows how XOR gates can be utilized to acquire the remaining half of the combination of OBC. It also necessitates the insertion of the initial term $P_{initial}$ during the shift-accumulation cycle. This approach provides several advantages, including a smaller area, lower power consumption, and faster LUT access. The OBC filter weight combinations, the precomputed values stored in LUTs of two kinds. The LSBs form the address lines to the W-LUT, holding the PPs from each register.

The proposed architecture's math details have been completed thus far. Consider the proposed architecture illustrated in Figure 8. It consists of two LUTs that keep their OBC combinations. X-LUT is the LUT for updating input samples, and W-LUT is the LUT for updating input weights.

X-LUT saves the OBC input sample combinations utilizing the proposed algorithm. According to equation (11), the suggested scheme's weight adaptation is made on the W-LUT content utilizing the X-LUT content.

$$\sum_{k=0}^{N-1} a_k^r h_k(n+1) = \sum_{k=0}^{N-1} a_k^r h_k(n) + \mu e(n) \sum_{k=0}^{N-1} a_k^r h_k(n-k) \tag{11}$$

Here address is r, in the N-bit form a_k^r is the k^{th} bit. Address r is depicted mathematically as in equation (12).





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$$r = \sum_{k=0}^{N-1} a_k^r 2^k \tag{12}$$

At time-instant n, X-LUT stores input samples, and later X-LUT is employed to update the W-LUT. The $\mu e(n)$ can be expressed as a power of two to simplify the process of multiplication by shifting.

PROPOSED METHOD

The proposed method is made up of a register-bank that stores the input samples. The LSBs form the address lines to the W-LUT, holding the PPs from each register. The output will be produced by successive content readings from W-LUT following the SA operation as indicated in equation (10). The input samples' word length determines the number of SA cycles. $P_{initial}$ should be utilized during the first cycle of SA. 2-to-1 multiplexer is demanded based on equation (10). The SA process is parallel with the X-LUT updates for B or 2^{N-1} clock cycles. The word length of the input samples is denoted by B. As per equation (2), the produced output is subtracted from $d(n)$. The content of X-LUT is then updated, and the product $\mu.e(n)$ is multiplied by the output. The related contents of W-LUT will be added to the terms $\mu e(n) \sum_{k=0}^{N-1} a_k^r x(n-k)$. As illustrated in Figure 9, both LUTs in the proposed design store equivalent OBC combinations at time instant n. As a result, the suggested design for LUTs does not need any external register or decomposition hardware. As an outcome, there is a substantial decrease in area and energy consumption. In the parallel operation of two LUTs, power consumption is more than a single LUT (non-decomposed) for the same algorithm. Figure 9 illustrates W-LUT and X-LUT contents at time instant n for a 4-tap filter.

Figure 10 depicts the X-LUT contents of an FIR filter with 4-tap at time instant n and time instant n+1. It also shows the update scheme. The contents are mapped using " $\frac{1}{2}[x(n+1)+x(n-3)]$ " as shown in the figure. For the subsequent iteration, the contents of LUT's address locations must be different. The following example demonstrates this. Figure 10 depicts a suggested 4th order filter using an X-LUT update strategy. At address 000 the LUT contents (n) is $\frac{1}{2} [x(n)+x(n-1) +x(n-2) +x(n-3)]$. The content of LUT becomes $\frac{1}{2} [x(n+1) -x(n) +x(n-1) -x(n-2)]$ when deducted using $\frac{1}{2}[x(n+1)+x(n-3)]$. The earliest sample $x(n-3)$ is not used in the updated term. All the address locations from 001 to 111 are updated utilizing the same external term $\frac{1}{2}[x(n+1) + x(n-3)]$.

The new entries $X_r(n+1)$ of the X-LUT.

$$X_r(n+1) = \frac{1}{2}[x(n+1)+x(n-3)] + X_{2^{r+1}}(n) \text{ with } r | r < 2^{N-1}$$

$$X_r(n+1) = \frac{1}{2}[x(n+1)+x(n-3)] - X_{2^{(2^{N-1}-r)}}(n) \text{ otherwise.} \tag{13}$$

Once the X-LUT update is completed, the W-LUT contents are updated utilizing the modified contents of X-LUT. Error signal $e(n) = d(n)-y(n)$ is computed and scaled with appropriate step size. W-LUT is used to accomplish the filtering process, and the content of X-LUT is modified to X-LUT(n+1) from X-LUT(n). As a result, at time n, one phase of the filtering operation and adaption is complete. A comparable explanation can be provided for every new sample for various time instances. A custom hardware multiplier can accomplish the multiplication needed in equation (11). A custom hardware multiplier can accomplish the multiplication needed in equation (11) but demands a substantial area on the chip. An approximation for the variable $\mu e(n)$ is utilized to simplify the multiplication. It specifies that only the MSB of the error signal has been considered. As a result, the area due to $\mu e(n)$ decreases, as shown in Figure 11, because it substituted the hardware multiplier with an uncomplicated barrel shifter. The right-shifted contents of the X-LUT are approximated by the merged product of $\mu e(n)$ and the contents of X-LUT. The suggested filter's throughput does not suffer as a result. However, the convergence suffers marginally.



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RESULTS AND DISCUSSIONS

Simulating in Verilog validates the proposed method. The architectures proposed in [19], [20], and [21] are compared with the proposed architecture. The filter is split into m smaller filters with k input lines each, yielding $N = m \times k$ and N is a composite number, and this is done to speed up LUTs access, especially for big taps. Weight adaption strategies for [20] and [21] are different. It should be emphasized that, like the [21] system, the suggested method stores OBC input sample and filter weight combinations. Nevertheless, unlike [21], the proposed architecture does not necessitate a decomposed LUT using multiplexers, resulting in substantial area and power savings. In comparison to conventional systems, this necessitates less hardware complexity. Furthermore, the proposed architecture does not demand an extra adder for weight-update and filtering operations, resulting in a shorter sampling interval for the proposed architecture than the [21] approach. The proposed design has 7.5 percent fewer adders and 20 percent fewer registers for the 32nd filter order. Table 1 shows LUT complexity, throughput, adders per cycle, and register details of several proposed architectures.

The proposed design requires " $4mk \times 1$ MUX" in the hardware mentioned above complexities, whereas the [21] approach requires " $2mk \times 1$ MUX." The proposed architecture's critical path is decreased by (LUT access time + 2×1 MUX delay time) units over the [21] scheme. Compared to [19], this will result in a higher sampling rate, lower power consumption. A "3:2" compressor coupled with a carry propagation adder [24-25] can further decrease the critical path. The proposed designs have the same clock cycles as the [19] architecture. Simulation in Verilog was performed to validate the proposed design. We used the Cadence RTL compiler to perform ASIC synthesis to evaluate the design's throughput, power, and area using the UMC180 nm CMOS library for $N = 32$ and $N = 16$. Filter weights and input samples were assigned an 8-bit word length. Table 2 shows the proposed and existing architectures' power consumption, throughput, and projected area.

The area values of the suggested architecture are lowered over the [21] architecture, specifically for extensive N and k , as shown by the findings. Like the [21] system, to minimize the size of a LUT, offset binary coding (OBC) is utilized, resulting in smaller memory size. For example, 4-tap FIR filter with 32nd order is compared with [21] architecture with 16th order of 4-tap and found that suggested architecture presents 20.56% less power consumption and 19.85% less area. The proposed 4-tap FIR filter with base order units greater than 32nd also offered less power consumption. Furthermore, compared to the [19] and [20] architectures, the proposed architecture utilizes less LUT. Synthesis on FPGA for a 4-tap FIR filter with $N = 32$ was executed. The FPGA used is EP3C55F484C6 (Altera-Cyclone-III). It is found that the number of sliced LUT is 16.69% less than [21] architecture and saving of 19.06% flip-flops over the [21] architecture.

CONCLUSION

This work presents an OBC-DA-based FIR architecture that reduces area, cost, and power needs, making the design suitable for practical deployment such as IoT applications. To minimize the size of a LUT, offset binary coding (OBC) is utilized, resulting in smaller memory size. The suggested method stores OBC input sample and filter weight combinations in two independent LUTs. OBC algorithm employed to optimize the structure of PPs, resulting in smaller memory size. Compared to prior techniques, the proposed architecture provides significant area and power reductions without breaking the LUT into two smaller LUTs. The OBC-DA-based architecture outperforms the traditional MAC-based architecture in terms of throughput. Also, it saves a large amount of sliced LUT and flip-flops compared to conventional systems. The proposed architecture of the adaptive FIR filter is extensively employed as a signal preprocessing stage in wireless sensor networks (WSN), which could assist in minimizing the amount of energy used in communication between nodes while also improving the efficiency of data transmission.





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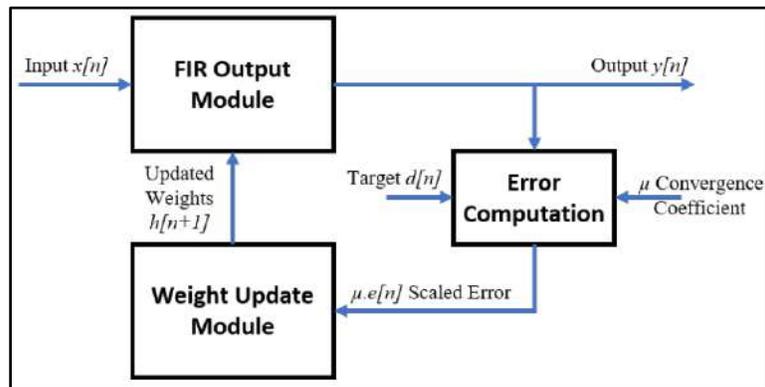


Figure 1. Traditional architecture of the LMS adaptive filter

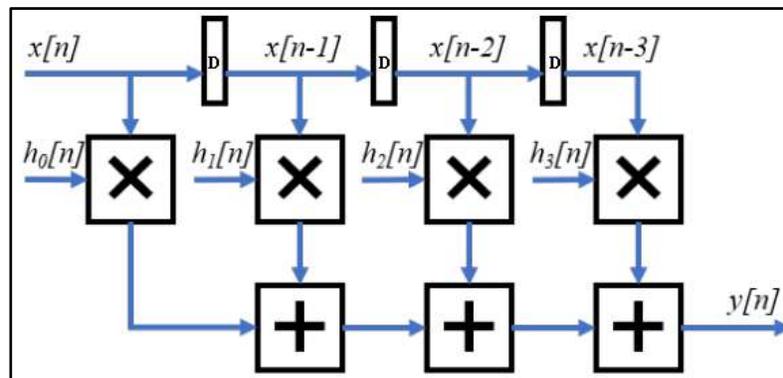


Figure 2. Output unit of the FIR filter





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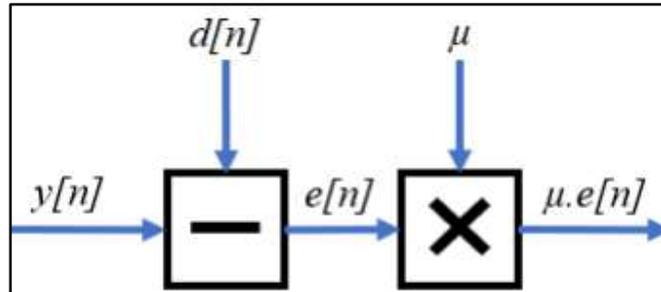


Figure 3. Module for calculating errors

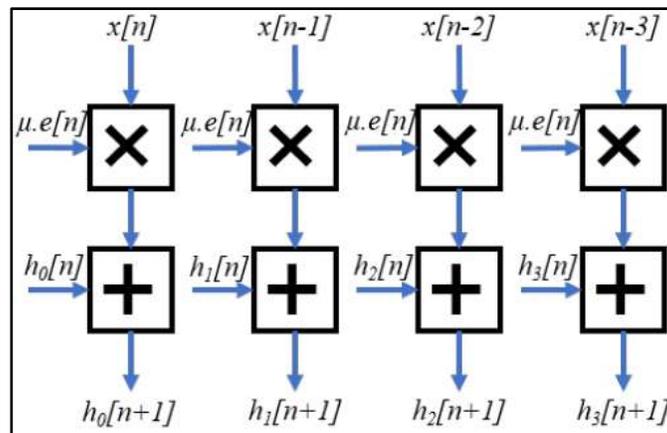


Figure 4. Module for weight updates

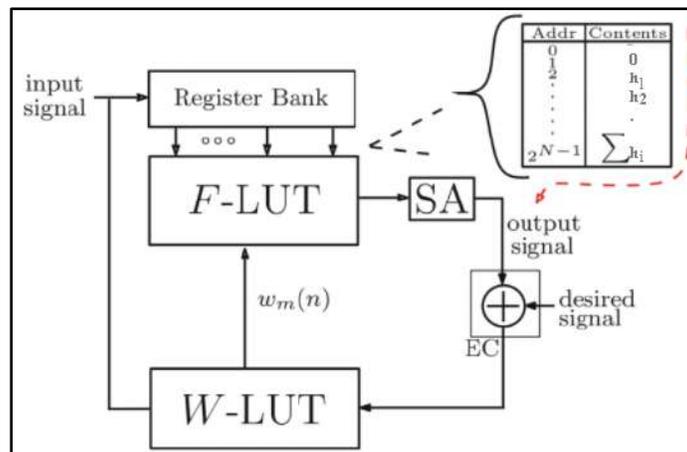


Figure 5. LMS adaptive FIR filter using OBC-DA





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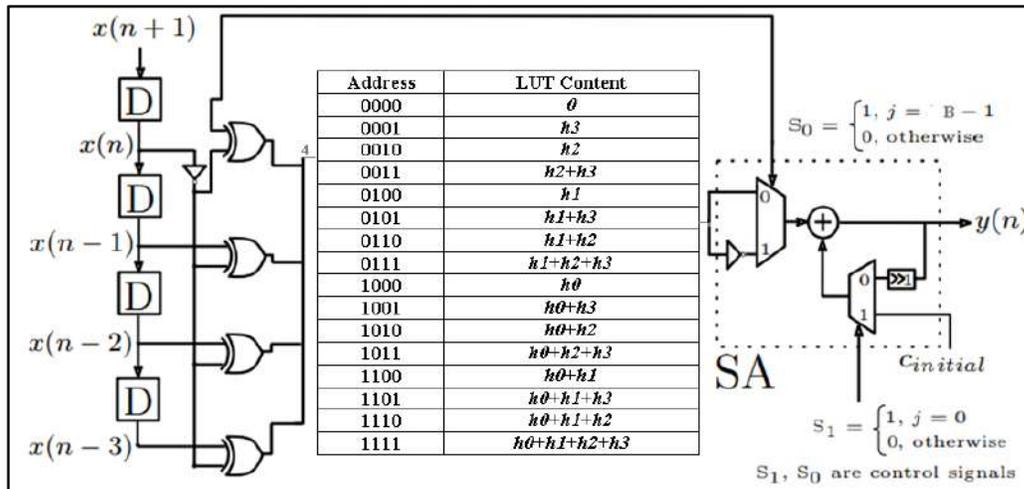


Figure 6. The 4-tap filter implementation using DA scheme.

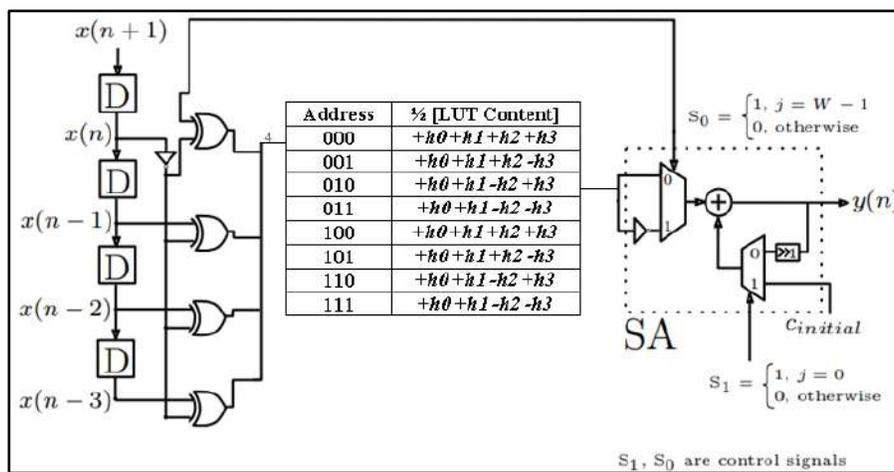


Figure 7. The 4-tap filter implementation using OBC-DA scheme.

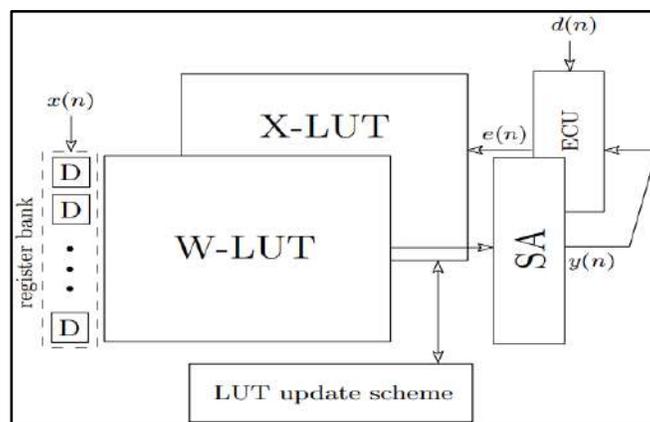


Figure 8. Proposed architecture of the OBC-DA based LMS adaptive filter





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W-LUT		X-LUT	
Address	LUT Contents(n)	Address	LUT Contents(n)
000	$\frac{1}{2} [+h0(n) +h1(n) +h2(n) +h3(n)]$	000	$\frac{1}{2} [x(n)+x(n-1) +x(n-2) +x(n-3)]$
001	$\frac{1}{2} [+h0(n) +h1(n) +h2(n) -h3(n)]$	001	$\frac{1}{2} [x(n)+x(n-1) +x(n-2) -x(n-3)]$
010	$\frac{1}{2} [+h0(n) +h1(n) -h2(n) +h3(n)]$	010	$\frac{1}{2} [x(n)+x(n-1) -x(n-2) +x(n-3)]$
011	$\frac{1}{2} [+h0(n) +h1(n) -h2(n) -h3(n)]$	011	$\frac{1}{2} [x(n)+x(n-1) -x(n-2) -x(n-3)]$
100	$\frac{1}{2} [+h0(n) -h1(n) +h2(n) +h3(n)]$	100	$\frac{1}{2} [x(n) -x(n-1) +x(n-2) +x(n-3)]$
101	$\frac{1}{2} [+h0(n) -h1(n) +h2(n) -h3(n)]$	101	$\frac{1}{2} [x(n) -x(n-1) +x(n-2) -x(n-3)]$
110	$\frac{1}{2} [+h0(n) -h1(n) -h2(n) +h3(n)]$	110	$\frac{1}{2} [x(n) -x(n-1) -x(n-2) +x(n-3)]$
111	$\frac{1}{2} [+h0(n) -h1(n) -h2(n) -h3(n)]$	111	$\frac{1}{2} [x(n) -x(n-1) -x(n-2) -x(n-3)]$

Figure 9. W-LUT and X-LUT contents at time instant n for a 4-tap filter

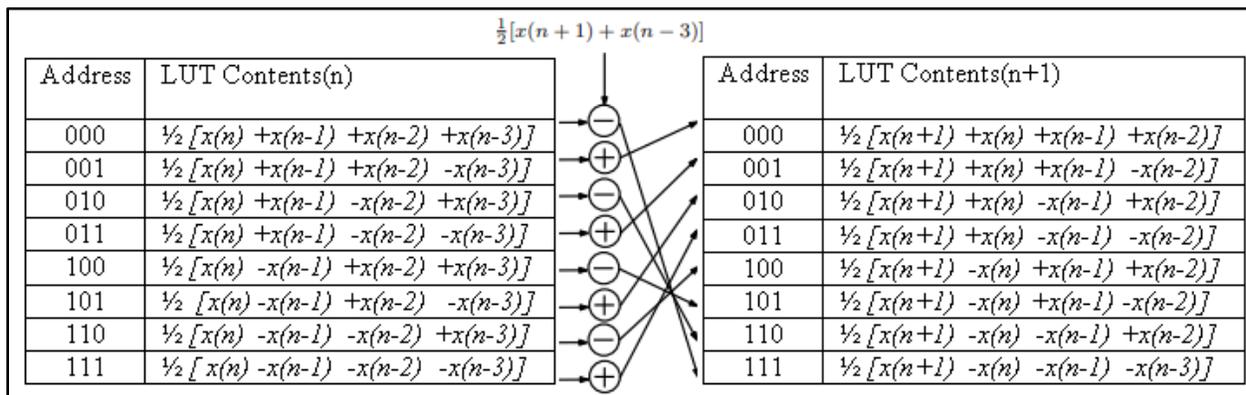


Figure 10. X-LUT contents of an FIR filter with 4-tap at time instant n and time instant n+1.

Table 1. LUT complexity, throughput, adders per cycle, and register details of several proposed architectures.

Architecture	LUT complexity (Memory)	Throughput	Adders per cycle	Registers
[19]	$(2^k-1).2m$	$1/[m_0(\text{LUT access time} + (k-1).2 \times 1 \text{ MUX delay} + \text{Adder delay})]$	$[(2^{k-1}+2^k) + B.m-1].m$	$[(1+k)+2].m$
[20]	$(2^k-1).m$	$1/[m_1(\text{LUT access time} + \text{Adder delay})]$	$[(2^{k-1}+k) + B.m-1].m$	$[(2+2k)+1].m$
[21]	$2^k.m$	$1/[m_2(\text{LUT access time} + (k+1).2 \times 1 \text{ MUX delay} + 2 \text{ Adder delay})]$	$[(2^{k/2+1} + 2^{k/2+2} + 2) + B.m + 1].m$	$[(4+k)+2].m$
Proposed	$2^k.m$	$1/[m_0(\text{LUT access time} + (k-1).2 \times 1 \text{ MUX delay} + \text{Adder delay})]$	$[(2^{k-1}+2^k) + B.m + 1].m$	$[(1+k)+2].m$

W and B is the word length of the filter weights and input samples respectively. $m_0=2^k+\max(W, 2^{k-1})+\log_2m$, $m_1=2^{k-1}+\log_2m+B+1$, $m_2=2^{k/2}+\max(W, 2^{(k/2)-1})+\log_2m+1$.





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Table 2. Proposed and existing architectures' power consumption, throughput, and projected area.

Architecture	Throughput per micro seconds		Area (mm ²)		Power (mW)	
	N=32	N=16	N=32	N=16	N=32	N=16
[19]	17.54	18.16	0.378	0.193	245.71	119.78
[20]	29.43	30.98	0.310	0.158	140.77	67.94
[21]	26.61	28.38	0.249	0.127	109.80	53.00
Proposed	19.69	20.56	0.200	0.104	87.25	44.15





A Hybrid RCGA–DGSA Algorithm for Maximum Power Point Tracking of PV System

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ABSTRACT

This paper proposes a hybrid Maximum Power Point Tracking algorithm composed of Real Coded Genetic Algorithm (RCGA) and Differential Gravitational Search Algorithm (DGSA) for Photovoltaic (PV) system. The hybrid algorithm integrates RCGA algorithm of global searching ability and DGSA of local search ability to increase maximum power point tracking ability. The MATLAB-SIMULINK simulations results under insolation change, load change, partially shaded conditions and extremely partial conditions shows lower tracking time, no steady-state oscillation, very good dynamic response and higher execution time. To show the effectiveness of the proposed method, it is compared with the Particle Swarm Optimization (PSO).

Keywords: Differential Gravitational Search Algorithm (DGSA), Maximum Power Point Tracking (MPPT), Partial Shaded Conditions (PSC), Particle Swarm Optimization (PSO) Photovoltaic (PV) and Real Coded Genetic Algorithm (RCGA).

INTRODUCTION

Due to the environmental issues, world nations focus towards the use of renewable sources of energy. The ready availability of solar energy and its friendliness to the environment made it to be recognized as the best alternative source of energy (1). However, PV system is non-linear in nature because of the varying environmental conditions like shading conditions and harvesting power from the PV system requires a Maximum Power Point Tracking



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(MPPT) algorithm (2). When the insolation is uniform, then all the PV panels act as PV generators and generate power but under shaded condition, it consumes the generated power and there exists an imbalance current. The bypass diodes are used to avoid this problem and it introduces multiple Local Maximum Power Point (LMPP). Among them, there exist one Global Maximum Power Point (GMPP) wherein the power extracted is maximum. The articles (3)(4) compares the various MPPT methods and it can be observed that the traditional MPPT algorithms fails under shaded conditions while modified version can guarantee convergence to GMPP. A ripple correlation control with time consuming differentiator is proposed in (5). An neural fuzzy based MPPT algorithm is proposed in (6) which would fail during aging. The INC method with estimation of the equivalent load line is proposed in (7). Line search algorithms with Fibonacci sequence dictating the step size is proposed in (8). A dividing rectangles algorithm is proposed in (9) which may fail as the rectangle search area may vary as partially shaded condition varies. an adaptive extremum seeking control is proposed in (10) and requires the P-V characteristics beforehand which is impractical.

The alternative approach to solving the above-mentioned drawbacks is to use optimization-based algorithms and has been proven effective (11)(12). Liang-Rui *et al.* (13) demonstrated biological swarm chasing behavior-based MPPT method uniform insolation conditions. The author in (14) realized a centralized MPPT with Particle Swarm Optimization (PSO) for PV systems and showed good performance under various PSC but the power generated from the photovoltaic panel which is not under shaded condition is affected. In (15), a PSO-based MPPT is proposed which has a large oscillation in the duty cycle. An improved PSO with two two-stage initialization is proposed in (16) which may track LMPP. Ishaque *et al.* (17) proposed a deterministic PSO removal of random numbers which will result in tracking the LMPP. Lian *et al.* (18) proposed a sequential P&O-PSO wherein the search space is limited. Sundareswaran *et al.* (19) proposed a Fireflies Algorithm (FA) which oscillates in output power before reaching GMPP. In (20), a grey wolf optimization based MPPT is proposed but dynamic changes in irradiance is not assessed. Ram *et al.* (21) proposed flower pollination algorithm for MPPT with high complexity in calculations. The authors in (22) proposed modified version of cat swarm optimization for MPPT which has high initial oscillation and tracking time.

This paper aims to develop a hybrid RCGA-DGSA-based MPPT for tracking GMPP. The hybrid algorithm integrates RCGA algorithm of global searching ability and DGSA of local search ability to increase maximum power point tracking ability. The simulations result under insolation change, load change, partial shaded conditions and extreme conditions, namely insolation change, load change, partial shaded condition and extremely partial shaded condition shows lower tracking time, no steady-state oscillation, very good dynamic response and higher execution time. The section 2 presents the modeling of the PV array. The section 3 describes the proposed hybrid RCGA-DGSA algorithm. The Section 4 validates the proposed MPPT algorithm. Finally, section 5 concludes the paper.

PV ARRAY

For simulating a PV system which is the series-parallel combination of PV cells, many single and two diode PV models have been published by researchers. Among them, the two diode model shown in Fig. 1 is used for modeling in the following section which is best known for its accuracy and simplicity with four parameters (23). The PV array consists of twelve PV modules connected four in series three in parallel and is configured with three different shaded conditions such as uniform, partially shaded and extremely shaded.

RCGA-DGSA BASED MPPT

The basic idea of a hybrid RCGA-DGSA is to combine the global searching ability in RCGA with the local search ability of DGSA. The RCGA is an optimization algorithm inspired by natural selection and genetic inheritance in biological organization (24) while the DGSA is a novel metaheuristics approach developed by the laws of gravitation (25) and motion and differential operator. This hybrid algorithm is used for MPPT. For many of the soft computing technique based MPPT like PSO, FLC, etc., the HC method acts as the basic structure for MPPT and it is applied for RCGA-DGSA based MPPT. The duty cycle given by





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$$d_i^{k+1} = d_i^k + \phi_i^{k+1} \tag{1}$$

Design of RCGA-DGSA Algorithm to MPPT

The photovoltaic system consists of a PV array boost converter and the load. The duty cycle generated from the proposed hybrid RCGA-DGSA MPPT algorithm is fed to boost converter for table operations.

The steps of the hybrid RCGA-DGSA algorithm are as follows:

Step 1: Parameter selection: The constants in algorithms, particle positions are set. $\alpha = 1$, $G_0 = 100$, $N_a = 4$. The duty cycle, d is particle position and the generated power, P_{PV} is the fitness function. The objective function is the current power greater than the previous power.

Step 2: Initialization of particles: The range of particles is initialized. Here d ranges from 30% to 72% of duty cycle.

Step 3: (Fitness Evaluation): The randomly generated duty cycles are generated from the MPPT algorithm and fed to the converter which produces a generated power, P_{PV} with size N_a . Using it, the fitness value is calculated for all the duty cycles.

Step 4: RCGA stage. The initial duty cycles act as the population of RCGA. These duty cycles undergo selection, crossover, and mutation of RCGA. The duty cycle undergoes tournament selection, BLX- α (Blended Crossover- α), uniform mutation operation with a constant mutation probability 0.01 and the final duty cycle is stored in G_{GA} .

Step 5: DGSA stage. With the duty cycles stored in G_{GA} , From F_{PV} , the force is calculated as,

$$F_i^k(t) = \sum_{j=1, j \neq i}^n rand_j G(t_0) \frac{t_0^\alpha}{t} \frac{M_i(t)M_j(t)}{\|d_i(t) - d_j(t)\|_2 + \varepsilon} (d_j^k(t) - d_i^k(t)), \alpha > 1 \tag{2}$$

where $rand_j$ takes the value [0 and 1], $M_i(t)$ and $M_j(t)$, masses of objects i and j . The duty cycle d is the G_{GA} and the mass $M_i(t)$ is calculated as follows:

$$M_i(t) = \frac{\frac{P_i(t) - w(t)}{b(t) - w(t)}}{\sum_{j=1}^n \frac{P_j(t) - w(t)}{b(t) - w(t)}} \tag{3}$$

where $q_i(t)$ is the strength of mass i at time t , $b(t) = \min(pi)$, $w(t) = \max(Pi)$ and P_i is the fitness function. The acceleration of the particle calculated from force F_{PV} is,

$$a_i^k(t) = \frac{F_i^d(t)}{M_i(t)} + \beta \cdot \delta \tag{4}$$

Step 6: Update: Update the velocity and position of each particle.

$$\phi_i^{k+1} = rand_i \phi_i^k + a_i^k \tag{5}$$

$$d_i^{k+1} = d_i^k + \phi_i^{k+1} \tag{6}$$

Step 7: Duty cycle calculation: The new duty cycle is,

$$d_{i,new}^k = [d_1 - Q_1, d_2 - Q_1 / 2, d_3 + Q_1 / 2, d_4 + Q_1] \tag{7}$$





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where Q_1 is the displacement factor and is taken as 0.05 (26) which helps to initialize the search area for the next iteration and send it to the converter and this process is repeated until the velocity becomes zero. The sampling interval is chosen to be 0.01 s (27).

COMPUTED RESULTS AND DISCUSSION

A simulation model is developed to analyze the proposed MPPT algorithm and is shown in Fig. 2. The array has 4×3 (series-parallel) modules with each module rated at 60W at STC (26). The boost converter is operated in the continuous current mode with parameters as in (26). The performance of the proposed method is compared with PSO (15) under irradiation change, load change, partial shaded and extremely shaded conditions.

Insolation change

At $t = 0.4$ s, the insolation is varied from $\lambda = 0.5$ kW/m² to $\lambda = 1.0$ kW/m² and at $t = 3.0$ s, the insolation varied from $\lambda = 1.0$ kW/m² to $\lambda = 0.5$ kW/m² with 25°C throughout the time. The voltage, current, power and duty cycle under the change in insolation (a) the PSO algorithm (b) RCGA-DGSA algorithm is shown in fig. 3. From fig. 3, for insolation change at $t = 0.4$ s, the duty cycle of PSO is slow stepped and takes 10 cycles to reach the MPP whereas the duty cycle of RCGA-DGSA suffers larger oscillation and takes 3 cycles to reach the GMPP due to the presence of gravitational element in GSA. And for insolation change at $t = 3.0$ s, the duty cycle of PSO is slow and linear stepped and takes 20 cycles to reach the MPP whereas the duty cycle of RCGA-DGSA suffers oscillation and takes 8 cycles to reach the GMPP. And both are stable after reaching the MPP.

Load change

The insolation is $\lambda = 1.0$ kW/m² and at $t = 1$ s, the load is varied from 100 % to 50 % then at $t = 3.2$ s, the load is varied from 50 % to 100 % with 25°C throughout the time. The voltage, current, power and duty cycle under the change in load (a) the PSO algorithm (b) RCGA-DGSA algorithm is shown in fig. 4. It can be observed from fig. 4, for load change at $t = 1$ s, the duty cycle of PSO takes one cycle to reach the MPP and the duty cycle of RCGA-DGSA takes one cycle to reach the MPP. And for load change at $t = 3.21$ s, the PSO takes a cycle to reach the MPP and the RCGA-DGSA takes a cycle to reach the MPP and both are under steady state after reaching the MPP.

Partial Shaded Conditions

The insolation is varied from high to low for below diagonal modules at $t = 1.6$ s and 25°C throughout the time. The voltage, current, power and duty cycle under Partial Shaded Conditions (a) the PSO algorithm (b) RCGA-DGSA algorithm is shown in fig. 5. It can be observed from fig. 5, for Partial Shaded Conditions at $t = 1.6$ s, the duty cycle of PSO oscillates and takes 15 cycles to reach the GMPP whereas the duty cycle of RCGA-DGSA suffers larger oscillation and takes 5 cycles to reach the GMPP due to exploration ability of RCGA.

Extreme Partial Shading

The insolation is varied from $\lambda = 1$ kW/m² at $t = 2$ s and 25°C throughout the time. The voltage, current, power and duty cycle under Partial Shaded Conditions (a) the PSO algorithm (b) RCGA-DGSA algorithm is shown in fig. 6. From fig. 6, for extreme Partial Shaded Conditions at $t = 2$ s, the duty cycle of PSO oscillates and takes 25 cycles to reach the GMPP whereas the duty cycle of RCGA-DGSA suffers larger oscillation and takes 10 cycles to reach the GMPP due to exploration ability of RCGA. The Table 1 shows the simulation results of PSO and RCGA-GSA based MPPT. It can be observed from table 1 that the RCGA-DGSA tracks the MPPT within a few tracking cycles while PSO suffers additional tracking cycles.

Further, Table 2 shows the comparison of proposed method with existing literature in terms of Tracking Speed, Steady-state response, dynamic response and Execution time. The RCGA-DGSA has high tracking speed, zero steady-state oscillation, a very good dynamic response such that till reaching the MPP, there is very little oscillation



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while suffering low execution time because of the hybrid nature. Lower oscillation gives the stability in tracking MPP and stable operating the power converter resulting in low power loss. The absence of steady-state oscillations makes it very suitable for handling extreme partial shading conditions. However, the hybrid RCGA-DGSA algorithm is little time consuming and complex than PSO. Therefore, it can be seen that the hybrid RCGA-DGSA is superior to all the other methods due to its additional exploration and exploitation ability.

CONCLUSION

In this paper, a hybrid RCGA-DGSA with the additional exploration and exploitation ability is implemented to track the GMPP under Insolation change, load change, partially shaded condition and extremely partially shaded condition. The hybrid RCGA-DGSA is robust with the global searching ability of RCGA to track the GMPP and additional local search capabilities of DGSA. The simulations result under Insolation change, load change, partially shaded condition and extremely partially shaded condition demonstrates the lower Tracking Speed, no Steady-state oscillation, very good dynamic response till reaching the MPP with higher execution time. This shows that the RCGA-DGSA is better than the PSO-based MPPT method.

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Table 1. The comparison of PSO and RCGA-DGSA

Shading Pattern	Tracking method	Voltage(v)	Current(A)	Power(W)	No of Tracking cycles
Insolation change	PSO	233.5	2.84	665	20
	RCGA-DGSA	240	2.93	705	8
Load change	PSO	113.9	1.65	188.7	1
	RCGA-DGSA	123.6	1.54	190.6	1
Partially shaded condition	PSO	110.8	1.31	145.7	15
	RCGA-DGSA	128	1.59	203.6	5
Extremely shaded condition	PSO	120.5	1.45	175.7	25
	RCGA-DGSA	130	1.54	200.4	10

Table 2. Qualitative comparison between different methods

Algorithms	Tracking speed	Steady-state oscillation	Dynamic response	Execution time
PSO (15)	Fast	Nearly zero	Oscillatory	Fast
IPSO (16)	Fast	Zero	Highly oscillatory	Fast
DPSO(17)	Fast	Zero	Highly oscillatory	Fast
Fireflies(19)	Fast	Zero	Oscillatory till reaching MPP	Fast
Proposed RCGA-DGSA	Fast	Zero	Few oscillation till reaching MPP	Slow





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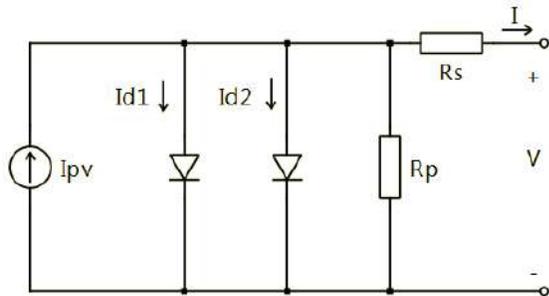


Fig 1. Two-Diode model of the PV cell.

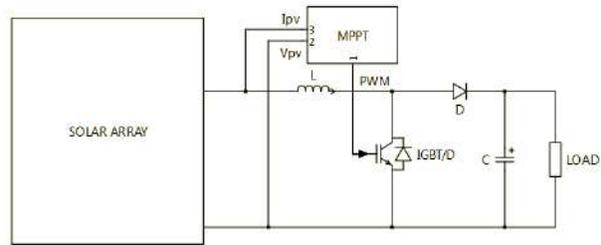
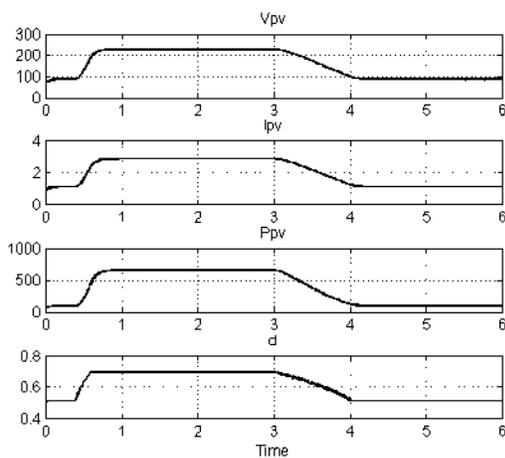
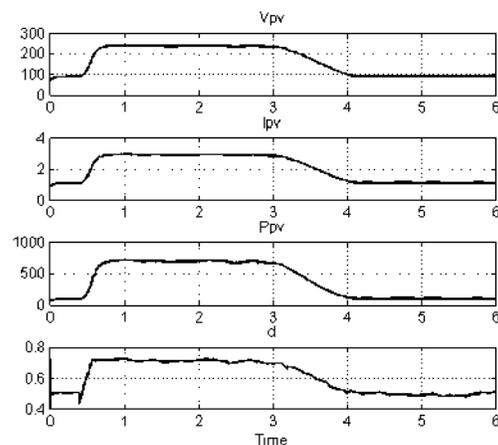


Fig 2. PV system with PV array, boost converter with MPPT controller and load.

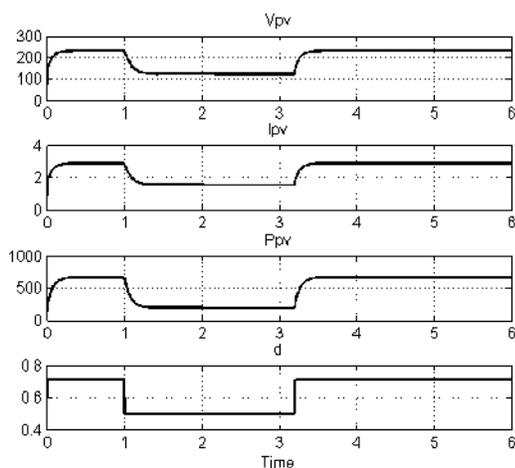


(a)

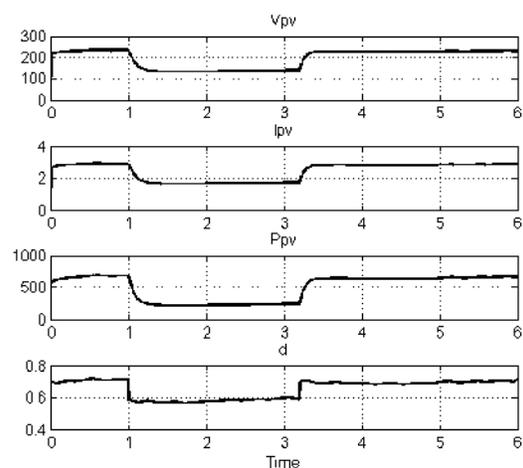


(b)

Fig 3. The voltage, current, power and duty cycle under the change in insolation (a) the PSO algorithm (b) RCGA-DGSA algorithm.



(a)



(b)

Fig 4. The voltage, current, power and duty cycle under change in load (a) the PSO algorithm (b) RCGA-DGSA algorithm.





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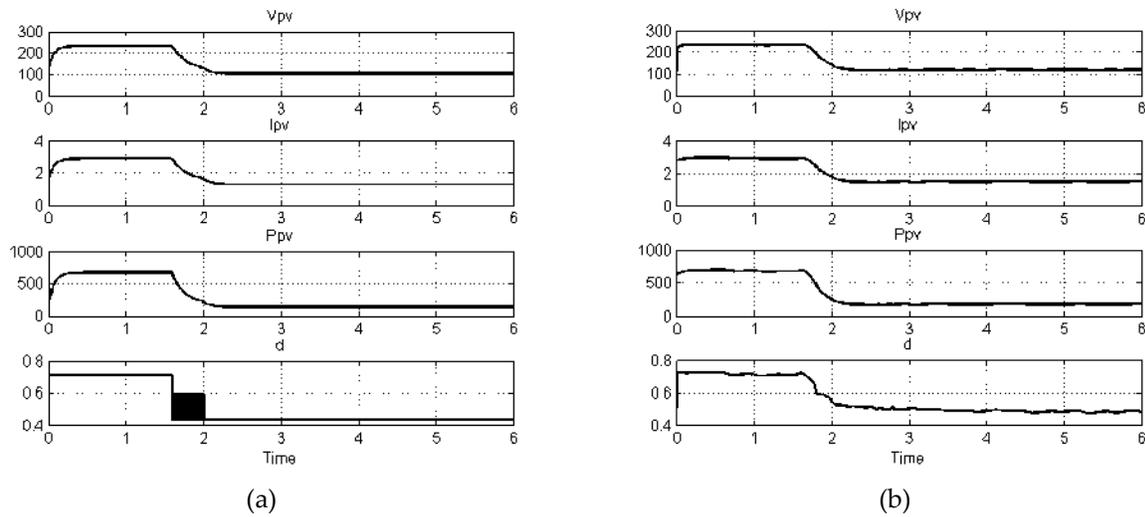


Fig 5. The voltage, current, power and duty cycle under Partial Shaded Conditions (a) the PSO algorithm (b) RCGA-DGSA algorithm.

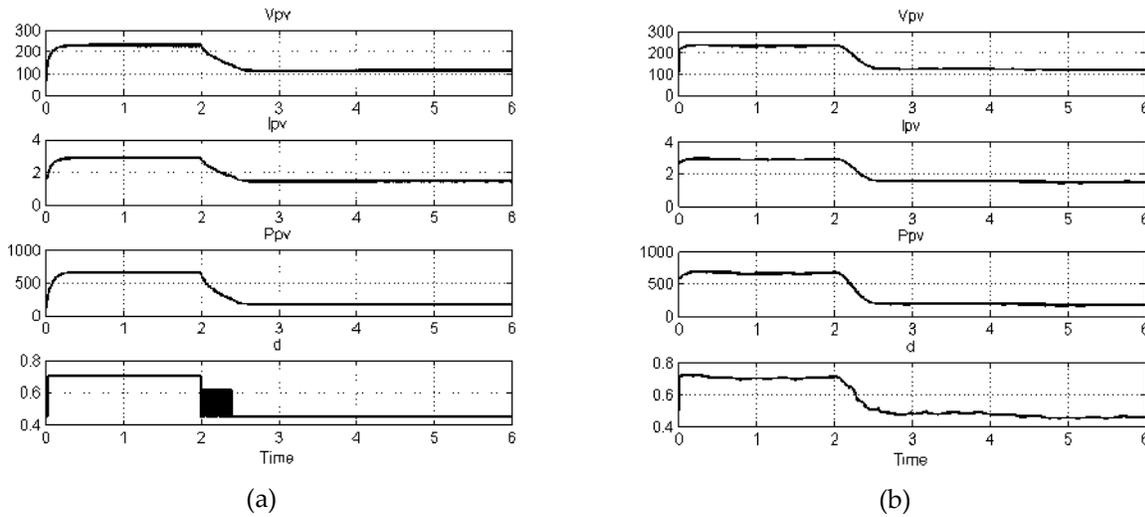


Fig 6. The voltage, current, power and duty cycle under Extreme Partial Shaded Conditions (a) the PSO algorithm (b) RCGA-DGSA algorithm.





Structural, Optical and Magnetic Properties in Rare-Earth Doped Multiferroic BiFeO₃

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ABSTRACT

Neodymium and Gadolinium co-doped samples are successfully synthesized by the solid-state reaction method. The X-ray diffraction patterns showed the Nd-Gd co-doped BiFeO₃ (BFO) ceramic exhibits rhombohedral R3c structure and did not suffer a more significant structural modification from BFO. Bismuth-rich residual phases such as Bi₂Fe₄O₉ and Bi₂₅FeO₄₀ are appeared along with the parent. Nd-Gd induces octahedra distortion in BFO due to changes in bond length and bond-angles, confirmed by Raman spectroscopy. The bandgap of Nd-Gd co-doped BFO decreases to 1.96 eV from the parent 2.08 eV of BFO. M-H plot for co-doped BFO shows that the magnetization reaches the value 0.0185 emu/gm. The increase in magnetic properties in the co-doped system may be due to the suppression of spiral spin modulated structure and magnetic interaction between the parent and foreign ions.

Keywords: BiFeO₃, Magnetic properties, Nd-Gd induces, BFO.

INTRODUCTION

In the recent past, the demand for multiferroic materials has been quite high due to their unique physical properties and ability to perform extraordinary multifunctional applications at room temperature. Multiferroic materials exhibit more than two ferroic orders, such as ferroelectricity, ferromagnetism, ferroelasticity, etc., simultaneously in a single phase. Due to such complexity, the number of these materials is very rare to date. Various materials show multiferroicity either at low-temperature or high-temperature; however, the room temperature multiferroics are always unique. Out of several multiferroics, BiFeO₃ (BFO) is one of the unique and well-studied multiferroic materials showing ferroelectricity at 845° C and anti-ferromagnetism at 370° C, which are well above room temperature [1, 2]. Ferro electricity in this material arises due to hybridization of Bi³⁺ lone pair with both vacant 6p⁰ orbitals of Bi³⁺ ion and the 2p⁶ orbitals of O²⁻ ion [3]. Whereas, ferromagnetic properties arise due to super-exchange interaction between Fe³⁺ ions through O²⁻ ions. The presence of high-leakage current and spin modulated cycloid of length 62nm hinders the ferroelectric and ferromagnetic properties of BFO [4, 5]. Multiferroic BFO also exhibits





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magnetoelectric properties, which have a broad extraordinary application in sensors, actuators, and spintronics [6]. The magnetic behavior in BFO originated from the Fe^{3+} ions and showed anti-ferromagnetic ordering along [111] direction [7]. Due to Dzyaloshinskii-Moriya interaction and the presence of spiral spin modulated structure (SSMS) of order 62 nm results in a weak ferromagnetic signal in BFO [5]. Methods such as doping either at A-site (Gd^{3+} , Nd^{3+} , Dy^{3+} , Ho^{3+} , Y^{3+} etc.) or B-site (Ti^{4+} , Sc^{3+} , Mn^{3+} , Zr^{4+} etc.) or co-doping at (A, B-site): (La, Ti), (Y, Zr), (Sm, Sc), (Gd, Ti) etc.) successfully enhance the magnetic properties in BFO [8-12]. Rare-earth Gd^{3+} ions exhibit a more significant magnetic moment ($7.88\mu_B$) and the highest number of shared pair electrons in their outermost shell. Similarly, Nd^{3+} ions also have a $3.62\mu_B$ magnetic moment. Combining these two unique rare-earth dopants may induce drastic physical properties of BFO for applications at room temperature. In this report, we explain the structural, optical and magnetic properties of BFO after Nd-Gd substitution.

Experimental Details

$\text{Bi}_{0.85}\text{Nd}_{0.05}\text{Gd}_{0.15}\text{FeO}_3$ (BNGFO) ceramic is prepared through the conventional solid-state reaction method. High purity of 99.99 % initial precursors such as Bi_2O_3 , Nd_2O_3 , Gd_2O_3 , Fe_2O_3 powders (99%, Sigma Aldrich) are used as starting materials. All the powders are taken in stoichiometry ratio before mixing. The mixed powders are grounded in a mortar-pestle for 2 hours. After complete mixing, the mixed powder is calcined at 810°C for 5 hours using a box furnace. The calcined powder was further mixed with poly-vinyl alcohol (PVA) as a binder to enhance the strengthening and density of the sample. Then, the final powers are pressed into 8 mm diameter pellets and sintered between 820°C - 850°C for 2 hours to obtain the desired phase. Finally, the furnace is cooled down to room temperature. The structure and phase purity of the Nd-Gd co-doped BFO samples are observed by X-Ray diffraction (XRD) employing $\text{Cu-K}\alpha$ radiation (Rigaku Smart Lab, 2012). The optical properties are investigated using Raman ($100\text{-}700\text{ cm}^{-1}$) and UV-visible spectroscopy (Perkin Elmer Lambda 1050) techniques. Magnetic measurements are carried out using Vibrating Sample Magnetometer (VSM) by applying a maximum magnetic field of 15 kOe at room temperature.

RESULTS AND DISCUSSION

Figure 1 shows the XRD patterns for BNGFO ceramic measured at room temperature (RT). The crystalline peaks in XRD are well matched with parent BFO and other experimental results (JCPDS no. 86-1518) [13]. After a rigorous analysis, the BNGFO film exhibits rhombohedral crystal structure with $R3c$ space group i.e. BNGFO retains its original structure after co-doping technique. The peak positions may evident a slight shift due to incorporation of doping, which will create a change in lattice parameters. The change in lattice parameters induces lattice distortion at FeO_6 octahedral cage, which in turns affects the physical properties of BNGFO from its pristine condition. The observance of octahedral distortion in BNGFO may be due to the slight mismatch in ionic radii between parent Bi^{3+} (1.03\AA) ions and foreign Gd^{3+} (0.93\AA) and Nd^{3+} (0.99\AA). From Fig. 1, the residual phases such as $\text{Bi}_2\text{Fe}_4\text{O}_9$ (* mark), $\text{Bi}_2\text{Fe}_4\text{O}_9$ (# hashtag) identified from the XRD pattern confirming the as-synthesize sample are polycrystalline in nature after sintering process, which has been also observed in other Gd-Nd doped BFO system [13]. From the previous results, the impurity percentage is found to be high at high Gd-doping concentration, which is also reflected in our studies [8]. Also, at higher Nd concentration, the impurity is minimum [13]. Hence, combination of such a system is interesting for further studies. These impurities can be suppressed by applying a temperature above the phase formation. In the present system, we applied 850°C to obtained lower percentage of impurity. However, methods such as thermal quenching, rapid thermal annealing, etc. helps to reduce the secondary phases [14-16]. Raman spectra are regarded as the figure-print of the sample, which provides vibrational modes present in the sample. Figure 2(a) elucidates the RT Raman spectra for the BNGFO sample showing the various vibrational modes at different wavenumbers. The Group theory calculations and other experimental results show that the rhombohedral $R3c$ multiferroic BFO exhibits 13 various phonon modes with the expression [17, 18]:

$$\Gamma R3c=13A1+9E$$

(1)





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where the A-modes and E-modes arise due to parallel polarization and cross-polarization, respectively [19]. However, some E-modes are missing from the BNGFO spectra may be due to the increase in oxygen chemical pressure at high sintering temperature. Also, the peak positions are slightly shifted towards the lower wave number due to a mismatch in the ionic radii. Some other studies reveal that Gd-Nd co-doping shifted the phonon modes towards the higher wave number when the doping concentration of Nd is higher [20]. The shifting of phonon modes supports the change in Bi-O and Fe-O bond length, which causes structural disorder and ultimately affects the ferroelectric properties of BFO. UV-visible spectroscopy is a useful technique to identify the bandgap of a sample. The bandgap of the as-prepared sample is calculated by using Tauc's plot expressed as:

$$E_g - hv = (\alpha hv)^2 \quad (2)$$

where E_g is the bandgap or energy gap, h and ν are the Planck's constant and frequency, respectively. α is the absorbance coefficient of the sample. The calculated bandgap is esteemed at around 1.96 eV, as shown in Fig. 2(b). The calculated E_g of BNGFO decreases from its parent compound BFO ($E_g = 2.08$ eV) [13]. The suppression of bandgap due to Nd-Gd co-doping may be due to the structural distortion, change in bond distance between Bi-O and Fe-O, and other defects created due to the appearance of secondary phases. However, the obtained bandgap is in the range for the application of photovoltaic solar cells.

The RT magnetic properties of BNGFO ceramic are explained through the M-H hysteresis curve by applying a maximum magnetic field of 15 kOe, as depicted in Fig. 3. The magnetic remanence (M_r) (0.0185 emu/gm) is found to be an increase from BFO (0.002 emu/gm) and other higher percentage Nd-doped BFO (0.0175 emu/gm) systems; however, the magnetization curve is not completely saturated, indicating a weak magnetization and requires a larger magnetization for saturation [13]. The contribution from impurity phases can be neglected due to their paramagnetic behavior [21]. The presence of a higher magnetic moment with a large number of shared pair electrons in the outermost shell of the Gd^{3+} ion may play a crucial role in increasing the magnetization in BNGFO. The presence of spin cycloidity suppresses the ferromagnetic properties of BFO; however, such effects can be eliminated by doping methods, which is reflected in the present studies where the higher Gd-doping enhances the magnetism. Also, the change in Fe-O bond length and Fe-O-Fe bond angle (evident from XRD and Raman), FeO_6 octahedral distortion help increase the BNGFO system's magnetic moments. The high coercivity (H_c) compared to BFO confirms the incorporation of Nd and Gd in the BFO lattice.

CONCLUSION

In summary, the Nd-Gd co-substituted $BiFeO_3$ (BNGFO) ceramics are synthesized by using solid-state reaction technique. XRD patterns confirm the BNGFO exhibits rhombohedral crystal structure with the $R3c$ space group. This ensures doping did not affect the crystal structure of BFO. Co-doping induces a change in bond length and bond angles, which distorts the octahedral cage and affects the physical properties of BFO. Nd-Gd helps to decrease the bandgap of BFO in the range for the application of photovoltaic solar cells. The magnetic properties are enhanced due to suppression of spiral spin modulated structure and other magnetic interactions between the ions.

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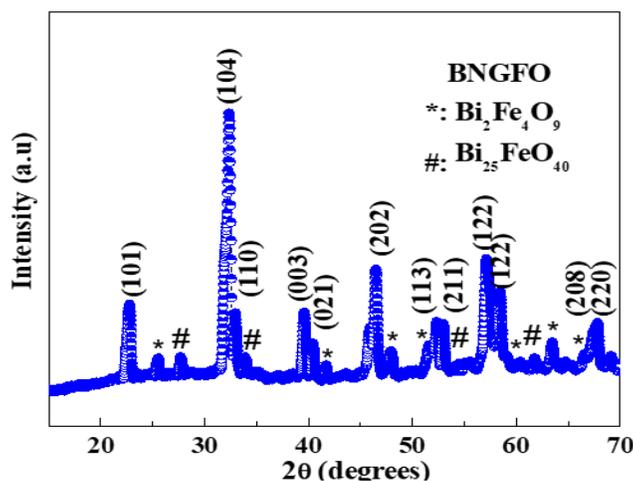


Figure 1. XRD patterns for Nd-Gd co-doped BFO (BNGFO) ceramics obtained by scanning the Bragg's angle between 100 to 700. The XRD pattern shows the origin of impurities phases along with BFO, which are marked with * and # symbols.





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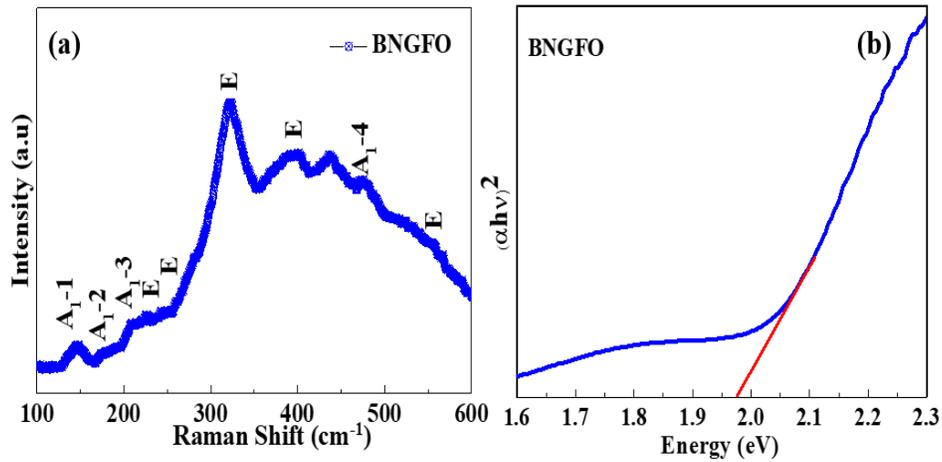


Figure 2. (a) Raman spectra showing the various vibrational modes of BFO after Nd-Gd co-doping. (b) UV-visible Tauc's plot showing the bandgap of the as-synthesize sample at room temperature.

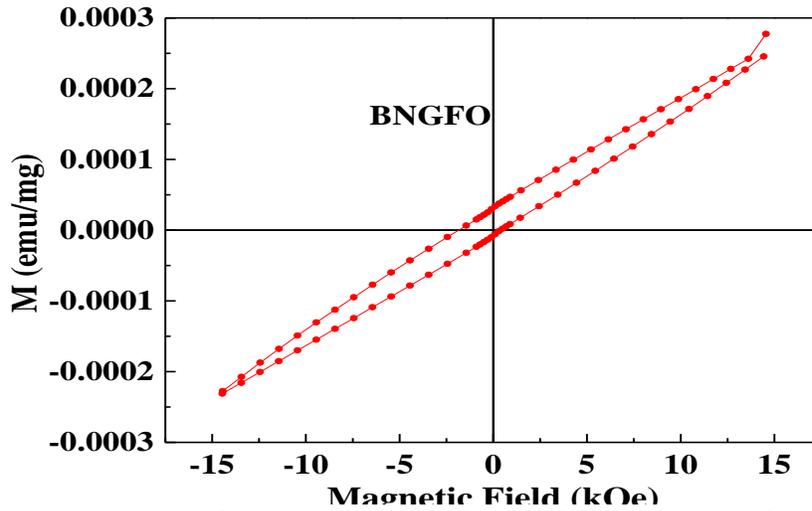


Figure 4. Room temperature magnetic hysteresis curve (M-H) for BNGFO ceramic is obtained by applying a maximum magnetic field of 15kOe. M-H curve showing weak ferromagnetic signal in BFO after co-substitution.





Efficacy of Task Oriented Training Versus Trunk Stabilization Exercises Trunk Control Ability Among Stroke Patients – A Comparative Study

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ABSTRACT

The purpose of the study is to investigate the effect of task oriented training and trunk stabilization exercise on trunk control ability among stroke patients. 44 patients were randomly divided into two groups. 22 patients in the experimental group A performed task oriented training (5times/week/16weeks) and 22 patients in the experimental group B performed trunk stabilization exercises; both the groups received conventional therapy. The Trunk Impairment Scale was used as outcome measure. On comparing pre and posttest mean values of Group A and Group B on TIS shows significant improvement in the post test Mean but Group A (18.6) and Group B (16.18) at p value <0.05 was significantly higher than before the intervention in both groups (p<0.05) and showed a greater improvement than the experimental group B. Task-oriented training and trunk stabilization exercises both showed improvement on trunk control among stroke survivors. Task oriented training group is more effective over trunk stabilization exercises on enhancing trunk control ability.

Keywords: Stroke, Trunk control, Task oriented training, Trunk stabilization exercises.

INTRODUCTION

Stroke is rapidly developing clinical signs of focal disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin. Worldwide, Stroke remains the



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second leading cause of death and the third leading cause of disability with permanent motor disability results in some degree of muscle weakness. In addition, muscle paresis and decreases in balance and mobility are also observed¹. Following a decrease in balance, stroke patients show postural sway, asymmetrical production of force, and migration of the center of mass to the unaffected lower extremity. The strong relationships among the measures of balance, gait and functional ability highlight the importance of trunk rehabilitation[1].

Globally, 70% of strokes and 87% of both stroke-related deaths and disability-adjusted life years occur in low- and middle-income countries. A WHO study which ahead of the world stroke day Friday, has quoted the incidence of the disease in India to be around 130 per 100,000 population every year and says about 20% of heart patients are susceptible to it. The disease has turned out to be the most common disability and dependence with more than 70% of stroke survivors remaining vocationally impaired and more than 30% requiring assistance with activities for daily living[2].

Trunk control is the ability to maintain the upright posture of the body, to adjust weight shifting, and to perform selective movements in the trunk to maintain the center of mass within the base of support[3]. The percentage of the variance of functional recovery after a stroke is explained by trunk control ranges from 45% to 71%. Trunk seems particularly important for balance as it stabilizes the pelvis and spinal column¹². Trunk muscles play an important role in the execution of daily activities, such as sitting, standing from a chair, transferring between different lying positions, and walking[4]. Activation of these trunk muscles has a relationship with gait speed and the Functional Independence Measure[5].

Trunk control needs to be preceded in order to control distal limb movements and is correlated with functional movements[6]. The trunk muscle weakness and the loss of proprioception concerning the affected side can interfere with balance, stability, and functional disability and may reduce ability to control posture. These patients have increased risk of falling toward the paretic side and limited functional abilities[7]. As a consequence, all these effects can bring difficulties in leading independent life. The trunk training exercises is an effective rehabilitation strategy for improving trunk performance with respect to gait performance[8]. Implementing core stability exercises may be a viable strategy for improving trunk performance and dynamic sitting balance, standing balance, and gait in post-stroke patients[9]. Contemporary task oriented approach are approaches to treat motor dysfunction, incorporating principles of motor learning during interventions focused on re-mediating motor control in persons with central nervous system dysfunction. Task oriented training is a method which focuses on specific functional tasks associated with musculoskeletal and neuromuscular systems[10].

Task oriented training involves a variety of practices to help patients to derive optimal control strategies for solving motor problems[10]. During task oriented training, many types of movement are practiced, to limit compensatory movements and increase adaptive movements. There is growing evidence that intensive, task oriented practice results in greater improvement than conventional therapy in walking competency of people with stroke[9]. In addition with task oriented approach, arm training using functional tasks such as grasping objects, CIMT and mental imagery are used. Such training is task and patient focused and not therapist focused. The strong relationships among the measures of balance, gait and functional ability highlight the importance of trunk rehabilitation[10].

Studies measuring trunk performance after a stroke have used various clinical tools, including the Trunk Control Test, The trunk impairment scale by Fujiwara (TIS-F) and Trunk Impairment Scale (TIS-V) by Verheyden in 2004. These three tools exhibit good psychometric properties and are suitable for use within the clinical setting; they do not require specialized equipment. The TCT measures trunk control in static positions, giving relatively minimal information. The TIS-V measures during selective movements of the trunk, in both static and dynamic positions, including flexion, extension, lateral flexion and rotation. The TIS-F has been used in less number of studies to assess impairment of the trunk in people with a stroke, neither of which applied trunk exercises. TIS that will be referred to throughout the rest of this paper is the TIS-V. The Rasch analysis version of TIS was established in 2010 and led to the elimination of the static sitting balance sub scale[10]. To





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investigate the comparative effects of task oriented training over trunk stabilization exercise along with conventional therapy on trunk control ability among stroke patients.

MATERIALS AND METHODS

This study was an pre and post experimental study design conducted at Department of Physiotherapy, Vinayaka Mission's Hospital, Salem, Tamilnadu, India. This study was done on a population of 44 subjects post stroke comprising 17 females and 27 males with an age group between 45 and 55 years. Duration of the study was 4 months.

Inclusion Criteria: Both the genders (male and female) with age group (45 – 55 years) patients who had experienced their first stroke, whether ischemia or hemorrhagic, who had the ability to walk 10m independently using an aid or orthotic with or without supervision/aid. Minimum score of 20 in MMSE subjects were included, and those who could sit independently for 30seconds on a stable surface, and subjects who were medically stable.

Exclusion Criteria: Subjects with chronic stroke (brainstem or cerebellar stroke), significant disability prior to stroke as evidenced by TIS Score >10. Other exclusion criteria included orthopedic/neurological impairments that could influence sitting balance, and presence of acute low back pain, inability to understand instructions assessed by a MMSE score<24, apraxia, hemi neglect, difficulty walking 10m distance, fracture of the musculoskeletal system. Materials: Inch tape, two standard chairs (one with arm rests, one without), stopwatch.

Procedure: Informed consent from the participants was taken before including the participants in the study. Based on inclusion and exclusion criteria the participants were recruited for the study. Selected 44 samples on the basis of inclusion and exclusion criteria were randomly divided equally by lottery method into two experimental groups A and B. Both groups were allocated 22 samples each. Pre and post assessment on Trunk control using trunk impairment scale was done. The experimental group A was trained with task oriented training program while the experimental group B was trained with trunk stabilization exercises.

Experimental group A: Task oriented training consisted of tasks designed to enhance trunk control and to strengthen the extremities along with walking balance, speed and distance. Duration of the training was 45mins/5days/week/16weeks. The tasks were –Reaching an object at different points; Catching a Swiss ball and throwing back; Ball twisting exercise; Step ups; Kicking a ball and crossing the obstacle; Stand-up and walk, and Sit to stand.

Experimental group B: Trunk stabilization exercise consisted of trunk stabilization exercises, weight shifting training and core stability exercises. Duration of the training was 45mins/5days/week for 16weeks.

In Supine: Pelvic Bridge; Unilateral Bridge; Upper trunk flexion rotation; Lower trunk flexion rotation (placing the soles of the feet on the ground and bringing the pelvis diagonally to the shoulder).

In Sitting: Upper trunk lateral flexion (descending the elbow to the table from the shoulder girdle); Lower trunk lateral flexion (raising the pelvis from the table in the direction of ribcage from the pelvic girdle); Upper trunk rotation (moving the shoulders forward and backward); Lower trunk rotation (moving the knees forward and backward)

In Prone:Weight shifting and cobra stretches. Both the groups had underwent conventional therapy program which was patient specific and consists mainly - tone facilitation, stretching, passive mobilization, and range of motion exercises for the hemi paretic side, and gait training.





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RESULTS AND DISCUSSION

The outcome value obtained from the data collection were tabulated on the spread sheets in the 'Microsoft Office Excel 07' and were exported to IBM SPSS version 16 for the statistical analysis of the data – Mean, median and standard deviation of the collected data done, using paired t-test to find statistical difference within the groups and Independent t-test (Student t-test) was used to find statistical difference between the groups. Post hoc test is done as an integral part of ANOVA. When you use ANOVA to test the equality of at least two group means, statistically significant results indicate that not all of the group means are equal. Both the genders, male and female with the mean age group of 45±20 were participated in this study. On comparing mean values of Group A TIS(18.6) showed significantly effective improvement post intervention. Hence, null hypothesis is rejected and alternative hypothesis is accepted. On comparing mean values of Group B TIS (16.18) showed significantly effective improvement post intervention. Hence, null hypothesis is rejected and alternative hypothesis is accepted. After the completion of 16 weeks of the intervention, the TIS was significantly higher than before the intervention in both the groups ($p<0.05$), and it showed a significant difference between the experimental group A and experimental group B ($p<0.05$). Hence, null hypothesis is rejected and alternative hypothesis is accepted.

This study examined the effects of task oriented training for stroke patients on ability of trunk control to suggest a proper approach for improving functional movements after stroke. In this study, TIS was used to evaluate the ability of trunk control. After the experiment, there was a significant difference in the ability of trunk control between the two groups. The increase in TIS was significantly larger in the experimental group than in the control group. Therefore, task-oriented training was effective at improving the ability of trunk control. In static control, there was some significant difference between two groups; this is because all subjects in this study were able to walk independently, so they had no difficulties in maintaining static sitting balance.

In the present study, the stand up and walk task was performed but a suitable training time and repetition for each subject were needed. Therefore, when starting task-oriented training, the training duration and repetition number for each item should be considered, and subjective and standardized criteria for the time and number of repetitions for each item will be needed in future studies.

CONCLUSION

Task-oriented training was effective at enhancing trunk control ability, and gait and balance also improved significantly. Therefore, we consider that task-oriented training with general physical therapy is an effective intervention for stroke patients. Future studies need to determine when a specific intensity task or standard loading task in a task-oriented training program should be performed. In addition, more study will be needed to examine the factors of trunk stability that affect the correlation of trunk control.

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Conflict of Interest

The authors have none to declare.





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Table 1. Dependent 't' Test Performed with Pre Test Values of Trunk Control Ability Using Trunk Impairment Scale for Significance With in Experimental Group A

VARIABLE	't'cal value	't'Table value
TRUNK CONTROL ABILITY	17.021	1.761

"t" calculated value > "t" table value
Significant at 5% level.

Table 2. Dependent 't' Test Performed With Pre Test Values of Trunk Control Ability Using Trunk Impairment Scale For Significance With in Experimental Group B

VARIABLE	't'cal value	't'Table value
TRUNK CONTROL ABILITY	16.098	1.761

"t" calculated value > "t" table value
Significant at 5% level.





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Table 3. Dependent 'T' Test Performed With Post Test Values of Trunk Control Ability Using Trunk Impairment Scale for Significance With in Experimental Group A

VARIABLE	't'cal value	't'Table value
TRUNK CONTROL ABILITY	21.097	1.761

"t" calculated value > "t" table value
Significant at 5% level.

Table 4. Dependent 't' Test Performed With Post Test Values of Trunk Control Ability Using Trunk Impairment Scale for Significance With in Experimental Group B

VARIABLE	't'cal value	't'Table value
TRUNK CONTROL ABILITY	20.012	1.761

"t" calculated value > "t" table value
Significant at 5% level.

Table 5. Independent 't' Test Performed With Pre & Post Test Values of Trunk Control Ability Using Trunk Impairment Scale for Significance Between Groups

VARIABLE	't'cal value	't'Table value
TRUNK CONTROL ABILITY	1.85	1.761

"t" calculated value > "t" table value
Significant at 5% level.





Study of Awareness and Knowledge towards Doping

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ABSTRACT

In today's modern era, the consumption of prohibited medicines in sports is increasing day by day, every fortnight a case of doping is found in headline of newspaper, which is proving very dangerous to the sports community in health, name and fame. The Present study was conducted to find out differences in Awareness and Knowledge of Doping between sportspersons and non-sportsperson of Haryana state. There were total 250 sportspersons and 250 non-sportspersons were randomly selected, who were ready to give their response and actively participated in the present study. After giving their consent at their training center the data were recorded and collected. The data was collected with the help of W. J. Kamenju's Questionnaire, which have comprises 08 Questions of Awareness in part 'B'. The 't' Test was used to interpret and tabulated the data. The results of study showed that. The analysis of results of study and after reviewing of others study, we can conclude that Sportsperson have more knowledge and awareness towards doping and it is significance difference at .05 level of significant. The results also showed that, after significant different it is concluded that Sportspersons have no proper knowledge and awareness towards doping and there are also shortage of literature and materials that whom educate the athlete about the deadly effects of doping. The study also suggests that latest and more literature of doping should be included in curriculum.

Keywords: Sports, Doping, Health, Awareness, Knowledge and Sportsperson.

INTRODUCTION

In today's modern era, the consumption of prohibited medicines in sports is increasing day by day, every fortnight a case of doping is found in headline of newspaper, which is proving very dangerous to the sports community in health, name and fame. Winning in sports is ultimate goal of every sportsperson, so to achieve top performance



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sportsperson are doing this act i.e. either fixing the competition or by using the illegal and unethically mean and methods which are not permitted in sports. It is well known that using of doping in sports affects every sportsperson while it is amateur sportsmen as well as professional sportsmen. It is also notable that over the past some decades the sportsperson have been competing on at par with their counterparts at the various level of sports at National and International level. It is very dangerous symptoms that at grass root level of sports many sportsperson do not know the how much hazardous effects of doping. The lack of data on the use and awareness of drugs by sportspersons prevents us from knowing the reality. As per the present situation, it is necessary to collect such data before preventive measures can be implemented, as appropriate. Kim T and Kim YH in (2015) conduct a cross-sectional study on 454 Korean elite adult and adolescents' athlete's adolescents in 22 events.

Data were collected by Performance Enhancement Attitude Scale (PEAS) to find out regarding doping practice, knowledge and use of performance-enhancing substances. They found that 57.00 % adult and 47.3 % adolescent's athletes got the information of their sports from Korea Anti Doping Agency, 39.00 % adult and 54.4% adolescent's had knowledge and lenient attitudes toward doping in comparison of unaware athletes. Adolescents 1.5% and adults 3.6%, respectively have accidentally. Adolescent 1.0% and adult 2.8%, athletes knowingly taken banned performance-enhancing substance. And 2.4% adolescents and 3.2 % adult athletes knew the sportsman who had taken the banned substance. The adolescent athletes in motor skill category (PEAS: 40.24±10.91) were more permissive toward doping than those in team category (PEAS: 35.08±10.21). An in-depth anti-doping education for Korean athletes should be more widely implemented, and effective anti-doping policy should meet the athletes' demographic characteristics, personalities, and values. Krzysztof Sas-Nowosielski (2017) conducted a twofold study first was aim to find out the attitude of athletes towards Performance Enhancing Substance second aim was to validation the Polish version of the Performance Enhancement Attitude Scale (PEAS). Total 340 athletes, 173 males and 167 females' athletes of 13 sport disciplines were selected to achieve this aim.

The validation of Polish version of PEAS is recommended in 11 items. According to the findings of the study, Polish athletes rather do not approve of doping behaviors. Men were more likely to use illegal substances than women. The most positive attitude towards doping was found for sport disciplines where there is no contact with the competitor. Masucci, Matthew A. et al (2019) conducted a study to investigate knowledge of doping practices and anti-doping education among American and Canadian female professional triathletes. The second aim of this study was to check the motive of taking the prohibited substance, as well as to identified the reason to engaged in doping practices. There were six Canadian and six American female professional triathletes (n=12) selected to avail the data, and semi-structured interviews (Sparkes & Smith, 2014) were used to get the response from athletes. The inductive thematic analysis of the transcript data yielded four major themes including, 1) changes in the sport over last 15 years; 2) perceptions of prevalence and availability of doping; 3) perceptions of anti-doping efforts and; 4) perceptions and experiences of anti-doping education approaches. These perceptions are in alignment with the findings of Bloodworth and McNamee (2010) and Mottram, Chester, and Gibson (2008), participants in this study also felt that shame and guilt were major factors in refraining from prohibited doping, rather than any supposed harmful health effects. The findings from this study certainly point to a number of possible improvements that might be made in the efforts to curb doping in triathlon.

Objectives of Study

The main objective of this study is to find out difference in Awareness and Knowledge of Doping between Sportspersons and Non-sportsperson of Haryana.

METHODOLOGY

The investigator were contacted the sportsman at their training center of district level for the present study, 250 sportspersons and 250 non-sportsperson of Haryana were randomly selected for the present study for the collection of data. The Questionnaire was developed W. J. Kamenju which was used for collection the data. Most of players feel



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comfort in Hindi so that the Questionnaire also translated into Hindi, because most of sportsman difficult to understand English. The questionnaire have two part A and B, A parts covers Attitude towards Doping and the 'B' part of Questionnaire comprises 08 Questions of Awareness and Knowledge of Doping, so that as per requirement of the study only B parts of the questionnaire was used. The respondents gave on 'Yes' or 'No' answers. The Questionnaire was filled up when the sportspersons in resting time and fresh and happy mood. The t-test and simple percentage was used to analyze the data results were assessed. The result was showed in tables and graphs.

RESULTS OF THE STUDY

Table No. 1 Showed that mean value of Awareness and Knowledge towards Doping of Sportspersons and Non-sportspersons of 30.02 and 25.37 respectively, whereas SD of Sportspersons and Non-sportspersons is 6.19 and 5.35. The calculated t-value is 6.682, which is significant at .05 level of significance. Therefore the results of study showed that there is significance difference found in Awareness and Knowledge between Sportspersons and Non-sportspersons, and null hypothesis of the study is rejected.

Table No 2 showed that the Sportspersons have more Awareness towards Doping than Non-sportspersons, table also show that (110) sportspersons have know about Anti Doping Code as compare to non-sportspersons which have only (50). In response to "I personally know Sportsperson who have used Performance-enhancing Substances" the non-sportspersons (90) was less than sportspersons (160). When asked about using of performance enhancing substance by their friends, the response of sportspersons is more compare to Non-sportspersons; it means more sportspersons used performance enhancing substance than non-sportspersons. In response to Question No. 4 the results was very drastic, because both sportspersons and non-sportspersons agreed to not use of Doping due to their health risk.

The answer of Question No. 5 was also showed that very high no. sportspersons and non-sportspersons want to proper knowledge and education of that substance which increased the sports performance. 150 sportspersons and 160 non-sportspersons are agreed to players should be tested for performance enhancing substances, it mean testing system of doping is not proper or systematic. As per Question No 7 the data showed very few players have learnt about performance enhancing Drugs. Last Question showed that a high numbers of players want to Govt. or others agencies, educate them and provide proper knowledge about Doping.

CONCLUSION AND DISCUSSION

The results of the showed that, sportspersons have more awareness than non-sportspersons. Morente, Sanchez and Zabala M. also studied that most of athletes have familiar anti-doping rules but they have no proper knowledge of doping as well as not have a proper knowledge of food supplements. Muwonge H., Zavuga R., Kabenge PA., (2015) studied that use of doping agents in this study was low, which may suggest that fewer athletes use doping agents in the African country of Uganda. However, they also suggest, there is still an urgent need for anti-doping programs to educate the athletes; there were knowledge gaps observed amongst athletes in their study.

They also concluded that there are necessary modification of existing Physical education and sports curriculum and need of inclusion of more content about doping in sport that provide the basis for doping prevention programs amongst young athletes in Ugandan's schools. The analysis of results of study and after reviewing of others study, we can conclude that Sportsperson have more knowledge and awareness towards doping and it is significance difference at .05 level of significant. The results also showed that, after significant different it is concluded that Sportspersons have no proper knowledge and awareness towards doping and there are also shortage of literature and materials that whom educate the athlete about the deadly effects of doping. The study also suggests that latest and more literature of doping should be included in curriculum.





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Authors Profiles



Dr. Ashok Kumar received the Master Degree in Physical Education (**Gold Medal**) in the year 2000 from MDU-Rohtak and M.Phil degrees in Physical Education from KUK, and Ph.D from MDU-Rohtak, B.P.Ed from Nagpur University-Nagpur, and Qualified UGC-NET in Dec 2004. Presently he is working as Associate Professor (Physical Education) in Department of Physical Education, Chaudhary Devi Lal University-Sirsa, Haryana (India). Presented more than 40 Research paper in National and International Seminar/ Coherences and also presented a paper at Cambridge University-London (UK)



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Table 1: Comparison of Awareness and Knowledge of Sportspersons and Non-sportspersons towards Doping

Variables	Mean	SD	SED	t-value	Level of Significance
Sportspersons (250)	30.02	6.19	.694	6.682*	0.05
Non-Sportspersons (250)	25.37	5.35			

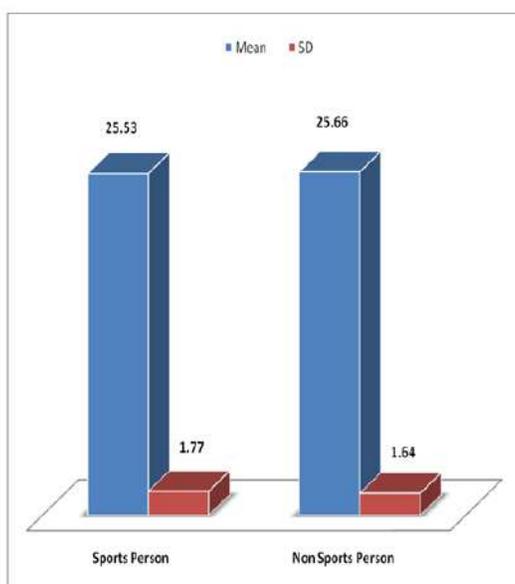
*Significance at .05 level significant

Degree of Freedom= .498

** Not Significance at .05 level significant

Table 2: Analysis of Awareness and Knowledge of Sportspersons and Non-sportspersons towards Doping

Sr. No.	Details of Questions	Sportspersons (250)		Non-Sportspersons (250)	
		Yes	No	Yes	No
1.	I am familiar with the World Anti-Doping Code.	110 (44%)	140 (56%)	50 (25%)	200 (75%)
2.	I Personally know Sportsperson who have used Performance-enhancing Substances.	160 (64%)	90 (36%)	70 (28%)	180 (72%)
3.	My peer Sportsperson use Performance enhancing Substance.	170 (68%)	80 (32%)	70 (28%)	180 (72%)
4.	It is the duty of Sportsperson to ensure they don't breach the Anti-Doping regulations.	190 (76%)	60 (24%)	220 (88%)	30 (12%)
5.	Sportspersons should be educated on Anti Doping regulations regularly.	220 (88%)	30 (12%)	230 (92%)	20 (8%)
6.	Athletes should be tested for Performance Enhancing-substance/ drugs at all levels of Competition.	150 (60%)	100 (40%)	160 (64%)	90 (36%)
7.	I have learnt about Performance-Enhancing Drugs.	30 (12%)	220 (88%)	10 (4%)	240 (96%)
8.	I consider my Awareness on Doping and Performance- Enhancing substance to be Adequate.	45 (88%)	205 (12%)	17 (92%)	223 (8%)



Graph 1: Mean and SD value of Awareness and Knowledge of Sportspersons and Non-sportspersons towards Doping





Comparative Study of Ethanol Production from Baker's Yeast and Toddy Yeast

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ABSTRACT

In this study, ethanol production from yeast isolated from palm toddy was compared with Baker's yeast. The parameter such as pH, temperature, incubation time, glucose concentration and medium components for the isolated yeast and baker's yeast was optimized. The enriched parameters was combined in a single medium and used for the enhanced ethanol production. The produced ethanol was recovered by distillation method. The isolated toddy yeast was identified by 18S rRNA sequencing. Among the parameters listed pH – 5, temperature around 30°C, incubation time of 48 hrs, glucose concentration of 400 g/L and medium component of yeast extract produced the maximum ethanol of 92% in toddy yeast and 87% in Baker's yeast. Ethanol production was produced from a novel yeast namely *Trichosporan asahii* with maximum ethanol when compared to Baker's Yeast.

Key words: Palm toddy, *Trichosporan asahii*, Ethanol, Glucose, Yeast.

INTRODUCTION

Toddy palm is one of the prominent naturally fermented seasonal traditional alcoholic beverages that is consumed in various regions of rural India since ages. Palm toddy is a naturally fermented sap from young inflorescences of *Borassus flabellifer* Linn. (Palmyra Palm), and they belong to the family Arecaceae [1]. Toddy palm with its substantial health benefits is consumed in parts of Africa, Asia, South America, Myanmar, Burma and Cambodia [2,3]. The local names of the drink are kallu in southern India, nsafufuo in Ghana, emu, and ogogoro in Nigeria and tuba in Mexico. The unfermented Toddy is a colorless sweet-flavored drink with a pH between 6.0 and 7.0, which comprises of various reducing and non-reducing sugars such as sucrose and trace amounts of fructose and glucose, amino acids,



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proteins and volatile fatty acids [4, 5]. Toddy is also called as Neera and is well known to consist of many minerals like phosphorus, calcium and iron and vitamins like niacin, vitamin A, vitamin C riboflavin, nicotinic acid and thiamin [3,6,7]. In the medicinal point of view, Toddy Palm Nectar (TPN) extracted from flowers of palm trees are used as a diuretic, stimulant, laxative, tonic and amebicide [3,8,9]. Despite the nutrients, toddy palm is rich in two major microbial groups: bacteria and yeast [10]. Microorganisms play a vital role in the fermentation of sugars in palm. Among the bacterial group, lactic acid bacteria, coliforms, acetic acid bacteria and mesophiles are predominantly present. Whereas in yeast, *Saccharomyces cerevisiae* is the dominant strain [11,12,13]. Most of the bacteria are the probiotic ones like *Lactobacillus*, *Enterococcus*, *Leuconostoc*, *Pediococcus*, *Bifidobacterium* and *Streptococcus* [10,14] and other bacteria like *Gluconobacter*, *Fructobacillus*, *Kluyvera* and *Zymomonas* [15]. Other Yeast such as *Hanseniaspora guilliermondii* and *Hanseniaspora uvarum* have also proven to increase the aroma of the wine [16]. Since ages, ethanol production is commercially prepared by fermentation of yeast. *Saccharomyces cerevisiae* is the well-known yeast due to its increased ethanol production and easy recovery. However, there are many reports for ethanol production from fermentation of sugar juices using *Kluyveromyces marxianus* [17], *Escherichia coli* strain KO11, *Zymomonas mobilis* [18], *Pichia kudriavzevii* [19], *Klebsiella oxytoca* strain P2 [20], *Thermoanaerobacter ethanolicus*, *Candida shehatae* and *Mucor indicus* [21].

Fermentation of toddy palm for a longer time yields bioethanol. In addition to this, there are many by-products formation in trace amounts. This totally depends on the microbial inoculum, seasonal conditions, pH and the variety of palm. The common by-products that are formed in toddy palm are lactic acid, acetic acid, citric acid, oxalic acid, ascorbic acid, tartaric acid and malic acid. These organic acids are produced in different stages of fermentation in very low quantities and its presence is acceptable in the palm wine [11,12,].

The substrates that are used for the ethanol production are the free sugar containing juices of crops such as sugarcane, sugarbeet, sweet sorghum and fruit juices [22]. Sucrose, the major sugar in fermentable juices is readily cleaved into monosaccharides like glucose and fructose during the initial stage of fermentation using invertase enzyme. This enzyme is found in the periplasmic space of yeast present in the palm toddy [23]. The main objective of this study is to isolate novel yeast from palm toddy to ferment glucose and produce ethanol. The ethanol efficiency will be compared with the commercial yeast to study the market value. The selected yeast strain was subjected to many parameters and evaluated for the ethanol production.

MATERIALS AND METHODS

Sample collection

Toddy sample (250 ml) was collected from Namakkal District at Tiruchengode and it was brought to the laboratory for the isolation of yeast strains to study the important process parameters which facilitate the fermentation of sugar to alcohol using isolated yeast strain.

Isolation and Characterization of yeast

The collected toddy was serially diluted for the isolation of yeast. The sample from 10^{-5} – 10^{-9} dilution was plated on the Yeast Extract Peptone Dextrose (YEPD) medium plates, which were incubated at 30°C for 3 days until visible colonies appeared. The visible colonies that were able to grow on the medium were selected and preserved as slants for further studies. The selected yeast colony was confirmed by motility, Sugar fermentation, Lactophenol cotton blue staining, Nitrate utilization, Surface growth test, Germ tube test and Urea hydrolysis test.

Inoculum preparation

The medium was prepared on 100 ml Erlenmeyer flask containing 1 g glucose, 0.2 g yeast extract, 0.5 g KH_2PO_4 , 0.2 g ammonium sulphate. The pH of the solution was adjusted to pH 5 by NaOH and then sterilized in an autoclave for 15 min at 15 psi pressure. After the broth was cooled to room temperature, colonies of yeast were introduced in it.



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Then the culture was kept for growth in an incubator at 30°C for 48hrs at 110 rpm. From this, 10% inoculum of optical density 0.4 at 610nm was added for fermentation.

Optimization of parameters

The concentrations of ethanol and glucose were monitored using UV-Visible spectrophotometer. Ethanol was determined by measuring Optical density (OD) at 600 nm after standard distillation by potassium dichromate method. The glucose was assayed by measuring OD at 550 nm using Dinitro salicylic acid method.

Effect of pH

The effect of pH of the fermentation media was studied with various pH ranging from 3.0-7.0 with 0.1N NaOH and 0.1N glacial acetic acid. The YEPD broth contains 400 g/L of sugar. It was then incubated in ordinary shaker at 110 rpm at 30°C for 24 h.

Effect of Temperature

The experiment was conducted by varying the temperature of YEPD medium. The medium contains glucose 400 g/L, pH 5. The fermentation media was incubated in thermostat shaker at 110 rpm at the temperature ranging from 30 to 40°C.

Effect of Glucose Concentration

The experiment was conducted by varying the glucose concentration from 240 to 480 g/L of glucose in YEPD medium. The fermentation media was incubated in ordinary shaker at 110 rpm at 30°C.

Variation in Incubation Time

The experiment was conducted by varying the incubation time of the inoculum. The inoculum for the fermentation was grown in incubator for different periods of time 12 h, 24 h, 48 h and 72 h. 4 vol% of inoculum was added for the fermentation. The medium contains 400 g/L glucose. The fermentation media was incubated in ordinary shaker at 110 rpm at 30°C.

Variation in Medium Constituents

The experiment was conducted by varying the medium constituents like yeast and beef extracts (2%) except other nutrients in YEPD medium. The medium contains 400 g/L glucose. The fermentation media was incubated in ordinary shaker at 110 rpm at 30°C.

Recovery of Ethanol

The cell mass was separated by centrifugation. Ethanol from the fermentation broth could be recovered by successive distillation. During the distillation process, the solvent-based waste is heated until it reaches the boiling point. It then evaporates and passes through the condenser where heat is removed from the vapor and it turns back into a cool, clean reusable liquid. Fortunately, contaminants are typically not volatile and stay behind in the distillation tank. By this process, ethanol of 95% purity could be achieved.

Ethanol Estimation and Glucose Estimation

The ethanol was estimated by 25% Chromic acid assay. 1ml of test sample was added with 25% chromic acid and optical density was measured at 350nm. Using the standard plot the percentage of ethanol present in the test sample was found out [24]. The glucose remnant in the fermented broth was estimated by DNS method. The optical density of the samples were read at 510nm. It was then plotted in a standard graph to examine the used glucose by yeast during the ethanol production.



**Ilakkia Sivaji and Deepika Jothinathan****DNA Sequencing**

The isolated DNA was subjected to polymerase chain reaction by the method followed by Saiki et al, 1988 [25]. The resultant PCR product was given for 18S rRNA sequencing.

RESULTS AND DISCUSSION**Isolation and Identification of Yeast from toddy**

Toddy was serially diluted and plated in PYN medium. Then the colonies streaked on PYN medium were observed to be white to cream colour, smooth, glabrous and yeast-like in appearance. Those organisms which can utilize glucose as carbon source alone are able to grow on PYN plate. Figure 1 shows the morphology of yeast colonies on PYN medium. Based on this, selected colonies were observed under microscope. The morphology of the strains was found to be large, globose to ellipsoidal budding structure with Lacto phenol cotton blue staining. This confirms the presence of isolated strain is yeast.

Biochemical tests**Fermentation Reactions**

Yeast is capable of using some, but not all sugars as an energy source. Yeast can metabolize sugar in two ways, aerobic or anaerobic. When the yeast respire aerobically, oxygen gas is consumed at the same rate that carbon dioxide is produced and hence there would be no change in gas pressure in the test tube. When yeast ferments the sugars in anaerobic condition, however, Carbon dioxide production will cause a change in the pressure of a closed test tube, since no oxygen is being consumed in pH 7. Table 1 shows sugar fermentation and sugar assimilation by toddy yeast. The table shows that the assimilation actually does occur during fermentation and that the quantitative results noted above may be due to the utilization of the stored carbohydrate after the exhaustion of the sugar.

Positive: Glucose, Fructose, Sucrose, Galactose, Mannitol, Maltose, Mannose

Negative: Lactose, Cellobiose

Nitrate assimilation test

Potassium nitrate (KNO₃) assimilation is a significant physiologic criterion for identifying yeasts. All strains of *Saccharomyces* can use ammonia and urea as a sole nitrogen source but cannot use nitrate, since it lacks the ability to reduce them into ammonium ions. Hence addition of peptone to the nitrate assimilation broth showed presence of growth and addition of potassium nitrate showed absence of yeast growth. This confirms that the isolated strain is yeast. Table 2 shows nitrate assimilation of yeast in peptone and potassium nitrate.

Surface growth test

Surface growth of yeast in malt agar was observed as pellicle, a ring around a surface of the tube and stabbed area. So, it is a facultative anaerobe. Figure 2 shows the surface growth of yeast colonies.

Germ tube test

A germ tube represents the initiation of hyphae directly from the yeast cell. It has parallel walls at their point of origin. A fresh normal pooled human serum was used for the assay. A short hyphal extension arising laterally from a yeast cell, with no constriction at the point of origin was observed.

Urea hydrolysis test

Christensen urea agar converts phenolphthalein indicator in the medium from yellow to pink or red color that denotes the alkaline change when urease liberates ammonia from urea. The presence of pink color differentiates the yeasts from *Candida albicans* and *Cryptococcus neoformans*.



**Ilakkia Sivaji and Deepika Jothinathan****Optimization of parameters for enhanced ethanol production****Variation in Incubation time**

In fermentation reactions, incubation time is very important in obtaining maximum ethanol production. In initial hours, yeast density will be low and the fermentation process might be incomplete. On the other side, higher incubation time can accumulate toxic products such as ethanol in yeast cells [26]. In the present study, 10% inoculum of OD value 0.4 for fermentation was grown in incubator for different periods of time namely 24h, 48h and 72h. The rate of ethanol production was increased with increased in growth time of 48 hours and with maximum sugar conversion. The high rate of ethanol production and substrate uptake with increase in incubation time is due to the high cell mass concentration achieved in 48h. The residual sugar concentration is 12g/L when incubated for 48 hours.

Effect of pH

The pH has the significant influence on fermentation due to its effect on yeast growth, fermentation rate and by product formation. It is one of the key factor in ethanol production which has direct impact on the yeast and their metabolic processes [27]. Therefore, maintenance of pH is of paramount importance in fermentation processes.

In the present study, the efficiency of the yeast strain was evaluated in the pH range 3 to 7. The results were shown in **Figure 5**.

From **Figure 5** it is observed that ethanol concentration was increased steadily with time in all pH values though the rate of production varied considerably. The maximum ethanol concentration of 85%/L was achieved with pH 5 followed by 78%/L ethanol with pH 4. The lower activity of the yeast strain at pH 3 is because the pH is too low to activate the enzymes to react. The rate of ethanol production initially was higher at higher pH but maximum ethanol concentration achieved was less than those obtained with pH 5. In 2007, Onsoy et al reported that *Zymomonas mobilis* yielded high ethanol when the pH of the broth was adjusted to 5.0 to 6.0 [28].

Figure 6 shows that the sugar concentration was reduced steadily with time for all pH values. However, percentage conversion was decreased drastically with pH 5. From yield calculation data, it is indicated that maximum ethanol yield of 85% was obtained with pH 5 which is higher than the yields obtained with pH values 3, 4, 6 and 7. The residual sugar concentration for 400g/L at pH 5 is 10g. The lower ethanol yield and sugar conversion obtained with higher pH values was possibly due to the formation of undesired products like glycerol or organic acids etc. at the expense of ethanol. Therefore, from the pH study, pH 5 was found to be the optimum pH value for ethanol fermentation using Yeast isolated from toddy.

Effect of Temperature:

The temperature has a marked influence on the production of biomass and ethanol. Usually, the rate of alcoholic fermentation increases with temperature ranging from 30°C to 40°C using conventional yeast. Fermentation experiments were conducted under varying temperature in the range 30-38°C to see the effect of the yeast strain towards ethanol production. The results were shown in **Figure 7**.

Though, the rate of production was initially found to be higher at the high temperatures 35°C and 38°C but the maximum ethanol concentration was achieved at 30°C. The lower efficiency of the yeast towards ethanol formation may be attributed to the loss of enzyme activity at higher temperatures. Similarly, when yeast is exposed to high temperature, it may produce heat-shock proteins and inactivate ribosomes. This may be the primary reason for the less ethanol production at higher production^[29]. Hence, 30°C was found to be optimum with respect to optimum ethanol concentration and rate of reaction.

The sugar concentration as shown in **Figure 8** initially reduced for 30°C and rapidly increased for higher temperatures. The residual sugar content is 12g/L at 30°C when compared to 30g/L and 40g/L at 35°C and 38°C respectively which indicates that the rate of substrate consumption became slow comparatively.



**Ilakkia Sivaji and Deepika Jothinathan****Effect of sugar concentration**

An interesting research field in alcoholic fermentation is the study of yeast strain's ability to utilize sugar solutions more concentrated than those generally fermented in usual practice and hence it is important to establish the limits of ethanol tolerance of the yeast strain. Therefore, the fermentation was conducted with sugar concentrations range from 240 to 480 g/L with an intention to obtain high yield of ethanol in reasonable time. The result shown in **Figure 9** indicates that ethanol concentration increased with increase in substrate concentration but there was wide variation in time taken for complete fermentation. It was observed that for the lower concentration of sugar, the production of ethanol was growth associated only for a short period of time and hence required less fermentation time. There was no difficulty in fermenting solutions containing up to 400 g/L sugar. When high sugar concentration (above 400g/L) is used, the ethanol production drops down due to low fermentation of yeast cells exposed to osmotic pressure [30]. Complete and efficient fermentation of such highly concentrated sugar solutions is beneficial from energy consumption point of view for distillation of the alcohol.

However, when a still higher concentration of sugar, i.e., 480 g/L was fermented, ethanol concentration and sugar conversion were observed to be lower than those obtained with 400 g/L sugar solution. The inhibitory effect of high sugar concentration for alcoholic fermentation may be due to plasmolysis of yeast cells as reported. Further, it was observed that ethanol became inhibitory when its concentration reached about 85%. So, it was found that the new yeast is tolerant of sugar concentration to at least 400 g/L with an ethanol yield of 85%. The residual sugar concentration for 400g/L is 10g. This denotes that 390g/L of sugar is converted into ethanol.

Variation in medium constituents

YEPD is the production medium for the ethanol fermentation. In YEPD medium, instead of Yeast extract, beef extract was used and checked for the ethanol production. Though, both the medium constituents favored the fermentation process, the maximum ethanol yield of 85% and 80% was obtained with yeast extract and Beef extract respectively. Therefore, it is evident that yeast extract can be used as medium component in alcoholic fermentation. The residual sugar concentration for yeast extract is 13g/L when compared to beef extract with 28g/L.

Ethanol Production Efficiency

Alcoholic fermentation was carried out to find the suitability of the toddy yeast over the performance of commercial yeast. Table 3 compares the productivity and efficiency of yeast strain obtained from toddy and commercial yeast. A hike of 5% ethanol was observed in toddy yeast compared to commercial yeast.

DNA sequencing

The isolated strain was identified as *Trichosporan asahii* by 18S rRNA sequencing.

CONCLUSION

Palm toddy is an easily available and cost-effective source of yeast. In this context, we have isolated many strains of yeast and subjected them to various parameters for the enhanced ethanol production. The Yeast developed from toddy has shown substantial alcohol fermentation activity. It was established in this work that the optimum pH and temperature were 5 and 30°C respectively. The novel yeast strain, *Trichosporan asahii* was able to ferment the sugar solution containing at least 400g/L sugar and 85% ethanol. Inhibition of ethanol production was observed when the sugar concentration is increased to 480g/L. High rate of ethanol was achieved in 48 hours with the ethanol concentration of 92%. Mass production of ethanol was carried out with the above ideal conditions for both commercial yeast and novel yeast and as a result 87% and 92% of ethanol was produced. This concludes that *Trichosporan asahii* showed better results in ethanol concentration in contrary to the commercial yeast. In near future, the ethanol production industries can bloom if novel yeast strains are explored with the cheap resource.





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Table 1. Sugar assimilation test and Sugar fermentation test of isolated yeast

S. No	Sugar Name	Sugar Fermentation		Sugar Assimilation	
		Gas production	Acid production	Gas production	Acid production
1.	Control	–	–	–	–
2.	Glucose	+	+	+	+
3.	Fructose	+	+	+	+
4.	Sucrose	+	+	+	+
5.	Galactose	+	+	–	+
6.	Mannose	+	+	–	+
7.	Maltose	+	+	–	+
8.	Mannitol	+	+	+	–
9.	Xylose	+	+	+	+
10.	Lactose	–	–	–	–
11.	Cellobiose	–	–	–	–





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Table 2. Nitrate assimilation test

Nitrogen source	Growth
Control	Nil
Peptone	+
Potassium nitrate	-

Table 3. Comparison of ethanol production by toddy yeast and commercial yeast

Yeast	Initial sugar conc.(g/L)	pH	Temperature	Fermentation Time (h)	Ethanol concentration (%)
Commercial yeast	400	5	30°C	48	87
Yeast strain (from toddy)	400	5	30°C	48	92



Figure 1: Organism growth on PYN medium



Figure 2: Surface Growth Test

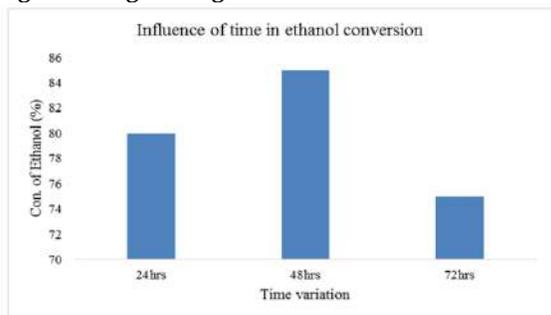


Figure 3: Effect of time variation on ethanol yield

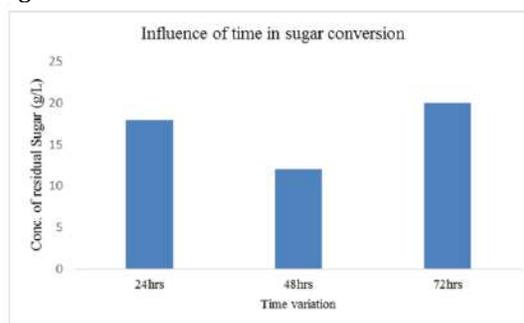


Figure 4: Effect of time variation on sugar conversion

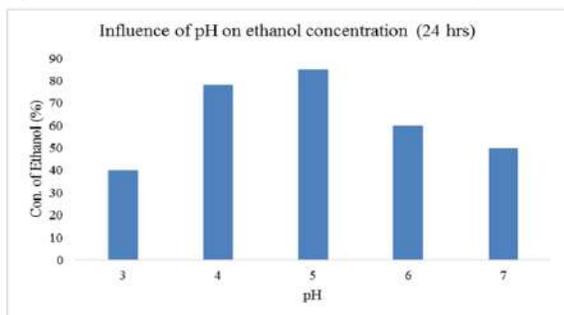


Figure 5: Influence of pH on ethanol concentration

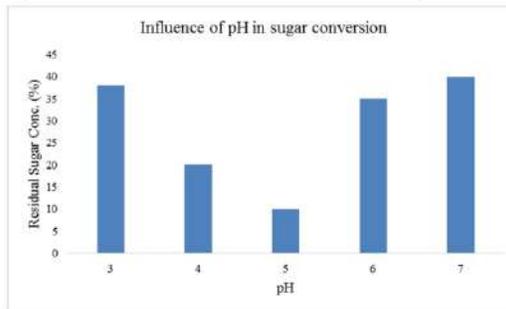


Figure 6: Effect of pH on sugar conversion





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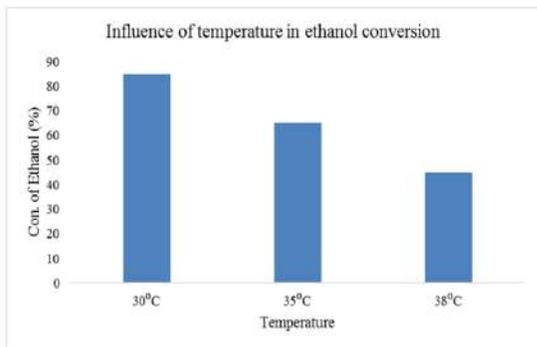


Figure 7: Effect of temperature on ethanol concentration

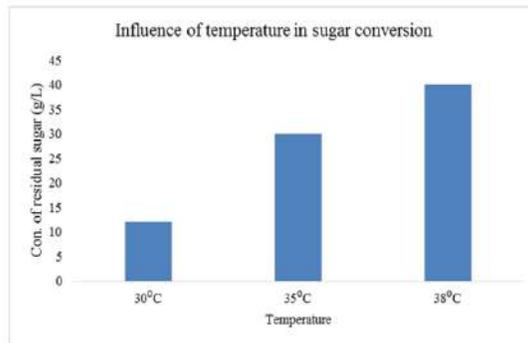


Figure 8: Effect of temperature on sugar conversion

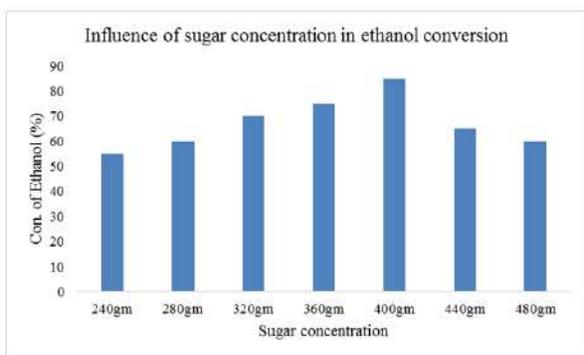


Figure 9: Effect of sugar concentration on ethanol production

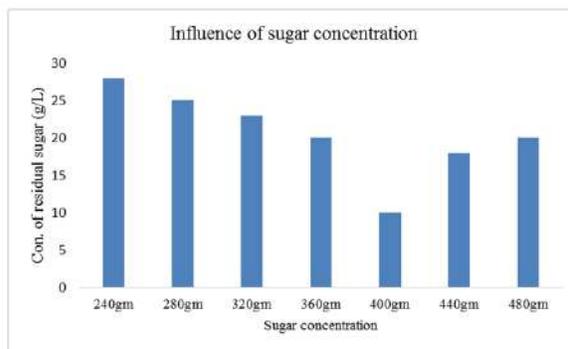


Figure 10: Effect of sugar concentration on sugar conversion

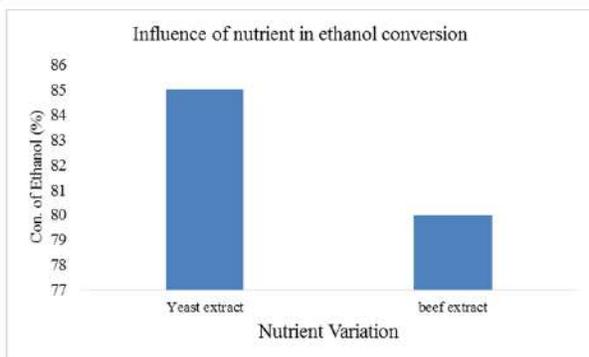


Figure 11: Effect of medium component on ethanol production

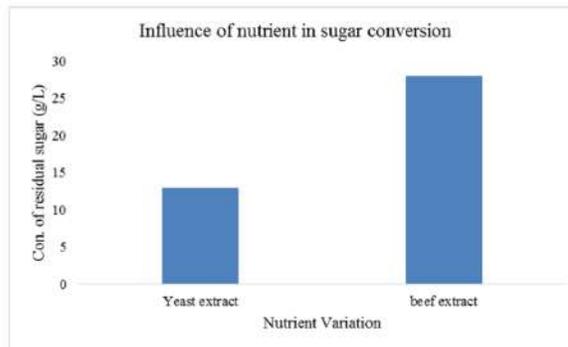


Figure 12: Effect of medium constituent on sugar conversion





High-Performance FIR adaptive filter Algorithm for Energy-Efficient IoT based Wireless Sensor Network using a Common Sub Expression Elimination Algorithm based on the Canonic Signed Digit

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ABSTRACT

Wireless communication systems are facing numerous obstacles due to their complexities and diversity. The finite impulse response (FIR) used in multi-standard wireless transmission technologies must meet specific fundamental characteristics such as minimal complexity, reconfigurable, and low energy consumption. Data dissemination in the wireless sensor network (WSN) utilized within an IoT system generally results in noisy values, inaccurate measurements, or incomplete data, all of which impact WSN behavior. FIR filters are used in the signal pre-processing phase of WSN because sensor nodes require extensive operating durations. As a result, it's critical to design a computationally robust, energy-efficient, rapidity, and area-optimized FIR filter architecture that can be reconfigured. Multiplying the filters' coefficients with input samples dominates the FIR filter's complexity. Filters use a lot of multipliers, which results in a lot of space and energy. However, the FIR filter coefficients are invariant, and multiplications are performed using a network of subtractors and adders. Minimal complexity FIR filters are designed with minimum adders and subtractors. An improved algorithm for eliminating common subexpression (CSE) is proposed. The filter coefficients are represented in canonical signed digit (CSD) format. The enhanced CSD-based CSE algorithm decreases the number of adders in multipliers, allowing for the economical implementation of dynamically reconfigurable filters. The proposed method combines horizontal CSE, vertical CSE, and look-forward techniques. FIR filter is implemented by selecting the highest number of commonly appearing subexpressions to prevent repetitive calculations in coefficient multiplication in the proposed method. The critical path length is not





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raised due to the adder reduction. Compared to other existing approaches, the proposed strategy improves the performance, energy efficiency, speed, and area reduction for the FIR filter implementation.

Keywords: WSN, IoT, Canonic signed digit (CSD), FIR filter, Common subexpression elimination (CSE).

INTRODUCTION

Filters are used to minimize noise and enhance data quality in communication and signal processing [1]. Filters are frequently used in signal processing and communication systems for channel equalization, noise removal, radar detection systems, biomedical data processing, and audio & video signal processing. Filters are used to remove noise from signals acquired by sensor nodes in IoT-based wireless sensor networks. Digital signal processing (DSP) devices with energy efficiency and increased speed are required by present mobile computing and communication applications [2]. FIR filtering is among the most significant DSP functions. The introduction of broadband technologies that enables increased data communication through networks has sparked interest in high-speed FIR filter design. FIR filter functions various tasks such as matched filtering, channel equalization, pulse shaping, and channelization. An FIR filter can have both stable and linear phases simultaneously. Higher-order digital filters must be used in wireless communication systems, and also it should consume less power and run at increased speed. Digital filtering with lower complexity for mobile communication and computing applications needs specialized architectures. The FIR filters must allow a significant sampling rate for exceptionally speedy digital transmission. The number of adders in multipliers grows linearly as the order of the filter is extended. Real-time implementation of a higher-order FIR filter in a resource-constrained setting is challenging. As a result, low-complexity FIR filter design has become increasingly important [3]. Channelizer demands increased speed, low energy requirement, and reconfigurable FIR filters. Even when implemented in completely custom ICs, the hardship of constructing FIR filters is dominated by numerous multiplications, which raises area and energy [4]. There are two steps in the multiplication process. One produces partial products, while the other adds these partial products. As a result, the speed of a multiplier is determined mainly by how quickly partial products are formed and added together. When the number of partial products to be generated is low, the speed of partial product generation is achieved. The multiplications are minimized by replacing multiplications with adding, subtracting, and shifting operations.

The coefficient multiplication dominates the complexities of FIR filters. Add, subtraction, and shift functions make up the multiplier unit. Multipliers account for most of the hardware complexity, as filters necessitate a considerable number of multiplications, resulting in delay, extreme size, and energy utilization. The number of adders employed in implementing the multipliers increases the number of resources needed to execute an algorithm of FIR filters. Coefficient multiplications are the most critical calculation in FIR filters. FIR filters use hardwired shifts and adders. The FIR filter's energy is decreased and speed is increased by employing the little logic operator (LO) and the minimum logic depth (LD) [5]. It is necessary to decrease LO (adders) and maintain shorter LD (critical path lengths). The formation of partial products and their addition are the two fundamental multiplication processes. High-speed operation of FIR filter is enabled by shorter critical path. Critical path is minimized using parallel processing or pipelining methods. Fast adder architectures are required to speed up the adding process among partial products. Because multipliers significantly impact overall system performance, various high-performance techniques and architectures have been developed. Many approaches are proposed to minimize the complexity of FIR filters. An effective arithmetic algorithm for coefficient coding reduces LO and LD [6]. The coefficient optimization approach is employed in which the number of addition/subtraction used will be the smallest if the multiplier is depicted in the canonical signed digit (CSD) format [7]. The distributed-arithmetic (DA) algorithm-based FIR filter architecture decreases the FIR filter's complexity [8]. Computation of constant multiplications with lookup tables and adders are used to reduce energy consumption and accelerate the application. With the advancement of VLSI technologies, the real-time implementation of FIR filters with minimal hardware needs and lower ultra-low latency has evolved crucial. The design and algorithms for memory-based (ROM) FIR filter implementation are presented to decrease total area, latency, and energy complexity [9-10]. The Common





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subexpression elimination (CSE) reduces the LO and LDin implementing the FIR filter [11-13].CSE is an optimization that looks for similar expressions and substitutes them with a single variable that holds the calculated value.The fundamental cause of the filter's difficulty is that as the filter order rises, adder steps in the multiplier block grow. The CSE method is utilized to minimize the complexity. The calculation of filter coefficients can be done in two ways. The first option is to express the tap values as binary numbers, whereas the second represents them as canonic signed digit (CSD) numbers. Using a CSD form of filter coefficients can minimize the hardware complexity necessary to implement an FIR filter [14-15]. Two types of CSE are vertical CSE and horizontal CSE [16]. The horizontal CSE finds out subexpressions within the same coefficient and the vertical CSE find out subexpressions among distinct coefficients. In the case of FIR filters, the vertical CSE reduces adders better than the horizontal CSE. Both the techniques can be coupled to decrease adders effectively [17].An optimization approach for lowering the number of LDs and LO in FIR filters is proposed in this study.The following is a breakdown of the paper's structure. The section-2 summarizes similar works, and section-3 gives the suggested architecture. Section-4 includes the findings and discussions, and section-5 provides conclusions.

RELATED WORKS

Authors have proposed several strategies for reducing the complexity of multiplications in FIR filters in the literature. Many proposed solutions decrease the number of LDs and LOs employed in multiplier implementation. M. Potkonjal et al. [18] proposed the multiple-constant-multiplication (MCM) problem that identifies how to apply subexpression elimination to a set of multiplication with constants to reduce the number of shifting and adding necessary for implementation. The number of adders and subtractors is minimized by exploiting redundancy between the coefficients, which results in a low-complexity performance. The MCM regards the multiplication of a single variable by many constants simultaneously. CSE is a widely known MCM approach that uses the most common bit patterns seen in the CSD to avoid duplicate computations in multiplier blocks [19]. M. Mehendale et al. [20] proposed removing the most common 2-bit common subexpression in the coefficients binary representations.M. Martinez-Peiro et al. [21] proposed a multiplier less fast-paced filter design utilizing a non-recursive signed CSE algorithm. While utilizing a CSE recursively results in a high logic depth in the digital structure, the suggested technique allows the developer to avoid this difficulty by only using each subexpression once. N. Sankarayya et al. [22] presented an approach in which differential coefficients were evaluated instead of optimizing the original filter coefficients. Differences among filter coefficients' absolute values were used to limit the dynamic range of calculation.However, overheads affect this differential coefficients method. Additional adders are needed to calculate the summation of the prior calculation's partial products kept in memory. Compensating for the impact of differential coefficients is required.K. Muhammad et al. [23] proposed a graph-based method to implement a multiplier-less digital filter using a differential coefficient. The proposed process involves rearranging computation using a graph to eliminate computational redundancy. In this method, coefficients are represented using vertices, and resources needed for calculation are defined utilizing edges. The difference of the vertices connected by the edge determines the differential coefficient. The complexity reduced in this method is significantly small compared to previous methods.

Yongtao Wang et al. [24] proposed a genetic algorithm-based approach to minimize complexity to design a multiplier-less FIR filter. Multiplier-less filters share CSE amongst coefficients to reduce the number of adders.This study investigates strategies for improving the architecture of CSD multipliers, with a focus on the benefits of shared subexpressions. However, overheads affect this method. Additional adders are needed to compensate for the impact of differential coefficients if differences in coefficients are considered or if the summation of coefficients is utilized, additional subtractors are necessary.R.Mahesh et al. [25] proposed a binary CSE technique that focuses on removing redundant binary common subexpressions from the coefficients. This approach effectively lowers the number of adders required to implement the multipliers of the higher-order FIR filter. This technique avoids repetitive calculations in coefficient multipliers by reusing the binary bit patterns that are most common in coefficients. Compared to other CSE approaches, the methodology dramatically lessens the number of LO while maintaining the





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same LD. Many bits are ungrouped in this method due to improper sequential verification and the formation of binary digits arranged in a sequence necessitating additional adders to implement them. Chia-Yu Yao et al. [26] proposed a vertical CSE technique to synthesize a fixed-point FIR filter. The redundancy between the LD in the multiplier block and CSD filter coefficients is considered in this method. Fei Xu [27] developed a CRA-2 algorithm for the CSE of the multiplication block of the FIR filter. It maintains a two-bit common subexpression with the primary purpose of achieving a minimal LD and reducing the number of LO's significantly. This method is the vertical CSE method. P. Vinod et al. [28] proposed extending the earlier techniques to remove repetitive calculations in the multiplier block utilizing the most CSE comprised of 2-non-zero-bits, 3-non-zero-bit and 4-non-zero-bit super-subexpressions are formed from 2-non-zero-bit in this method utilizing vertical CSE and horizontal CSE methods by using similar repetitious shifts among them. The super-subexpressions reduce the number of adders by eliminating unnecessary computations of 2-non-zero bit common subexpressions. The technique did not select the highest number of subexpressions because super-sub expression and common subexpressions are grouped sequentially without any look-forward. D.R Bull et al. [29] developed a vertical CSE strategy for minimizing the amount of LOs based on a graph-synthesis method for the multiplier block. This presented method adds the partial sums serially produced by multiplication. This approach makes multipliers with high LDs, which significantly raises the multiplier's delay. Even though the suggested technique uses fewer LOs in the coefficient-multipliers than other methods, the LD is high. A.G Dempster et al. [30] presented an updated approach to lower the number of LO even more than the "Bull-Horrocks" algorithm. Even though this method produces the fewest adders, the algorithm ignores the LD. For implementing FIR filters, the horizontal CSE offers reduced adders and LD than vertical CSE. A.P. Vinod et al. [31] proposed combining vertical CSE and horizontal CSE to implement an FIR filter with a minimum number of adders. R.Mahesh et al. [32] proposed a reconfigurable design of minimal complexity FIR filters. Nevertheless, the length of the binary CSE chosen in this technique makes the architecture ineffective by raising the adder step and hardware complexity. Indranil Hatai et al [33] suggested a method that minimizes the number of adders and multiplication per input sample, and a 2-bit binary-CSE technique to create an effective constant multiplier has been presented.

The Proposed architecture

Review of binary CSE Approach

Generally, coefficient values are represented in binary or signed digits and find common bit-patterns known as common subexpressions to remove. The binary CSE algorithm extracts the common subexpression in binary format. It's employed to implement an effective constant multiplier, making it suitable for low-complexity reconfigurable FIR filters in the binary CSE approach. In the binary CSE, approach coefficients of the filter are represented in binary format and eliminate repetitive binary common subexpressions. Frequently occurring common binary-bit patterns found in coefficients are reused in this strategy. It lowers the number of adders needed to implement multipliers. Compared to other methods, binary coefficients have few bits (not paired) that do not create common subexpressions. The binary CSE approach entails a binary vertical subexpression approach, binary horizontal subexpression approach, and adders hardwiring to make adders smaller. The horizontal binary CSE method uses similar subexpressions found inside each coefficient to eliminate repetitive calculations. Vertical binary CSE eliminates superfluous calculations using common subexpressions identified across neighboring coefficients. An "m" bit binary number can be converted into a totality of $2^m - (m+1)$ binary common subexpressions. For "m" bit binary common subexpressions, the number of adders necessary to create partial-products is $2^{(m-1)} - 1$. The length of binary common subexpressions increases the adder step and makes hardware design expensive. For instance, the binary HSE is created from the coefficients' binary representation depicted in **Table1**.

Here "k" is the input signal. The straightforward realization of the binary horizontal CSE needs 12 adders. By using shift operation or using adders, there are only seven adders required to realize the binary horizontal CSE. The significant drawbacks of the binary subexpression elimination strategy are that after obtaining the binary horizontal common subexpression, multiple bits do not form or belong to a group. The coefficient values represented in binary





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have nonzero bits that are extensively high compared to CSD. The drawback of the binary CSE approach is that it starts with many non-zero bits in the optimization area than CSE approaches based on CSD. Binary CSE approaches require a look-forward strategy to circumvent these disadvantages.

The Look-forward, Horizontal CSE method, and Vertical CSE method

The core idea behind our method is to use a look-forward algorithm to find and pick patterns that maximize the grouping of common subexpressions while leaving a small number of non-zero bits ungrouped. The group is accomplished to keep the multiplier's LD to a minimum. The proposed CSE employs CSD, and the initial optimization region has fewer non-zero bits. For instance, the suggested CSE approach is demonstrated in Table 2 utilizing 12-tap FIR filter coefficients. It shows that the common subexpressions are grouped in conventional sequential order, for example, filter h_1 and h_0 . It is found that two ungrouped bits are in the traditional method. Table 3 shows the grouping of common subexpressions utilizing the look-forward method. Because of the look-forward-based selection strategy, all non-zero bits are grouped. It is found that there are no ungrouped bits in the look-forward technique, and it cuts down on the number of adders needed.

The horizontal common subexpressions are $k_6 = [1\ 0\ 0\ -1]$, $k_5 = [1\ 0\ 0\ 1]$, $k_4 = [1\ 0\ -1]$ and $k_3 = [1\ 0\ 1]$. $k_2 = [1\ 1]$ is the vertical common subexpressions are shown inside colored rectangles and the rejected version is also shown in uncolored boxes which is depicted in Table 4. It depicts the CSD representation of filter coefficients. The vertical common-subexpression pattern $[1\ 1]$ is only utilized out of all the potential patterns. Symmetric FIR filter's coefficients are used entirely. The presented approach can be demonstrated utilizing 16-tap FIR filter coefficients. The first row's digits denote the number of bit-wise right shifting. The filter's coefficients $h_0, h_1, h_2, h_3, h_4, h_5, h_6$ and h_7 are displayed and rest of the filter's coefficients $h_8, h_9, h_{10}, h_{11}, h_{12}, h_{13}, h_{14}$ and h_{15} are symmetric to h_0 to h_7 . Here h_0 is the first coefficient. By putting the relevant pattern numbers in the respective places, Table 5 is generated from Table 4. Table 6 is the representing the coefficients after taking horizontal common-subexpressions and vertical common-subexpressions. Here $k_6 = 6$, $k_5 = 5$, $k_4 = 4$, $k_3 = 3$ and $k_2 = 2$.

The final representation of coefficients utilizing common subexpressions and super subexpressions are shown in Table 6 which is obtained from Table 5. The patterns $[1\ 0\ -4]$, $[1\ 0\ 4]$, $[1\ 0\ 3]$ and $[1\ 0\ -3]$ are grouped to form super subexpressions. From these super subexpressions: $[1\ 0\ -3] = [4\ 0\ 0\ 0\ -1] = 10 = k_{10}$, $[1\ 0\ 4] = [3\ 0\ 0\ 0\ -1] = 9 = k_9$, $[1\ 0\ 3] = [3\ 0\ 0\ 0\ 1] = k_8$.

The filter's outcome can be represented as illustrated as shown below:

$$Y_i = 2^{-3}.k_2 + 2^{-5}.k_6 + 2^{-11}.k_5 + 2^{-5}.k_2[-1] + 2^{-7}.k_6[-1] + 2^{-13}.k_3[-1] + 2^{-10}.k_8[-2] + 2^{-4}.k_9[-3] + 2^{-11}.k_2[-3] + 2^{-3}.k_2 + 2^{-5}.k_6 + 2^{-11}.k_5 + 2^{-5}.k_2[-1] + 2^{-7}.k_6[-1] + 2^{-13}.k_3[-1] + 2^{-10}.k_8[-2] + 2^{-4}.k_9[-3] + 2^{-11}.k_2[-3] + 2^{-4}.k_9[-4] + 2^{-13}.k_3[-4] + 2^{-2}.k_5[-5] + 2^{-7}.k_2[-5] + 2^{-11}.k_{10}[-5] + 2^{-1}.k_{10}[-6] + 2^{-9}.k_5[-6] + 2^{-14}.k_3[-6] + 2^{-2}.k_5[-7] + 2^{-9}.k_8[-7].$$

Utilizing the straightforward approach, 44 LOs are needed to attain the filter coefficients, illustrated in Table 4. Only 19 adders are required in the multiplier adder block to implement the filter in the proposed technique shown in Table 6. The sub expressions require eight adders, but the actual implementation requires 11 adders. It is observed that there is a 57 percent reduction of adders, and the complexity of the filter can be significantly decreased.

The algorithm of the suggested technique

This division explains the suggested CSE approach. Both horizontal sub expressions and vertical sub expressions are employed. The horizontal common sub expressions are $k_6 = [1\ 0\ 0\ -1]$, $k_5 = [1\ 0\ 0\ 1]$, $k_4 = [1\ 0\ -1]$ and $k_3 = [1\ 0\ 1]$. $k_2 = [1\ 1]$ is the vertical common sub expressions are shown inside colored rectangles and the rejected interpretations are considered. The vertical common-sub expression pattern $[1\ 1]$ is only utilized out of all the potential patterns. Symmetric FIR filter's coefficients are used entirely.





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1. Create an L-length filter that meets the required specifications. Filter Length is represented as "L".
2. For the desired word length, get the CSD depiction of the coefficients.
3. The algorithm check for bits that aren't zero at $(1+z, 2+b)$, $(z, 2+b)$, $(1+z, b)$, and (z, b) . Here "b" represents the bit's position, and z represents the filter coefficient.
4. **Case-1:** When there are horizontal subexpressions and vertical common subexpressions at (z,b) , and bits that aren't zero at $(z, 2+b)$, $(1+z, b)$, and (z, b) .
 - 4.1. The algorithm checks for bits that aren't zero at $(1+z, b)$, and (z, b) have an identical sign.
 - 4.2. The vertical common-subexpression at (z, b) is evaluated if the sign is identical. The algorithm finds the number of subexpressions and bits left in the (z) coefficient that cannot be paired. The combining of subexpressions with vertical common subexpression is shown in **Table 7**.
 - 4.3. The horizontal common subexpression at (z, b) is evaluated. The exact approach as in (4.2) is utilized. **Table 8** shows the combining of subexpressions with horizontal common subexpression
 - 4.4. These two approaches are compared in terms of the number of bits that have not been grouped and the number of subexpressions. The technique with the highest number of patterns is selected to group the remaining bits left in the (z) coefficient.
 - 4.5. If the number of bits that have not been grouped and the number of subexpressions are equal, the horizontal common subexpression approach is used because it is more straightforward to implement.
 - 4.6. If the horizontal common subexpression at (z, b) is considered, the value of bit position "b" is incremented by 3. When the value of "b" is less than or equal to filter length-1, proceed to step 3; else, move to step-9.
 - 4.7. If the vertical common subexpression at (z, b) is considered, the value of bit position "b" is incremented by 1. When the value of "b" is less than or equal to filter length-1, proceed to step 3; else, move to step-9.
 - 4.8. The algorithm checks for bits that aren't zero at $(1+z, b)$, and (z, b) havenot an identical sign then the vertical common-subexpression at (z, b) is evaluated if the sign is not identical. The value of bit position "b" is incremented by 3. When the value of "b" is less than or equal to filter length-1, proceed to step 3; else, move to step-9.
5. **Case-2:** When there are horizontal subexpressions at $(1+z, b)$ and vertical common subexpressions at (z, b) , and bits that aren't zero at $(1+z, 2+b)$, $(1+z, b)$, and (z, b) .
 - 5.1. The algorithm checks for bits that aren't zero at $(1+z, b)$, and (z, b) have an identical sign.
 - 5.2. The vertical common-subexpression at (z, b) is evaluated if the sign is identical. The algorithm finds the number of subexpressions and bits left in the $(z+1)$ coefficient that cannot be paired to form common subexpressions. The combining of subexpressions with vertical common subexpression is shown in **Table 9**.
 - 5.3. The horizontal common-subexpression at (z, b) is evaluated if the sign is identical. The algorithm finds the number of subexpressions and bits left in the $(z+1)$ coefficient that cannot be paired to form common subexpressions. The combining of subexpressions with horizontal common subexpression is shown in **Table 10**.
 - 5.4. These two approaches are compared in terms of the number of bits that have not been grouped and the number of subexpressions. The technique with the highest number of patterns is selected to group the remaining bits left in the $(z+1)$ coefficient.
 - 5.5. If the horizontal common subexpression at $(z+1, b)$ is considered, the value of bit position "b" is incremented by 3. When the value of "b" is less than or equal to filter length-1, proceed to step 3; else, move to step-9.
 - 5.6. If the vertical common subexpression at $(z+1, b)$ is considered, the value of bit position "b" is incremented by 1. When the value of "b" is less than or equal to filter length-1, proceed to step 3; else, move to step-9.
 - 5.7. The algorithm checks for bits that aren't zero at $(1+z, b)$, and (z, b) havenot an identical sign then the horizontal common-subexpression at $(z+1, b)$ is evaluated if the sign is not identical. The value of bit position "b" is incremented by 3. When the value of "b" is less than or equal to filter length-1, proceed to step 3; else, move to step-9.





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6. **Case-3:** When there are only horizontal subexpressions and no vertical common subexpressions at (z, b) , and bits that aren't zero at $(z, 2+b)$, and (z, b) . The value of bit position "b" is incremented by 3. When the value of "b" is less than or equal to filter length-1, proceed to step 3; else, move to step-9.
7. **Case-4:** When there are only vertical subexpressions and no horizontal common subexpressions at (z, b) , and bits that aren't zero at $(z, 1+b)$, and (z, b) . The value of bit position "b" is incremented by 1. When the value of "b" is less than or equal to filter length-1, proceed to step 3; else, move to step-9.
8. **Case-5:** When there are different combinations, the value of bit position "b" is incremented by 1. When the value of "b" is less than or equal to filter length-1, proceed to step 3; else, move to step-9.
9. When the value of "b" is greater than filter length (L) - pattern length (p), the value of bit position "b" is incremented by one, and the value of filter coefficient "z" is incremented by 1. Proceed to step 3. When the value of "b" is greater than filter length (L) - pattern length (p) and $z = \text{filter length (L) } / 2$, proceed to step 10.
10. The coefficients are verified for super subexpressions like $[1\ 0\ -1\ 0\ -1]$, $[1\ 0\ -1\ 0\ 1]$, $[1\ 0\ 1\ 0\ -1]$, $[1\ 0\ 1\ 0\ 1]$ and their negated counterparts after the horizontal common subexpressions and vertical common subexpressions have been grouped. Execute the super-subexpressions only if they appear in the coefficient-matrix a minimum of two times. LO is kept in check because super-subexpressions will try to rise multiplier's LD and affect the delay of multipliers. Once the super-subexpressions are executed, the value of bit position "b" is incremented based on the pattern and proceeds to step 9. When the value of "b" is greater than filter length (L) - pattern length (p), the value of bit position "b" is incremented by one, and the value of filter coefficient "z" is incremented by 1. Proceed to step 9. When the value of "b" is greater than filter length (L) - pattern length (p), and $z = \text{filter length (L) } / 2$, end the program.

RESULTS AND DISCUSSION

The minimization of adders in five FIR filters [35], [36], [37], and [38] with different stop-bands and pass-band frequencies was investigated. The suggested algorithm's average decrease in LDs and LOs is compared to existing algorithms. Table 11 shows the pass-band frequency and stopband frequency of the benchmark FIR filters, shown in Table 11. The proposed method is compared with [7], [9], [11], [30], and [32]. Compared to all existing CSE approaches, the suggested method reduces the number of logic operators (LO) while maintaining nearly the same logic depth (LD). Table 12 compares the reduction of the logic operator (LO) in the proposed method with other CSE methods. Table 13 compares the proposed method's logic depth (LD) with other CSE methods. The proposed technique reduces LO by 27 percent compared to [32], 67 percent compared to [9], and 44 percent compared to [30], 64 percent compared to [11], and 69 percent compared to [7].

The FIR filter considered is [37]. The passband edge is 30 KHz and stopband edges is 30.5 KHz. The proposed strategy significantly reduces the number of adders while having no increase on the LDs. The proposed technique gives a 20% reduction over [30] and a 62% reduction over [11], as shown in Table 14. The suggested method's LDs are nearly identical to [32], shown in Table 15.

CONCLUSION

The proposed method combines horizontal CSE, vertical CSE, and look-forward techniques to improve the CSE algorithm based on CSD. The approach is designed to implement FIR filters with a minimal level of complexity. The strategy maximizes subexpression grouping, leaving the fewest possible unpaired non-zero bits. FIR filter is implemented by selecting the highest number of commonly appearing subexpressions to prevent repetitive calculations in coefficient multiplication in the proposed method. The proposed technique reduces LO by 27 percent compared to [32], 67 percent compared to [9], 44 percent compared to [30], 64 percent compared to [11], and 69 percent compared to [7]. The critical path length is not raised due to the adder reduction. Compared to other existing approaches, the proposed strategy improves the performance, energy efficiency, speed, and area reduction for the FIR filter implementation.





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Table 1. Formation of binary horizontal CSE from binary representation of coefficient

Binary representation of coefficients	Formation of binary horizontal CSE (Straight forward realization)	Shift operation or using adders.
[1 1]	$k_2 = k + 2^{-1}.k$	-
[1 0 1]	$k_3 = k + 2^{-2}.k$	-
[1 1 1]	$k_4 = k + 2^{-1}.k + 2^{-2}.k$	$k_4 = k_2 + 2^{-2}.k$
[1 1 1 1]	$k_5 = k + 2^{-1}.k + 2^{-2}.k + 2^{-3}.k$	$k_5 = k_2 + 2^{-2}.k + 2^{-3}.k = k_2 + 2^{-2}.[k + 2^{-1}.k]$ $k_5 = k_2 + 2^{-2}.k_2$
[1 1 0 1]	$k_6 = k + 2^{-1}.k + 2^{-3}.k$	$k_6 = [k + 2^{-1}.k] + 2^{-3}.k = k_6 = k_2 + 2^{-3}.k$
[1 0 1 1]	$k_7 = k + 2^{-2}.k + 2^{-3}.k$	$k_7 = k + 2^{-2}.k + 2^{-3}.k = k + 2^{-2}.[k + 2^{-2}.k]$ $k_7 = k + 2^{-2}. [k + 2^{-2}.k] = k + 2^{-2}. k_3$
[1 0 0 1]	$k_8 = k + 2^{-3}.k$	-

Table 2. The grouping of common subexpressions (CSE) in sequential order.

	4	5	6	7	8	9	10	11	12	13	14
h_0	0	1	0	1	0	1	0	-1	0	-1	0
h_1	0	1	0	0	1	0	0	-1	0	0	0

Table 3. The grouping of common subexpressions utilizing look-forward method.

	4	5	6	7	8	9	10	11	12	13	14
h_0	0	1	0	1	0	1	0	-1	0	-1	0
h_1	0	1	0	0	1	0	0	-1	0	0	0

Table 4. Canonical signed digit (CSD) representation form of coefficients of FIR filters.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
h_0	0	0	1	0	1	0	0	-1	0	0	1	0	0	1	0	0
h_1	0	0	1	0	1	0	1	0	0	-1	0	0	1	0	1	0
h_2	0	0	0	0	1	0	0	0	0	1	0	1	0	1	0	0
h_3	0	0	0	1	0	1	0	1	0	0	1	0	0	0	0	0
h_4	0	0	0	1	0	1	0	-1	0	0	1	0	1	0	1	0
h_5	0	1	0	0	1	0	1	0	0	0	1	0	-1	0	-1	0
h_6	1	0	1	0	1	0	1	0	1	0	0	1	0	1	0	1
h_7	0	1	0	0	1	0	0	0	1	0	1	0	1	0	0	0

Table 5. Representing the coefficients after taking horizontal common-sub expressions and vertical common-sub expressions

Bit-shift	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
$h(n)$																
h_0	0	0	2	0	6	0	0	0	0	0	5	0	0	0	0	0
h_1	0	0	0	0	2	0	6	0	0	0	0	0	3	0	0	0
h_2	0	0	0	0	0	0	0	0	0	1	0	3	0	0	0	0





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h ₃	0	0	0	1	0	4	0	0	0	0	2	0	0	0	0	0
h ₄	0	0	0	3	0	0	0	-1	0	0	0	0	3	0	0	0
h ₅	0	5	0	0	0	0	2	0	0	0	4	0	0	0	-1	0
h ₆	1	0	-3	0	0	0	0	0	5	0	0	0	0	3	0	0
h ₇	0	5	0	0	0	0	0	0	3	0	0	0	1	0	0	0

Table 6. The final representation of coefficients utilizing common sub expressions and super sub expressions

Bit-shift	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
h(n)																
h ₀	0	0	2	0	6	0	0	0	0	0	5	0	0	0	0	0
h ₁	0	0	0	0	2	0	6	0	0	0	0	0	3	0	0	0
h ₂	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0
h ₃	0	0	0	9	0	0	0	0	0	0	2	0	0	0	0	0
h ₄	0	0	0	9	0	0	0	0	0	0	0	0	3	0	0	0
h ₅	0	5	0	0	0	0	2	0	0	0	10	0	0	0	0	0
h ₆	10	0	0	0	0	0	0	0	5	0	0	0	0	3	0	0
h ₇	0	5	0	0	0	0	0	0	8	0	0	0	0	0	0	0

Table 7. The combining of subexpressions with vertical common subexpression

	4	5	6	7	8	9	10	11	12	13	14
h ₀	0	1	0	1	0	1	0	1	0	-1	0
h ₁	0	1	0	0	0

Table 8. The combining of subexpressions with horizontal common subexpression.

	4	5	6	7	8	9	10	11	12	13	14
h ₀	0	1	0	1	0	1	0	-1	0	-1	0
h ₁	0	1	0	0	0

Table 9. The combining of subexpressions with vertical common subexpression.

		3	4	5	6	7	8	9	10	11	12	13
h ₀	1	0	0	0
h ₁	1	0	1	0	0	1	0	1	0	0	-1

Table 10. The combining of subexpressions with horizontal common subexpression.

		3	4	5	6	7	8	9	10	11	12	13
h ₀	1	0	0	0
h ₁	1	0	1	0	0	1	0	1	0	0	-1





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Table 11. The passband frequency and stopband frequency of the benchmark FIR filters

FIR Filter	Passband frequency (π)	Stopband frequency (π)
[35]	0.021	0.07
[35]	0.15	0.25
[36]	0.6836	0.6973
[37]	0.6173	0.6276
[38]	0.5	0.37

Table 12. Comparison of logic operators (LO) reduction in the proposed method with other CSE methods.

FIR Filter	Coefficient of word length "C"	Filter Length "N"	Proposed method	[34]	[7]	[9]	[11]	[30]	[32]
[35]	14	25	26	86	70	60	55	55	40
[35]	9	59	7	23	21	18	19	18	9
[36]	12	230	30	227	162	164	139	162	44
[37]	13	200	42	224	171	154	150	152	61
[38]	17	120	48	205	116	121	105	112	57

Table 13. Comparison of logic depth (LD)in the proposed method with other CSE methods.

FIR Filter	Coefficient of word length "C"	Filter Length "N"	Proposed method	[34]	[7]	[9]	[11]	[30]	[32]
[35]	14	59	3	2	4	3	3	5	3
[35]	9	25	2	2	3	2	2	2	3
[36]	12	230	3	3	3	4	4	5	3
[37]	13	200	3	3	3	4	3	6	3
[38]	17	120	4	3	4	4	4	7	4

Table 14. Comparison of logic operators (LO) reduction in the proposed method with other CSE methods with 610-taps.

Word length	Proposed method	[32]	[11]	[7]
24	441	496	872	1007
20	282	306	680	774
16	125	168	462	525
12	30	47	247	260

Table 15. Comparison of logic depth (LD) in the proposed method with other CSE methods with 610-taps.

Word length	Proposed method	[32]	[11]	[7]
24	5	4	5	4
20	4	4	4	4
16	3	3	4	4
12	3	3	2	3





Utilization of Waste Newspaper for the Production of Bioethanol using *Pseudomonas fluorescens*

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ABSTRACT

Paper, which is one of the largest constituent of Municipal solid waste, has become a severe problem for disposal in developed and developing countries due to the shrinking landfill capacity. It is very important and challenging task in managing the solid waste. Newspaper, which is a cellulosic feed stock, is emerging as an attractive option for the production of bio-ethanol because of lower feedstock costs, higher potential for fossil fuel displacement and also there will be reduction in greenhouse gas emission as compared to production of ethanol from corn. The main objective of the current study is to minimize the newspaper load on municipal solid waste by efficiently utilizing the waste newspaper in the production of bio-ethanol. Experimental studies have been carried out to optimize the pre-treatment process for increasing the efficiency of bacterial hydrolysis, the efficient conversion of cellulose to sugars from cellulose degrading bacteria, are isolated from soil and its undergoes standard biochemical tests then, its used to convert the sugars released to Ethanol by using Fermentation process. Pretreatment, hydrolysis and fermentation are the steps involved in the production of Bioethanol. In the pre-treatment process, the Lignin, Hemicellulose and Cellulose are separated to enhance the hydrolysis process. The optimized condition for the pre-treatment was found to be 1.3% concentration of H₂SO₄ at 121 °C and 45 minutes. The bacteria *Pseudomonas fluorescens* was used for hydrolysis process, which helped in converting the cellulose to sugars and was analysed using Dinitro salicylic acid. The reducing sugars were fermented to produce Bioethanol using the Yeast *Saccharomyces Cerevisiae* and the yield was estimated after the conversion of bioethanol that analyzed 0.2875 absorbance and 0.1134 sugar concentration by UV spectrometer at 575nm.



**Uma maheswari and Abirami****Keywords:** Bioethanol, cellulose degrading bacteria, newspaper, UV spectrometer.

INTRODUCTION

News-papers, which is a cellulosic feed stock, is emerging as an attractive option for the production of bioethanol because of lower feed stock costs, higher potential for fossil fuel displacement and also there will be reduction in greenhouse gas emission as compared to production of ethanol from corn. The frequent recycling of newspaper has resulted in low grade quality of the paper and recycling of paper needs the high processing cost. The main objective of the current project is to minimize the newspaper load on municipal solid waste by efficiently utilizing the waste newspaper in the production of bioethanol with the help of microorganism at low cost. (Shruthi *et al.*, 2016). The need of energy is increasing continuously due to rapid increase in the number of industries and vehicles owing to population explosion. The sources of this energy are petroleum, natural gas, coal, hydrocarbon and nuclear. The major disadvantages of using petroleum based fuels are atmospheric pollution created by the use of petroleum diesel. The petroleum diesel combustion emits several greenhouse gases. Apart from the emissions, petroleum diesel is also a major source of these air contaminants: particulate matter and volatile organic compounds.

Bioethanol is simply ethanol is a renewable energy source which is made by fermenting the sugar and the starch components of plant. It is produced from the agriculture product such as corn, sugarcane, potatoes, rice, beetroot and recently using grapes, banana, dates and other wastes. This is due to the decreasing amount of fossil fuel, alternative energy source need to be renewable, sustainable, cost efficient, convenient and safe. The demand for oil is expected to 57% from 2002 to 2030. The average price of gasoline in 2005 was \$2.56 per gallon, which was \$0.67 higher than the average price of gasoline in the previous year. Yet in June 2008, the average price of gasoline in the US reached \$4.10 per gallon. Rise in energy demand worldwide and the progressive depleting of oil reserves motivate the search for alternative energy resources, especially for those derived from renewable material such as biomass.

As a result, they come up ethanol production as substitutes to fossil fuel. The lower cost to produce bioethanol comes from biomass waste because the raw material are available in abundance. Lower carbon dioxide emission helps reduce the impacts of global warming. Energy balance and security bioethanol production and use at home bioethanol helps reduce the need of foreign. Less toxicity, biodegradable, safety and can recycle, so the bioethanol has minimal environmental impact. Environmental pollution and diminishing supply of fossil fuel are the key factors leading to search for alternative sources of energy. Today, 86% of the world energy consumption and almost 100% of the world energy needed in the transportation sector is met by fossil fuel (Ananda *et al.*, 2007). Since the world's accessible oil reservoirs are gradually depleting, it is important to develop suitable long-term strategies based on utilization of renewable fuel that would gradually substitute the declining fossil fuel production. In addition, the production and consumption of fossil fuel have caused the environmental damage by increasing the CO₂ concentration in the atmosphere (Westerman *et al.*, 2007).

MATERIALS AND METHODS

Isolation and identification of *Pseudomonas fluorescens*:

The soil sample was collected from agricultural area in and around Thiruvavur district, Tamilnadu, India. Soil samples were stored in polythene bags, labeled and took to laboratory.



**Uma maheswari and Abirami****Media and its Composition:****Nutrient Agar:**

Peptone 0.5g, beef extract 0.2g, sodium chloride 0.5g, agar 1.5g and 100ml of distilled water were taken in 250ml of conical flask and mixed gently with sterile stirrer without making air bubble. And it sterilized in autoclaving at 121°C for 15 minutes.

Serial Dilution Technique:**Procedure:**

After sample collection, serial dilution was performed for isolating bacterial growth from the collected samples by crushing and blending with Mortar and Pestle, then its sample was undergoes sterile dilution. For this 10ml of sterile distilled water was taken in attest tube. 1g of soil sample was added. The tubes were vigorously vortexed 3 times to obtain uniform suspension of organisms. A series of tube labeled as 10^{-2} upto 10^{-8} were filled with 9ml of sterile distilled water. 1ml of diluted sample were transferred into the 10^{-1} marked tube. It is further continued upto 10^{-8} dilution and from last dilution 1ml was discarded. The nutrient agar medium plates was inoculated with 10^{-4} and 10^{-5} dilution for bacteria and incubated at 27°C for 24 to 48 hours.

Plating of culture:

Nutrient medium was prepared and it poured in sterile petriplates then it was kept for sterilization for 20 minutes. After sterilization, the plates was kept in laminar air flow chamber and allowed to cool, for sometime the mixture can be poured into plates and allowed it to solidify. Using streaking method the 10^{-4} of serial dilution culture can be streaked in the solidified plates using loop aseptically and sealed with paraffin and stored in refrigerator.

Gram staining:**Procedure:**

The smear of the isolated *Pseudomonas fluorescens* culture was dropped in a clean glass slide. The smear was allowed to air dried and fixed with heat. The slide was placed on the slide rack for staining. The smear was flooded with crystal violet and followed it for 30 seconds or 1 minute. Iodine solution was washed with 95% ethyl alcohol. Ethyl alcohol was added drop wise, until more colour flows from the smear. The slide was washed with distilled water and dried properly. The smear was finally with counter stain saffranin for 30 seconds. The slide was washed with distilled water and dried properly. The slides was observed under low and high power objectives of the compound microscope.

Biochemical Tests (Cappuccino and Shermann,1998):

The biochemical tests were conducted by the following methods as described by Norries and Ribbens (1972) to identify the bacteria.

Indole Test

Tryptone broth was prepared by 10g of peptone in 100ml of distilled water. the pH of the medium was adjusted to 7.3 and sterilized. The test tubes containing trypton broth were inoculated and uninoculated tubes were maintained at 35°C for 48hours. After 48 hours, 1ml of Kovac's reagents was added to each tube, including control. The tubes were gently shaken at interval of 10-15 minutes and allowed to stand until the reagent reached to top. Cherry red ring was formed may indicated that indole positive. Absence of red colour ring formation is considered as negative result.

Methyl Red Test

MR-VP broth was prepared by mixing peptone-7.0g, dextrose-5.0g and potassium phosphate -5.0g in 100ml of distilled water. The pH of the medium was adjusted to 6.9. 5ml of the broth was poured into each tube and sterilized MR-VP tubes were inoculated with the isolated separately and the control was maintained. All tubes were incubated at 28°C for 48 hours. After 48 hours, few drops of methyl red indicator was added to each tube, red colour observed (throughout tube) which indicated positive result. If it remains yellow it is said to be negative.



**Uma maheswari and Abirami****Voges Proskauer Test**

5 ml of MR-VP broth was poured into each tube and sterilized. The tubes were inoculated separately with the isolates. The uninoculated tubes were as control. All the tubes were incubated at 28°C for 48 hours. A few drops of deep rose colour. If tubes remain yellow it is considered as negative result.

Citrate Utilization Test

Simmon's citrate agar slants were prepared by mixing ammonium dihydrogen phosphate-1.0g, dipotassium phosphate - 1.0g, sodium chloride-5.0g, sodium citrate-5.0g, magnesium sulphate-0.2g, bromothymol blue-0.08g and agar- 15.0g in 1000ml of distilled water, and it was sterilized. The slants were incubated with the isolates and control was maintained without inoculums, the tubes were when incubated at 28c for 24 hours and the colour change was observed from green to blue which indicates positive results. Otherwise it is to be negative.

Oxidase Test

Tryptase soy agar plates were prepared, and single line streak of inoculated bacterial isolates were made separately on the agar surface and incubated at 28c for 24 hours. then 2 or 3 drops of aminodimethyl aniline oxalate were added to the surface of the incubated plants and observed for the colour change indicates positive results. otherwise it is said to be negative.

Catalase Test

The isolate of *Pseudomonas fluorescens* were picked up aseptically from the slants and a smear was made on a clean glass slide. Then a drop of hydrogen peroxide was placed on the slides with bacterial culture and observed for the production of gas bubble indicates positive results. If there is no bubble formation, that indicates negative results.

Collection of substrate:

Newspaper, which was used as a substrate for the production of bioethanol, was collected from the households. The substrate was collected in a dust free and fungus-free state and dried in sunlight and was made into small pieces and stored in sealed plastic bags.

Chemical Pretreatment

Newspaper was dried in autoclaving at 65°C for 24 hours and pulverized in electric blender to form fluffy wool like substrate. 20 g of pulverized substrate and 300ml of 1% NaOH solution are mixed in 500ml conical flask and kept it for hydrolysis for 6 hours.

Estimation of Cellulose (Updegraff, 1969)

Cellulose, a major structural polysaccharide in plants, is the most abundant organic compound in nature, and is composed of glucose units joined together in the form of the repeating units of the disaccharide cellobiose with numerous cross linkages. It is also a major component in many of the farm wastes.

Procedure

3mL acetic/nitric reagent were added to a known amount (0.5g or 1g) of the sample in a test tube and mix in a vortex mixture. Placed the tube in a water bath at 100°C for 30min. Cooled and then centrifuge the contents for 15-20min. The supernatant was discarded. The residues was washed with distilled water. 10mL of 67% sulphuric acid was added and allowed it to stand for 1h. 1mL of the above solution was diluted to 100mL. To 1mL of this diluted solution, was added 10mL of anthrone reagent and mixed well. The tubes was heated in boiling water bath for 10min. And it was cooled and measure the color at 630nm. Set a blank with anthrone reagent and distilled water. 100mg cellulose I was taken in a test tube and it proceeded for standard. Instead of just taking 1mL of the diluted solution, take a series of volume and it develop color. The cellulose can estimated by the turbidometry method at 600nm it is based on the cellulose density content in the sample.



**Uma maheswari and Abirami****Optimization of pretreatment process (Zahid Anwar 2011)**

Pre-treatment optimization for the substrate was carried out by using different combination dilute sulphuric acid ranging from 0 to 6% and heating period of 30, 45 and 60 minutes at 121°C and 15lb pressure. 1gm of substrate was added with 10 ml of dilute sulphuric acid (1:10). Cellulose released during this optimization process was analysed by anthrone method, (Zahid Anwar *et al.*, 2011). Maximum amount of cellulose was released during pre-treatment process, the solution was taken for hydrolysis.

Hydrolysis of the pretreated substrate (Zahid Anwar 2011).

Maximum cellulose released during the pretreatment was hydrolysed by the isolated *pseudomonas fluorescens*. The pretreated substrate was washed with distilled water several times to neutralise the acid concentration. The substrate was oven dried till constant weight and the pH was adjusted to 7.0. Reducing sugars release during substrate hydrolysis were analysed by Dinitrosalicylic Acid (DNS) method every 24hr from zero hour, for both the organisms. Maximum sugars released during this period were further taken for fermentation to produce bioethanol.

Sugar analysis by Dinitrosalicylic acid (DNS) method (Warwick 1982)

One liter of water was added to 20 g of the hydrolysed pretreated substrate. The medium composition are 6.60g of potassium sulfate, 3.0g of monopotassium phosphate, 0.50g of magnesium sulfate, 1.0g of calcium chloride dehydrate, 5.0g of peptone was added into the hydrolysed pretreated substrate. The mixture of substrate was autoclaved at 121°C for 15 minutes, and then the medium was cooled to room temperature. After it cools down the isolated *Pseudomonas fluorescens* were added into the substrate and finally the substrate was kept in orbital shaker at 35°C for 140rpm for 48 hours.

Procedure

3ml of prepared reagent was added to 3ml of pretreated substrate in the lightly capped test tubes. The mixture was heated at 90°C for 5-15 minutes till red-brown coloration is developed. 1ml of 40% Na-K tartarate solution was added to stabilize the color.

Fermentation and centrifugation (Sahail 2011)

The cellulose were filtered, mixed, and diluted with water to adjust the initial concentration. After 48 hours of substrate, it is taken from shaker and kept in a laminar air flow chamber and take yeast cultured plates and dilution in 250ml conical flask and keep in incubator for about 72 hours. After fermentation the sugar molecules that can be centrifuged at 5000rpm for 10 minutes. During centrifugation the yeast containing substrate that can be separated into pellet and supernatant. For distillation purpose, the supernatant that is poured in the round bottom flasks by setting at 70rpm until the supernatant of sample that can be filtered and converted into bioethanol

Distillation and Analysis (Hernandez Lopez 2018)

After the centrifugation process, the supernatant was taken in round bottom flask to set at 70rpm for distillation purpose. Then filter the sample and that can be analysed by UV- spectrometer at 575nm.

RESULTS

Our research findings was highlighted that minimize the newspapers load on municipal solid waste by efficiently utilizing the waste newspapers in the production of bioethanol by pretreatment, hydrolysis and fermentation with *Pseudomonas fluorescens*.

Isolation and Identification of *Pseudomonas fluorescens*

The isolated *Pseudomonas fluorescens* was a common Gram-negative, rod-shaped bacterium. It appear white, smooth edge colony in nutrient agar plates. Under the sun light, it was seen a little greenish yellow fluorescent. The



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identification of *Pseudomonas fluorescens* was done by Biochemical tests. The *Pseudomonas fluorescens* was positive for voges proskaur, citrate, oxidase, catalase tests. And it showed negative test for indole and methyl red tests (Table-1).

Chemical Analysis of the Substrate

The chemical analysis of the substrate showed that the showed consisted of 40% cellulose. Ash content and moisture content were found to be 5.14% and 5% respectively.

Estimation of Cellulose

Cellulose was estimated by using a method described by Updegraff (1969), Where the fiber is dissolved in acetic and nitric acid to remove lignin, hemicelluloses and xylosans. The resulting cellulose is allowed to react with anthrone in sulfuric acid. That resulted the coloured compound is assayed turbidometrically at the wavelength was approximately 80% transmittance for the 5ml of estimated cellulose (Table-2).

Optimization of Pretreatment of the Substrate

In the present study, pre-treatment optimization of substrate was carried out at various concentration of dilute sulphuric acid (0 to 5%) and at varying heating periods (30,45 and 60 minutes). Acetic acid furfural and other inhibitors of yeast metabolism are usually present in the pre-treated substrate was washed with distilled water several times. The graphical representation of the maximum recovery of cellulose was 55% with dilute sulphuric acid concentration of 2% and a heating period of 45minutes at 121° C. In the literature also it has been reported that dilute sulphuric acid with concentration below 3% has been used to make the pre-treatment process inexpensive and effective. (Table-3)

Hydrolysis of the pretreated substrate

In the hydrolysis process the cellulose present the substrate was converted into ethanol using the cellulose degrading organism after the pre-treatment process. In the present process, bacteria was used for hydrolysis instead of enzymes, since the enzymes are expensive and it helps to increase the cost of bioethanol production. Hydrolysis was carried out at neutral pH. The pure culture of isolated an identified *Pseudomonas fluorescens* was used for the hydrolysis of the pretreatment substrate.(Table-4)

Fermentation and Centrifugation

Fermentation of sugars released during hydrolysis of substrates was carried out by yeast *Saccharomyces cerevisiae* at pH 4.6 and 34°C temperature, to convert released sugar into bioethanol. Fresh inoculum of yeast (5%v/v) was added to the hydrolysed broth. Fermentation was carried out for six days with fermented samples being collected every twenty four hours for analysis of reducing sugar by Dinitrosalicylic acid(DNS) method for substrate utilization. The cellulose and dinitrosalicylic acid was added and its indicated yellow colour. Yellow colour was changed into reddish orange when added cellulose, dinitrosalicylic and cellulase. Then effluent was filtered and taken for centrifugation for 5000rpm for 10 minutes (Table-5)

Distillation and analysis

During centrifugation the yeast containing substrate are separated into pellet and supernatant. the supernatant was poured in round bottom flask to setted at 70rpm until the supernatant of sample that filtered and converted into bioethanol. After the conversion of bioethanol that analyzed 0.2875 absorbance and 0.1134 sugar concentration by UV-Spectrometer at 575nm.

SUMMARY AND CONCLUSION

Newspaper is emerging as an attractive option for the production of bioethanol because of lower feedstock costs. It also has higher potential for fossil fuel displacement due to which there will be reduction of the house gas emission. The present work deals with the studies on production of bioethanol from waste newspaper which is one of the





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largest constituent of Municipal solid waste. Experiments was carried out for pretreatment of the newspaper substrate with dilute sulphuric acid and the cellulose was released during this optimization process, it analysed by anthrone method. The optimized condition for the pretreatment was found to be 1.5% concentration of H₂SO₄ at 121°C and 45 minutes of constant time. The maximum amount of cellulose obtained under these optimum parameters was 55%.The study of hydrolysis of pretreated subset was carried out by using *Pseudomonas fluorescens* and it was isolated from soil sample and identified by biochemical test. Then these *P.fluorescens* are well effective to convert the cellulose into reducing sugar as the result it was observed that 0.146g of reducing sugar was obtained per gram of the substrate. Yeast *Saccharomyces cerevisiae* was used to ferment the reducing sugar into bioethanol. Based on these results, it can be concluded that the biological hydrolysis of cellulose by endophytic bacteria *Pseudomonas fluorescens* was more effective to converting cellulose into reducing sugar for the major compound for the production of ethanol. Further study is to be planned to isolate *Pseudomonas* species able to produce bioethanol from newspapers at low cost, identify bioactive compound and make able to use in coming generation when the demand for other fuel.

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Table-1: Biochemical Tests For *Pseudomonas fluorescens*

Biochemical test	Positive or Negative
Indole	Negative
Methyl red	Negative
Voges proskaur	Positive
Citrate	Positive
Oxidase	Positive
Catalase	Positive

Table – 2 Estimation of Cellulose By Turbidimetry Method

S.No	value of the estimated cellulose (ml)	OD value (%)	percent transmittance (nm)
1	0	0	90
2	1	0.08	89
3	2	0.09	86
4	3	0.4	84
5	4	0.7	82
6	5	0.9	80

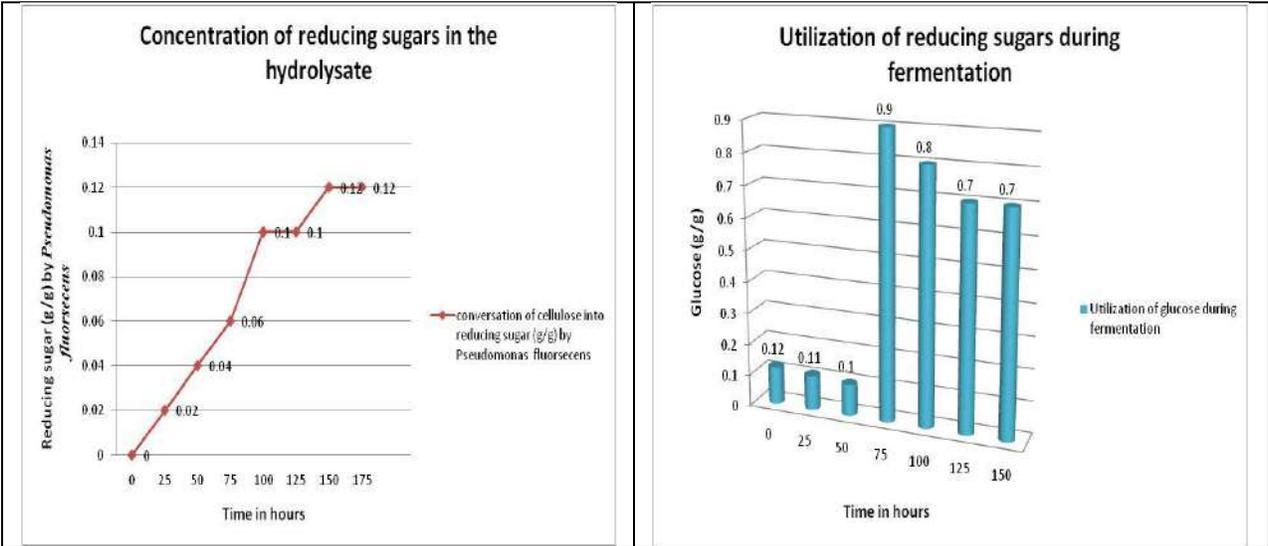
Table-3 Optimization of pretreatment of the substrate

Time duration (minutes)	Concentration of H ₂ SO ₄ (%)	Cellulose released (g/g)
30	1	0.12
	2	0.16
	3	0.17
	4	0.15
	5	0.13
45	1	0.17
	2	0.21
	3	0.19
	4	0.18
	5	0.16
60	1	0.22
	2	0.21
	3	0.19
	4	0.18
	5	0.16





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To Identify The Peripheral Artery Disease and Association with QOL, Physical Activity and Body Composition Among Diabetic Patients In VMMC Hospital, Karaikal.

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ABSTRACT

Peripheral artery disease refers to any disorder of the circulatory system outside of the brain and heart. It is "silent killer" disease. As stated to "save India" screening programme for vascular disease on "national vascular week", in India one in every 20 Indians over the age of 40 has peripheral arterial disease and it is extent to affect more than 9 million people in India. 1.To identify the peripheral arterial disease (PAD) among diabetic patients in VMMC hospital, Karaikal.2.To identify the relation between peripheral arterial disease (PAD) with quality of life (QOL), physical activity and body composition.3.To find the association of quality of life, physical activity and Body composition with selected demographic and clinical variables. Quantitative descriptive research design was used. Hundred patients were selected using purposive sampling technique and study conducted in VMMC, Karaikal, The demographic data and clinical data were collected. The peripheral artery disease assessed by using ankle brachial index stander scale and quality of life assessed by using six item vascular Quality of life, physical activity assessed by PAD Walking impairment questionnaire and body composition assessed by Body mass index. The finding reveal that among the total number of 100 subjects, ankle brachial index scale was used this study to identify the peripheral artery disease (PAD) among diabetic patients 34%& 33% were



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mild and moderate level PAD, 27% were normal level, 6 % were severe level PAD. Results shows that regarding QOL majority of the study subjects 54% were better QOL, 42% were moderate QOL and 4% were mild QOL. Regarding physical activity majority of the study subjects 69% were no physical activity, 25% were much difficulty and 6% were some difficulty. Regarding body composition majority of the study subjects 71% were overweight, 19% were obesity, 8% were normal and 2% were extreme obesity.

Keywords: Peripheral Artery Disease, QOL, Physical Activity, Diabetic, Patients, VMMC Hospital, Karaikal.

INTRODUCTION

Diabetes mellitus is one of the leading non-communicable diseases of the 21st century. India with more than 62 million diagnosed diabetics is fast earning the title of 'Diabetes Capital of the World. Diabetes imposes a heavy toll on the vascular system, with both macro vascular and micro vascular complications. PAD is one of the macrovascular complications of Type 2 DM. Prevalence of PAD is higher among diabetics and has a predilection for lower limbs. Peripheral arterial disease is a term used to describe the impairment of blood flow to the extremities usually as a result of atherosclerotic occlusive disease. Generally speaking, the presence of symptoms in PAD depends on the metabolic demands of the ischemic tissue during exercise, the degree of collateral circulation and the size and location of the affected artery. The incidence of PAD varies in the general population from 3% to 10% in people younger than 70 years to 15–20% in people older than 70 years. However, 40% of PAD patients are asymptomatic, while only 10% of them present with typical intermittent claudication (IC).^{3, 4} One third of PAD patients will have a complete occlusion of a major artery to the leg at first presentation. Peripheral arterial disease is an important circulatory system disorder similar in prevalence to stroke and coronary heart disease. Although 65–75% of patients with PAD are asymptomatic, the classic presenting symptom is IC which is usually described as muscle cramps, fatigue or pain in the lower legs induced by exercise and rapidly relieved by rest; often the symptom location indicates the level of arterial involvement. Less commonly, patients may present with critical limb ischemia.

Around 20–40 million are likely to have intermittent claudication and 100 million atypical leg symptoms. Pain and limited mobility lead to a diminished quality of life. However, even asymptomatic people with peripheral artery disease have impaired lower extremity functioning, increased mobility loss, and faster functional decline than individuals without peripheral artery disease. Studies have been conducted both in health care centers as well as in the urban population for estimation of the prevalence of PAD among Type 2 diabetics. The studies conclude that a large proportion of diabetics have a decreased ABI below 0.9 [13,14]. However, there are no studies to establish diabetes as an independent risk factor for PAD in asymptomatic patients. Early diagnosis of PAD can help patients to effectively manage the condition and prevents its long term sequelae. The purpose of this study mainly to identify the peripheral artery disease among diabetic patients. The outcomes of PAD in diabetic patients are also associated with QOL, Physical Activity and Body composition. Early Diagnosis and treatment of PAD in diabetic patients was important for risk factors modification, reduction of its prevalence, prognosis and improvement of its outcome.

Statement of the Problem

A study to identify the peripheral artery disease and association with QOL, Physical activity and body composition among diabetic patients in VMMC, Karaikal.

Objectives

- To identify the peripheral arterial disease (PAD) among diabetic patients in VMMC, Karaikal.
- To identify the relation between peripheral arterial disease (PAD) with quality of life (QOL), physical activity and body composition.
- To find the association of quality of life, physical activity and Body composition with selected demographic and clinical variables.



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- To find the association of Peripheral artery disease with selected demographic variables and clinical variables

MATERIAL AND METHODS

The Quantitative descriptive research design was selected for the study to identify the peripheral artery disease and association with QOL, Physical activity and body composition among diabetic patients. The study setting was Vinayaka Mission's Medical college and Hospital, Karaikal. The population for this study was Diabetic Patients. Hundred diabetic patients were selected by purposive sampling technique. Using the demographic variables basic information was collected. Peripheral artery disease identified by Ankle Brachial Index Scale, Quality of Life assessed by Six item vascular QOL, physical activity assessed by PAD walking impairment questionnaire and Body Composition assessed by Body mass index.

Data Collection Procedure

Formal permission was obtained from the dean of Vinayaka Mission's Medical College and Hospital at Karaikal, considering all ethical principles. Purpose of the study was explained to the sample, the confidentiality of their responses was assured and their written consent was taken prior to the study. The standardized ABI scale was administered to the Diabetic patient to identify the peripheral arterial disease (PAD). ABI scale to assess the highest pressure in right or left foot and highest pressure in both arms recorded. Six item Vascular QOL and PAD walking impairment questionnaire used to collect the data regarding QOL and physical activity. Height and weight checked to assess the Body mass index.

RESULTS AND DISCUSSION

- In the above table, shows that correlation value for Peripheral artery disease and Quality of Life score is 0.116. It is evident that there is a positive relationship between Peripheral artery disease and Quality of Life
- In the above table shows that correlation value for Peripheral artery disease and Physical activity score is 0.48. It is evident that there is a positive relationship between Peripheral artery disease and Physical Activity.
- In the above table shows that correlation value for Peripheral artery disease and Body composition is 0.20. It is evident that there is a positive relationship between Peripheral artery disease and Body composition.

CONCLUSION

According to the result, diabetic patients had the risk to develop peripheral artery disease. There was association with quality of life, physical activity and body composition with demographic variables and clinical variables which is statically proved. Hence the researcher concluded that study Diabetes Mellitus is a one of the risk and major causes of peripheral artery disease (PAD) and this study results also supported Peripheral Artery Disease affects the quality among diabetic patients.

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Table.1: Identify the Peripheral Artery Disease Among Diabetic Patients

ABI score	Frequency	Percentage (%)
Severe PAD	06	6
Moderate PAD	33	33
Mild PAD	34	34
Normal	27	27
Total	100	100

Table.2: Identify the Relation between Peripheral Artery Disease with Quality Of Life (QOL)

S. No	Aspects	Sample size (N)	Mean	' r'
1	Peripheral Artery Disease	100	3.56	0.116
2	QOL	100	2.52	

Table 3: Identify the Relation Between Peripheral Artery Disease With Physical Activity

S.No	Aspects	Sample size (N)	Mean	' r'
1	Peripheral Artery Disease	100	2.18	0.48
2	Physical Activity	100	3.63	

Table 4: Identify the Relation between Peripheral Artery Disease with Body Composition

S.No	Aspects	Sample size (N)	Mean	' r'
1	Peripheral Artery Disease	100	2.18	0.20
2	Physical Activity	100	2.15	

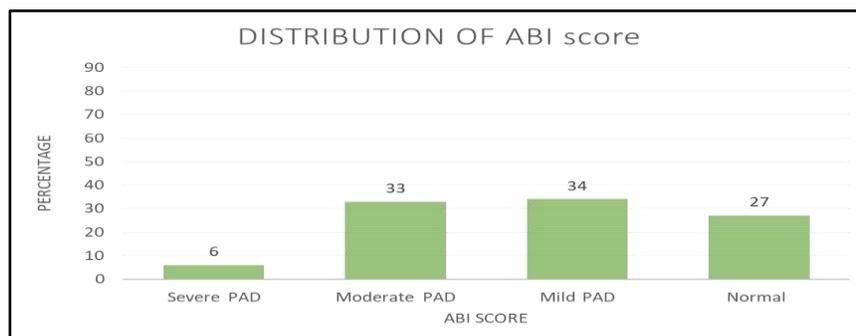


Fig.1. Distribution of ABI Score





Effectiveness of Myofascial Release Technique in Improving Blood Oxygen Level in Athletes

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ABSTRACT

The purpose of the study is to evaluate the effect of myofascial release technique on blood oxygen level. 15 young aged athletes between the age of 18 and 22 within the Vinayaka Mission's College Of Physiotherapy were selected as the samples using simple random sampling technique. Pre-test measurement of blood oxygen level were taken using pulse oximeter. After the Pre-test assessment, all the subjects received myofascial release technique for a period of 15 days and on the 15th day, post-test measurement were taken similar to that of pre-test measurement. The result showed that myofascial release technique was significantly effective in improving blood oxygen level

Keywords: myofascial, Pre-test assessment, blood oxygen, Physiotherapy.

INTRODUCTION

The myofascial release is now recognized by many in the training industry as an accepted component to an overall regimen of training and competition. "Myofascial release is a mechanical stimulation of the tissues by means of slow, steady pressure along with sustained stretching to gently soften that fascia over and through the muscles, more effectively releasing the tension throughout" [1-3]. Myofascial release is incredibly beneficial when used by athletes to improve range of motion, reduce soreness and help assist the tissue recovery process, help the body relax overall, improve circulation, release tension knots and even stress [4-5]. Supply of oxygen and nutrients will improve in blood by myofascial release. Blood flow into muscle is vital in creating new tissue and increase strength and stamina [6]. Increased blood oxygen level is essential for athletes and in sports endurance sometime plays a decisive



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role in victory and help in preventing injuries related to poor fitness [7]. The aim of the study is to find whether there is any significant changes post myofascial release technique in improving blood oxygen level in athletes.

MATERIAL AND METHODS

The study design is a Pre and Post experimental trial. 15 samples were selected using simple random sampling method. Medically and psychologically fit female athletes of VMRF deemed university between the age group of 18-22 years were only included for the study. Informed consent was obtained from the group. The purpose and methods of study were explained to them. The parameter for measuring blood oxygen level was pulse oximeter. Pre-test assessment of blood oxygen level was taken for the group using Queens college step test for all the 15 samples using pulse oximeter. Queens college step test has been proved to be reliable tool to measure blood oxygen level. In Queens college step test the students stepped up and down on the platform/step with a height of 41.3 cm at a rate of 24 steps per minute. The subjects were asked to step using a four-step cadence, 'up-up-down-down' for 3 minutes. The students stopped immediately on completion of the test, once they completed the test, their oxygen level was recorded using pulse oximeter.

After the pretest assessment was over all the subjects underwent myofascial release technique for a period of 15 days. Myofascial release technique was given for six sittings in a period of 15 days where it covered the upper limb & neck, trunk and back and lower limbs. The study was conducted in the Vinayaka Mission's College of Physiotherapy, Salem. On the 15th day post – test measurement were taken for group in a similar fashion as that of pre – test measurement. The data collected through pre and post treatment assessment were subjected to statistical analysis using paired 't' test. Paired 't' test was used to understand whether there is a significant difference between the pre and post treatment assessment in the group. The statistical results following paired 't' is as follows

't' calculated value > 't' tab value.

RESULTS AND DISCUSSION

The data was subjected to statistical analysis and the following results were obtained. Myofascial release technique is significantly effective in improving blood oxygen level in athletes. The present study was designed to determine the effectiveness of myofascial release technique in improving blood oxygen level in athletes. The female athletes selected for the study received for a period of 15 days. A pre and post test measurement of blood oxygen level was tested using Queens college step test. The results are in favour of myofascial release technique may be because of the improved lymphatic and venous drainage which in turn improves the amount of blood in circulation. It also improves the interfascial mobility and thereby flexibility [8-9].

CONCLUSION

The results of this study make us to conclude that myofascial release technique is a effective to improve blood oxygen level in athletes.

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Conflict of interest

The author have none to declare

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**Table 1. Paired 't' test value for experimental group
Myofascial Release Technique**

Variable	t- cal value	t- tab value
Cardiovascular endurance	15.58	2.145





Effectiveness of Warm Up Exercises and Reciprocal Inhibition in Improving Hamstring Flexibility in Middle Aged Women

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ABSTRACT

The purpose of the study is to evaluate the effect of Warm Up Exercises and Reciprocal Inhibition on Hamstring Flexibility. 15 middle aged women between the age group of 35 and 45 years in and around Salem who had Hamstring Tightness were selected as samples using simple random sampling technique. Pre-test measurements of Hamstring Flexibility were collected using Passive Knee Extension Test. After the Pre-test assessment, all the subjects received Warm up exercises and Reciprocal Inhibition for a period of 15 days and on the 15th day, Post-test measurements were collected similarly to that of Pre-test measurement. The results showed that Warm up exercises and Reciprocal Inhibition were significantly effective in improving Hamstring Flexibility in Middle Aged Women.

Keywords: Hamstring Flexibility, Warm Up Exercises, Reciprocal Inhibition, Passive Knee Extension Test

INTRODUCTION

Hamstrings are the group of muscles located on the posterior aspect of the thigh. These muscles play a critical role in human activities, ranging from standing to explosive movements like sprinting and jumping (1). The Hamstring group plays a prominent role in hip extension and knee flexion (2). While walking, the work of the hamstring begins at the final 25% of the swing phase, which generates extension force at the hip and resists knee extension. The hamstring plays as a dynamic stabilizer of the knee (3). Tight muscles are always linked with postural disturbances. It can contribute to multiple musculoskeletal conditions (4). Limited extensibility of the hamstring results in various injuries to the lower back and the lower limbs (5). A tight hamstring causes limited mobility in the knee joint. It is commonly occurring in many individuals (6). Hamstring tightness is defined as the inability of the knee to extend more than 160°, with the hip flexed at 90° (7). A study done by Koli et al. in 2018 identified that females have a high

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prevalence of hamstring tightness than males (8). Warm-up and stretching are advocated to increase the length of the muscle and reduce stiffness (9). Muscle energy technique (MET) is an osteopath-developed manual technique that is now used in various manual therapy professions. MET has received little attention, with only a few published studies supporting its use (10). Reciprocal inhibition (RI) inhibits the antagonist's muscle when isometric contraction occurs in the agonist's muscles. This happens due to stretch receptors within the agonist muscle fibers and muscle spindle (11). RI mechanism states that when a muscle contracts isometrically, its antagonist is inhibited, and there is reduced tone immediately after this (12). The aim of the study is to find out the effectiveness of Warm Up Exercises and Reciprocal Inhibition in improving hamstring flexibility in middle aged women. The need of the study is as many of the middle-aged women who have a sedentary life style have a tight hamstrings problem, which hinder their works in day-to-day activities and end with back pain, knee pain and all. So, improving the flexibility and maintaining that will reduce their problem to maximum level. Hence, we work on finding whether introducing newer physiotherapeutic technique like Reciprocal Inhibition when given in addition to Warm Up Exercises will improve the hamstring flexibility.

MATERIALS AND METHODS

The study design is a Pre and Post experimental trial. 15 samples were selected using simple random sampling method. The subjects who had hamstring tightness and are medically and psychologically fit and also who fulfil the selection criteria of between age group of 35 and 45 in and around Salem were only included for the study. The parameter for hamstring tightness was kept as passive knee extension angle above 19.2 degrees. Informed consent was obtained from the subjects and then only they were included for the study. Pre-test assessment was taken using Passive Knee Extension Test for all the 15 samples for both the legs using goniometer for both groups in the following fashion. The subjects were positioned in supine lying with hip and knee at 90-degree Flexion. Neutral hip rotation was maintained throughout the test. The fulcrum of the goniometry was placed over lateral intercondylar area of knee joint. The stable arm is placed along the lateral border of femur and the movable arm is placed along the lateral border of fibula and with this alignment of the goniometer passive ROM of knee extension was measured.

Then all the subjects were given warm up exercise for 16 mins with rest periods in between. The warm-up exercises included, walking for 7 minutes, marching in the standing position itself with the upper limb reaching outwards for 3 minutes, moving back and front from the standing position one step ahead with upper limb reaching forwards for 3 minutes, moving one step side wards from the standing position and coming back to normal with both upper limbs reaching sideward for 3 minutes.

After the Warm-up exercises, Reciprocal inhibition was provided for hamstring muscle. The treatment technique was given to both legs. The subjects were made to lie in supine lying with contralateral hip and knee supported by a pillow below the knee. The therapist stood on the side of the leg to which reciprocal inhibition needed to be given. The hip is flexed to 90 degrees, and the knee is fully flexed. Then the knee was extended until the restriction barrier was identified. Then placed the leg on the shoulder of the therapist, and the patients were asked to contract the muscle sub maximally by pushing upwards away from the shoulders of the therapist. In contrast, the therapists gave submaximal resistance (a counterforce). This made the knee extensors and the hip flexors contract, which are the antagonists for the hamstring muscle. The contraction is kept held for 7-10 counts, and then it is relaxed for ten counts. After that, again, the limb was passively moved by the therapists and identified a new barrier. Then the procedure was repeated from the new barrier, giving the technique six times. The same protocol was repeated for the other leg. The treatment technique was given once daily for six days a week, for two weeks consecutively for both sides (13), (16).

The collected data was subjected to statistical analysis using paired "t" test for both the legs separately (Table No. 1).



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RESULTS AND DISCUSSION

The data subjected to statistical analysis using paired “t” test for 9 degrees of freedom at 95% confident level had revealed that the Warm up exercises and Reciprocal Inhibition was found to be significantly effective in increasing Hamstring Flexibility in Middle Aged Women.

The results of the study shows that Warm up exercises and Reciprocal Inhibition is significantly effective may be because, reciprocal inhibition inhibits the antagonist's muscle when isometric contraction occurs in the agonist's muscles the reciprocal inhibition mechanism encourages the activation of the opposite muscle group and effectively ensures the tightened muscle to relax. The nerve complex reinforces the spindle to ensure a sound lengthening of the desired muscles. This work in the cohesion of the muscle work (14), (15). Few kinds of literature cited to support the RI is effective in improving hamstring flexibility (10), (15). Reciprocal inhibition is an active contraction of the opposite muscle, facilitating the hamstring muscles. The mechanism is by the neural inhibition associated with pre-stretch contractions (17). Voluntary contraction of the opposing muscles can lead to reducing activation levels. Opposite muscles are activated by stimulating the motor neurons, which provide excitatory input to the Ia-inhibitory interneurons. Thereby the motor neuron of the target muscles is treated for lengthening (18), (19). There is an increased Ia- afferent input from the opposite muscles commonly reported in the literature and which contribute to muscle elongations. However, there is a lot of evidence suggesting that greater activation of the muscle results in a greater level of presynaptic inhibitions of the Ia-afferents targeting the target muscles in the motor neuron pool (14). In the current study, the impact of warm up exercises and one of the muscle energy techniques reciprocal inhibition was analyzed on hamstring flexibility. The outcomes showed that the two techniques essentially improved the hamstring flexibility.

CONCLUSION

The result of the study makes us to conclude that Warm Up and Reciprocal Inhibition are significantly effective in improving Hamstring Flexibility in Middle Aged Women.

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Conflict of Interest

The authors have none to declare.

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Table. 1. Passive Knee Extension test data with paired ‘t’ test

Test	Variable	t calculated value	t table value
Paired “t” test for Warm up exercises and Reciprocal Inhibition for Right leg	Flexibility	15.78	2.145
Paired “t” test for Warm up exercises and Reciprocal Inhibition for Left leg	Flexibility	14.92	2.145





Characterization of (λ, μ) - Multi Fuzzy Quotient Group of A Group

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ABSTRACT

In this paper, we have defined (λ, μ) -multi fuzzy quotient group of a group G and discussed some of their properties under homomorphism and anti- homomorphism .The purpose of the study is to implement the (λ, μ) -fuzzy set theory and group theory in multi fuzzy set theory.

Keywords: (λ, μ) -fuzzy set ((λ, μ) -FS) , (λ, μ) -multi fuzzy set ((λ, μ) -MFS), (λ, μ) -multi fuzzy subgroup ((λ, μ) -MFSG), (λ, μ) -multi fuzzy coset.

INTRODUCTION

Since Zadeh [17] introduced the concept of a fuzzy set in 1965, various algebraic structures have been fuzzy field. In 1971, Rosenfeld [9] introduced the notion of a fuzzy subgroup and thus initiated the study of fuzzy groups. In recent years, some variants and extensions of fuzzy groups emerged. In 1996, Bhakat and Das proposed the concept of an $(\epsilon, \in \vee q)$ -fuzzy subgroup in [5] and investigated their fundamental properties. They showed that A is an $(\epsilon, \in \vee q)$ -fuzzy subgroup if and only if $A \alpha$ is a crisp group for any $\alpha \in (0, 0.5]$ provided $A \alpha \neq \emptyset$. A question arises naturally: can we define a type of fuzzy subgroups such that all of their nonempty α -level sets are crisp subgroups for any α in an interval $(\lambda, \mu]$? In 2003, Yuan et al. [15] answered this question by defining a so-called (λ, μ) -fuzzy subgroups, which is an extension of $(\epsilon, \in \vee q)$ -fuzzy subgroup. As in the case of fuzzy group, some counterparts of classic concepts can be found for (λ, μ) -fuzzy subgroups. For instance, (λ, μ) - fuzzy normal subgroups and (λ, μ) -fuzzy quotient groups are defined and their elementary properties are investigated, and an equivalent characterization of (λ, μ) -fuzzy normal subgroups was presented in [16].





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S.Sabu and T.V.Ramakrishnan [11] proposed the theory of multi fuzzy sets in terms of multi dimensional membership functions and investigated some properties of multi level fuzziness. An element of a multi-fuzzy set can occur more than once with possibly same or different membership values. R.Muthuraj and S.Balamurugan [9] proposed the characterization of quotient multi-fuzzy subgroup. The notion of t-intuitionistic multi fuzzy set and t-intuitionistic multi fuzzy group has already been introduced by the KR. Balasubramanian and R.Rajangam in [3]. Here in this paper, we define and establish some related properties of new algebraic structure (λ, μ) - multi fuzzy quotient group of a group.

PRELIMINARIES

We first recall some Definitions for the sake of completeness of the topic under study.

Definition: 2.1

Let X be a non-empty set. A fuzzy subset A of X is defined by a function $A : X \rightarrow [0,1]$.

Definition: 2.2

Let X be a non-empty set. A multi fuzzy set A in X is defined as the set of ordered sequences as follows,
 $A = \{ (x, A_1(x), A_2(x), \dots, A_k(x), \dots) : x \in X \}$. Where $A_i : X \rightarrow [0,1]$ for all i .

Definition: 2.3

Let X be a non-empty set. A k -dimensional multi fuzzy set A in X is defined by the set
 $A = \{ (x, (A_1(x), A_2(x), \dots, A_k(x))) : x \in X \}$. Where $A_i : X \rightarrow [0,1]$ for $i = 1, 2, 3, \dots, k$.

Definition: 2.4

Let A be a fuzzy subset of G . Then a (λ, μ) - fuzzy subset $A^{(\lambda, \mu)}$ of a fuzzy set A of G is defined as $A^{(\lambda, \mu)} = (x, A \vee \lambda \wedge \mu : x \in G)$.

Definition: 2.5

Let A be a fuzzy subset of G . A is called a (λ, μ) -fuzzy subgroup of G if, for all $x, y \in G$,

- (i) $A(xy) \vee \lambda \geq A(x) \wedge A(y) \wedge \mu$
- (ii) $A(x^{-1}) \vee \lambda \geq A(x) \wedge \mu$

Clearly, a $(0, 1)$ -fuzzy subgroup just a fuzzy subgroup, and thus a (λ, μ) -fuzzy subgroup is a generalization of fuzzy subgroup.

Definition: 2.6

Let A be a multi fuzzy subset of G . Then a (λ, μ) - multi fuzzy subset $A^{(\lambda, \mu)}$ of a fuzzy set A of G is defined as $A^{(\lambda, \mu)} = (x, A \vee \lambda \wedge \mu : x \in G)$. That is, $A_i^{(\lambda_i, \mu_i)} = (x, A_i \vee \lambda_i \wedge \mu_i : x \in G)$.

Clearly, a $(0, 1)$ -multi fuzzy subset is just a multi fuzzy subset of G , and thus a (λ, μ) - multi fuzzy subgroup is a generalization of fuzzy subgroup, where $(0, 1)$ -multi fuzzy subset A is defined as $A^{(0,1)} = (A_i^{(0,1_i)})$

Definition: 2.7

A MFS $A = \{(x, A(x)) : x \in X\}$ of a group G is said to be a (λ, μ) -multi fuzzy sub group of G ((λ, μ) -MFSG), if it satisfies the following: For $\lambda, \mu \in [0,1]^k, 0 \leq \lambda_i \leq \mu_i \leq 1, 0 \leq \lambda_i + \mu_i \leq 1$

- (i) $A(xy) \vee \lambda \geq \min\{A(x), A(y)\} \wedge \mu$
- (ii) $A(x^{-1}) \vee \lambda \geq A(x) \wedge \mu$ for all $x, y \in G$. That is,
- (i) $A_i(xy) \vee \lambda_i \geq \min\{A_i(x), A_i(y)\} \wedge \mu_i$
- (ii) $A_i(x^{-1}) \vee \lambda_i \geq A_i(x) \wedge \mu_i$ for all $x, y \in G$.

Clearly, a $(0, 1)$ -multi fuzzy subgroup is just a multi fuzzy subgroup of G , and thus a (λ, μ) - multi fuzzy subgroup is a generalization of multi fuzzy subgroup.





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Definition: 2.8

A (λ, μ) – MFSG $A^{(\lambda, \mu)}$ of a group G is said to be an (λ, μ) –multi fuzzy normal subgroup ((λ, μ) – MFNSG) of G , it satisfies $A^{(\lambda, \mu)}(xy) = A^{(\lambda, \mu)}(yx)$ for all $x, y \in G$

Theorem: 2.9

A (λ, μ) –MFSG $A^{(\lambda, \mu)}$ of a group G is normal, it satisfies $A^{(\lambda, \mu)}(g^{-1}xg) = A^{(\lambda, \mu)}(x)$ for all $x, y \in G$ and $g \in G$

Proof : Let $x \in A^{(\lambda, \mu)}$ and $g \in G$.

Then $A^{(\lambda, \mu)}(g^{-1}xg) = A^{(\lambda, \mu)}(g^{-1}(xg)) = A^{(\lambda, \mu)}((xg)g^{-1})$, since $A^{(\lambda, \mu)}$ is normal.

$A^{(\lambda, \mu)}((xg)g^{-1}) = A^{(\lambda, \mu)}(x(gg^{-1})) = A^{(\lambda, \mu)}(xe) = A^{(\lambda, \mu)}(x)$. Hence its true.

Definition: 2.10

Let G be a group and A be FSG of group G . Let $x \in G$ be a fixed element. Then for every element $g \in G$, we define $(xA)(g) = (A)(x^{-1}g)$.

Then Ax is called fuzzy left coset of G determined by A and x also we have

$(Ax)(g) = (A)(gx^{-1})$. Then Ax is called the fuzzy right coset of G determined by A and x .

Definition: 2.11

A FSG A of a group G is FNSG of G if and only if $xA = Ax$ for all $x \in G$

Definition: 2.12

Let X and Y be two non-empty sets and $f : X \rightarrow Y$ be a mapping. Let A and B be FS's of X and Y respectively. Then the image of A under the map f is denoted by $f(A)$ and is defined as,

$$f(A)(y) = \begin{cases} \vee \{A(x) : x \in f^{-1}(y)\} & \text{and} \\ 0 & \text{otherwise} \end{cases}$$

Also the pre-image of B under f is denoted by $f^{-1}(B)$ and is defined as $f^{-1}(B)(x) = B(f(x))$

Note: 2.13 For any $x \in X$, we have $f(A)(f(x)) \geq A(x)$.

Definition: 2.14

Let X and Y be any two non-empty sets and $f : X \rightarrow Y$ be a mapping. Let $A^{(\lambda, \mu)}$ and $B^{(\lambda, \mu)}$ be any two (λ, μ) –MFSs of X and Y respectively having the same dimation k . Then the image of $A^{(\lambda, \mu)}(\subseteq X)$ under the map f is denoted by $f(A^{(\lambda, \mu)})$, is defined as : $\forall y \in Y$,

$$f(A^{(\lambda, \mu)})(y) = \begin{cases} \max_{x \in f^{-1}(y)} A^{(\lambda, \mu)}(x) & : x \in f^{-1}(y) \\ \lambda_k & : \text{otherwise} \end{cases}$$

Also, the pre-image of $B^{(\lambda, \mu)}(\subseteq Y)$ under the map f is denoted by $f^{-1}(B^{(\lambda, \mu)})$ and it is defined as: $f^{-1}(B^{(\lambda, \mu)})(x) = (B^{(\lambda, \mu)})(f(x)), \forall x \in X$.

Definition: 2.14

Let A be a normal multi fuzzy subgroup of G with identity element 'e'. Let $K = \{x \in G / A(x) = A(e)\}$. Consider $\bar{A} = (\bar{A}_1, \bar{A}_2, \dots, \dots, \bar{A}_n)$ which is defined by $\bar{A}(xK) = \sup_{k \in K} A(xk)$ for all $x \in G$, each $\bar{A} : G/K \rightarrow [0,1]$. Then the normal multi fuzzy subgroup \bar{A} of G/K is called a multi fuzzy quotient group or quotient multi fuzzy subgroup of A by K .

Definition: 2.15

Let A be a multi fuzzy subgroup of a group G . For any $a \in G$, define $(aA)(x) = A(a^{-1}x)$ for all $x \in G$ is called a multifuzzy coset of a multi fuzzy subgroup A of the group G determined by the element $a \in G$.





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Properties of (λ, μ) –Multi Fuzzy Quotient Group $\overline{A^{(\lambda, \mu)}}$ of a group G determined by $A^{(\lambda, \mu)}$ and N

In this section, we discuss some of the properties of (λ, μ) – Multi Fuzzy quotient group $\overline{A^{(\lambda, \mu)}}$ of a group G determined by $A^{(\lambda, \mu)}$ and N .

Theorem: 3.1

Let A^t be a (λ, μ) – multi fuzzy normal subgroup of a group G with identity e . Let $= \{x \in G \mid A^{(\lambda, \mu)}(x) = A^{(\lambda, \mu)}(e)\}$. Consider $\overline{A^{(\lambda, \mu)}} = (\overline{A_1^{(\lambda_1, \mu_1)}}, \overline{A_2^{(\lambda_2, \mu_2)}}, \dots, \overline{A_k^{(\lambda_k, \mu_k)}})$ which is defined by,

$$\overline{A^{(\lambda, \mu)}}(xN) = \left(\overline{A_i^{(\lambda_i, \mu_i)}}(xN) \right) = \left(\sup_{n \in N} A_i^{(\lambda_i, \mu_i)}(xn) \right) \text{ for all } x \in G, \text{ where each } \overline{A_i^{(\lambda_i, \mu_i)}} : G/N \rightarrow [0,1]. \text{ Then}$$

- (i) N is normal subgroup of G
- (ii) $\overline{A^{(\lambda, \mu)}}$ is well defined and (λ, μ) –MFSG of G/N

Proof: Given A is (λ, μ) –MFNSG of G and $N = \{x \in G \mid A^{(\lambda, \mu)}(x) = A^{(\lambda, \mu)}(e)\}$. Let $x \in G$ and $y \in N$. Then $A^{(\lambda, \mu)}(y) = A^{(\lambda, \mu)}(e)$. Since $A^{(\lambda, \mu)}$ is (λ, μ) -MFNSG of G , $A^{(\lambda, \mu)}(xyx^{-1}) = A^{(\lambda, \mu)}(y) = A^{(\lambda, \mu)}(e)$. Hence, $(xyx^{-1}) \in N$. Hence, $N = \{x \in G \mid A^{(\lambda, \mu)}(x) = A^{(\lambda, \mu)}(e)\}$ is a normal subgroup of G . Also, consider $\overline{A^{(\lambda, \mu)}} = (\overline{A_1^{(\lambda_1, \mu_1)}}, \overline{A_2^{(\lambda_2, \mu_2)}}, \dots, \overline{A_k^{(\lambda_k, \mu_k)}})$ which is defined by $\overline{A^{(\lambda, \mu)}}(xN) = \left(\sup_{n \in N} A^{(\lambda, \mu)}(xn) \right) = \left(\sup_{n \in N} (A_1^{(\lambda_1, \mu_1)}(xn), A_2^{(\lambda_2, \mu_2)}(xn), \dots, A_k^{(\lambda_k, \mu_k)}(xn)) \right)$ for all $x \in G$,

Where, each $\overline{A_k^{(\lambda_k, \mu_k)}} : G/N \rightarrow [0,1]$.

Let $xN = yNK$ for some $x, y \in G$. Then $xy^{-1} \in N$

That is, $A^{(\lambda, \mu)}(xy^{-1}) = A^{(\lambda, \mu)}(e)$

That is, $\overline{A^{(\lambda, \mu)}}(xN) = \overline{A^{(\lambda, \mu)}}(yN)$

Hence, the map $\overline{A^{(\lambda, \mu)}}$ is well defined.

Now $\overline{A^{(\lambda, \mu)}}(xNyN) = \overline{A^{(\lambda, \mu)}}(xyN) = \left(\sup_{n \in N} A^{(\lambda, \mu)}(xyn) \right)$ for all $x, y \in G$.

$$\sup_{n \in N} A^{(\lambda, \mu)}(xyn) \geq \sup_{n_1, n_2 \in N} \{ \min\{A^{(\lambda, \mu)}(xn_1), A^{(\lambda, \mu)}(yn_2)\}$$

$$\geq \min \{ \sup_{n_1 \in N} A^{(\lambda, \mu)}(xn_1), \sup_{n_2 \in N} A^{(\lambda, \mu)}(yn_2) \}$$

$$\geq \min \{ \overline{A^{(\lambda, \mu)}}(xN), \overline{A^{(\lambda, \mu)}}(yN) \}$$

$$\overline{A^{(\lambda, \mu)}}(xN yN) \geq \min\{ \overline{A^{(\lambda, \mu)}}(xN), \overline{A^{(\lambda, \mu)}}(yN) \}$$

Also, $\overline{A^{(\lambda, \mu)}}((xN)^{-1}) = \overline{A^{(\lambda, \mu)}}(x^{-1}N) = \left(\sup_{n \in N} A^{(\lambda, \mu)}(x^{-1}n) \right)$, for all $x \in G$

$$= \left(\sup_{n \in N} A^{(\lambda, \mu)}(xn) \right) = \overline{A^{(\lambda, \mu)}}(xN).$$

Definition: 3.2

Let $A^{(\lambda, \mu)}$ be a (λ, μ) – multi fuzzy normal subgroup of G with identity element ‘ e ’. Let $N = \{x \in G \mid A^{(\lambda, \mu)}(x) = A^{(\lambda, \mu)}(e)\}$. Consider $\overline{A^{(\lambda, \mu)}} = (\overline{A_1^{(\lambda_1, \mu_1)}}, \overline{A_2^{(\lambda_2, \mu_2)}}, \dots, \overline{A_k^{(\lambda_k, \mu_k)}})$ which is defined by $\overline{A_k^{(\lambda_k, \mu_k)}}(xN) = \left(\sup_{n \in N} A_k^{(\lambda_k, \mu_k)}(xn) \right)$





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for all $x \in G$, each $\overline{A_k^{(\lambda, \mu)}} : G/N \rightarrow [0,1]^k$. Then the (λ, μ) - multi fuzzy subgroup $\overline{A^{(\lambda, \mu)}}$ of G/K is called a (λ, μ) - multi fuzzy quotient group or (λ, μ) - quotient multi fuzzy subgroup of $A^{(\lambda, \mu)}$ by N .

Remark: 3.3

1. $\overline{A^{(\lambda, \mu)}}$ is not a (λ, μ) -multi fuzzy normal subgroup of G/N , Since $\overline{A^{(\lambda, \mu)}}(xNyN) \neq \overline{A^{(\lambda, \mu)}}(yNxN)$.
2. Consider $A^{(\lambda, \mu)} = (\overline{A_1^{(\lambda_1, \mu_1)}}, \overline{A_2^{(\lambda_2, \mu_2)}}, \dots, \overline{A_k^{(\lambda_k, \mu_k)}})$ which is defined by $\overline{A^{(\lambda, \mu)}}(xN) = A^{(\lambda, \mu)}(x)$, for all $x \in G$, where each $\overline{A_i^{(\lambda_i, \mu_i)}} : G/N \rightarrow [0,1]$. Then $\overline{A^{(\lambda, \mu)}}$ is a (λ, μ) -normal multi fuzzy quotient group of G/N .

Theorem: 3.4

Suppose $A^{(\lambda, \mu)} = (\overline{A_1^{(\lambda_1, \mu_1)}}, \overline{A_2^{(\lambda_2, \mu_2)}}, \dots, \overline{A_k^{(\lambda_k, \mu_k)}})$ is a (λ, μ) -multi fuzzy quotient group G/N if and only if each $\overline{A_i^{(\lambda_i, \mu_i)}}$, $i = 1, 2, \dots, k$ is a (λ, μ) - fuzzy quotient group of a group G/N .

Proof:

Let $\overline{A^{(\lambda, \mu)}} = \{\overline{A_i^{(\lambda_i, \mu_i)}}\} = (\overline{A_1^{(\lambda_1, \mu_1)}}, \overline{A_2^{(\lambda_2, \mu_2)}}, \dots, \overline{A_k^{(\lambda_k, \mu_k)}})$ be a (λ, μ) - multi fuzzy quotient group of a group G/N .
 $\Leftrightarrow \overline{A^{(\lambda, \mu)}}(xyN) \geq \min \{ \overline{A^{(\lambda, \mu)}}(xN), \overline{A^{(\lambda, \mu)}}(yN) \} \Leftrightarrow \overline{A_i^{(\lambda_i, \mu_i)}}(xyN) \geq \min \{ \overline{A_i^{(\lambda_i, \mu_i)}}(xN), \overline{A_i^{(\lambda_i, \mu_i)}}(yN) \}$. Also we have
 $\overline{A^{(\lambda, \mu)}}(x^{-1}N) = \overline{A^{(\lambda, \mu)}}(xN)$
 $\Leftrightarrow \overline{A_i^{(\lambda_i, \mu_i)}}(x^{-1}N) = \overline{A_i^{(\lambda_i, \mu_i)}}(xN)$
 $\Leftrightarrow \overline{A_i^{(\lambda_i, \mu_i)}}$, $i = 1, 2, 3, \dots, n$ is a (λ, μ) - fuzzy quotient group of a group G/N .

Remark: 3.5

If $\overline{A^{(\lambda, \mu)}} = (\overline{A_1^{(\lambda_1, \mu_1)}}, \overline{A_2^{(\lambda_2, \mu_2)}}, \dots, \overline{A_n^{(\lambda_n, \mu_n)}})$ is not a (λ, μ) - multi fuzzy quotient group of a group G/N , then there is at least one $\overline{A_i^{(\lambda_i, \mu_i)}}$, $1, 2, \dots, n$ is not a (λ, μ) - fuzzy quotient group of a group G/K .

Theorem: 3.6

If $\overline{A^{(\lambda, \mu)}}$ is a (λ, μ) - multi fuzzy quotient group of a group G/N , then $\overline{A^{(\lambda, \mu)}}(xN) \leq \overline{A^{(\lambda, \mu)}}(eN)$ for all $x \in G$, where e is the identity element of G .

Proof: Let e is the identity element of G . Then for any $x \in G$ then
 $\overline{A^{(\lambda, \mu)}}(eN) = \overline{A^{(\lambda, \mu)}}(xx^{-1}N) \geq \min \{ \overline{A^{(\lambda, \mu)}}(xN), \overline{A^{(\lambda, \mu)}}(x^{-1}N) = \overline{A^{(\lambda, \mu)}}(xN) \}$ for all $x \in G$.

Theorem: 3.7

$\overline{A^{(\lambda, \mu)}}$ is a (λ, μ) - multi fuzzy quotient group of a group G/K if and only if $\overline{A^{(\lambda, \mu)}}(xK y^{-1}K) \geq \min \{ \overline{A^{(\lambda, \mu)}}(xK), \overline{A^{(\lambda, \mu)}}(yK) \}$ for all x and y in G .

Proof:

Let $\overline{A^{(\lambda, \mu)}}$ be a (λ, μ) – multi fuzzy question group of a group G/N .

Then, $\overline{A^{(\lambda, \mu)}}(xNy^{-1}N) \geq \min \{ \overline{A^{(\lambda, \mu)}}(xN), \overline{A^{(\lambda, \mu)}}(y^{-1}N) \} \geq \min \{ \overline{A^{(\lambda, \mu)}}(xN), \overline{A^{(\lambda, \mu)}}(yN) \}$ for all $x, y \in G$.





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Conversely, If $\overline{A^{(\lambda, \mu)}}(xNy^{-1}N) \geq \min \{ \overline{A^{(\lambda, \mu)}}(xN), \overline{A^{(\lambda, \mu)}}(yN) \}$, Then

$$\overline{A^{(\lambda, \mu)}}(x^{-1}) = \overline{A^{(\lambda, \mu)}}(ex^{-1}N) \geq \min \{ \overline{A^{(\lambda, \mu)}}(eN), \overline{A^{(\lambda, \mu)}}(xN) \} = \overline{A^{(\lambda, \mu)}}(xN)$$

That is, $\overline{A^{(\lambda, \mu)}}(x^{-1}N) \geq \overline{A^{(\lambda, \mu)}}(xN)$

Hence, $\overline{A^{(\lambda, \mu)}}(xN) = \overline{A^{(\lambda, \mu)}}(x^{-1}N) \geq \overline{A^{(\lambda, \mu)}}(x^{-1}N)$, for all x in G

Therefore, $\overline{A^{(\lambda, \mu)}}(x^{-1}N) = \overline{A^{(\lambda, \mu)}}(xN)$, for all x in G

Now, we replace y by y^{-1} , then

$$\overline{A^{(\lambda, \mu)}}(xyN) = \overline{A^{(\lambda, \mu)}}(x(y^{-1})^{-1}N) \geq \min \{ \overline{A^{(\lambda, \mu)}}(xN), \overline{A^{(\lambda, \mu)}}(y^{-1}) \}$$
, for all x and y in G .

Hence, $\overline{A^{(\lambda, \mu)}}$ is a (λ, μ) - multi fuzzy quotient group of a group G/N .

Theorem: 3.8

If $\overline{A^{(\lambda, \mu)}}$ and $\overline{B^{(\lambda, \mu)}}$ are two (λ, μ) - multi fuzzy quotient groups of a group G/N , then $\overline{A^{(\lambda, \mu)}} \cap \overline{B^{(\lambda, \mu)}}$ is a (λ, μ) - multi fuzzy quotient group of G/N .

Proof:

Let $xN, y^{-1}N$ in $\overline{A^{(\lambda, \mu)}} \cap \overline{B^{(\lambda, \mu)}}$

$$\begin{aligned} \text{Then, } \overline{A^{(\lambda, \mu)}} \cap \overline{B^{(\lambda, \mu)}}(xNy^{-1}N) &= \overline{A^{(\lambda, \mu)}}(xNy^{-1}N) \wedge \overline{B^{(\lambda, \mu)}}(xNy^{-1}N) \\ &\geq \min \{ \overline{A^{(\lambda, \mu)}}(xN), \overline{A^{(\lambda, \mu)}}(y^{-1}N) \} \wedge \min \{ \overline{B^{(\lambda, \mu)}}(xN), \overline{B^{(\lambda, \mu)}}(y^{-1}N) \} \\ &= \min \{ \overline{A^{(\lambda, \mu)}}(xN), \overline{B^{(\lambda, \mu)}}(xN) \} \wedge \min \{ \overline{A^{(\lambda, \mu)}}(y^{-1}N), \overline{B^{(\lambda, \mu)}}(y^{-1}N) \} \\ &= \min \{ \overline{A^{(\lambda, \mu)}}(xN), \overline{B^{(\lambda, \mu)}}(xN) \} \wedge \min \{ \overline{A^{(\lambda, \mu)}}(y^{-1}N), \overline{B^{(\lambda, \mu)}}(y^{-1}N) \} \\ &= \overline{A^{(\lambda, \mu)}} \cap \overline{B^{(\lambda, \mu)}}(xN) \wedge \overline{A^{(\lambda, \mu)}} \cap \overline{B^{(\lambda, \mu)}}(yN), \text{ for all } x, y \text{ in } G. \end{aligned}$$

Corollary: 3.9

The intersection of a family of (λ, μ) - multi fuzzy quotient groups of a group G/N is a (λ, μ) -multi fuzzy quotient group of a group G/N .

Properties of (λ, μ) -multi fuzzy quotient group $\overline{A^{(\lambda, \mu)}}$ determined by $A^{(\lambda, \mu)}$ and N under Homomorphism and Anti – Homomorphism.

Theorem: 4.1

Let G and G' be any two groups. Let $f : G \rightarrow G'$ be a onto homomorphism. Let $\overline{A^{(\lambda, \mu)}}$ be a (λ, μ) – multi fuzzy quotient subgroup of G/N . Then $f(\overline{A^{(\lambda, \mu)}})$ is a (λ, μ) – multi fuzzy quotient group of G'/N' , if $\overline{A^{(\lambda, \mu)}}$ has supremum property and $\overline{A^{(\lambda, \mu)}}$ is f -invariant and $f(\overline{A^{(\lambda, \mu)}}) = \overline{f(A^{(\lambda, \mu)})}$.





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Proof:

Let $\overline{A^{(\lambda, \mu)}}$ be a (λ, μ) – multi fuzzy quotient group of G/N

$$f(\overline{A^{(\lambda, \mu)}})(f(x)f(y)N) = (f(\overline{A^{(\lambda, \mu)}})) f(xy)N = \overline{A^{(\lambda, \mu)}}(xy)N$$

$$\overline{A^{(\lambda, \mu)}}(xy)N \geq \min\{\overline{A^{(\lambda, \mu)}}(xN), \overline{A^{(\lambda, \mu)}}(yN)\}$$

$$= \min\{f(\overline{A^{(\lambda, \mu)}})(f(x)n), f(\overline{A^{(\lambda, \mu)}})(f(y)n)\}$$

$$f(\overline{A^{(\lambda, \mu)}})(f(x)f(y)N) \geq \min\{f(\overline{A^{(\lambda, \mu)}})(f(x)n), f(\overline{A^{(\lambda, \mu)}})(f(y)n)\}$$

Also,

$$f(\overline{A^{(\lambda, \mu)}})([f(x)]^{-1}N) = f(\overline{A^{(\lambda, \mu)}})([f(x^{-1})]N) = \overline{A^{(\lambda, \mu)}}(x^{-1}N) = \overline{A^{(\lambda, \mu)}}(xN)$$

$$= f(\overline{A^{(\lambda, \mu)}})([f(x)]N). \text{ Hence, } f(\overline{A^{(\lambda, \mu)}}) \text{ is a } (\lambda, \mu)\text{- multi fuzzy quotient group of } G'/N'.$$

Also, $\overline{f(A^{(\lambda, \mu)})}(yN) = \sup_{n \in N} f(A^{(\lambda, \mu)})(yn)$, for all $y \in G'$

$$\sup_{n \in N} f(A^{(\lambda, \mu)})(yn) = \sup_{n \in N} f(A^{(\lambda, \mu)})(f(x)n), \text{ } f \text{ is onto and } x \in G$$

$$= \sup_{n \in N} A^{(\lambda, \mu)}(xn), \text{ for all } x \in G$$

$$= \overline{A^{(\lambda, \mu)}}(xn)$$

$$= f(\overline{A^{(\lambda, \mu)}})(xn)$$

$$= f(\overline{A^{(\lambda, \mu)}})(f(x)n)$$

$$= f(\overline{A^{(\lambda, \mu)}})(yn)(\overline{v_A^t})(yn).$$

Theorem 4.2

Let G and G' be any two groups. Let $f: G \rightarrow G'$ be a homomorphism. Let $\overline{B^{(\lambda, \mu)}}$ be a (λ, μ) -multi fuzzy quotient group of G'/N' . Then $f^{-1}(\overline{B^{(\lambda, \mu)}})$ is a (λ, μ) -multi fuzzy quotient group of G/N . Then $f^{-1}(\overline{B^{(\lambda, \mu)}})$ is a (λ, μ) -multi fuzzy quotient group of G/N and $f^{-1}(\overline{B^{(\lambda, \mu)}}) = \overline{f^{-1}(B^{(\lambda, \mu)})}$.

Proof:

Let $\overline{B^{(\lambda, \mu)}}$ be a (λ, μ) -multi fuzzy quotient group of G'/N' , for all x, y in G

$$f^{-1}(\overline{B^{(\lambda, \mu)}})(xyN) = \overline{B^{(\lambda, \mu)}}(f(xy)N) = \overline{B^{(\lambda, \mu)}}(f(x)f(y)N)$$

$$\geq \min\{\overline{B^{(\lambda, \mu)}}(f(x)N), \overline{B^{(\lambda, \mu)}}(f(y)N)\}$$

$$\geq \min\{f^{-1}(\overline{B^{(\lambda, \mu)}})(xN), f^{-1}(\overline{B^{(\lambda, \mu)}})(yN)\}$$

That is,

$$f^{-1}(\overline{B^{(\lambda, \mu)}})(xyN) \geq \min\{f^{-1}(\overline{B^{(\lambda, \mu)}})(xN), f^{-1}(\overline{B^{(\lambda, \mu)}})(yN)\}$$

Hence, $f^{-1}(\overline{B^{(\lambda, \mu)}})$ is a (λ, μ) – multi fuzzy quotient group of G/N





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Also,

$$\begin{aligned} \overline{f^{-1}(B^{(\lambda,\mu)})}(xN) &= \sup_{n \in N} f^{-1}(B^{(\lambda,\mu)})(xn) \text{ for all } x \in G \\ &= \sup_{n \in N} B^{(\lambda,\mu)}(f(x)n) \text{ for all } x \in G \\ &= \overline{B^{(\lambda,\mu)}}(f(x)N) \\ &= f^{-1}(\overline{B^{(\lambda,\mu)}})(xN) \end{aligned}$$

Hence, $\overline{f^{-1}(B^{(\lambda,\mu)})}(xN) = f^{-1}(\overline{B^{(\lambda,\mu)}})(xN)$

Theorem 4.3

Let G and G' be any two groups. Let $f: G \rightarrow G'$ be an anti homomorphism on G . Let $\overline{A^{(\lambda,\mu)}}$ be a (λ, μ) -multi fuzzy quotient group of G/N . Then $f(\overline{A^{(\lambda,\mu)}})$ is a (λ, μ) - multi fuzzy quotient group of G'/N' if $\overline{A^{(\lambda,\mu)}}$ has supremum property and $\overline{A^{(\lambda,\mu)}}$ is f -invariant and $f(\overline{A^{(\lambda,\mu)}}) = \overline{f(A^{(\lambda,\mu)})}$.

Proof:

Let $\overline{A^{(\lambda,\mu)}}$ be a (λ, μ) -multi fuzzy quotient group of G/N and if

$$\begin{aligned} f(\overline{A^{(\lambda,\mu)}})(f(x)f(y)N) &= f(\overline{A^{(\lambda,\mu)}})(f(yx)N) \\ &= \overline{A^{(\lambda,\mu)}}(yx)N \\ &\geq \min\{\overline{A^{(\lambda,\mu)}}(yN), \overline{A^{(\lambda,\mu)}}(xN)\} \\ &\geq \min\{\overline{A^{(\lambda,\mu)}}(yN), \overline{A^{(\lambda,\mu)}}(yN)\} \end{aligned}$$

$$f(\overline{A^{(\lambda,\mu)}})(f(x)f(y)N) \geq \min\{\overline{A^{(\lambda,\mu)}}(f(x)N), f(\overline{A^{(\lambda,\mu)}})(f(y)N)\}$$

Hence, $f(\overline{A^{(\lambda,\mu)}})$ is a (λ, μ) -multi fuzzy quotient group of G'/N'

Also,

$$\begin{aligned} f(\overline{A^{(\lambda,\mu)}})(yN) &= \sup_{n \in N} f(A^{(\lambda,\mu)})(yn), \text{ for all } y \in G' \\ &= \sup_{n \in N} f(A^{(\lambda,\mu)})(f(x)n), \text{ f is onto and } x \in G \\ &= \sup_{n \in N} A^{(\lambda,\mu)}(xn), \text{ for all } x \in G \\ &= \overline{A^{(\lambda,\mu)}}(xN) = f(\overline{A^{(\lambda,\mu)}})(f(x)N) \\ &= f(\overline{A^{(\lambda,\mu)}})(yN) \end{aligned}$$

Therefore, $\overline{f(A^{(\lambda,\mu)})}(yN) = \overline{f(A^{(\lambda,\mu)})}(yN)$

Hence, $\overline{f(A^{(\lambda,\mu)})}(yN) = \overline{f(A^{(\lambda,\mu)})}(yN)$

Theorem 4.4

Let G and G' be any two groups Let $f: G \rightarrow G'$ be an anti – homomorphism Let $\overline{B^{(\lambda,\mu)}}$ be a (λ, μ) - multi fuzzy quotient group of G'/N' . Then $f^{-1}(\overline{B^{(\lambda,\mu)}})$ is a (λ, μ) -multi fuzzy quotient group of G/N and $f^{-1}(\overline{B^{(\lambda,\mu)}}) = \overline{f^{-1}(B^{(\lambda,\mu)})}$.





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Proof :

Let $\overline{B^{(\lambda, \mu)}}$ be a (λ, μ) - multi fuzzy quotient group of G/N .

$$\begin{aligned} f^{-1}(\overline{B^{(\lambda, \mu)}})(xyN) &= \overline{B^{(\lambda, \mu)}}(f(xy)N) \\ &= \overline{B^{(\lambda, \mu)}}(f(y)f(x)N) \\ &\geq \min \{ \overline{B^{(\lambda, \mu)}}(f(y)N), \overline{B^{(\lambda, \mu)}}(f(x)N) \} \\ &\geq \min \{ \overline{B^{(\lambda, \mu)}}(f(x)N), \overline{B^{(\lambda, \mu)}}(f(y)N) \} \\ &\geq \min \{ f^{-1}(\overline{B^{(\lambda, \mu)}})(xN), f^{-1}(\overline{B^{(\lambda, \mu)}})(yN) \} \end{aligned}$$

That is,

$$f^{-1}(\overline{B^{(\lambda, \mu)}})(xyN) \geq \min \{ f^{-1}(\overline{B^{(\lambda, \mu)}})(xN), f^{-1}(\overline{B^{(\lambda, \mu)}})(yN) \}$$

And, $f^{-1}(\overline{B^{(\lambda, \mu)}})(x^{-1}N) = \overline{B^{(\lambda, \mu)}}(f(x^{-1})N)$

$$\begin{aligned} &= \overline{B^{(\lambda, \mu)}}(f(x)^{-1}N) \\ &= \overline{B^{(\lambda, \mu)}}(f(x)N) \\ &= f^{-1}(\overline{B^{(\lambda, \mu)}})(xN) \end{aligned}$$

$$f^{-1}(\overline{B^{(\lambda, \mu)}})(x^{-1}N) = f^{-1}(\overline{B^{(\lambda, \mu)}})(xN)$$

Hence, $f^{-1}(\overline{B^{(\lambda, \mu)}})$ is a (λ, μ) -multi fuzzy quotient subgroup of G/N

Also $f^{-1}(\overline{B^{(\lambda, \mu)}})(xN) = \sup_{n \in N} f^{-1}(B^{(\lambda, \mu)})(xn)$, for all $x \in G$

$$\begin{aligned} &= \sup_{n \in N} B^{(\lambda, \mu)}(f(x)n) , \text{ for all } x \in G \\ &= B^{(\lambda, \mu)}(f(x)N) \\ &= f^{-1}(\overline{B^{(\lambda, \mu)}})(xN) \end{aligned}$$

Hence, $f^{-1}(\overline{B^{(\lambda, \mu)}})(xN) = f^{-1}(B^{(\lambda, \mu)})(xN)$

CONCLUSION

In this paper, we define a new algebraic structure of (λ, μ) -multi fuzzy quotient subgroup of a group and discussed some of its related for properties under homomorphism and anti-homomorphism. The purpose of this study is to implement the special type (λ, μ) -fuzzy set theory and group theory in multi fuzzy set .

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Effectiveness of Flax Seed and Counseling on Quality of Life among Menopausal Women at Selected area, Karaikal – A Pilot study Analysis

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ABSTRACT

Menopause is the new start. Don't let your estrogens decide everything. Learn to live and enjoy the new beginning. Menopause is not an evidence of maladjustment but a proof of the adjustments process itself. A study was conducted to evaluate the Effectiveness of flax seed and Counseling on Quality of Life among menopausal women at Selected area, Karaikal. The objectives of the study are to assess the quality of life among menopausal women before intervention in control and experimental group, to assess the effectiveness of flax seed and counseling on Quality of Life among menopausal women in control and experimental group, to find the association between of Quality of life with selected demographic variables and menopause Variables in control and experimental group. The Study was conducted in selected areas of Karaikal. Purposive sampling technique was used to select 20 sample who met the inclusion criteria. Pretest was conducted using MENQOL tool was used to assess the Quality of life of menopausal women. Counseling session I was conducted on the day pre test and encouraged intake 5 grams of roasted flaxseed for 45 days. Counseling session II was conducted three weeks after the counseling session I. Post test was conducted using the same MENQOL tool. The analysis and interpretation of the data proves $p < 0.05$ value which was statistically significant. However mean difference between control and experimental was higher in post tests than the pretest which can be attributed to the effectiveness of flaxseed and counseling on improving the QOL among menopausal women

Keywords: flaxseed, counseling, Quality of life, Menopausal Women.



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INTRODUCTION

The word 'Climacteric', is a Greek derivation of the meaning 'Ladder' or 'steps of a ladder'. Over many years, middle - aged women had been viewed as the extremes of either climbing up or down the ladder. The word 'Menopause' / Climacteric refers to the final or last menstrual period which every women experiences. Women is considered to be Postmenopausal when she had no periods for 12 consecutive months. During menopause period women can experience many symptoms including hot flashes, night sweats, sleep and mood disorders, impaired memory, lack of concentration, nervousness, depression, insomnia, bone and joint complaints, and reduction of muscle mass. The duration, severity, and impact of these symptoms vary extremely from person to person, and population to population. According to the latest world Health organization (WHO) data which is published in 2018, in India, life expectancy for a female in 70.3 years, which is expected to increase to 77 years by 2050. Current population in India is 1.2 billion. Approximately 10% of India's population i.e., more than 100 million of population in aged over 50 years. By 2025, there will be over 1 billion women experiencing menopause in the world, which will be 12% of the entire world population of 8 billion. Using age 50 as a proxy for menopause, about 25 million women pass through menopause each year, and we estimate that in 1990 there were 467 million post-menopausal women in the world, with an average age of about 60 years. By 2030, the world population of menopausal and postmenopausal women is projected to increase to 1.2 billion, with 47 million new entrants each year. Some women have severe symptoms that greatly affect their personal and social functioning, and quality of life. Phytoestrogens help naturally to cope with climacteric symptoms that occurs due to hormone imbalances and it is beneficial for the women in menopause period. Phytoestrogen is very essential for menopausal women to rebalance their hormones during premenopausal period. The lignans in flax have estrogenic properties. After menopause, estrogen levels in women drop to abysmal levels. Use of flax seeds can shore-up estrogen levels and provide protection for bones, heart and other organs.

Menopause is the normal physiological process for which medical treatments are individual – based and may lead to anxiety. Hence non-pharmacological treatments are considered as first level of care. The initial steps of treatment for manipulating the mind, body and communication issues is education and counseling. Counseling and psychological interventions can be promising and proactive interventions for the treatment of sexual dysfunction.

Statement of the Problem

A Study to evaluate the Effectiveness of flax seed and Counseling on Quality of Life among menopausal women at Selected area, Karaikal.

Aim and Objectives of the study

- To assess the quality of life among menopausal women before intervention in control and experimental group.
- To assess the effectiveness of flax seed and counseling on Quality of Life among menopausal women in control and experimental group.
- To find the association between of Quality of life with selected demographic variables and menopause Variables in control and experimental group.

Hypothesis

H₁ :There is a significant difference in posttest on Quality of life of menopausal women after the Intervention Programme.

H₂ :There is a significant association between quality of life of menopausal women with selected demographic variables and menopausal variables.





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METHODOLOGY

Quantitative approach and Quasi experimental design was used in this study. The setting of the study were Kezhakasakudimedu for Experimental group and Kilinjalmedu for control group. The menopausal women aged 45 -55 years with menopausal symptoms who met the inclusion criteria were chosen as sample from the selected area Karaikal. Purposive sampling technique was used to select 20 sample. Experimental group -10 and control group – 10. The tool consists of 3 sections. Section A - Demographic characteristics of menopausal women. Section B - Menopausal variables of women. Section C - MENQOL Tool was used to assess the quality of life of menopause women. The scale consists of four domains which includes vasomotor symptoms, psychosocial symptoms, physical symptoms and sexual symptoms. Each domain is scored separately.

Ethical approval was obtained from Ethics Review Committee from our Institution. Permission obtained from District collector as adopted villages of our institution. Permission obtained from respective village heads to conduct the study. During Pre test, Demographic and menopausal variables were assessed using structured Interview schedule for both control and experimental group and duration of Interview lasts for about 15 – 20 minutes. Quality of life is assessed using MENQOL tool. Immediately after the pretest Counseling session I was conducted regarding menopause symptoms and self help tips for about 40 – 50 minutes. Then 5 grams of roasted flaxseed was administered to the menopause women on the day of pretest and continued till 6 weeks. Counseling session II was conducted 3 weeks after I counseling session. Post test-I was conducted after 6 weeks of pretest for both control and experimental group. Post test-II was conducted after the one month of intervention for the experimental group and control group.

RESULTS AND DISCUSSIONS

- Demographic variables of the control and experimental group of menopause women. Age of the menopause women 45 – 50, 50-55 yrs is equal (50%, 50%), majority of the women were married (90%, 80%), had higher secondary education (40%, 30%) and belong to Hindu religion (70%, 70%) and most of the menopause women were home maker (50%, 70%) and their spouse is employed in private sector (40%, 40%) with the family income of Rs.10,000 to Rs.20,000 (70%, 90%) by following the food habits of Non vegetarian (80%, 90%) and living in a joint family (50%, 60%) in control and experimental group of menopause women respectively. This two groups are comparable and homogenous with respect to the demographic variables such as age of menopause women, educational status, occupation of the spouse, type of family.
- Majority of the women sleeps during night time (70%, 90%), with the height of 145cm – 155cm (50%, 30%), and weight of 60kg – 70kg (40%, 70%) in control and experimental group respectively. Majority of the women had pulse rate between 72 – 80bpm (40%, 50%), systolic BP in the range of 120 – 140 mmhg (40%, 60%), Diastolic BP between 80 – 90 mmhg (40%, 60%) in control and experimental group. Majority of the women had regular menstrual cycle (80%, 90%), attained natural menopause (100%, 80%) and duration of menopause is less than 2 years (50%, 40%) in control and experimental group respectively.
- Comparison of Mean and SD of Quality of Life (ANOVA) among Menopausal Women between Assessments in Control Group (n=10) denotes Post test 1 and post test 2 of Global scores are (128.10 ± 7.32, 132.20 ± 8.79) were lower than pretest Global scores (121.40 ± 8.67) both within the subjects and between subjects at p< 0.005 level. Hence Hypothesis H₁ stating that there will be significant improvement in Quality of Life among menopause were accepted in control group.
- Comparison of Mean and SD of Quality of Life (ANOVA) among Menopausal Women between Assessments in Experimental Group (n=10) denotes Post test 1 and post test 2 of Global scores are (73.00 ± 19.27, 59.10 ± 15.10) were lower than pretest Global scores (111.60 ± 29.69) both within the subjects and between subjects at p< 0.005 level. Hence Hypothesis H₁ stating that there will be significant improvement in Quality of Life among menopause were accepted in experimental group.





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- Post hoc analysis reveals that in control group of menopause women there was a significant difference in Global scores between pre test and post test 1(mean difference = -6.7) at $p < 0.05$, pre test and post test 2 (mean difference = -10.8) at $p < 0.05$, post test 1 and post test 2 (mean difference = -4.1) at $p < 0.05$ level Hence Hypothesis H_1 stating that there will be significant improvement in Quality of Life among menopause were accepted in control group.
- Post hoc analysis reveals that in experimental group of menopause women there was a significant difference in Global scores between pre test and post test 1(mean difference = 38.6) at $p < 0.05$, pre test and post test 2 (mean difference = 52.5) at $p < 0.05$, post test 1 and post test 2 (mean difference = 13.9) at $p < 0.05$ level Hence Hypothesis H_1 stating that there will be significant improvement in Quality of Life among menopause were accepted in experimental group.
- Independent 't' test of Comparison of Mean and SD of Quality of Life of Menopausal Women Between Control and Experimental Group shows post test 1 and 2 t value ($t = 13.228, t = 8.450$) at 0.000 level is greater than pretest t value ($t = 1.002$) at 0.330 level. Hence hypothesis H_1 is accepted as there is an improvement in Quality of Life of Menopause women after Intervention.
- There is an significant association Between Quality of Life of Menopausal Women and Selected Demographic Variable such as type of family in pretest of Control Group at $p < 0.05$. Hence H_2 stating that there is an significant association between Quality of Life of Menopausal Women and Selected Demographic Variable is accepted.
- There is a significant association between Quality of Life of Menopausal Women and selected Menopausal Variables such as type of menopause in posttest 2 of Experimental Group. Hence hypothesis H_2 stating that there will be a significant association between Quality of life of menopause women with selected menopause variables is proved and hypothesis H_2 is accepted.

Recommendations

- The similar study can be replicated to large sample size for generalizing the findings
- A study to assess the effect of different teaching strategies regarding non pharmacological therapies for menopausal problem among menopausal Women.
- An experimental studies can be undertaken in different settings and in different population
- A study can be performed by developing a self instructional module which enables the care givers to become aware of consumption of flax seed on reduction of menopausal problem

CONCLUSION

The pilot study shows the feasibility for conducting main study and the analysis and interpretation of the data proves $p < 0.05$ value which was statistically significant. However mean difference between control and experimental was higher in post tests than the pretest which can be attributed to the effectiveness of flaxseed and counseling on improving the QOL among menopausal women. Hence pilot study was found to be feasible and reliable to conduct the main study.

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Migraine: An Overview with Emphasis on Pathophysiological Mechanisms

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ABSTRACT

Migraine is a neurovascular disorder that affects over 1 billion people across the world. Its widespread pervasiveness and affliction have a range of negative and considerable effects not only on those affected but also on their families, colleagues, employers, and society. To reduce this universal laden collective efforts are needed to control migraine by understanding the pathophysiology and mechanism. Significant advancement has been made in explaining the pathophysiological systems of headache, related hereditary elements that might impact, and physical changes during the movement of a headache assault or the change of verbose to ongoing headache. In this study we summarized the triggers and risk factors of migraine and management of migraine targeting the biomarkers.

Keywords: Migraine, pathophysiology, CGRP, Substance P

INTRODUCTION

Migraine is considered as a complex neurological disease with primary headache leading to disabling conditions. The 3rd edition of International classification of Headache Disorders describes migraine as a recurrent headache sickness manifesting as a unilateral and throbbing headache with pain intensity from mild to intense [1]. There are many types of migraines. Two of the most common types of them are migraine without aura and migraine with aura, which means any sensory changes that happen before a migraine headache. Some people have both these types. Migraine without aura is also called common migraine and most people with migraine do not experience an aura. Migraine with aura is also known as classic migraine, complicated migraine, and hemiplegic migraine. Other



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types include chronic migraine which is used to be called mixed headache because it can have features of migraine and tension headaches[3]. It can also be caused by medication overuse and acute migraine which aren't diagnosed as chronic. People who have episodic migraines have headaches up to 14 days in a month. Vestibular migraine is also known by migraine-associated vertigo. About 40 percent of people who have migraines are having some vestibular symptoms that may affect the balance, cause dizziness, or both. Optical migraine is also called as ocular migraine, monocular migraine, ophthalmic migraine and retinal migraine, eye migraine. It is a rarer type of migraine with aura, but unlike other visual auras, it affects only one eye [4,5,6,7].

Migraine is a debilitate neurovascular disorder with unilateral throbbing head ache and evident symptoms includes hypersensitivity to light, sound and smell, nausea, and a variety of autonomic, cognitive, emotional and motor disturbances. Although the induction of a migraine attack is usually connected with a variety of internal and external factors such as stress, hormonal changes, sleep disturbances, skipping meals or sensory overload. The vascular and neural mechanisms underlying the development of this primary condition remain to be explained. It is believed that migraine headache is a demonstration of the brain state of altered excitability [8,9]. Approximately 16% of the worldwide population suffer from migraine, and about one third of those migraines are due to neurological symptoms associated with a transient cortical malfunction, known as aura. Such cortical disturbances arise due to cortical spreading depression (CSD), which occurs in the human cortex before the onset of the headache. The circumstance for its occurrence likely depends on genetic factors that makes the cerebral cortex hyperexcitable through abnormal excitatory/inhibitory balance. Even though there is a large degree of evidence that support the role of CSD as a vital event for the activation of the trigeminovascular system and the scientific evidence of symptom less cortical spreading depression like events in migraine without aura remain to be determined. This review on relevant clinical and preclinical data that widens our understanding about the pathophysiology[8].

Signs And Symptoms

The signs and symptoms of migraine differ with age. Migraine attacks in kids and children vary from the ones in adults, as the more youthful regularly have attacks of shorter duration and bilateral vicinity[10]. These assaults usually persist 4 to 72 hours if left untreated. The diagnosis requires a mix of characteristics, but not all characteristics are present in every attack or in every patient. These symptoms distinguish migraine from tension-type headache, which is the most frequent type of primary headache and is defined by the absence of concomitant symptoms. Any severe and recurrent headache is almost always a form of migraine that responds to antimigraine medication[11]. Migraine attack is of four phases that include premonitory prodrome, aura, headache and postdrome. If the frequency is one or two attacks per months is a common pattern. Repeated episodes of headache with any two of the features i.e. Unilateral, throbbing, worsened by motion, slight or severe or any one i.e. photophobia nausea or vomiting, and phonophobia are the maximum traits of migraine. Typically pain is unilateral and significant quantity of cases will become bilateral frequently, overdue in an attack. Headache can be over about 30 minutes and last from hours to one or two days. In majority of cases nausea occurs however in about 50% of the cases vomiting occurs. Vasomotor changes manifested as pale face, cold extremity, may be subconjunctival haemorrhage or bruising around eyes. Visual disturbance usually lasts for 15 to 20 minutes. Symptoms of paraesthesia and numbness arise in cortical distribution, concerning the periphery of the limbs and circumoral plane. The lip, face, and tongue can be eventually affected on one facet on one or each sides. Migraine assaults leads neurological disturbance [12,13,14]. Generally, migraine provides itself as a sluggish ache after which maintains to progress as a continuous pulsating pain near the temples, or at the front or again of the one aspect of the head (unilateral). However, it may be on the each facets of the pinnacle (bilateral), but its incidence is low. The pain is usually accompanied through nausea, vomiting, and sensitivity to mild and any sort of sound. An individual with migraine headache prefers to stay in a dark and quiet place to rest. In most instances, the ache is relieved after vomiting. Many migraine sufferers may experience two to four attacks each month. This, however, varies every patient, ranging from a few days between attacks to only one or two occurrences per year [15].



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Migraine attacks are commonly preceded or accompanied by brief focal neurologic symptoms, which are typically visual, in 15% of patients; these individuals suffer migraine with aura (previously known as classic migraine). According to a recent large population-based study, 64% of migraine patients had only migraine without aura, 18% had only migraine with aura, and 13% had both forms of migraine, the remaining 5 percent had aura without headache [11].

Risk Factors And Triggers

Major risk factors included in migraine are the following:

- Family history: A person is likely to have migraines if they are having a positive family history. A migraineur with a positive family history is more prone to earlier onsets of migraine headache [16].
- Age: Migraine is more common in adolescent, but migraines can start at any age most commonly before age of 40 [17].
- Sex: Women are more likely to have migraine than men. Despite the fact that migraine occurrence is same in both girls and boys during childhood but it is found to be more in girls after puberty [17, 15].

Common Migraine Triggers include

- Food and Drink: Migraines can be brought on by way of positive ingredients including, elderly cheeses, chocolate, aspartame, and nuts. Certain chemicals present in food consisting of monosodium glutamate (MSG) which is used in Asian foods, caffeine and alcohol, specially beer and purple wine are also feasible culprits. Skipping a meal or fasting might increase the threat of attacks [18].
- Hormone changes: Menstruation, pregnancy, use of hormonal contraceptives and hormone replacement treatment affects migraine occurrence. The onset of migraine is usually after menarche, occurs more frequently in the days of menstruation, during pregnancy and menopause. These variations are arbitrated by fluctuation of oestrogen. Furthermore, administration of exogenous hormones may worsen the condition [19].
- Emotional triggers: emotional triggers, such as stress, depression, anxiety, and excitement have an impact on migraine headaches. Stress due to work, stress at home also cause migraine [20].
- Medicine: Certain medicines can escalate migraine attacks. Towards the end of the birth control pill cycle, women may encounter migraine due to the halting of metabolism of oestrogen components in the pills. Use of certain pain killers like aspirin, acetaminophen may also trigger migraine headache [15].
- Environment: Various environmental factors are included that are considered to be trigger in migraine headaches. It includes bright sunlight, flickering lights, air quality, and odors etc [21].
- Illness: Infections, like cold or flu, may trigger migraines, especially in children.

Pathophysiology Of Migraine

During a migraine attack, the trigeminal nerve i.e. cranial nerve 5 becomes activated. This results in the release of neuropeptides from cranial nerve 5. The neuropeptides include vasoactive inhibitory peptide or VIP, substance P and calcitonin gene related peptide or CGRP. These neuropeptides cause painful neurogenic inflammation in the meningeal vasculature, causing mast cell degranulation, plasma protein extravasation, vasodilation and activation of nociceptors, all which contribute to the migraine headache. These neuropeptides plays an important role in trigeminal vascular pain transmission in migraine headaches. On the initiation of a CNS disorder, there are 3 key tactics in the era of migraine headache. These mechanisms were cautiously studied in the health center and experimental models. The first key procedure is notion to involve meningeal blood vessel dilation [22, 23, 11]. As the arteries swell, they set off perivascular sensory trigeminal nerves inflicting pain impulses to be transmitted to the caudal brain stem nuclei. Activation of these nerve terminals triggers the 2nd key technique in migraine – a release of vasoactive neuropeptides [24, 25]. These materials worsen and perpetuate vasodilation, setting up a vicious cycle that increases meningeal blood vessel diameter similarly, thereby increasing nerve activation and intensifying headache representing the 3rd key tactic. The greatest of these peptides is substance P, neurokinin A and calcitonin gene-related peptide (CGRP) [26]. Substance P released i) from the trigeminal sensory neurones within the dura mater : induces plasma extravasation and vasodilatation, ii) centrally inside the trigeminal



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caudalis nucleus: cause the significant transmission of pain [27, 28]. Consequently, it's been suggested that trigeminal-related pain is mediated through substance P which acts at NK1 receptors and the antagonists of these receptors could be a capacity treatment of migraine headache [29].

CGRP is a 37-amino acid peptide with an N-terminal disulphide bond and amidated C terminus, both of which can be required for CGRP receptor activation. CGRP has been shown to regulate the cardiovascular system, mediate neurogenic irritation, and modulate nociceptive centre. CGRP is expressed in greater than half of the trigeminal neurons. Upon trigeminal nerve activation, CGRP released into the plasma from trigeminal afferent nerve fibre. It is readily degraded, suggesting that the CGRP-mediated consequences are more Prominent in proximal areas. The release of CGRP promotes non endothelium-mediated vasodilation. Elevated CGRP levels are visible peripherally and centrally in patients with migraine. CGRP antibodies and antagonists are concept to reduce migraine via reducing the CGRP levels or by blocking the action of CGRP [30, 31]. The third vital event is the relay of migraine headache impulse originating from the activated peripheral sensory nerves to the second order sensory neurones inside trigeminal nuclei within the caudal brain stem and higher cervical spinal cord (trigemincervical complex). It has been hypothesized that these relevant sensory neurones can also end up sensitized during migraine. This sensitization may be liable for the intensification of migraine headache as an assault progresses. The trigeminal sensitization can also involve the facial sensory disturbances which might be sometimes associated with migraine and perhaps additionally hold up its transformation in some people to a severe condition. The trigeminal nuclei are then answerable for relaying incoming pain alerts to higher cortical centres wherein migraine pain is registered [32, 33].

Diagnosis

Diagnosis of migraine is attributed to characteristics of headache and other symptoms associated with it [34]. Migraine characteristics change over time in some patients. Such patients experience chronic headaches daily rather than episodic attacks. It is known as refractory or intractable migraine[35]. Criteria for diagnosis mainly follow the information from the International Headache Society. But in almost 50 percent of patients, migraine remains undiagnosed. Under-reporting to physicians by patients about symptoms and lack of simplified diagnostic tests are two main reasons for underdiagnosis and further delay in treatment. International Headache Society in 1988, established criteria for headache including migraine. But many physicians and clinicians prefer other assessment tools instead of these. Headaches are of two types- primary and secondary. The classification is successfully done via understanding the clinical functions and pattern of migraine[36]. Rational migraine remedy necessitates correct diagnosis, identification and elimination of potential triggering elements and a pharmacological intervention[37]. There are certain key approaches to differentiate migraine from tension-type headache. Firstly, migraine is a headache with special features while tension or anxiety type headache is featureless. Secondly, migraine has biological signatures that tension type headache does not manifest IHS criteria for classification consequences in a practical check that generally yields a beneficial scientific solution. [38].

Several devices are designed to improve the prognosis of migraine and to identify the comorbid mental disturbances. A structured intake form and a headache diary are encouraged in drawing near sufferers with headaches. In addition, 3-item screening test, ID- migraine test have been established for rapid diagnosis. Diagnosis of migraine with aura is carried out by Visual Aura Rating Scale (VARs)[39]. Great advances were made in diagnosing and reporting migraine over the past numerous years. Tools including the International Classification of Headache Disorders help with diagnoses [40]. Optometrists can help their migraine sufferers with a thorough examination, recommendation, proper referrals, and optical management when suitable. If in doubt of diagnosis or assigning a nonmigraine diagnosis, attention should be made in using a diary to verify primary headache analysis[41].

Prevention

Preventive remedy ought to be taken into consideration for patients with migraine who robotically have extra than 6 headache days per month or in other special instances. Choices for preventive therapy are based on patient



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preferences about effects and evidence of efficacy. The evidence degree and generally used doses for selected classes of migraine preventive medication are reviewed, which includes antiepileptic pills, antihypertensive capsules, and antidepressants. Timolol, propranolol, topiramate, and divalproex sodium are authorized for migraine prevention by the USA FDA. OnabotulinumtoxinA is approved for the prevention of chronic migraine. Several off-label capsules, in particular Lisinopril, candesartan, and amitriptyline additionally have appropriate proof of benefit. Preventive treatment need to be started at a low dose and doses multiplied slowly until therapeutic gain is accomplished or outcomes preclude persisted use [42]. Preventive measures for migraine encompass life-style changes (e.g., keeping off migraine triggers and keeping normal sleep etc.) and drug therapy. Beta-blockers, calcium channel blockers, tricyclic antidepressants, and anticonvulsants are the more common drugs used for migraine prophylaxis, however preventive remedies ought to be individualized, considering efficacy, checking unfavourable results, co-present clinical conditions, and drug prices. Many medications are available for the intense remedy of migraine, inclusive of prescription drugs and over-the-counter analgesics. Of the former, triptans, are the maximum currently used ones. Each of the known triptans (sumatriptan, zolmitriptan, naratriptan, and rizatriptan) is powerful in finishing a migraine assault and the proportion of patients who achieve ache alleviation[43].

Preventing migraines, lowering their frequency, intensity, or severity, or maybe making them extra aware of acute medicinal drugs are important desires for every migraine victim. Ideally, the selection of preventive strategy need to be matched to the individual patient[44]. Effects of exercise on migraine has also been studied. Biologically, workout suppresses inflammatory modulators, including many cytokines, stress hormones, cortisol and growth hormones etc. Exercise has additionally been proven to affect microvascular health. Randomized manage trials have tested that a sufficiently rigorous aerobic workout routine is sufficient to yield a statistically giant reduction in migraine frequency, depth, and length. Higher-intensity training appears to confer extra advantage. Special populations, like those with neck ache or tension headache, may additionally benefit from workout; and patients who can't tolerate high-effect exercising may even advantage from low-effect workout like yoga [45]. Migraine is a multiplex and multifactorial brain disorder. Until this date, there have been no medications that were designed with the unique motive to lower the variety of migraine attacks, which prompts a search for alternative interventions that might be treasured, which includes acupuncture. Acupuncture appears to be as effective as traditional drug preventative remedy for migraine and is secure, long lasting, and economic[46]. There's a growing demand for herbal treatment like nutrients and dietary supplements for migraines. The use of magnesium, Petasites hybridus, feverfew, coenzyme Q10, riboflavin, and alpha lipoic acid are recommended[47].

Epidemiology Of Migraine

The epidemiological data of migraine suggests that migraine with aura is much more intense than migraine without aura. Nausea, phonophobia and photophobia are some common symptoms associated with migraine headache. [48]. The prevalence of migraine is focused on most of the epidemiological studies. Prevalence is common in women between the ages of 25-55. Even though migraine prevalence is clinically significant, most of the patients do not make an attempt to consult a physician. [49]. Migraine usually begins in childhood and can become worse in adulthood. After the age of 50, migraine becomes rare. The migraine headache that occurs usually at this stage without any symptoms is referred to as silent migraine. Food triggers should be considered if it is causing migraine. Medication errors can also contribute to migraine. Emotional triggers are part of the system and the approach must be to handle these triggers in order to control and relieve them. [50].

Migraine is a global incapacitating condition that affects the health quality of adolescents to adults. In a study of prevalence of migraine involving medical students, it was observed that 40 % of the students were experiencing some kind of headache. Among this, 40.2% students having headache was found to be suffering from migraine. Women were affected more commonly. The major finding was that only 7.1% of the students had undergone medical treatment. Considering the adolescents and students, there have been a rise in prevalence of migraine over the past few years. The recurring headaches in this category are a challenging one. A little attention is only given to children regarding the condition. The result will be the impaired quality of life in their social aspects, school education, and



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career. In 2016, Migraine was ranked first among the disabling conditions from 15-49 age group. Diagnosis and treatment is very rare among the children and adolescents. Recent studies show that in the patients with migraine, only 5% consult health practitioners [51,52]. In contrast to episodic migraine, chronic migraine lasts from 15 days to 3 months. It is not common as episodic migraine. Its prevalence is approximately 0.9% to 2.2% among general population. Hence chronic migraine is underdiagnosed and undertreated. We concluded that a strong need of detection and pharmacotherapy is relevant in chronic migraine [53].

Management Of Migraine**Prophylaxis**

Migraine attacks can be usually recurrent one and preventive migraine therapy is employed to reduce the regularity and timespan of these attacks. Whenever other therapies are not effective, occurrence of drastic reactions associated with the drugs, cases where contraindicated; all these occasions prophylaxis using specific agents are considered. The agents chosen for the treatment are propranolol, timolol, divalproex sodium and topiramate, which are FDA approved. The administration of these drugs is based upon the synchronization of disease characteristics in different patients and ADR profiles. It takes almost a period of six months in order to achieve complete therapeutic effect.

Abortive treatments**Analgesics and NSAIDS**

For mild attacks of migraine, simple analgesics and NSAIDS are commonly used. Aspirin, ibuprofen, acetaminophen and diclofenac are recommended. Combination of acetaminophen plus aspirin and caffeine have established efficacy in treating migraine headache. Acute NSAIDS therapy is beneficial where gastrointestinal side effects are persistent.

Serotonin receptor agonists (Triptans)

5-HT receptor agonists, triptans recommended for treating moderate to severe migraine. Sumatriptan is the first member of this class, and examples for second generation are zolmitriptan, naratriptan, rizatriptan, frovatriptan, and eletriptan. The former member of the class is available in oral, intranasal and subcutaneous administration. Sumatriptan along with the second generation agents are selective agonists of the 5-HT_{1B} and 5-HT_{1D} receptors. Selection of a triptan depends upon peculiarity of headache, dosing convenience, and patient characteristics. Common adverse reactions associated with serotonin receptor agonists include dizziness, flushing, fatigue, paresthesias, warm sensations and somnolence.

Ergot alkaloids and derivatives

These drugs are nonselective 5-HT₁ receptor agonists that constrict intracranial blood vessels thereby inhibiting the development of neurogenic inflammation in the trigeminovascular system. Therapeutic doses produce arterial and venous constriction. Ergotamine tartarate exerts more potent arterial effects than dihydroergotamine. Nausea, vomiting pain, and weakness are the common observed side effects of these agents.

Anti-emetics

Adjunctive anti-emetic therapy is used to counteract against adverse reactions associated with the migraine headache including nausea and vomiting. Metoclopramide is beneficial to reverse gastroparesis and also thereby improving absorption from GI tract. Prochlorperazine and chlorpromazine are also effective in relief from migraine headache when administered parenterally.

5-HT_{1F} and CGRP as treatment target

The ditan class of drugs including Lasmiditan is highly specific for 5-HT receptor agonist, targeting central and peripheral 5-HT receptors. They act by the activation of 5-HT receptor; hence trigeminal neuronal activation is blocked. Subsequently migraine pathway is inhibited. Peripheral and central neurons are the site for CGRP. Delayed migraine type headache is associated with exogenous CGRP. On this basis, CGRP is a significant target for migraine pharmacotherapy. The gepant class of drugs has gain popularity due to their potential mechanism. Small molecule





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CGRP receptor antagonists include ubrogepant and rimegepant. CGRP mAbs are large molecule CGRP receptor antagonists. These agents are primarily involved in inhibiting vasodilation and neurogenic inflammation. This is carried out by the blockade of release of CGRP in the migraine pathway [54].

CONCLUSION

Migraine is a complex primary brain condition that involves a series of events that result in recurrent trigeminocervical pain. While many elements of migraine pathophysiology remain unknown, much research has been done in recent years to improve our understanding of the complicated mechanisms involved in all stages of a migraine attack, which has led to the discovery of therapeutic targets such as CGRP, Substance P, and VIP for acute and preventive treatment. These are involved in the activation and transmission of sensory signals within trigeminovascular pathways. The subject of research in this study will be useful in future prophylactic therapies.

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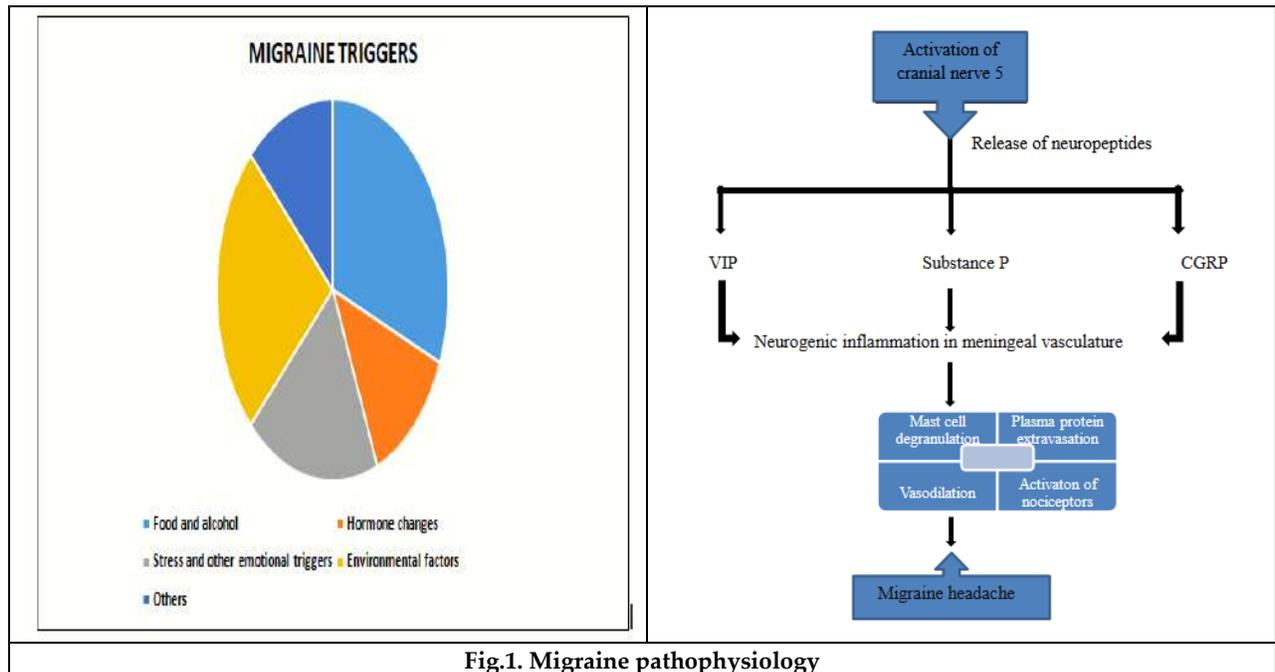


Fig.1. Migraine pathophysiology





Effect of Myofascial Release in Improving Agility of Athletes

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ABSTRACT

The aim of the study is to find the effects of Warm-up and Myofascial Release in improving the agility among young athletes. Myofascial release (MFR) is a manual soft tissue technique that stretches the restricted fascia. In immobilized muscles, the connective tissue in these muscles changes, and the ratio of collagen increases. Warm up is a common activity used by athletes, older adults, rehabilitation patients, and anyone participating in a fitness program. Flexibility is needed for the relatively easy performance of everyday and sporting activities. Enhanced flexibility may boost performance, muscle strength, agility, endurance and decrease the chance of injury. They have to use the full length of the muscle to demonstrate power and energy to reach optimum performance. This Quasi experimental study was conducted on the college level male athletes belonging to the constituent colleges of Vinayaka Mission's Research Foundation – Deemed to be University, Salem. 40 athletes in the age group of 17-23 years were selected and included in this study. Independent Variables such as Warm-up drills and Myofascial release were studied with the dependent Variable Agility (T-Test). The results were statistically analyzed by using SPSS version 25. The Experimental group who received Myofascial release along with warm-up drills showed significant improvement in Agility, The improvement gained by the Experimental group I was significantly effective better than the other two groups with regard to all the outcome measures i.e Agility, Flexibility, Explosive power, and Muscular Endurance. The results of this study conclude that Myofascial release enhances agility.

Keywords: Warm-up, Myofascial Release, Agility, Fitness, Athletes



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INTRODUCTION

Athletes are defined as individuals who are adept in any sort of physical activity or sporting activity. Athletes are often considered to be in excellent physical condition and motivated to maintain their fitness. The fact of the matter is that they must be physically fit to attain success in this extremely competitive world of sports. When it comes to athletes' fitness, they place a high value on it, and their fitness routines are believed to go for many hours each day. A significant amount of time is spent preparing their bodies to withstand the strains and expend the energy required to accomplish the desired results. It is commonly believed that athletes' outstanding performances are the result of a unique combination of talent and physical fitness, as well as technical, tactical, and psychological attributes[1]. Out of all of these factors, physical fitness is believed to be the most important factor in determining an athlete's competitive abilities[2]. The ability to maintain and improve one's physical fitness is critical for improving one's technical and tactical level and performance, and it is the fundamental prerequisite for athletes who compete in high-intensity training[3]. Athletes' ability to compete might be jeopardised if they lose their physical fitness component, which can result in sports injuries[4].

Agility is an important fitness component which is defined as the capacity to quickly change direction, accelerate, or decelerate one's body. It is influenced by several factors, including balance, strength, coordination, and skill level. Athletes' agility can be improved by first establishing a strong base of strength and conditioning that is appropriate for the level of hardship that they will be experiencing. After this has been accomplished, drills designed to improve reactive and explosive motor abilities can be gradually included in the programme.

METHODOLOGY

Research Design

This is a quasi experimental study.

Criteria for Selection

a) Inclusion criteria

- All the college level male athletes belonging to the constituent colleges of Vinayaka Mission's Research Foundation – Deemed to be University, Salem
- Subjects between the age group of 17 and 23 years were only included.
- Subjects with Psychological and Medical fitness for participation in sports were only included.

b) Exclusion criteria

- Athletes belonging to the off-campus of Vinayaka Mission's Research Foundation – Deemed to be University, Salem were excluded.
- Athletes with recent injuries were excluded.
- Athletes who are not willing to participate in this study were excluded.

Study Population

All the college-level athletes between the age group of 17 and 23 years at Vinayaka Missions Research Foundation – Deemed to be University, Salem who fulfilled the selection criteria were taken as the population of the study. This is an quasi experimental study. 40 athletes were selected using simple random sampling method from 72 athletes, who fulfilled the selection criteria. Young male athletes aged between 17 and 23 who met the inclusion criteria and who are members of Vinayaka Mission Research Foundation's Constituent Colleges - Deemed University in Salem - participated in the randomized control trial. Before being included in the research, a competent doctor checked and confirmed physical and mental fitness. The nature of the research was described to the athletes before they participated in the study and signed consent was acquired. The Pre-Test assessment scores of Agility were collected



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using the T-test. The athletes started from cone A. At the timed instruction, the subject sprinted to cone B and with their right hand contacted the cone. They then turned left and went sideways onto cone C, and this time contacted the cone with their left hand. Then they stirred laterally to cone D to the right and contacted the cone using the right hand. They then shuffled back to cone B and contacted the cone with their left hand and moved backwards to cone A. When they travelled over cone A, the timer is stopped.

Scoring: The test was not counted if the individual crossed one foot before the other while shuffling and if failed to contact the cones. The best time of three successful attempts was recorded as the score. Subjects received Myofascial release and warm-up exercises over two weeks. The subjects received Myofascial release in the following manner: Subjects in the control group underwent the following warm-up exercises as per FIFA 11+ protocol. The warm-up phase lasted 20 minutes and included the following five components: Ten minutes of warming up, two minutes of muscle activation, two minutes of balance exercises, four minutes of strengthening activities, and four minutes of core stability exercises. The athletes were taught a range of self-myofascial release methods using a standard tennis ball or foam roller. In a standing position, the athletes were made to roll the plantar fascia using a tennis ball. Using the foam roller from the supine body position, the rolling progression targeted the calf region (gastrocnemius, soleus), hamstring region (semitendinosus, semimembranosus, biceps femoris), the gluteal region (gluteus maximus, gluteus medius, gluteus minimus), and thoracic/lumbar region (erector spinae, multifidus). From the prone body posture, the quadriceps (rectus femoris, sartorius, psoas major, iliacus) were followed by the pectoral region (pectoralis major, pectoralis minor). Each muscle group was rolled across its full surface area five times every 30 seconds. Each method was carried out bilaterally. Post Intervention scores for Agility were collected at the end of 2 weeks similar to that of the pre-test score collection procedure

Observation and Analysis

The collected data were subjected to paired “t” test. Statistical analysis using a paired t-test revealed that Myofascial release is significantly effective in improving Agility of athletes.

RESULTS AND DISCUSSION

Myofascial release is significantly effective in improving the Agility of Athletes. Using foam rolling for self-myofascial release has been shown to have numerous beneficial therapeutic effects (e.g., increased vascular plasticity and soft tissue regeneration) on performance and recovery in a variety of sports. In response to foam rolling self-myofascial release, there is an increase in myogenic and endothelial dilation, as well as an increase in NO₂. In addition to serving as a mood enhancer, it also potentiates exhaustion, making it a useful ergogenic aid. The reduction of spasms, the breaking up of adhesions, the increase in blood flow, and the lymphatic drainage all contributed to the improvement in soft tissue extensibility. Fascia's thixotropic qualities, which allow it to soften when disturbed, have also been postulated as a possible explanation for greater soft tissue extensibility. This may be due to the ground substance being less viscous as a result of the manipulation of soft tissues.

Deep sustained pressure is believed to activate the autonomic nervous system by stimulating Ruffini endings in the fascia, which respond to deep sustained pressure. Proponents of SMR say that activating these receptors lowers the overall sympathetic tone, increases gamma motor neuron activity, and promotes the relaxing of intra-fascial smooth muscle cells, among other effects[5]. Also believed to be the case is that the autonomic nervous system stimulated vasodilation and local fluid dynamics, which altered the viscosity of fascia by transforming the ground substance into a more gel-like condition. Allegedly, the combination of these effects will produce a perceptible release of the trigger point while simultaneously improving muscular function[6,7]. The stimulation of the mechanoreceptors causes the autonomic nervous system and the central nervous system to become active at the same time. Because of the localized pressure, the central nervous system responds by reducing the tonus of adjacent striated muscle fibres, which in turn contributes to the release felt after applying SMR to the affected area[8].





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CONCLUSION

The results of the study make us conclude that Myofascial release is effective in improving the agility of athletes

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Conflict of Interest

The authors have none to declare.

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Efficacy of Self Myofascial Release Technique on Hamstring Flexibility in Participants With Acute Non Specific Low Back Pain

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ABSTRACT

Low back pain (LBP) is a most common worldwide problem and Nearly 60% of Indians have significant back pain at some time in their life. As hamstring is a biarticular and postural muscle, it has tendency to shorten even under normal situations and Hamstring tightness is considered to be a causative factor for low back pain. A “schematic map” of the entire fascial system known as anatomy trains has been suggested that any tension placed on particular part of fascial system may cause detrimental effects and can decreased global flexibility. Studies have also proven the existence of connections and continuity between muscle or fascia which are located anatomically distant from one another. So purpose of the present study to see the effect of self myofascial release (SMR) technique on hamstring flexibility in participants with acute non specific low back pain. An pre-post experimental study was conducted at Physiotherapy department, Civil Hospital Ahmedabad. 52 Participants of both genders with age group of 18-35, LBP with no specific pathology, Active Knee Extension (AKE) measurement more than 15 degree and LBP less than 6 weeks were included in the study by convenience method. Participants of LBP with trauma and specific pathology (e.g., infectious and inflammatory diseases, fracture, tumor and structural deformity), any neurological symptoms involving intervertebral disc herniation and radiculopathy, history of recent abdominal or back surgery and pregnancy, psychological risk factor, participants apprehensive for stretching techniques were excluded. Outcome measurements in this study are pain measured by VAS, functional disability score measured by Oswestry Disability Index (gujarati version), Hamstring flexibility measured by AKE. All three outcome measures were assessed prior to technique. All participants were received self myofascial release technique and along with that SWD and exercises also. All outcomes were reassessed on 7th day after treatment. The results will be recorded and





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statistically analyzed. Significant improvement were seen in all outcome measures that is pain on VAS, Hamstring flexibility on AKE and ODI score for functional disability after self myofascial release technique. Present study results concludes that Self myofascial release technique is effective to improve pain, hamstring flexibility and functional disability in acute non specific low back pain participants.

Key words: Self myofascial release, Active knee extension, Acute non specific low back pain, Hamstring flexibility.

INTRODUCTION

Low back pain (LBP) is a major public health issue that has reached pandemic levels in recent years. Back pain is the leading cause of disability and incapacity to work in the globe, and it is projected to impact up to 90 percent of the world's population at some point throughout their lives [1,2]. Nearly 60% of Indians have significant back pain at some time in their life [3]. Based on the etiology LBP is classified as Specific LBP and Non-specific LBP. Specific LBP causes are nerve root compression, vertebral fracture, tumor, infection, inflammatory diseases, spondylolisthesis or spinal stenosis [4]. Non-specific low back pain is defined as low back pain which is not due to recognizable specific cause (e.g., infectious and inflammatory etiology, malignancy, osteoporosis, vertebral fracture, spinal deformity, radiculopathy.) [5]. Of all the LBP patients 90% are attributed to Non-specific causes, a disorder which is a health problem of high economic importance [5]. Based on the duration Non-Specific LBP (NS-LBP) is classified Acute (Less than 6 weeks), Subacute (6 weeks – 3 Months) and Chronic (More than 3 Months) [6]. Several risk factors for development of Low Back Pain include increased lumbar lordosis, reduced abdominal muscle length and strength, decreased back extensor muscle endurance, back extensor muscle flexibility, length of iliopsoas, hamstring muscle flexibility, body composition etc [7,8,9,10]. Poor Hamstrings muscular flexibility, Poor abdominal strength and Increased level of physical activity and work related postural stress are considered as risk factors for NSLBP [11].

Hamstring tightness is a common source of discomfort in asymptomatic individuals. Biarticular and postural Hamstring muscles tend to shorten even under normal circumstances. Injuries to the hamstrings were most often caused by inflexibility. Tight hamstrings restrict hip flexion range of motion (ROM). Knee flexion contractures may result from hamstring strain (inability to completely extend knee joint). Patella femoral syndrome is caused by a tight hamstring. Hamstring inflexibility has been related to low back pain in both adolescents and adults [12]. The hamstring's primary function is to maintain the low back structure in a mechanically favourable posture. A frequent anomaly that decreases the normal lumbar lordotic curve is excessive posterior pelvic tilt caused by a tight hamstring. Inadequate pelvic mobility results in compensatory lumbar motion, which produces recurrent microtrauma and low back pain. FJ Reis and colleagues discovered that LBP patients had less pelvic and trunk flexion but more compensatory lumbar movement [12,13].

Fascia, which is made up of connective tissue sheets, is lying just below the skin. Fascia encircles and connects every neuron, blood vessel, and muscle fibre in the body, allowing bones and muscles to communicate with other organs and the development of large networks throughout the body. Previous investigations, which were conducted in accordance with the tensegrity principle, have shown the existence of continuity and connection between fascia or muscle that may be anatomically distant from one another. This paper presents a "anatomy train" diagram of the body's fascia connections, also known as a "schematic map," and it is argued that any stress placed on a particular segment of a "anatomy train" may have detrimental effects, culminating in overall decreased flexibility [14,15]. With Myofascial release (MFR), the patient's fascia or muscle length is restored while the patient's fascia or muscle length is continuously stretched. Self-MFR is MFR performed by the client with the use of a tool rather than a therapist. Patients can self-MFR to alleviate muscle and fascia soreness and maintain flexibility. Self-MFR equipment include a





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foam roller and roller massage [16,17]. So present study aimed to find out effectiveness of self myofascial release technique in participants with acute non specific low back pain.

METHODOLOGY

A pre-post experimental study was conducted at Physiotherapy department, Civil Hospital Ahmedabad from January 2021 to October 2021. Ethical clearance was taken from the Institutional Ethical Committee. Out of 60 subjects, 52 subjects were included in the study based on inclusion criteria by convenience method. Informed consent were taken from all subjects. Subjects of both gender with age group 18-35 years, low back pain with no specific pathology, LBP less than 6 weeks duration and Active Knee Extension [18,19]. (AKE) angle greater than 15 degree were included in the study. LBP with trauma and specific pathology (e.g, infection, tumor, inflammatory disorder, fracture and structural deformity), any neurological symptoms involving prolapsed intervertebral disc and radiating pain, history of knee injury and knee deformity, History of any recent Abdominal, Back Surgeries and Pregnancy, Psychological risk factor, Participants apprehensive for the Stretching Techniques subjects were excluded.

Baseline Measurements prior the treatment was conducted on participants that is VAS [20] for Pain, ODI for functional disability index, AKE measurement for hamstrings flexibility.

SELF MYOFASCIAL RELEASE TECHNIQUE

Participants were told to roll a tennis ball on the soles of their feet for two minutes, starting from below the metatarsal heads and ending at the heel, with emphasis on the medial arch of each foot. Along with this participants were received SWD for lower back region for 10 minutes, isometric back exercise, bridging, abdominal curl-up, supine SLR, prone back extension, prone SLR and quadruped arm and leg lift in cross manner and all outcomes were assessed again on 7th day post treatment.

DATA ANALYSIS

Data were entered and analysed through Epi Info 7. Continuous variables were expressed as mean and standard deviation. Association between continuous variables were assessed with t test. A p-value less than 0.05 was considered as statistically significant.

RESULTS

Significant improvement were seen in all outcome measures that is pain on VAS, Hamstring flexibility on AKE and ODI score for functional disability after self myofascial release technique.

DISCUSSION

The purpose of the current research was to evaluate the effectiveness of Self myofascial release on hamstring flexibility in individuals with acute non specific low back pain who were also included in the study. Hamstring tightness or shortness begins to start as child starts sitting school career at the age of 5 to 6 years. The degree of tightness grows as children get older, and it reaches a peak at the age of 25 years when a person enters the workforce or becomes occupationally related. Participants receiving self-myofascial release method were on average 27.85 years old according to the findings of this research. In the research conducted by Phansopkar PA [21] et al, the mean age among patients with hamstring tightness was 29.1±5.13 years and 27.2±6.50 years among those without tension which supports our findings. In this research, 59.6 percent of study participants were males who received self-myofascial technique treatment and 40.4 percent were females.





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When back pain patients received self-myofascial release method in addition to conventional treatment, the mean value of the visual analogue scale was substantially lower (2.17 as opposed to 6.81). Visual analogue scale is widely used in nationally and internationally. In many studies it is proved that VAS [20] is a reliable scale but for validity it showed moderate to strong correlation for pain measurement. A myofascial release technique helps to assess restrictions and allows fascial release. Bad posture, Injury, surgical procedures or soft tissue inflammation can lead to myofascial restrictions which create pain and pressure on sensitive areas. With help of MFR, adhesions can be break and improve flexibility and decrease pain. MFR can accelerate blood circulation and temperature which allows more nutrients to tissues and remove waste products. Improved circulation also helps to break fascial adhesions and prevents incorrect functioning of fascial system. In a research conducted by Phullaya [22] p et al, it was discovered that there was a statistically significant difference in pain (VAS) between the groups treated with myofascial release method.

After treating patients with self-myofascial release method, the reading on the goniometer while conducting an active knee extension test on the right leg was substantially reduced (17.10 degrees as opposed to 25.98 degrees). After treating patients with self-myofascial release method, the reading on the goniometer while conducting an active knee extension test on the left leg was substantially reduced (16.85 degrees as opposed to 25.00 degrees). AKE test is a reliable tool for measuring hamstring muscle tightness [18]. According to the findings of a research conducted by Phullaya p [22] et al, the group treated with myofascial release method showed a statistically significant difference in improvement in hamstring flexibility (AKT). Myofascial release helps increase movement. Muscle and fascia tissue may begin to short and restrict joint range of motion after injury. The MFR techniques can break these adhesions and relax fascia or muscle tissue to restore joint range of motion.

Previous studies have proven the existence of connections and continuity between muscle or fascia which are located anatomically distant from one another. This paper presents a "anatomy train" diagram of the body's fascia connections, also known as a "schematic map," and it is argued that any stress placed on a particular segment of a "anatomy train" may have detrimental effects, culminating in overall decreased flexibility [14,15]. Myers (2012) [23] also suggested that tension along entire fascial line can be modified by applying massage on the superficial back line muscle group including muscles of the posterior muscle chain. Hotfiel et al., 2017 [24] have proposed that colloidal substance of fascia is sensitive to mechanical stress and becomes more soft fluid like structure after massage which can be contributing positive effect of SMR. These modifications can reduce the adhesion between the different layers of the fascial tissues and increase connective tissue's extensibility and compliance (Kalichman and Ben David, 2017; Schleip, 2003) [25]. Barnes MF [26] found that MFR can alter the viscosity of fascial ground substance and muscle which lead to regain proper biomechanics and alignment of muscle fibres and improve flexibility.

Other theoretical explanation to improve flexibility following foam roller massage is due to change in fluid like form and thixotropic property of fascia which covers muscle [27]. When fascia which is made up of colloidal substance, is disturbed by mechanical stress or heat, it becomes more gel like soft structure but fascia become thick, solid and more viscous when it left undisturbed [28]. Repeatedly stress applied on soft tissues because of overactivity of inactivity leads to abnormal cross links and scar tissue formation in fascia which can prevent the proper biomechanical function and decrease joint ROM. SMR can realign abnormal cross links, breaks scar tissues and restore fascia's gel like state [29]. Once the fascia becomes more soft gel like state, it improves joint ROM by increasing soft tissue compliance [26]. After undergoing self-myofascial release method, the level of impairment (as assessed by the Oswestry Disability Index) among the patients was substantially reduced (10.71 as opposed to 46.62). Myofascial release treatment, according to research conducted by Arun B [30] et al, is helpful in decreasing pain, increasing activities of daily living (ADL) activities, and assisting in the improvement of sleep quality and depression. Shannon [31] et al and Addison [32] et al studied the effects of hamstring tightness on functional problems and hence with results obtained from the present study proved positive effect of SMR to restore the normal functional biomechanics for a healthy lower back.

In contrast to present findings, Roylance et al (2013) [33] found no statistically significant difference in Sit and Reach test scores of hamstring, calf and low back flexibility in individuals treated by either SMR, postural alignment



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exercises or static stretches. Along with SMR technique, SWD and other included exercises also helps to decrease pain and improve function. SWD is deep heating modality and helps to increase vasodilatation and compliance of connective tissue, decrease inflammation and muscle spasm, reduce pain and improve flexibility also. In a study done by Shabana Khan [34] et al, it is concluded that both intervention SWD and Exercise group are more effective in pain reduction compared to exercise alone. The present work findings suggest that facial release has good impact on hamstring flexibility which is prerequisite for healthy back also. So along with other treatments we can add self myofascial release technique also to improve hamstring flexibility. Treatments which includes hamstring flexibility program will add higher value. Various therapeutic techniques can also be studied and compare in future to treat hamstring tightness in LBP subjects effectively.

CONCLUSION

Present study results conclude that SMR technique along with exercise and SWD is effective to improve pain, hamstring flexibility and functional disability in acute non specific low back pain participants. As hamstring muscle tightness is contributing factor to develop LBP, we need to include treatment techniques to improve hamstring flexibility in treatment plan. SMR technique is easy to learn and practice to improve hamstring flexibility.

CONFLICT OF INTEREST : Nil

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Table 1: Age distribution of study participants undergoing Self-myofascial release techniques (n=52)

	Mean	Standard deviation
Age (in years)	27.85	5.70

In present study, mean age of participants undergoing self-myofascial release technique was 27.85 ± 5.7 years.

Table 2: Pre-post Comparison of Pain, hamstring Flexibility and ODI score after self myofascial release technique

Outcome measures	Pre technique (Mean ± SD)	Post technique (Mean ± SD)	p-value
VAS	6.81 ± 1.14	2.17 ± 1.2	<0.001
Right -AKE test	25.98 ± 3.91	17.10 ± 3.37	<0.001
Left – AKE test	25.00 ± 3.73	16.85 ± 3.60	<0.001
Oswestry Disability Index	46.62 ± 12.84	10.71 ± 8.45	<0.001

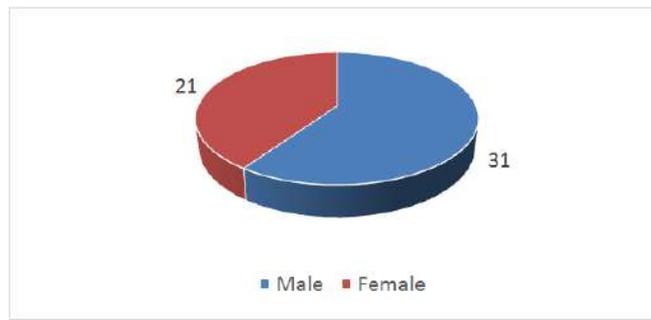


Chart 1. Sex distribution of study participants undergoing Self-myofascial release techniques (n=52)





“To Evaluate the Effect of Different Levels of NPK on Yield of China Aster (*Callistephus chinensis* (L.) Nees) under Chhattisgarh Plains”

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ABSTRACT

The present investigation entitled “Standardization of different concentrations of plant growth regulators and levels of nitrogen, phosphorus on growth, flowering, yield and quality of China Aster (*Callistephus chinensis* (L.)Nees) under Chhattisgarh conditions” was conducted at the Horticultural Research cum Instructional Farm, Department of Floriculture and Landscape Architecture, College of Agriculture, IGKV, Raipur (C.G.). The Experiment-II entitled “Effect on different levels of nitrogen and phosphorus on growth, flowering, yield and quality of China Aster The experiment was conducted in Factorial Randomized Block Design with 24 treatments” comprising of two varieties *viz.* Arka Shashank and Arka Archana as factor A and four levels of Nitrogen, *i.e.* (120, 140, 160, 180 kg/ha), and three levels of Phosphorus (100, 120, 140 kg/ha) as factor B. The doses of Nitrogen and Phosphorus were applied in two splits with constant dose of potassium (60kg/ha) were applied in the field. The result of experiment- II, were indicated that China aster *cv.* Arka Archana performed superior as compare to Arka Shashank and the F₁₂ (N₁₈₀: P₁₄₀) performed significantly better on yield and quality characters *viz.* flower yield per plot (kg), flower yield per hectare (qt/ha), Stalk length of flowers (cm), Diameter of flowers (cm), Vase life of flowers (days).

Keywords: Shashank, Archana, Vase life, Stalk length





INTRODUCTION

China aster (*Callistephus chinensis* L. Ness) belongs to 'Asteraceae' family and is native to China. It is one of the most important annual flower crops grown in most parts of the world. Among annual flowers, it ranks third next only to Chrysanthemum and Marigold. Increased flower quantity and quality with perfection in the form of plants are important objectives to be reckoned in commercial flower production. Although, there are sufficient number of cultivars under cultivation but their performance are region specific and varies from place to place. The quality of flowers is primarily a varietal trait, besides being influenced by nutritional and climatic conditions that prevail during the growing period. It is therefore essential to study the performance of cultivars in a particular place before recommending for commercial cultivation. In view of these, an investigation was conducted to study the growth, flowering and yield characters of two China aster cultivars under Chhattisgarh conditions and their effect on different plant growth regulators. In India, it is being grown on a large scale in Karnataka, Tamil Nadu, Andhra Pradesh, Maharashtra and West Bengal. The exact area of its cultivation and production are not available. However, it is evident from the statistics that Karnataka alone has a production of about 20,846 Mt from 2,199 ha with a productivity of 9.45 t ha⁻¹ (Anonymous 2018). Aster is also an important commercial flower crop of Siberia, Russia, Japan, North America, Switzerland and Europe. The commercial importance of China aster is growing day by day in India, particularly in Karnataka, Tamil Nadu, West Bengal and Maharashtra. It was discovered that its cultivation was a profitable venture for eastern Uttar Pradesh. It is cultivated in an area of 500 and 400 ha, respectively, in the peri-urban areas of Bangalore (Karnataka) and Pune (Maharashtra) alone (Sonu kumar *et al.*, 2018).

METHODS AND MATERIALS

The experiment was conducted at the Horticulture Research cum Instructional Farm, Department of Floriculture and Landscape Architecture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G) during two rabi seasons of 2018-19 and 2019-20. The study consisted The experiment was conducted in Factorial Randomized Block Design with 24 treatments" comprising of two varieties *viz.* Arka Shashank and Arka Archana as factor A and four levels of Nitrogen, *i.e.* (120, 140, 160, 180 kg/ha), and three levels of Phosphorus (100, 120, 140 kg/ha) as factor B. The doses of Nitrogen and Phosphorus were applied in two splits with constant dose of potassium (60kg/ha) were applied in the field. 45 days old healthy and uniformly grown seedlings were used for transplanting with a spacing of 30 cm x 30 cm @ one seedling per hill." There are twelve fertilizer doses which were applied in two varieties of China Aster, hence there are 24 treatments combinations. V₁F₁ (Arka Shashank+ N₁₂₀: P₁₀₀), V₁F₂ (Arka Shashank+ N₁₂₀: P₁₂₀), V₁F₃ (Arka Shashank+ N₁₂₀: P₁₄₀), V₁F₄ (Arka Shashank+ N₁₄₀: P₁₀₀), V₁F₅ (Arka Shashank+ N₁₄₀: P₁₂₀), V₁F₆ (Arka Shashank+ N₁₄₀: P₁₄₀) V₁F₇ (Arka Shashank+ N₁₆₀: P₁₀₀), V₁F₈ (Arka Shashank+ N₁₆₀: P₁₂₀), V₁F₉ (Arka Shashank+ N₁₆₀: P₁₄₀), V₁F₁₀ (Arka Shashank+ N₁₈₀: P₁₀₀), V₁F₁₁ (Arka Shashank+ N₁₈₀: P₁₂₀), V₁F₁₂ (Arka Shashank+ N₁₈₀: P₁₄₀), V₂F₁ (Arka Archana+ N₁₂₀: P₁₀₀), V₂F₂ (Arka Archana + N₁₂₀: P₁₂₀), V₂F₃ (Arka Archana + N₁₂₀: P₁₄₀), V₂F₄ (Arka Archana + N₁₄₀: P₁₀₀), V₂F₅ (Arka Archana + N₁₄₀: P₁₂₀), V₂F₆ (Arka Archana + N₁₄₀: P₁₄₀) V₂F₇ (Arka Archana + N₁₆₀: P₁₀₀), V₂F₈ (Arka Archana + N₁₆₀: P₁₂₀), V₂F₉ (Arka Archana + N₁₆₀: P₁₄₀), V₂F₁₀ (Arka Archana + N₁₈₀: P₁₀₀), V₂F₁₁ (Arka Archana + N₁₈₀: P₁₂₀), V₂F₁₂ (Arka Archana + N₁₈₀: P₁₄₀) and statistical analysis of variance was followed according to the method described by Panse and Sukhatme.

Meteorological data on temperature, rainfall, relative humidity and sunshine hours was recorded during the cropping period at the Meteorological Observatory Unit, Department of Agro-meteorology, IGKV, Raipur. During the cropping season namely *Rabi season* during two consecutive years *i.e.*, (2018-19) and (2019-20) the total amount of annual rainfall received was 83.40 mm and 309.40 mm, respectively. The maximum and minimum temperatures were 40.8°C in 3rd week of April and 8.5°C in 1st week of January respectively during the year (2018-19). The maximum and minimum temperatures were 35.1°C in 4th week of April and 10.8°C in 2nd week of January, respectively during the year (2019-20). The average wind velocity ranges from 3.8-4.4 (Kmph) during (2018-19) and 3.9-4.5 (Kmph) during (2019-20) respectively, Apart from this, other weather parameters were recorded in respective



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seasons of both years. Relative humidity was ranged from 71.00 to 92.00 per cent during first year (2018-19) and 76.00 to 94 per cent second during year (2019-20). During the experimentation no define pattern was observed both the years regarding sunshine hours. The weather conditions during the course of investigation remained favourable for the growth, development and production of China Aster.

RESULTS AND DISCUSSION

The data on Yield of flower per plot were recorded and are presented in Table 1 In case of cultivar, the maximum yield of flower per plot were observed in cultivar (V₂) Arka Archana (4.30, 4.20 and 4.20 kg) and minimum yield of flower per plot (3.70, 3.60 and 3.70 kg) respectively were recorded in cultivar (V₂) Arka Shashank in both the years as well as on pooled mean analysis. As far as plant growth regulators showed significant response on yield of flower per plot, the highest yield of flowers per plot (5.20, 5.10 and 5.20 kg) was observed under treatment P₆ (GA₃ 200 ppm) and it was found significantly superior over other plant growth regulators, during both the year as well as on pooled mean analysis and for rest of the other treatment it was showed significant difference. Whereas, the minimum yield of flowers per plot (2.80, 2.90 and 2.90 kg) respectively was recorded under treatment P₀ (Control). The interaction between cultivars and plant growth regulators was found non-significant during both the years as well as on pooled mean basis under this investigation. It is clear from the data presented in Table 2 that the Yield of flower per hectare were significantly influenced by different levels of cultivars and nitrogen and phosphorus.

In case of cultivar, the maximum yield of flower per hectare were observed in cultivar (V₂) Arka Archana (18.90, 18.70 and 18.80 qt/ha) respectively and minimum yield of flower per plant (16.40, 16.00 and 16.20 qt/ha) respectively were recorded in cultivar (V₂) Arka Shashank in both the years as well as on pooled mean analysis. In respect to plant growth regulators, the highest yield of flowers per hectare (23.00, 22.80 and 22.90 qt/ha) was observed under treatment P₆ (GA₃ 200 ppm) and it was found significantly superior over other plant growth regulators, during both the year as well as on pooled mean analysis and for rest of the other treatment it was showed significant difference. Whereas, the minimum yield of flowers per hectare (12.6, 12.7 and 12.7 qt/ha) respectively was recorded under treatment P₀ (Control). The interaction between cultivars and plant growth regulators was found non-significant during both the years as well as on pooled mean basis under this investigation.

CONCLUSION

On the basis of two years study, it was concluded that optimum doses of nitrogen and phosphorus are necessary for maximizing growth, yield and quality of China aster under Chhattisgarh condition. Among the treatments, the treatment F₁₂ (N₁₈₀: P₁₄₀) recorded flower yield per plot, flower yield per hectare, stalk length of flowers, Diameter of flowers, Vase life of flowers. The study suggested that China aster cv. Arka Archana performed superior as compare to Arka Shashank and application of NPK @ 180:140 and 60 kg ha⁻¹ is the most appropriate dose for attaining high growth, yield and quality of these cultivar of China aster under Chhattisgarh condition. The interactions between two cultivar and application of nitrogen and phosphorus were found mostly non- significant for different yield and quality attributes of China aster. This may be due to the similar effect of nitrogen and phosphorus on both the varieties of China aster under Chhattisgarh conditions.

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Treatments	Table 1: Effect of Varieties and Nitrogen and phosphorus on Yield per plot		
	2018	2019	Pooled
V ₁	3.61	3.70	3.6
V ₂	4.30	4.44	4.34
SEM±	0.04	0.03	0.02
CD at 5%	0.08	0.07	0.05
F ₁	3.21	3.02	3.2
F ₂	3.40	3.41	3.4
F ₃	3.40	3.44	3.4
F ₄	3.59	3.70	3.6
F ₅	3.74	3.71	3.7
F ₆	3.97	4.00	3.9
F ₇	3.90	4.10	4.0
F ₈	4.11	4.29	4.2
F ₉	4.20	4.47	4.3
F ₁₀	4.44	4.52	4.5
F ₁₁	4.61	4.90	4.7
F ₁₂	4.87	5.00	4.9
SEM±	0.10	0.09	0.06
CD at 5%	0.20	0.19	0.14
V ₁ F ₁	2.80	2.76	2.786667
V ₁ F ₂	2.99	2.96	2.98
V ₁ F ₃	3.07	3.11	3.09





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V ₁ F ₄	3.24	3.33	3.28
V ₁ F ₅	3.32	3.32	3.32
V ₁ F ₆	3.45	3.61	3.53
V ₁ F ₇	3.50	3.76	3.63
V ₁ F ₈	3.65	3.93	3.79
V ₁ F ₉	3.77	4.22	3.99
V ₁ F ₁₀	4.13	4.38	4.25
V ₁ F ₁₁	4.43	4.54	4.48
V ₁ F ₁₂	4.63	4.61	4.62
V ₂ F ₁	3.58	3.57	3.58
V ₂ F ₂	3.71	3.83	3.77
V ₂ F ₃	3.74	3.78	3.76
V ₂ F ₄	3.84	4.00	3.92
V ₂ F ₅	4.11	4.13	4.12
V ₂ F ₆	4.28	4.31	4.30
V ₂ F ₇	4.37	4.41	4.39
V ₂ F ₈	4.5	4.52	4.51
V ₂ F ₉	4.60	4.58	4.59
V ₂ F ₁₀	4.72	4.70	4.71
V ₂ F ₁₁	4.75	5.19	4.97
V ₂ F ₁₂	4.88	5.31	5.09
SEm±	0.14	0.13	0.09
CD at 5%	NS	NS	NS

Treatments	Table 2: Effect of Varieties and Nitrogen and phosphorus on Yield per hectare		
	2018	2019	Pooled
V ₁	15.90	16.50	16.20
V ₂	18.90	19.40	19.20
SEm±	0.45	0.42	0.30
CD at 5%	0.91	0.84	0.62
F ₁	14.20	14.10	14.20
F ₂	14.90	15.10	15.00
F ₃	15.20	15.30	15.20
F ₄	15.80	16.30	16.00
F ₅	16.50	16.60	16.60
F ₆	17.20	17.60	17.40
F ₇	17.50	18.20	17.90
F ₈	18.10	18.80	18.50
F ₉	18.60	19.60	19.10
F ₁₀	19.70	20.20	20.00
F ₁₁	20.40	21.70	21.00
F ₁₂	21.20	22.10	21.60
SEm±	0.18	0.17	0.12





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CD at 5%	0.37	0.34	0.25
V ₁ F ₁	12.49	12.31	12.40
V ₁ F ₂	13.32	13.2	13.26
V ₁ F ₃	13.66	13.83	13.74
V ₁ F ₄	14.43	14.83	14.63
V ₁ F ₅	14.78	14.77	14.77
V ₁ F ₆	15.38	16.06	15.72
V ₁ F ₇	15.59	16.73	16.16
V ₁ F ₈	16.23	17.51	16.87
V ₁ F ₉	16.77	18.76	17.76
V ₁ F ₁₀	18.36	19.52	18.94
V ₁ F ₁₁	19.70	20.21	19.96
V ₁ F ₁₂	20.62	20.49	20.55
V ₂ F ₁	15.95	15.90	15.92
V ₂ F ₂	16.53	17.08	16.80
V ₂ F ₃	16.65	16.84	16.74
V ₂ F ₄	17.09	17.8	17.44
V ₂ F ₅	18.31	18.40	18.35
V ₂ F ₆	19.05	19.21	19.13
V ₂ F ₇	19.47	19.61	19.54
V ₂ F ₈	20.00	20.14	20.07
V ₂ F ₉	20.46	20.39	20.42
V ₂ F ₁₀	21.00	20.95	20.97
V ₂ F ₁₁	21.13	23.10	22.12
V ₂ F ₁₂	21.72	23.61	22.67
SEm±	0.64	0.59	0.43
CD at 5%	NS	NS	NS





Design and Fabrication of Solar Power Weeder for Agricultural Use

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ABSTRACT

Power weeders are used for removing weeds and loosening the soil thereby preventing them from the main crop. A weed is any unwanted plant or vegetation which interferes with farming. Most of the power weeders use fossil fuels to carry out their operation. Due to the scarcity of fossil fuels producing high emissions, an alternative source of energy is required. The main objective of this project is to design and fabricate a solar-operated power weeder used for farming. As solar energy is readily available and all the operations are generally carried out during the daytime, a solar-operated power weeder has been developed. This will reduce the dependency on fossil fuels, harmful emissions, and cost of operation. 3-D modeling of the power weeder has been created using CATIA V5, and the physical model has been fabricated in the mechanical workshop. The power source includes a solar photovoltaic panel that charges the battery. A geared D.C motor run by the battery has been used for power transmission. It is found that the weeding efficiency and digging of soil

Keywords: Power weeder, Solar, Weeding efficiency, Fossil fuels, Emissions, Supercapacitor, Conventional, Non-conventional

INTRODUCTION

Agriculture is the backbone of our country, and we are the second largest country producer of agricultural products. Nowadays, the Automobile sector has moved on to electrical vehicles to reduce emission levels and attain an eco-friendly environment. Weeding is one of the most important farm operations in the crop production system. Weed growth is a major problem for land crops. Weeding is generally done 15-20 days after sowing. The weed should be controlled and eliminated at its early stage depending upon the weed density. 20-30% loss in grain yield is quite usual which might increase up to 80% if adequate crop management practice is observed. Weeds compete with crop plants for nutrients and other growth factors and in the absence of effective control measures and remove 30 to 40% off applied nutrients resulting in significant yield reduction. Delay and negligence in weeding operation affect the crop yield and the loss in crop yield due to weeds in upland crops varies from 40-60% and in many cases cause



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complete crop failure. Timely weeding is very much essential for a good weeder which can reduce the time spent on weeding (man-hours), cost of weeding, and drudgery involved in manual weeding. Mechanical weed control not only uproots the weeds between crop rise but also keeps the soil surface loose, ensuring better soil aeration and water intake capacity. Weeding is simple with solar operated power weeder instead of reaching down to pool weeds, the job can be done from a comfortable standing position. Lightweight is quite easy to use. It makes quick work of small weeds.

India was the second biggest growth driver of primary energy consumption in the world, behind China, in 2019 even though it witnessed a fall in demand for oil and coal. India's energy needs are high and being a developing country, the requirements are growing further. According to the world energy report, India gets around 80% of energy from conventional fossil fuels like oil (36%), natural gas (21%), and coal (23%). It is well known that the time is not so far when all these sources will be completely exhausted. So, alternative sources should be used to avoid an energy crisis in the nearby future. A large amount of solar radiation falls on the earth. In most parts of our country, very few days are without sunshine. India lies within the latitude of 8-37°N with an annual average intensity of solar radiation of 1361 W/m².

This project aims to

Provide and implement non-conventional sources of resources to provide costless operation than the conventional type weeding process. Design, construct and test automatically operated portable weeder, to provide the best opportunity to farmers' to easily control and remove the weed from farm thereby bringing the fertility of the soil.

LITERATURE REVIEW

Hegazy *et al.* [1] discussed the development and evaluation of a small-scale power weeder based on weeding efficiency, depth of operation, moisture content of soil, and forward speed of blades of the weeder. The weeder used fossil fuels and evaluated *triple hybrid 314 variety of maize* crop. Ikechukwu *et al.* [2] focused on the planning and fabrication of a weeder operated by hand single row maize planter capable of delivering seeds exactly in an exceedingly line with uniform depth within the furrow and with uniform spacing between the seeds. Pundkar and Mahalle [3] presented the innovations done on seed sowing machines available for plantations. They discussed seed metering devices and developed high precision pneumatic planters for many varieties of crops, for uniform seed distribution along the travel path, in seed spacing. Raut *et al.* [4] discussed the problems that farmers face while doing farming and developed a machine that could carry out weeding operations as well as perform spraying operations reducing human effort and making it easy for farmers to carry out cheaper operations. Naque *et al.* [5] designed, developed, and fabricated a soil tiller and weeder which reduced human effort, increased labor productivity, reduced cost of operation, and improved timeliness of operations. The operating procedure is self-guided which is beneficial for farmers to easily learn how to use it. Their main focus was to reduce the manpower and the working time.

Jadhav and Sawale [6] designed and fabricated a manually operated weeder with a multi-nozzle pesticide sprayer giving optimum results in less time. To reduce the intense effort of the pushing mechanism, a three-wheel mechanism was used which also gave proper balancing to the machine. Singh *et al.* [7] used the mechatronics concept to develop a battery-assisted four-wheel weeder for weeding operations to be used by small farmers with reduced drudgery. The machine constructed was simple, low cost, and environmentally friendly. It operated satisfactorily for wide-row crops. Kyadaand and Patel [8] have discussed basic requirements for small-scale cropping machines suitable for small farms, simple in design and technology, and versatile for use in different farm operations. A manually operated template row planter was designed and developed to improve planting efficiency and reduce the drudgery involved in the manual planting method. Manjunatha *et al.* [9] discussed the development and evaluation of a manually operated sprocket weeder based on its performance. They observed that the weeding efficiency was much better, and the cost of operation was much cheaper as compared to the traditional methods. Kachhot *et al.* [10]





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discussed the development of solar operated walking-type power weeder which could be used successfully to carry out weeding operations. Plant damage increased with an increase in the forward speed of the operation. The objective of the present work in this paper is to design, analyze and fabricate a solar-operated power weeder for use in agriculture and farming.

MATERIALS AND METHODS

The development of solar operated power weeder is designed and developed at the Mechanical Department, Centurion University of Technology and Management, Bhubaneswar, Odisha. A field experiment has been conducted at B.Sc. Agriculture compound of the college. 3-D modeling of each component of the power weeder has been done using CATIA V5 as shown in Fig. 1. The material of the frame is fabricated by welding, drilling, grinding, and hand tools. The material of the frame is made up of structural iron.

Working Principle

Solar power is the main source of energy for the developed weeder machine. This non-conventional source of energy is harvested by employing a suitable electrical device. The capacity of the solar panel shown in Fig. 2(e) utilized in the weeder is 40 W. The solar panel works on the principle of the photovoltaic effect. The photovoltaic effect is a process that generates current or voltage at a solar cell when exposed to sunlight. It is due to this effect that the cells of the solar panels convert sunlight to voltage. The electricity generated by the panel is then transmitted to the battery of 12 A as shown in Fig. 2(a) for storage purposes. The batteries send the electricity to a circuit card which transmits power to different components of the developed power weeder. The DC motor of capacity 24 V and 250 W shown in Fig. 2(d) converts electrical energy into mechanical energy. The rotational power from the DC motor is transferred to blades by a rotating mechanism with the help of a chain and sprocket drive shown in Fig. 2(b). By this process, the sprocket which is already fixed at the rotating shaft will start to rotate. The blades shown in Fig. 2(c) are also fixed to the shaft with the help of blade fitting. From this process, the blades will rotate and cut the unwanted plants and dig the soil. Arc soldering, welding, and grinding were carried out throughout the fabrication process. The entire weeder assembly has been shown in Fig. 2(f).

RESULTS AND DISCUSSION

A D.C. motor with a capacity of 24 V and 250 W (0.3351 HP) has been used for the operation of a power weeder. To maintain a constant voltage while digging, we have taken a 12 A battery because there is always a possibility of voltage up or down while starting a motor as the battery drains out and an immense amount of current gets discharged. Taking the speed of the shaft as 300 rpm at the motor end, the load torque at the motor end can be calculated using Eq. (1). It is found that the torque produced is 7.9617 Nm.

$$Power = \frac{2\pi NT}{60} W \quad (1)$$

Where, N = speed of shaft in rpm

T = Torque in N.m

The speed of the shaft at the rotary end is 150 rpm with a gear ratio of 1:2. Considering the efficiency as 60% due to transmission loss of power due to relative motion, the power and torque available at the rotary of the shaft are calculated as 150 W and 9.55 Nm respectively. It is seen that the torque is increased because the speed of the rotor is reduced. It means the torque is inversely proportional to speed.

Before field testing, using a digital tachometer, the speed of the motor end & speed of the rotary blade was recorded.

The moisture content of the soil has been calculated using Eq. (2):





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$$\text{Moisture content} = \frac{\text{Weight of moist soil} - \text{Weight of dry soil}}{\text{Weight of dry soil}} \quad (2)$$

The working depth of the cut of the rotary blade is found to be 10 cm and the width of the cut is found to be 18 cm. The effect of forwarding speed on the heart rate of the person is also observed to be 105 beats per minute in Table 6. Weeding is carried out in the same field to determine weeding efficiency (WE). It is calculated using Eq. (3).

$$WE = \left(\frac{W_1 - W_2}{W_1} \right) \times 100\% \quad (3)$$

where WE = Weeding efficiency

W_1 = Number of weeds before weeding

W_2 = Number of weeds after weeding

The comparison of weeding efficiency to that of the soil texture is shown in table 5 below.

Fig. 3 shows the graphical representation of the speed at the motor and the speed of the rotary blade. At maximum throttle, the speed of the motor and rotary blade is maximum and vice versa. Fig. 4 shows the graphical representation of the torque at the rotary end and cut depth by the rotary blade. On full throttle, the rotary blade digs the soil attaining full cut depth with maximum cutting force. Fig. 5 shows the graphical representation of the soil texture and the depth of cut by the rotary blade. The depth of cut is maximum in the case of peat soil as it contains maximum moisture content and minimum in the case of sandy soil as it has very less water content. Fig. 6 shows the graphical representation of weeding efficiency to the moisture content present in the soil. The weeding efficiency increase with the increase in the soil moisture content. Fig. 7 shows the statistical analysis of the mean values of the heart rate for all three forward speeds. The heart rate was recorded to be 105 beats per minute at 2.0-2.5 km/hr.

CONCLUSION

The fabrication and performance evaluation has confirmed that it has less operating cost than conventional type weeder. The machine maintains space between rows of crops and digging of the soil doesn't harm the crop. The machine can be utilized to burrow soil as well as remove weeds. The well-being of the client is a fundamental need. We haven't utilized any kind of petroleum or diesel motor. It absolutely works with the assistance of a powerful D.C Motor, battery, and solar panel. The segment must be utilized for planting little pieces of land. The client of this item can be anybody as its condition is amicable and no need for any additional source. It can likewise be utilized for multi-reason work, for example, water system and leveling the dirt bed. The segment is anything but difficult to utilize and can without much of a stretch move starting with one spot and then onto the next. It would be an incredible lift to the innovation of weeder due to its security measures and multi-reason.

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Table 1: Speed and torque of shaft at the motor end and rotor end

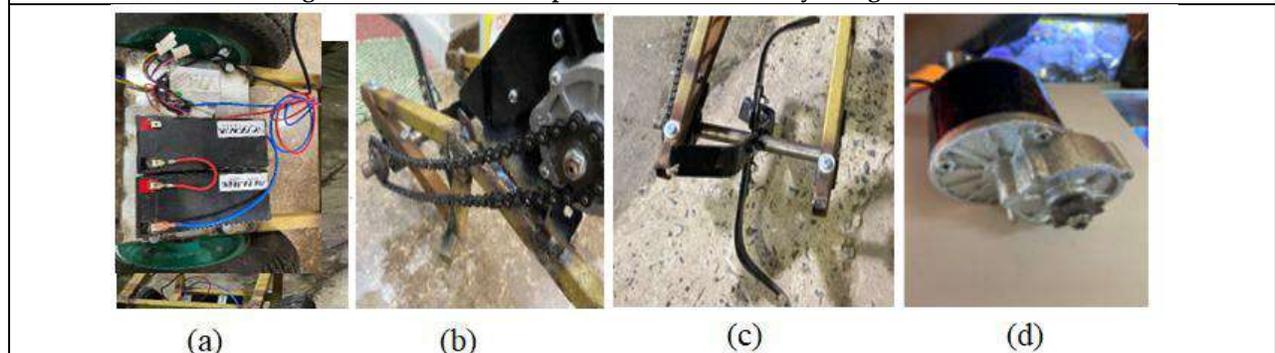
Throttle (%)	Speed at the motor end (rpm)	Torque at the motor end (Nm)	Speed at the rotor end (rpm)	Torque at the rotor end (Nm)
25	130	1.1942	72	1.3337
50	238	2.3885	140	2.7563
100	500	4.7770	260	5.5126

Table 2: Soil type, number of weeds before and after weeding, and weeding efficiency

Soil Type	W1	W2	WE
Peat Soil	45	4	91.11
Clayey Soil	39	10	74.35
Sandy Soil	46	18	61.37



Fig. 1: 3D model of solar power weeder assembly using CATIA V5





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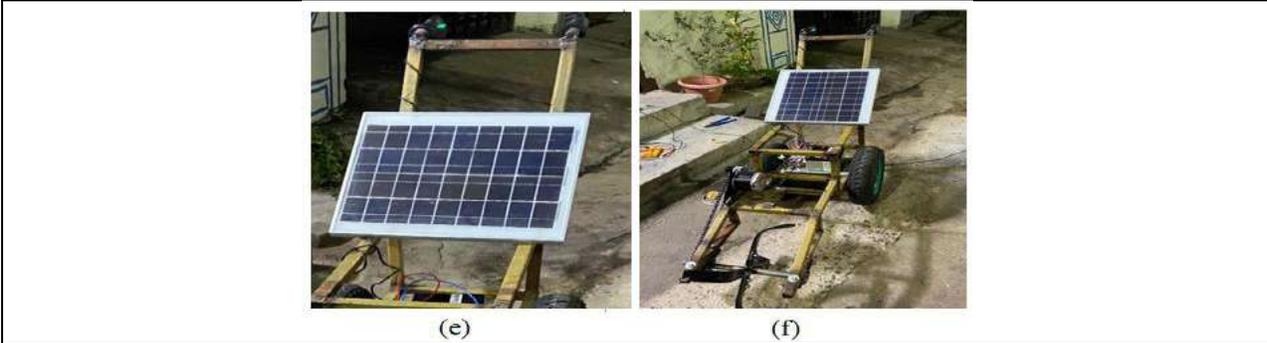


Fig. 2: (a) Batteries, (b) Chains sprocket, (c) Rotary blade, (d) D.C. motor, (e) Solar panel, (f) Weeder assembly

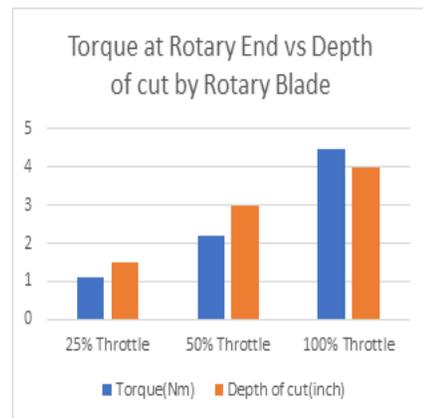
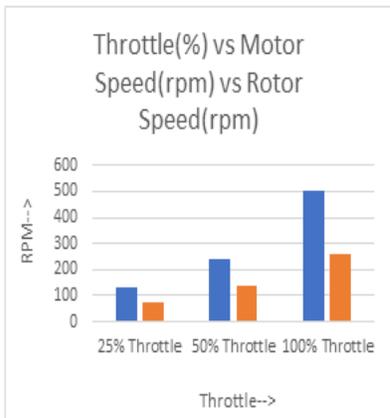


Fig. 3: Throttle vs motor and rotor speed

Fig. 4: Torques vs depth of cut

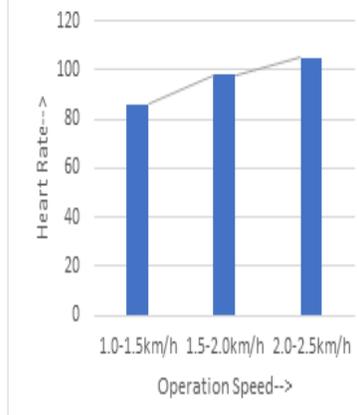
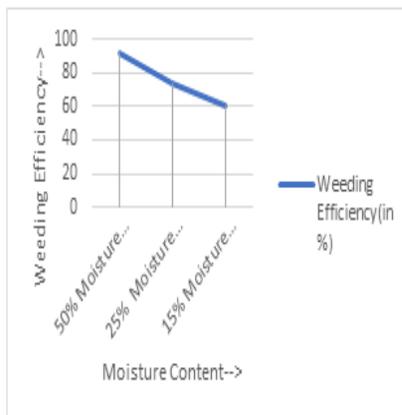
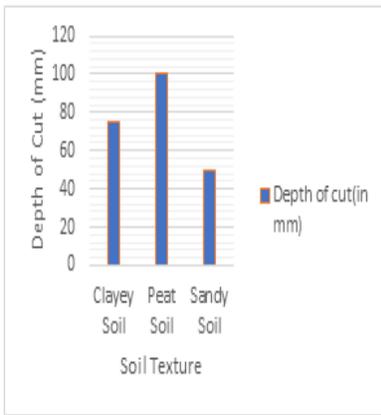


Fig. 5: Depth of cut vs soil texture

Fig. 6: Weeding eff, vs moisture content

Fig. 7: Heartbeat vs operation speed





Effect of Dual Task Training on Motor and Cognitive Function in Subjects with Parkinson's Disease

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ABSTRACT

Parkinson's disease (PD) is degenerative neuropathology characterized by motor slowdown, rigidity, and tremors, with decreased muscular strength and balance, a negative trend of risk of falls and injuries, fear of falling, a decline in physical activity and decreased quality of life. Dual task performance is also known as concurrent performance and involves the execution of a primary task, which is the major focus of attention, and a secondary task performed at the same time. This study aims to find out the effect of dual task training on motor and cognitive function in subjects with Parkinson's disease. 30 subjects with PD were selected for the study by convenience sampling. Subjects were instructed to rise from a chair with an armrest, walk 5 meters at a comfortable pace, turn around and walk back to the chair and sit down. An indication was marked on the floor at 5 meters away from the chair to indicate where the subject should turn. Subjects were made to walk on a hard, level surface. Along with walking subjects performed dual tasks of motor and cognitive activities simultaneously. This study showed statistically significant improvement in subjects from pre to post-test measurements in UPDRSi.e.; $p < 0.05$. Dual Task Training on Motor and Cognitive Function in Parkinson's Disease subjects proved to be more effective and can be used clinically for better improvement in managing Parkinson's disease.

Keywords: Parkinson's disease, Dual task, Dual task training, Motor, Cognitive, Unified Parkinson's Disease Rating Scale.





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INTRODUCTION

Parkinson's disease (PD) is degenerative neuropathology characterized by a motor slowdown, rigidity, and tremors, with decreased muscular strength and balance, and also many risks of falls and injuries, fear of falling, back fall in physical activity, and difficulty in maintaining the quality of life. [1,2]The clinical features of this neurodegenerative disorder include resting tremor, muscular rigidity, bradykinesia, and postural instability. Also, other clinical features include secondary motor symptoms (e.g., Dysarthria, dysphagia, micrographia, shuffling gait), non-motor symptoms (e.g.,Autonomic dysfunction, cognitive/neurobehavioral abnormalities, sleep disturbances). Cognitive deterioration and dementia are common in PD and should occur early or late. Major depression is associated in patients with PD [3]. These motor and non-motor symptoms can impact function to a variable degree.Non-motor features of PD, like dementia, could also be an intrinsic feature of the disorder and persist no matter the medication state (i.e., they still manifest within the "on" or "off" state); some non-motor features, like psychotic symptoms, maybe iatrogenic complications of pharmacologic intervention for the treatment of the motor manifestations of PD [4].In addition to these abnormalities, gait impairments and walking limitations are common among people with PD. While gait abnormalities are not pronounced in the early stages of PD, their prevalence and severity increase with disease progression. The consequences of gait impairments in PD are significant and include increased disability, increased fall risk, and reduced quality of life[5].Falling is one of the most serious and disabling features of Parkinson's disease[6].Falls are a leading cause of fractures, head trauma, soft tissue injuries, and joint dislocations which increase morbidity and mortality in older adults[7].

Levodopa is the mainstay of symptomatic treatment for Parkinsonism[8].Rehabilitation can have a vital impact on reducing functional limitations and disability. The perfect program would be restorative rehabilitation which is targeted at the improvement of strength, range of motion, functional performance, endurance, etc. These individuals also get pleasure from functional maintenance programs designed to manage progressive disease results.[9]In several activities of daily living, quite one task is executed at an identical time. The power to perform dual tasks is very advantageous and may be a prerequisite to normal life. A stroll, for instance, allows for communicating with somebody, transporting objects from one place to different, and monitoring the environment to avoid accidents. In normal circumstances, the concomitant executions of motor and cognitive tasks are common, in such situations, motor activities are performed automatically, and no efforts of conscious attention are required. Such an autonomous stage of performance of motor ability is achieved through a process of motor learning, during which practice and its variability originate the formation of action programs[10].

Dual task performance is additionally referred to as concurrent performance and involves the execution of a primary task, which is the major focus of attention, and a secondary task performed at an identical time[11].Gait deficits are exacerbated during the performance of the dual task in Parkinsonism because the requirement to focus on both walking and concurrent task exceed available attentional resources. In Parkinson's disease, the movement becomes slow, and hence conscious attention to the task and extra assistive cues are required to interchange the same old automatic control. Dual task interference has been demonstrated in people with Parkinsonism[12].

MATERIALS AND METHODS

Subjects diagnosed with Parkinson's disease who fulfilled the inclusion criteria were recruited for the study by Convenience Sampling. The inclusion criteria were: (1) Subjects of both genders (2) Age group above 55 years (3) Subjects graded between II-IV stage on HOEHN AND YAHR SCALE (4) Mini-Mental Status Examination (MMSE) score with 24 and above.

A total of 30 subjects met the criteria. Once the approvals were received, each subject was assessed using a general neurological proforma (Annexure-III) and a detailed explanation was given about the procedure to be done. Before the intervention, the motor and cognitive function of the subjects was assessed using the Unified Parkinson's Disease





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Rating Scale (UPDRS)(I-IV components). The intervention was conducted 3 days a week for 30 minutes for 4 weeks during the “on” state of subjects who were under the administration of Levodopa. Subjects were instructed to raise from the chair with the armrest, walk 5 meters at a comfortable pace, turn around and walk back to the chair and sit down. An indication was marked on the floor at 5 meters away from the chair to indicate where the subject should turn. Subjects were made to walk on a hard, level surface. Along with walking subjects were asked to perform dual tasks of motor and cognitive activities simultaneously. The following were the motor and cognitive tasks: (1) carrying an empty tray and serial 3 subtraction (2) carrying a tray with sponge balls and repeating the days of the week (3) transferring coins from the tray and repeating a phrase[8,13]. At the end of 4 weeks subjects were assessed for cognitive and motor function using the same outcome measure as earlier.

DISCUSSION

This study aimed to evaluate the effect of dual task training on motor and cognitive function in subjects with Parkinson’s disease. In several activities of daily living, more than one task is executed at the same time [10]. Cognitive-motor interference refers to the phenomenon that occurs when 1 or 2 tasks that interfere with each other are being performed, such as engaging in cognitive and motor tasks simultaneously. Interference between cognitive tasks and motor control activities (such as walking) is important for functional improvement in patients with neurological deficits[14].

Statistical analysis was done using frequency and percentage analysis for describing gender of the subjects, mean and SD to assess the pre and post-test outcome measures, unpaired t-test for normally distributed data, Wilcoxon test for skewed data, and chi-square test for categorical data where p-value less than 0.05 was considered significant. An analysis was conducted based on gender where there were 23(76.7%) males and 7(23.3%) females. It was found to be statistically not significant; i.e., $p > 0.05$. It evidenced the baseline characteristics of gender are homogenous in both groups. Epidemiological studies have shown that both incidence and prevalence of PD are 1.5-2 times higher in men than in women when asked at a disease duration of 9 years [15] which supports the finding of more males than females.

An additional analysis was conducted based on age where the mean age for males was 63.00 ± 7.78 and the mean age of females was 62.57 ± 8.42 . Although the age range of subjects in the study was above 55 years, most of the subjects fall under the age group of 55-77 years. PD a neurodegenerative disease affects approximately 1% of the elderly population over 60 years. The peak age of onset is in the ‘60s (range is 35-85) and the course of illness ranges between 10 & 25 years. [16] The mean age at onset is 61 years, but the disease can range from juvenile cases to cases within the 8th or 9th decade of life. [17] It shows similar findings within the study. Out of the entire number of subjects taken, 15 subjects were in Hoehn &Yahr stage 2, 13 subjects were in Hoehn &Yahr stage 3, and 1 subject was in Hoehn &Yahrstage 4. It was found that the Hoehn &Yahr score of the 2-4 staging scale is homogenous in both genders and was statistically not significant.

The range of male subjects was 24-30 with a mean and SD of 26.35 ± 1.69 and also the range of female subjects was 24-29 with a mean and SD of 24.29 ± 1.79 . It was found that the range of MMSE of most of them was 26-28. Although the MMSE scores were normal a number of them had attention deficits and were distracted easily during the treatment sessions. At other times, they had difficulty recalling a name or an exercise taught to them. Forgetfulness, difficulty in finding words, and getting lost were also seen based on the disease severity during the therapy sessions. It had been evidenced that mild cognitive deficits are common in PD patients with normal cognition supported MMSE performance and are observed with increasing disease severity[18].

The pre and post-score of UPDRS (I-IV components), the Mean with SD of the Pre-test was 66.30 ± 24.01 and also the Mean and SD of the Post-test were 51.70 ± 121.9 . In which, 5 female subjects had the pre-test scores within the range of 74-118 and post-test scores within the range of 43-106. 2 female subjects had the pre-test scores of 45,87 and the post-





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test scores of 43, 60. 11 male subjects had the pre-test scores within the range of 24-88 and the post-test scores within the range of 22-86. 11 male subjects had the pre-test scores within the range of 30-106 and the post-test scores within the range of 18-74. One male subject had a pre-test score of 74 and a post-test score of 70. The Wilcoxon test was carried out to compare the pre and post-values and it showed a statistically significant difference in UPDRS. During the beginning of the intervention, subjects of Hoehn &Yahr stage 3 had difficulty rising from the chair and needed little assistance and also postural sway was observed in most of the subjects. Because the intervention was practiced, subjects were able to raise from the chair and there was a reduction in the postural sway. Geraldine L. Pellecchiadiscovered in her study that, attentional demands of the cognitive task impacted postural sway. [19]This correlates with the study. Initially, subjects had difficulty turning and were only able to perform with support and verbal instructions. Gradually, subjects were able to turn and perform the activities with ease, without verbal instructions. Spildooren J et al mentioned in their study that turning is the most important trigger in freezing of gait in PD[20] which correlate with the study. While turning, the subjects failed to lift their foot initially and seemed confused over which leg to initiate the turning. In Parkinson's disease, falls and freezing of gait are the two "episodic" phenomena that are commonly observed[21].Within the early stages' subjects hesitated to step forward because of fear of falling and sudden freezing of gait. Also, hallucinations of objects being obstacles for them to move forward and most subjects presumably fall while performing the tasks.

The motor function of the subjects played a significant role in carrying out the task as some of them faced difficulty in grasping the tray and also the coins and maintaining their posture due to their rigidity, muscle weakness, and fatigue. While carrying the empty tray, they showed more interest in cognitive tasks than carrying a tray with sponge balls. Because the subjects were involved in the task, making it easier for them to perform better in each session. Some failed to do so because of loss of interest, depression, drug administration, sleep disturbances, no support from the family, and also due to muscle weakness, tremors, postural instability, rigidity, and other non-motor symptoms. M. A. Hely concluded that the age of onset of symptoms of Parkinson's could be a major determinant of the course of the disease and response to treatment[22].And also the subjects with a history of smoking and quitting smoking before the onset of the disease showed very less improvement in the cognitive tasks. M. Doiron concluded in their study that smoking history was associated with global cognitive impairment in PD even in patients who had quit smoking [23].

Based on the UPDRS I-IV components, subjects from Hoehn &Yahrstage 2 showed better improvement in intellectual impairment and motivation than the Hoehn &Yahrstages 3 and 4. Subjects from Hoehn &Yahrstages 2 and 3 showed a major progression in speech, handwriting, dressing, and walking. Subjects from Hoehn &Yahrstage 2 showed a reduction in the frequency of falling, freezing when walking, and tremors. Subjects from Hoehn &Yahr stage 4 showed improvement only in speech. Subjects from Hoehn &Yahr stage 2 showed better progression in speech and there was a reduction in tremors and muscle rigidity while performing the task. Improvement was seen in finger taps, hand movements, rapid alternating movements of hands, leg agility, and arising from a chair. Subjects from Hoehn &Yahrstage 3 showed improvement in speech, finger taps, hand movements, rapid alternating movements of hands, and leg agility, arising from a chair. They also showed a reduction in muscle rigidity and tremors during the action. Subjects of Hoehn &Yahrstages 2 and 3 had mild disabling dyskinesias mostly 25% - 50% of the day but Hoehn &Yahr stage 4 had severely disabling dyskinesias almost 75% of the day and they also had early morning dystonia.

Dual task training is beneficial in improving the lives of Parkinson's subjects for better performance of their ADL. Wang XQ et al discovered that cognitive-motor intervention is effective for gait and balance [24].These dual task training has improved the motor skills as well as cognitive skills in the subjects. Giselle M Petzinger et al mentioned that interventions in individuals with Parkinson's disease incorporate goal-based motor skill training to engage cognitive circuitry is important in motor learning[25].Some factors that limited the findings may be due to the environmental distractions that hampered the patient's concentration. Since the patient had to perform dual tasks (walking and performing the activities) at the same time, the interlimb coordination may not have synchronized. The above-mentioned studies have clear evidence of dual task interference, fear of falling, or risk of falling, and





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sometimes the drugs administered were slowing down the activities and the hallucinations for some subjects were the obstacles to moving forward. It is therefore evidenced that Dual task training has a significant effect on motor and cognitive function, in subjects with Parkinson's disease.

CONCLUSION

The objective of the study was to determine the Effect of Dual Task Training on Motor and Cognitive Function in subjects with Parkinson's disease. The results of the study showed statistically significant improvement in subjects from pre to post-test measurements within the Unified Parkinson Disease Rating Scale (I-IV components). Thus, the study accepts the alternative hypothesis and rejects the null hypothesis concluding that "There is a significant effect of Dual Task Training on Motor and Cognitive function in subjects with Parkinson's disease." Thus, it can be stated that the application of Dual Task Training on Motor and Cognitive Function in Parkinson's Disease subjects can be used clinically for better improvement in managing Parkinson's disease. As it is a simple, time-saving, cost-effective method that can be done at home under the supervision of a caretaker.

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Hybrid Power Quality Enhancement Techniques for Grid Synchronized Renewable DG Systems

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ABSTRACT

Globally, electric power networks are gradually transitioning away from conventional fossil fuel-fired generating units and toward green renewable energy sources. Environmental and economic issues are driving this transition. Additionally, current power systems are becoming increasingly overloaded on a daily basis as a result of population growth, resulting in the overloading of transformers, transmission, and distribution lines. Despite the overwhelming benefits of renewable energy sources, there are a few significant drawbacks. Power quality is a critical element of renewable DG systems since modern loads are more susceptible to PQ disruptions and renewable energy and nonlinear loads are increasing their penetration into distribution power networks. As a result of the continuing reformation of traditional distribution networks through the incorporation of renewable energy, the necessity for innovative power quality improvement (PQI) solutions becomes unavoidable. This study emphasises theoretical representation of the critical power quality difficulties associated with renewable energy grid integration, and it includes a comprehensive survey of all PQI solutions introduced to date, as well as potential for future research. Additionally, all critical power quality issues, including the influence of high renewable energy penetration and mitigation measures on power quality, are presented using a grid-integrated PV-based DG system simulation in MATLAB/Simulink. This article is intended to assist academics and industry professionals in gaining a better understanding of current PQ issues, PQI methodologies, and future research directions for renewable energy technology.



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Keywords: Renewable energy, power quality, harmonics mitigation, Distributed generation (DG), AI techniques

INTRODUCTION

Renewable energy sources (RESs) have seen a substantial increase in popularity in recent years, owing to rising energy consumption and increased awareness about environmental conservation. It goes without saying that RES is a pollution-free, nontoxic, adaptable, and sustainable source of energy, and its penetration is increasing at a breakneck pace throughout the world [1] –[4]. Solar thermal, photovoltaic, hydro, wind, geothermal, and biomass were all examined in this review. However, the benefits outlined above come with specific power quality difficulties when utilizing electricity from RESs. Power quality is a vital factor in the reliability of smart distribution grids, and as such, it is unavoidable to pay attention to it. Utilizing the energy generated by RESs necessitates the integration of generating units with distribution network grids. Stable operation of grid-connected renewable distributed generation systems is a difficult task due to the associated PQ challenges posed by environmental intermittency and generation technological differences compared to fossil fuel-based distributed generation systems that provide a constant source of energy. Only the seamless integration and steady functioning of such integrated DG systems will enable the realization of future smart grids. The widespread integration of power electronic inverters used to connect DG units to the grid over the last few years has created significant challenges for distribution power networks, most notably harmonic distortion and difficulties achieving frequency stability due to the decrease in overall inertia.

The power electronic (PE)–based generation technologies rather than the traditional synchronous generators one and the intermittent nature of RESs are primarily liable for PQ issues with renewable DG systems. The foremost concern is fluctuations in voltage and frequency, which originate due to no controllable inconsistency in renewable energy. The inconsistent behavior of renewable energy because of frequently varying weather characteristics results in voltage and frequency fluctuations at the grid of integration. The study showed that intermittent solar irradiation resulting from cloud motion causes voltage, frequency, and power fluctuations. The purpose of this work is to provide a detailed analysis of the power quality difficulties related with the integration of renewable distributed generation systems, as well as a systematic review of the available literature on associated mitigating measures. To begin, all of the PQ difficulties confronting renewable DGs are thoroughly reviewed. Second, each type of mitigation approaches is explained in detail, including their operation, advantages, and disadvantages, as well as the present state of research and future research potential. Additionally, all critical power quality issues, the impact of high renewable energy penetration, and the impact of specific mitigation methods are presented using a grid integrated PV-based DG system simulation in MATLAB/ Simulink. Indices for five critical power quality issues are calculated for three bus types: grid bus, load bus, and DG bus. The magnitudes of the indices indicate the state of power quality at each bus category under various operating conditions, including intact system, high penetration, VQI techniques, CQI techniques, and a combination of VQI and CQI.

POWER QUALITY CHALLENGES AND MITIGATION TECHNIQUES

While grid integration of DG systems is necessary for more effective use of renewable energy sources, it introduces various difficulties such as voltage, frequency fluctuation, and harmonic distortion. Voltage, frequency, voltage flicker, and current harmonic distortion are four critical characteristics, and their indices should be utilised to evaluate the PQ performance of photovoltaic DGs, according to the IEEE Standard 929-2000 specification. The electricity generated by renewable energy sources such as wind or solar energy oscillates mostly due to changes in climatic conditions, which can result in excessive voltage and frequency fluctuations on the grid. Numerous consumer appliances are susceptible to such variations, which may manifest as changes in the peak and RMS voltages on the distribution line.



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Numerous PQI studies have been conducted with an emphasis on mitigating PQ difficulties and promoting seamless grid integration of renewable energies; nevertheless, each mitigation strategy has certain limitations; hence, it will continue to be an important topic of research in the future as well. PQI techniques stressing renewable energy integration can be broadly classified as voltage quality improvement (VQI) and current quality improvement (CQI), as seen in Figure 5. VQI approaches are primarily concerned with mitigating voltage and frequency fluctuations in DGs, whereas CQI techniques are concerned with compensating for current harmonics generated by DG systems or load bus and grid bus, respectively. VQI techniques can be further classified as custom power devices (CPDs), energy storage (ES) methods, energy conversion optimization (ECO), spinning reserve (SR), and a few other specialised techniques that utilise the variable frequency transformer (VFT) and virtual synchronous machine (VSM) concepts. CQI approaches can be further characterised as passive filters (PFs), shunt and series active power filters (APFs), hybrid filters, smart impedance, and multifunctional digital gate arrays (DGs). The capabilities of each technique kind, as well as its operation, benefit, drawback, and present status, as well as the future scope of study, are painstakingly reviewed in succeeding sections.

VOLTAGE QUALITY IMPROVEMENT TECHNIQUES

The development and implementation of feasible and affordable voltage quality improvement strategies capable of mitigating grid contingencies, reactive power, and voltage fluctuations, as well as load demand fluctuations, have garnered considerable attention in recent years. Voltage and frequency regulation are also performed manually, based on routine operating procedures and experience in diverse distribution power networks throughout the world. However, industrialized economies, in particular, have introduced hierarchical voltage and frequency management mechanisms at the distribution power level. All of the strategies for mitigating PQ concerns are explained in detail below.

Custom power devices

The integration of renewable energy and traditional distribution networks has affected power utilities' policies about network control, dependability, management, PQ, and protection. A high PQ is critical for the reliable distribution of electricity to loads. CPDs are critical in enhancing PQ in both traditional (without renewable energy) and modern (with renewable energy) distribution networks.

Energy storage technologies

Energy storage technology benefit both consumers and suppliers. Consumers can save money by storing energy purchased during off-peak hours and using it during high-priced peak hours. During grid outages, suppliers can boost the reliability of their power distribution. Electrical energy storage (EES) systems can be utilised to increase the PQ of DG systems, as well as the stability of the power support in DG systems, and hence the PQ. Figure 7A illustrates the role of EES in on-grid renewable DG systems. As illustrated in Figure 7B, energy storage devices can be classified into five categories based on the energy forms they store. Electric power companies are required to provide electricity to their customers within allowed voltage and frequency restrictions. Simultaneously, due to the unpredictable nature of climate conditions, it is unavoidable to balance the surplus and shortage in renewable energy. This fluctuation in the output of the RES hampers system voltage and frequency management, which ESSs are capable of performing well.

CURRENT QUALITY IMPROVEMENT TECHNIQUES

Due to the widespread penetration of renewable energy sources in current power grids, the harmonics associated with interfacing power inverters, as well as their problematic interaction with the grid, necessitate significant research in this sector. While the properties of harmonics generated by the majority of nonlinear loads have grown quite obvious over the last two decades of research, the characteristics of harmonics generated by renewable DG systems remain hazy, and mitigation strategies are still in their infancy. The next sections cover each stream of mitigating approaches in detail.





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SIMULATION AND DEMONSTRATION OF PQ CHALLENGES AND MITIGATION TECHNIQUES

A test distribution system with an integrated PV-based DG system and a mix of load types is studied to demonstrate the power quality difficulties and mitigation approaches. The single line diagram (SLD) of the test distribution system is depicted in Figure 11. The sole modification to the regularly used conventional test distribution networks is the integration of a PV-based DG system at the PCC and the addition of a nonlinear load to the load bus in addition to a linear load. A transformer and feeder are used to integrate a 100 KW three phase DG unit at PCC. For simulation purposes, a three phase induction motor drive rated at 200 HP is configured as a nonlinear load, while a three phase unbalanced RL load is configured as a linear load.

CONCLUSION AND FUTURE RESEARCH DIRECTIONS

The study discusses the power quality issues associated with grid integration of renewable distributed generation systems in detail. The challenges relating to power quality are categorised into two major categories (voltage quality and current quality), and a full assessment of associated improvement strategies is done. All available PQI techniques for grid-connected renewable energy systems are covered in detail and in equal measure, including their operation, advantages and disadvantages, and current area of research. Additionally, all PQ concerns have been clarified through the use of a test distribution system simulation, and the importance of implementing PQI approaches has been demonstrated. Without a doubt, it will be more cost effective to power integrated DG systems with self-protection capabilities such as voltage and frequency stabilisation (VFI) in DGs via the VSM method and current harmonic mitigation (CFI) in DGs via advanced interfacing inverter controlling techniques such as CCM, VCM, and HCM, rather than using separate CPDs or filters for PQI objectives. Additionally, better control strategies for grid-interfacing inverters are required to improve DGs' ability to ride through grid and load side disturbances, hence minimising the requirement for supplementary CPDs. Thus, if the aforementioned points are taken into account in future study, they will undoubtedly enhance the hassle-free operation of renewable DG systems and aid world economies in meeting their renewable energy targets.

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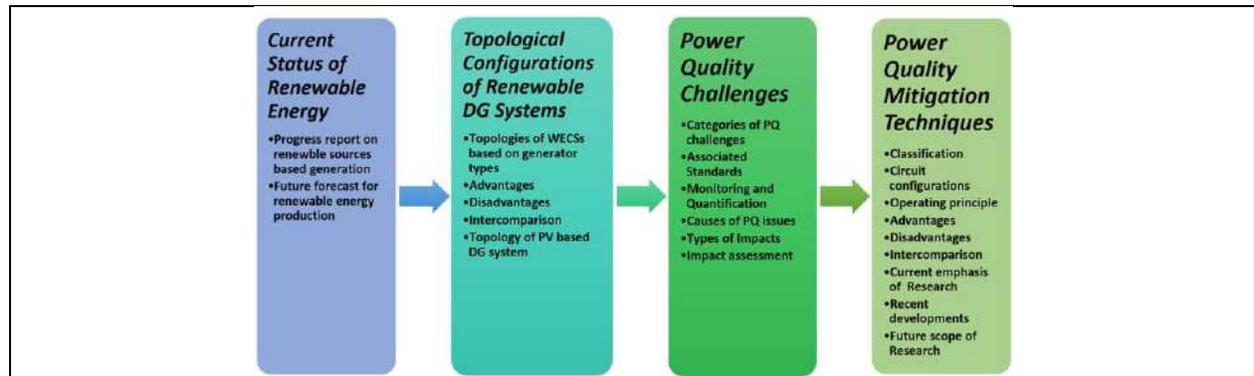
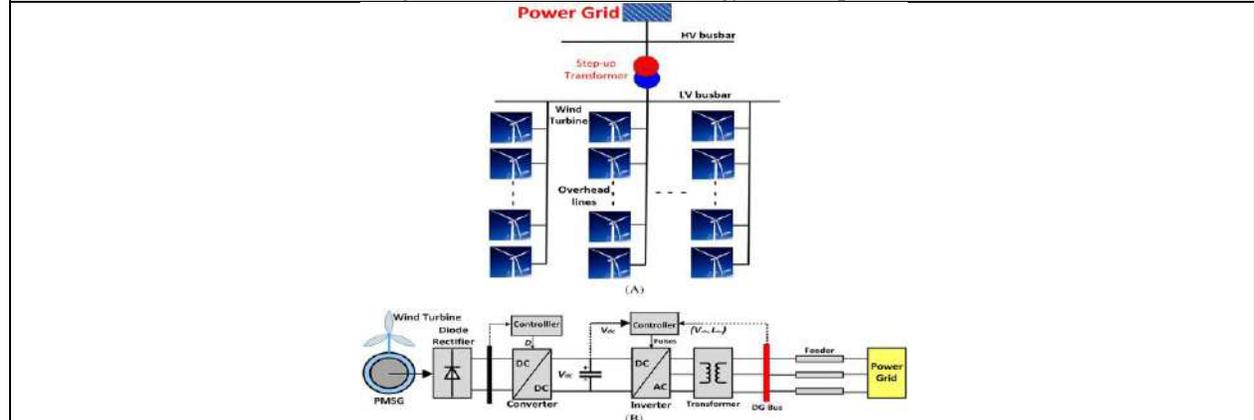


Figure 1 The review methodology used in present work





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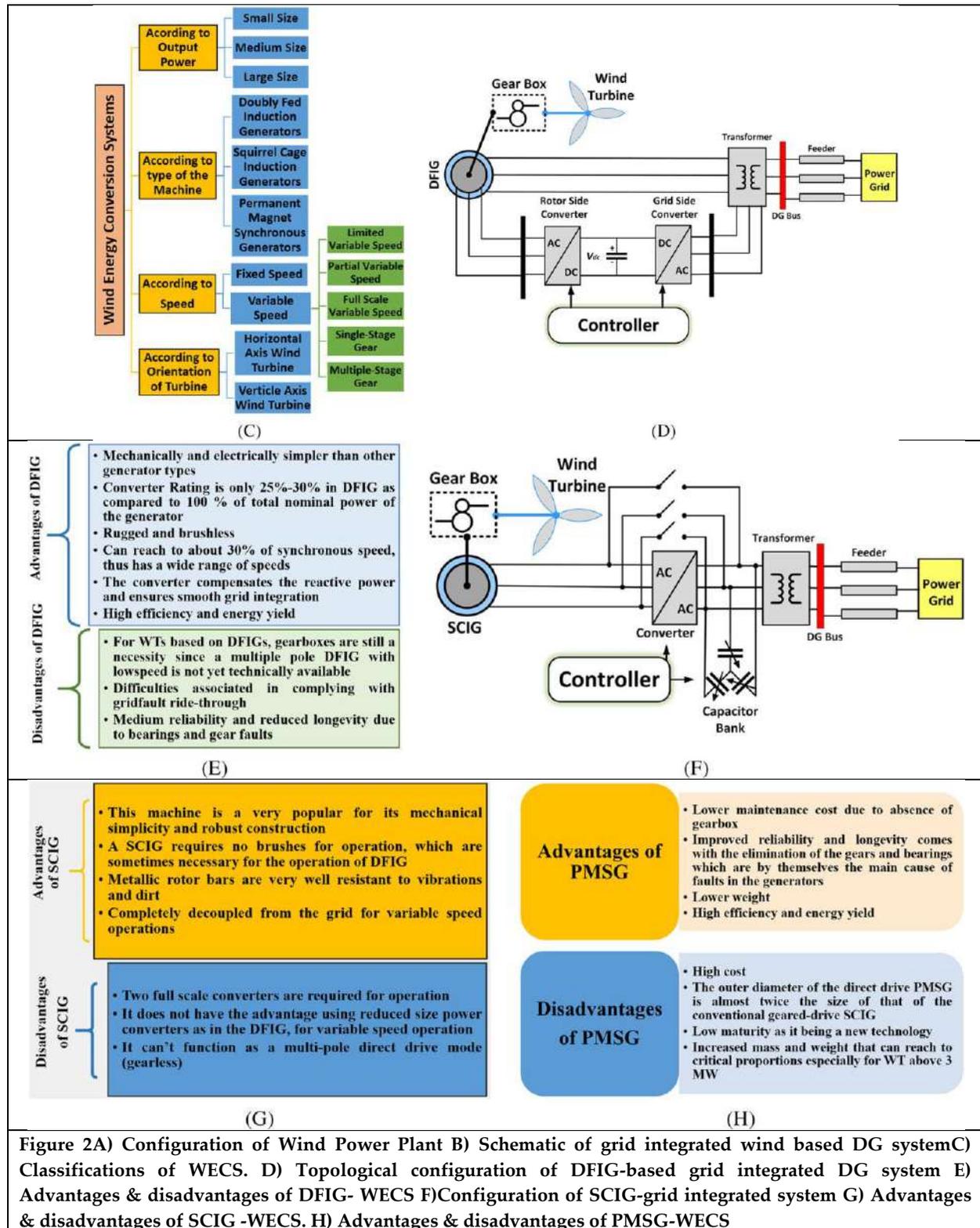


Figure 2A) Configuration of Wind Power Plant B) Schematic of grid integrated wind based DG system C) Classifications of WECS. D) Topological configuration of DFIG-based grid integrated DG system E) Advantages & disadvantages of DFIG- WECS F) Configuration of SCIG-grid integrated system G) Advantages & disadvantages of SCIG -WECS. H) Advantages & disadvantages of PMSG-WECS





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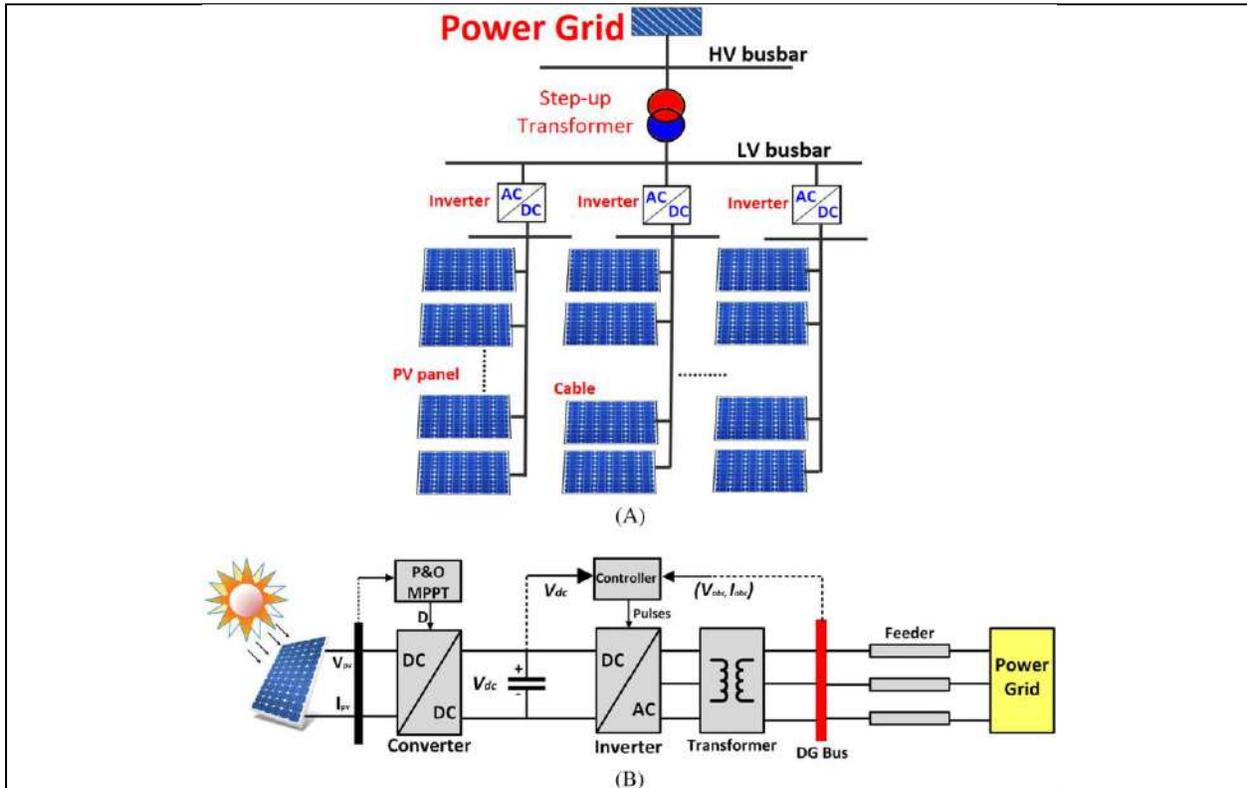


Figure 3 A) Topological configuration of PV power station B) Schematic of grid integrated PV-DG system

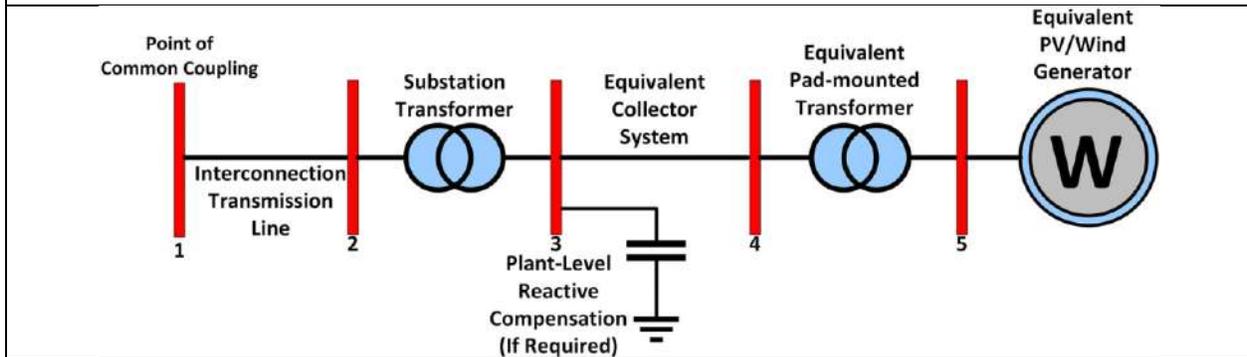


Figure 4 Representation of renewable DG systems in bulk system studies





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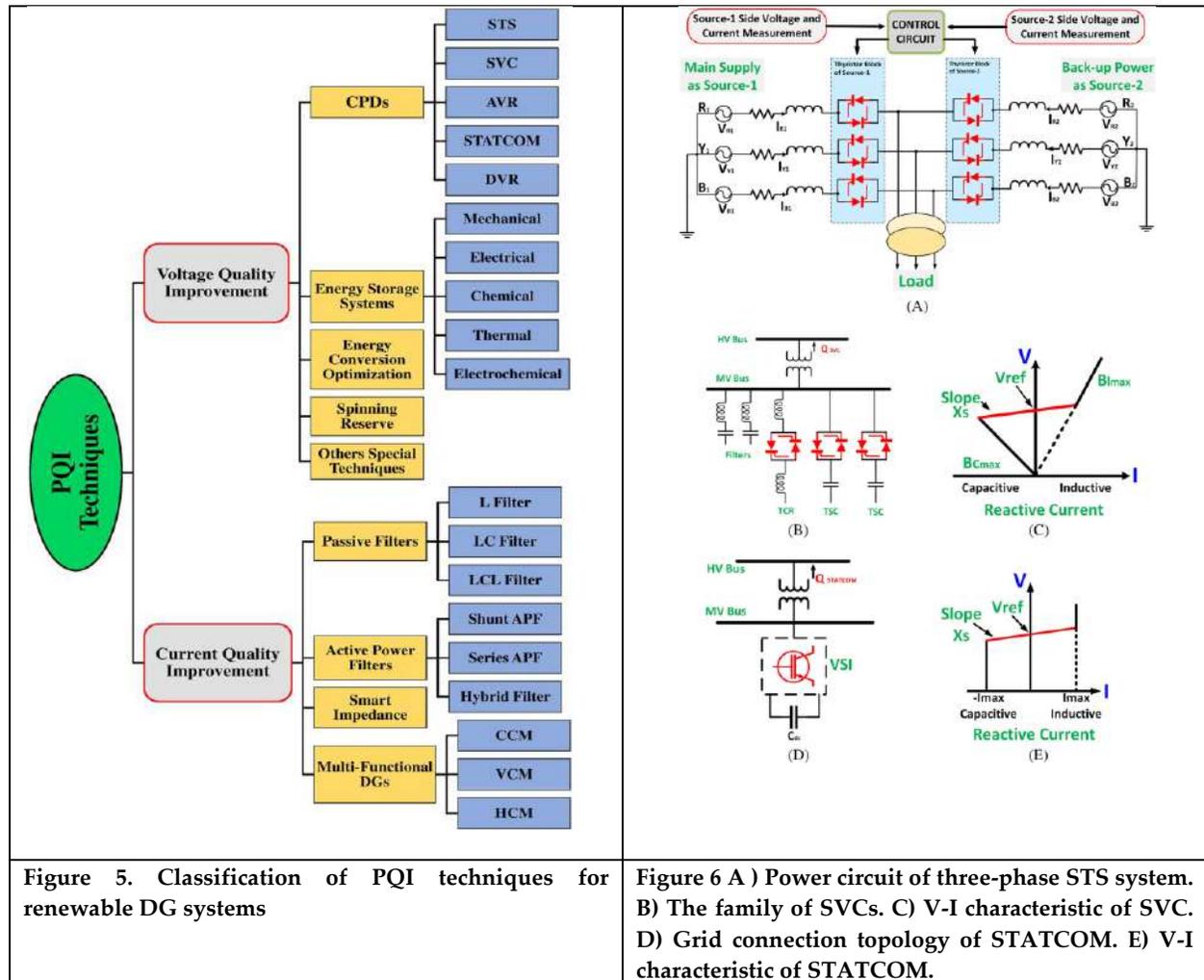


Figure 5. Classification of PQI techniques for renewable DG systems

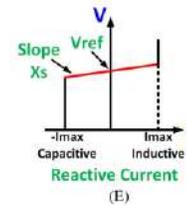
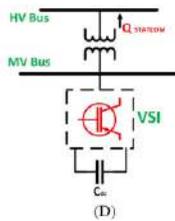
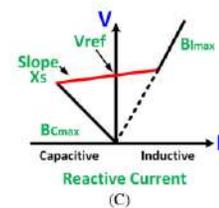
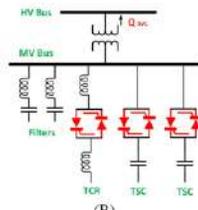
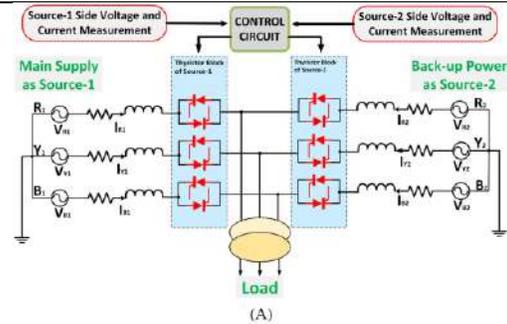


Figure 6 A) Power circuit of three-phase STS system. B) The family of SVCs. C) V-I characteristic of SVC. D) Grid connection topology of STATCOM. E) V-I characteristic of STATCOM.





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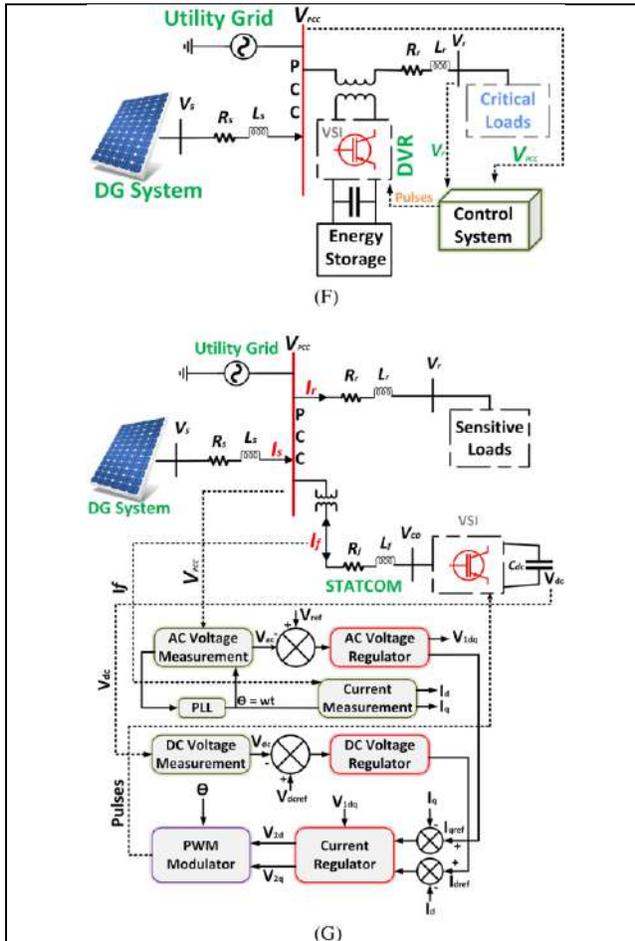


Figure 6 F) Distribution system configuration with DG and STATCOM. G) Topological configuration of DVR with DG system

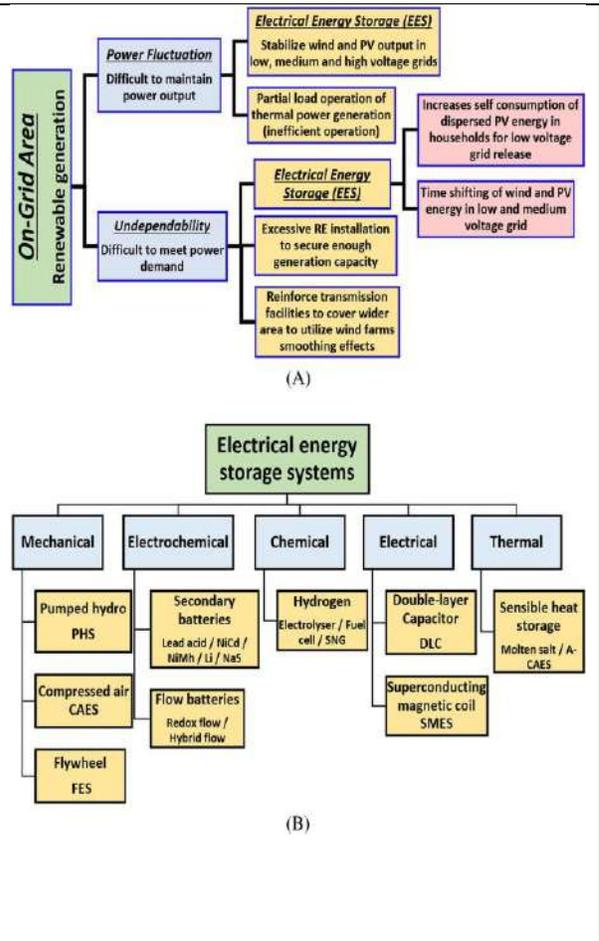


Figure 7 A) Role of EES for on-grid renewable DG systems. B) Categorization of EES systems

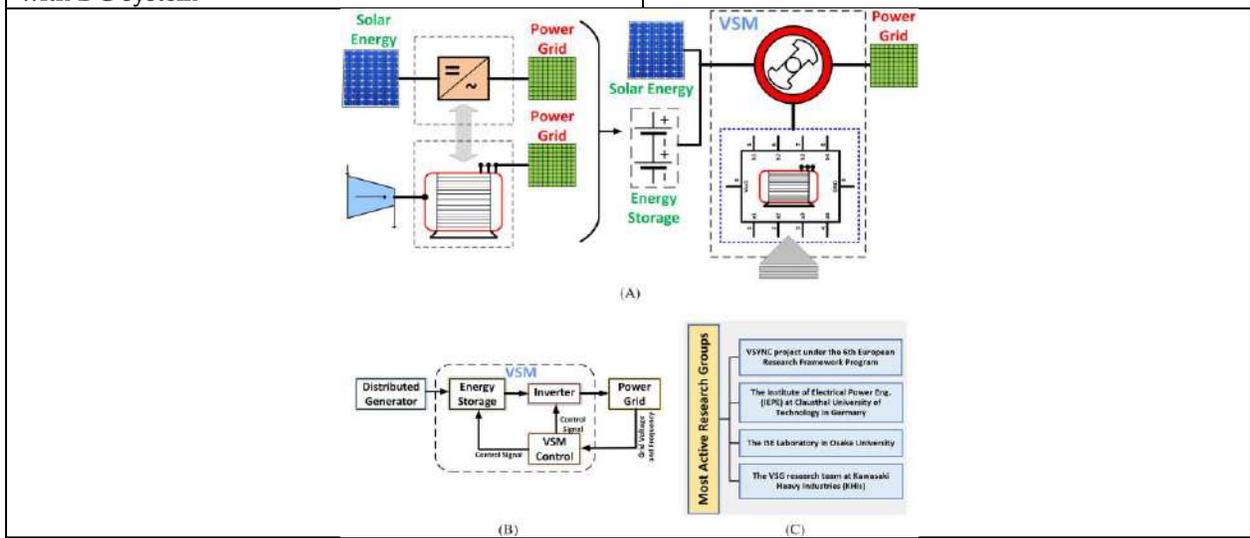


Figure 8 A) Fundamental concept of the VSM. B) Block diagram for a VSM C) List of research groups in VSM





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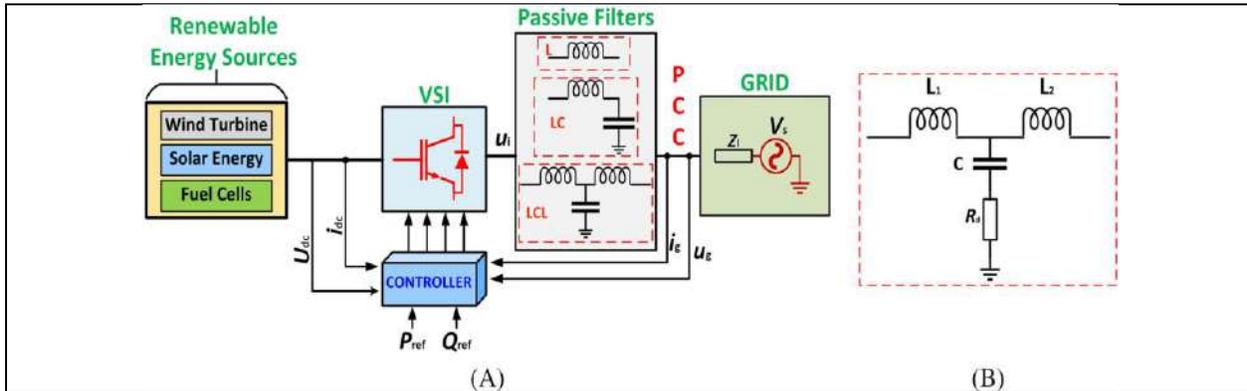


Figure 9A) Renewable DG connected to grid through passive filters B) LCL filter with a damping resistor Rd

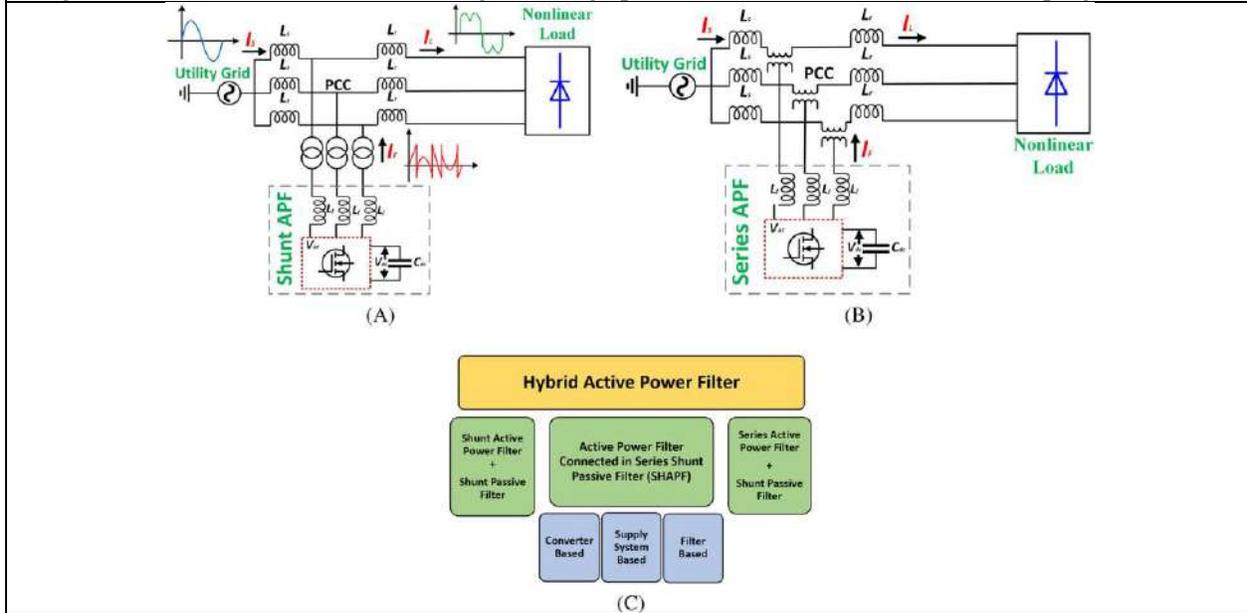


Figure 10A) Shunt active power filter. B) Series active power filter. C) Categories of hybrid APFs

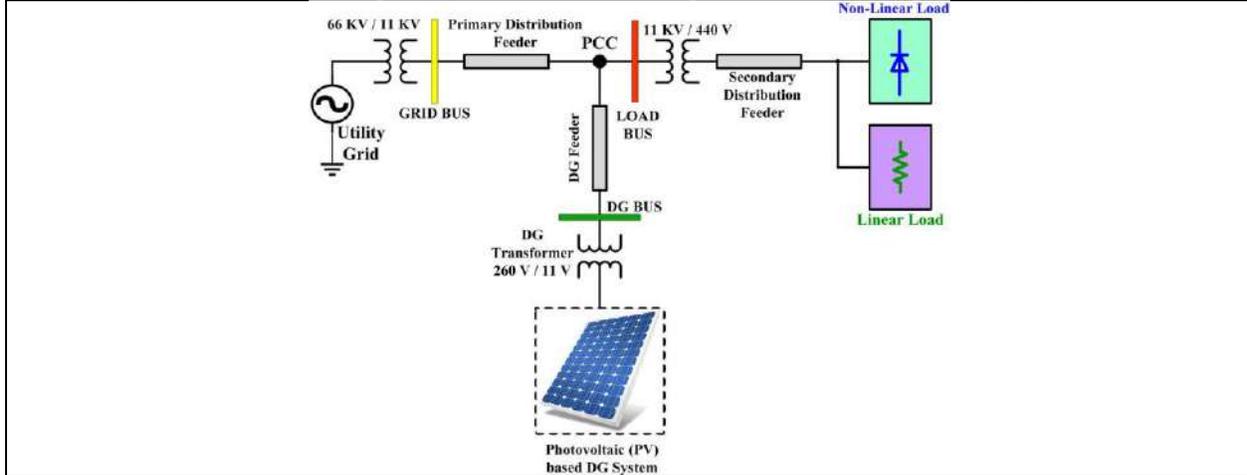


Figure 11 SLD of test distribution system





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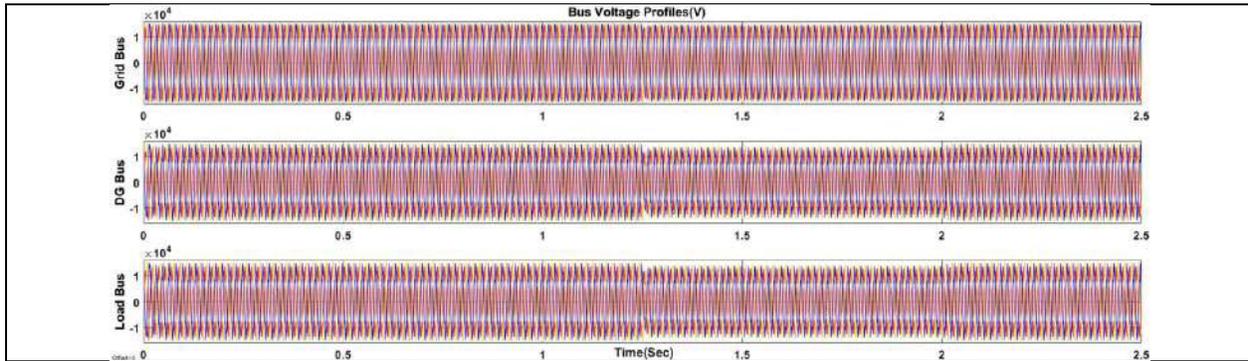


Figure 12 Bus voltage profiles

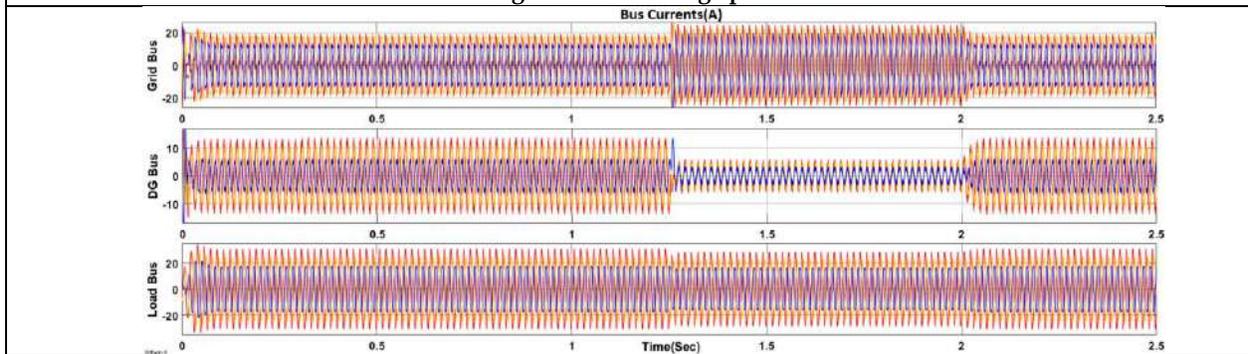
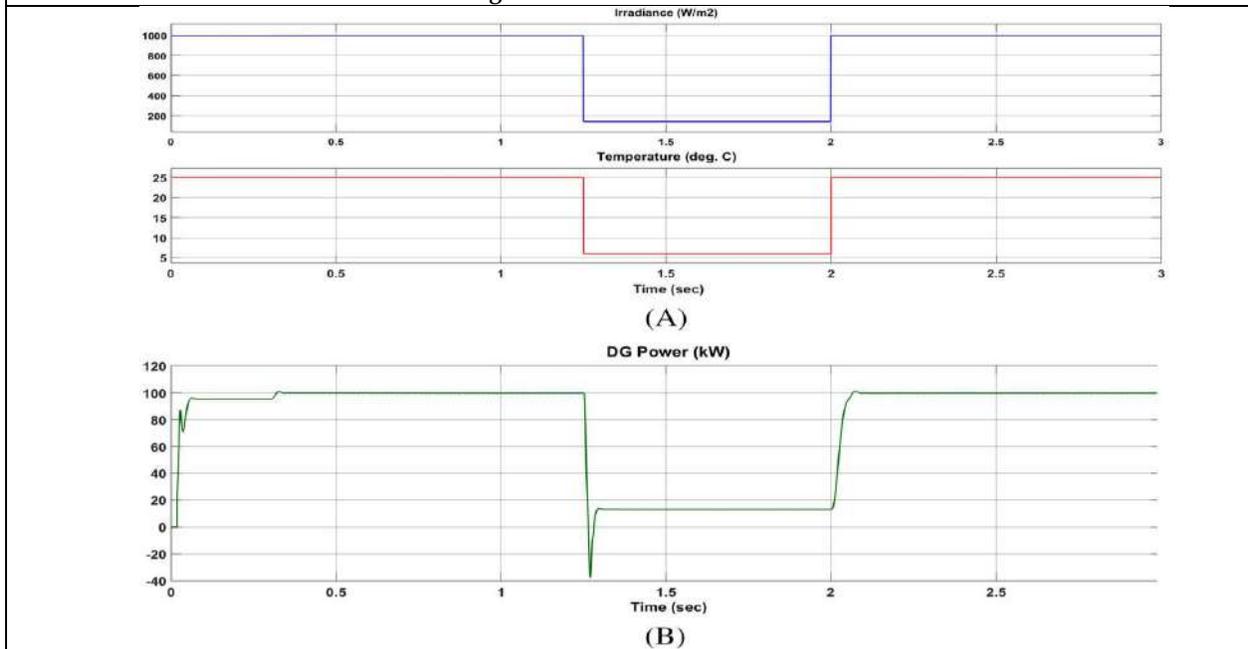


Figure 13 Bus currents waveforms





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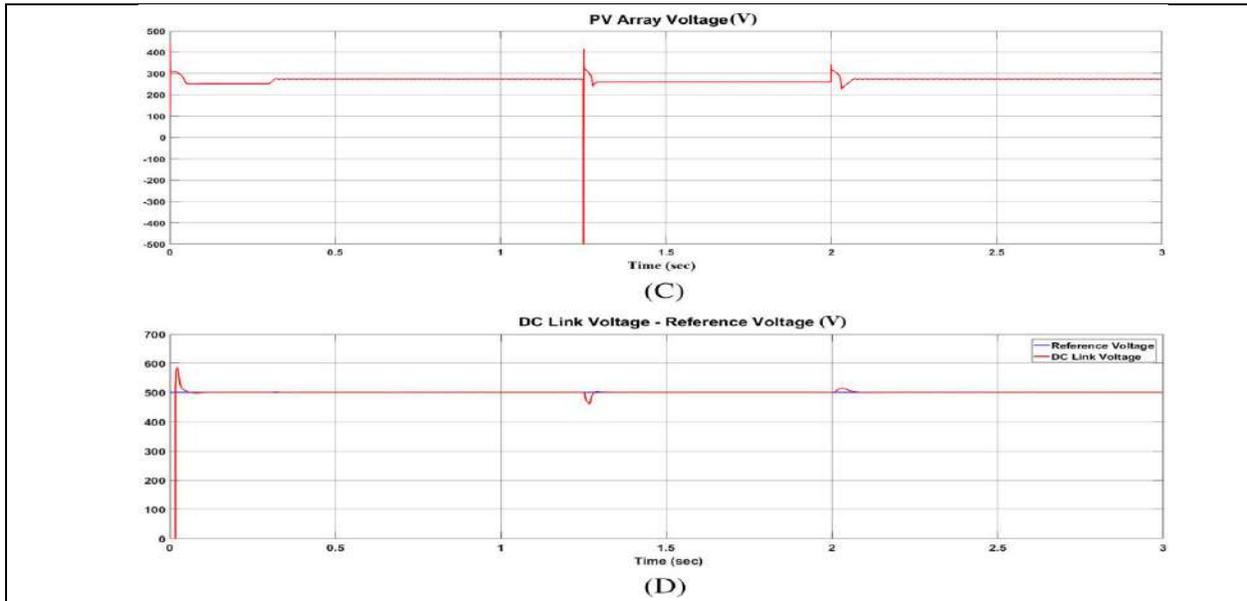


Figure 15 A)-D) Waveforms corresponding to renewable DG system

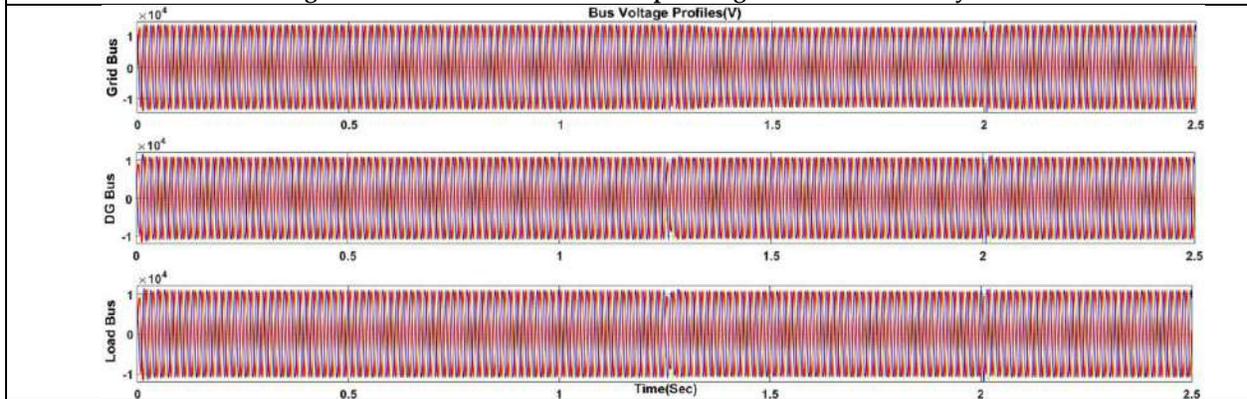


Figure 16 Bus voltage profiles after application of both PQI techniques

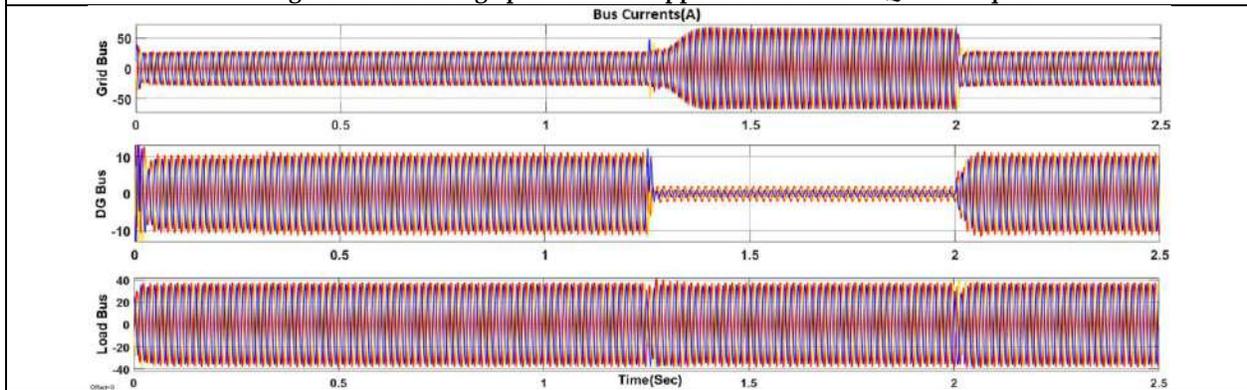


Figure 17 Bus currents waveforms after application of both PQI techniques





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Figure 18. Bus frequency waveforms after application of both PQI techniques





To Assess Nerve Conduction Velocity Declination Rate of Ulnar Nerve According to Age in Healthy Population

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ABSTRACT

The aging process (normal aging) represents a global biological change that occurs with age and is not affected by diseases that have a negative impact on the clinic. Studies have shown that aging affects the anatomy and structural features and function of the peripheral nervous system. There is significant decrease of myelinated and unmyelinated nerve fibers. There is a strong documented effect on the function and electrophysiological properties of peripheral nervous System. Which includes a reduction of nerve conduction velocity, muscle strength discrimination, autonomic responses and endoneurial blood flow. Declinations of conduction velocities may be begin after 30-40 years of age but the values normally change by less than 10m/s at 6th or even in 8th decade to evaluate the nerve conduction velocity of Ulnar Nerve 2) to find the declination rate of Ulnar nerve according to age. 120 healthy subjects having the ages of 31-70 were included in this study, divided into four age groups. Subjects suffer from metabolic, traumatic or nervous disorders affecting the nerves were excluded. Ulnar Nerve conduction Velocity was evaluated. Significant statistical decrease was found in the nerve conduction velocity of the Ulnar nerve with increasing age, after analyzing the data with SPSS version twenty. Declination rate of Ulnar Nerve (MNC) is 1.62 m/s per decade and Ulnar Nerve (SNC) is 1.91 m/s per decade. Significant decrease in the Motor and Sensory conduction velocity of the Ulnar nerves as age increases.

Keywords: Aging, Ulnar Nerve, NCV,SNC,MNC





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INTRODUCTION

Ageing is a process that is often accompanied by physiological changes. The physiological changes include slowing in muscle contractibility, alteration in muscle metabolism and neuromuscular junction as well reduction in nerve conduction velocity (NCV) [1]. There is loss of both myelinated and unmyelinated nerve fibers due to aging. There is potentially a well documented effect on the function and electrophysiological properties of peripheral nerves. These include a decline in nerve conduction velocity, muscle strength discrimination, autonomic responses and endoneurial blood flow [2]. The peripheral nervous system as well as the central nervous system is implicated in many age dependent disorders. Functional deficits may be consequences of structural and bio chemical changes that result in a slowly progressive loss of neuron and nerve fibers. This loss is not compensated because of the decreased regenerative and innervating capabilities of nerve fibers in aged subject [3]. Functional, structural and biochemical changes have been reported in peripheral nerves of aged subjects. Electrophysiological studies found lower nerve conduction velocity values in older subjects than in younger subjects. However, there are some discrepancies among reports regarding the rate of decline. Some studies showed a linear decline in nerve conduction velocities with age.³ With aging, peripheral nerves demonstrate a progressive reduction in myelinated fibers, most notably in the largest diameter fibers. Ventral roots appear to be more affected than dorsal roots and lumbosacral segments more than cervical segments. While the epineurial and perineurial sheath thicken, the endoneurial sheath demonstrates fibrosis due to an increased presence of collagen. The microcirculation of peripheral nerves is particularly vulnerable to aging, and atherosclerosis has been suggested as a mechanism. Accordingly, there appears to be direct relation between dysfunction of the vasa nervosum and the loss of myelinated fibers [4].

Aging significantly affects some morphological and functional characteristics of the peripheral nervous system. Motor and sensory axons, which have the highest conductivity (they innervate type II muscle fibers), disappear the fastest with age. Conduction velocities decrease at an average rate of 0.5–4 m/s per decade [5]. Past the age of 60, motor and sensory summation potential amplitudes decline significantly with age. Conversely, motor unit action potential durations increase with aging. This is probably due to re-innervation after motor axon loss. Beginning in the third decade of life, approximately 1 % of motor units are lost per year. Their loss is accelerated even more at ages over 60 to 66. In normal aging, some myelin sheaths degenerate as a consequence of their axon degenerating, but in other cases myelin sheaths degenerate even though the axon is intact. In the latter category there are two kinds of myelin sheath alterations. The most common age-related degenerative alteration is an accumulation of dark cytoplasm in pocket that is produced by splitting of the major dense line. Another but less common myelin alteration associated with aging is the formation of myelin balloons [5]. As humans ages, the amplitude of sensory-evoked response wane and the response latency increases. Changes in the latency of transmission through the sensory system could contribute to age-related impairments in the temporal processing of sensory information. Studies in aged animals indicate that conduction velocity (i.e. the speed at which an action potential travels down the axon) decreases for neocortical, cerebellar and peripheral sensory and motor neurons. The cause of this reduced conduction velocity is unclear but it may result from alteration in the myelin sheath [6].

There is a decrease in the total number of the motor unit with age. The average motor neuron loss from the second to tenth decade is approximately 25%. The loss of motor unit neuron seems to be uniform within and between the segments. The decrease in motor unit number is accompanied by an increase in size or innervation ratio, such that on average each motor neuron innervates more muscle fibers in the older adult [4]. Electrophysiological studies have shown a reduced number of functioning motor unit with increasing age, mainly after 60 years in both proximal and distal muscles [4]. Nerve conduction studies have been performed in animals since 1850. Techniques for the examination of motor nerve function in man has been established since 1948 and for sensory nerve function since 1956 and later with improved techniques [7]. Hodes et al first developed the technique for calculating the conduction velocity of the ulnar nerve in 1948. Down and Scott further refined the procedure after a year [8].

A number of physiological and technical variables can influence the result of nerve conduction studies. Many factors have been found to influence nerve conduction velocity results. They include biological factors such as age, height



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and gender as well as physical factors such as body temperature and particular study technique being used [9]. Neural conduction studies can be affected by intraneural temperature. Nerve impulse conduction is faster at a higher body temperature as is seen after physical activity. The conduction velocity increases almost linearly by 2.4 m/sec or approximately 5 percent per degree [10, 11]. Both motor and sensory fibers conduct substantially more slowly in the leg than arms. Longer nerves generally conduct more slowly than shorter nerve [13,14,15] An inverse relation between height and nerve conduction velocity suggests that the longer nerve conduct slower than the shorter nerve [10,12]. The conduction velocities begin to decline after 30-40 years of age but the values normally change by less than 10m/s at 6th or even in 8th decade [10]. Median and ulnar nerves are two important nerves in the upper limb. They are responsible for the movements as well as sensation of the hand. The Current research has been designed to evaluate the Nerve conduction declination Rate in Ulnar nerve.

MATERIAL AND METHODS

This study was carried after the obtaining Institutional Ethical Clearance certificate form institute (PUIECHEER/PIMSR/00/081734/2610 Date: 23.07.2020). The study included 120 moderately healthy people aged 31–70 years (30 in Each Group). They are divided into four groups (Group 1: 31–40 Group 2: 41–50, Group: 51–60, Group: 61–70. Total Subjects were excluded if they were bothered with the report of history of diabetes or any metabolic disorders, neurological disorders, peripheral neuropathy, cognitive impairment, recent surgery. Those who smoked and consumed alcohol in the past 24 hours were not included [3,4,5,6].

Before obtaining the data collection informed consent were taken from the all the subjects. Items that met the inclusion criteria were selected and informed of the study protocol. All subjects were considered for demographic data on age, height, weight, gender, dominance, and occupation before the procedure. The nerve conduction velocity test was performed only on the dominant limb only. The test protocol was explained to all the subjects. Height (cm) and weight (kg) were recorded using a stature meter and weight machine. Skin temperature (°c) was recorded by an infrared thermometer. Room Temperature was maintained 21° to 23°C [10]. Skin temperature (°C) had been recorded through a digital thermometer and Limb's Skin Temperature was maintaining to 34°C. Limb was warmed by infrared lamp if skin Temperature was less than 34°C [10]. According to the protocol, the body parts were cleaned with spirit before the Nerve Conduction Velocity test. Ground electrode, recording electrodes and stimulating electrodes were used to record the sensory nerve conduction velocity. The conduction distance is measured using flexible measuring tape.

Neural conduction velocity (m/s) was measured.

Using the following formula.

Nerve conduction velocity (m/sec) had been measured by using following formulae

Motor nerve conduction velocity (MNCV) = $D/PL-DL$

Sensory nerve conduction velocity (SNCV) = D/L

Where: - D= distance, PL= proximal latency in ms, DL= distal latency in ms and L= latency in ms

Ulnar Motor Nerve Conduction

The Individual were positioned supine lying with arm abduction with 90 degree elbow flexion & forearm in supination, the palm facing upward with wrist slight Extension during stimulation. Recording had been done from abductor digiti minimi and reference electrode had placed just distal to it. Supramaximal stimulation had been given at wrist, at elbow joint. The conduction distance between the stimulated points had measured by a measuring tape.



**Gaurav Jagjivanbhai Patel and Rameshchandra Chavda****Ulnar Sensory Nerve Conduction**

The sensory conduction of the Ulnar nerve can be recorded by both orthodromic and antidromic stimulation. In Current research the sensory conduction of the Ulnar nerve had been recorded by antidromic stimulation. The Individual position is the same as for the Ulnar motor nerve conduction. Ring electrode were placed on fifth digit. The stimulator had placed 3cm proximal to distal crease at the wrist. The conduction distance between the recording and stimulating electrode has been measured by using measuring tape. Peak to Peak amplitude and Onset Latency were considered for both MNCV and SNCV. Data were collected. The data was recorded with the use of RMS EMG.EP MARK II (Recorder and Medicare System). Data was analyzed on statistical software SPSS.

RESULT

Declination rate of Ulnar Nerve (MNC) is 1.62 m/s per decade and Ulnar Nerve (SNC) is 1.91 m/s per decade. The results of this study showed that with increasing age, the Motor and sensory nerve conduction velocity of the Ulnar nerve ($P < 0.001$) gradually decreased.

DISCUSSION

Nerve conduction study is an important method used in clinical practices as a diagnostic tool and has been thoroughly validated [13]. There are many published studies and reviews on nerve conduction. These include the factors that affect nerve velocities. These factors can be divided into biological factors (age, height, gender) and physical factors (body temperature, study technique) which are related to the physical state of the nerve and muscle [13,14,15]. The focus of this study was on the effect of age on nerve conduction velocity. The result obtained from data analysis proved that there is significant declination of Ulnar nerve Motor Nerve Conduction Velocity & Ulnar Nerve Sensory Nerve Conduction Velocity.

NCV is an age dependent variable and there are a number of studies showing the NCV values according to the different age groups. It was observed, that most of the studies are derived from Caucasians subjects, however in this study an attempt is made to study the influence of age on nerve conduction velocities of ulnar in healthy participant of Indian Population [1]. The result of this study showed that as the age advances there is a progressively decrease in the sensory nerve conduction velocity of ulnar ($p < 0.001$) as well as in the motor nerve conduction velocity of ulnar nerves ($p < 0.001$).

These results correlate with the studies done by S. Saeed (2008), Mohamed Saufi Awang (2007)¹, Mohamed Saufi Awang (2006) [13], Henry C.Tong, (2004) [16], Frank J.E. Falco et al (1994) [17].

The reduction in the average conduction velocity in older subjects can be explained by following factors

- i. Local ischemia due to vascular changes.
- ii. Metabolic depression associated with changes in permeability or trans-membrane transfer mechanisms of nerve fibres.
- iii. Selective degeneration of fastest conducting fibres [18].
- iv. Decrease in axon diameter than of fibre diameter.
- v. Tapering of the axon size.
- vi. Morphological changes such as loss of myelinated nerve fibre, decrease in size and myelin of the remaining myelinated fibers [19].

There is strong evidence that age and myelin integrity reduces conduction velocity along nerve fibers and the age related alteration in structure of myelin conduction velocity [20]. In human peripheral nerves, it has been proved that with an increase in the age there is an increase in connective tissue and a reduction in the patency of the blood vessels. This begins in the fourth decade of life as an endothelial proliferation and hyalinization of the vessels with



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an increase in the endoperineurium invading and apparently replacing areas of the nerve bundles by connective tissue elements. This gradual reduction in blood supply and increase in connective tissue seems to be a gradual alteration and reduction of the nerve fibers, especially the larger ones [21]. The study shows the Ulnar Nerve Motor Nerve Conduction velocity declination rate in Group 1 to Group 2 is 0.81 m/s per Decade. Group 1 to Group 3 is 1.40 m/s per Decade & Group 1 to Group 4 is 1.63 m/s per decade.

Similarly we have also observed that Ulnar Nerve Motor Nerve Conduction velocity declination rate in Group 2 to Group 3 is 1.29 m/s per Decade. Group 2 to Group 3 is 1.62 m/s per Decade and Group 3 to Group 4 is 1.15 m/s per Decade. The Nerve declination has been observe more in age group 60-70 years in compare to other age groups. This is also statistically significant. And show declination after age 50 years . Konishi studied the effect of age on motor conduction velocities of both the median and ulnar nerves. A comparison was made between two age groups: a young group defined as subjects in their third decade and an older group consisting of subjects in their ninth decade of life. The study reported a significant reduction in motor conduction velocities in the older group. In addition, Ganeriwat et al., who studied seventy-five healthy male subjects divided into different age groups, also reported that they observed a slowing in motor conduction velocities in the older age groups [1]. Diana et al., observed that for the upper extremity conduction velocities shown, there was about a 1 m/s decrease in conduction velocity per decade increase in age group of 60-70 years [14]. The study showed maximum decline ulnar motor nerve conduction at the rate of 0.3385 m/s/yr. This finding may be useful to confirm the diagnosis of peripheral neurological diseases or any entrapment neuropathy with clinical correlation. The study shows the Ulnar Nerve Sensory Nerve Conduction velocity declination rate in Group 1 to Group 2 is 0.89 m/s per Decade. Group 1 to Group 3 is 1.52 m/s per Decade & Group 1 to Group 4 is 1.91 m/s per decade. Similarly we have also observed that Ulnar Nerve Sensory Nerve Conduction velocity declination rate in Group 2 to Group 3 is 1.39 m/s per Decade. Group 2 to Group 3 is 1.96 m/s per Decade and Group 3 to Group 4 is 1.54 m/s per Decade. The Nerve declination has been observe that after age of 50 years the Sensory conduction velocity has been show more declination and increase according to age. This is also statistically significant.

Tong et al. found in their prospective cohort study that median sensory velocities decrease at a rate of 0.14 m/s per year of age. Stetson et al. estimated a reduction in sensory NCV of 0.13 m/s per year, and a study by Letz and Gerr reported a decrease of 0.13 m/s per year. These last two studies were cross-sectional studies. However, our study also show significant reduction in ulnar sensory velocities across different age groups [1]. Diana S. Stetson⁵⁶ reported the decrease in ulnar SNC velocity by 0.099m/s per year of age and Letz R. Gerrf⁶³ reported decrease in ulnar SNC velocities by 0.16m/s per year of age. The study showed maximum decline ulnar sensory nerve conduction at the rate of 0.31 m/s/yr. It has been observed that environment factor like climate change [22] and geographic factor are also influencing the nerve Conduction Velocity declination rate [23]. Height has negative correlation with NCV. It has been also observed that there is height variation due to the geographical area. So Whenever there is comparison of any data this factor also has to be taken for consideration for Normative value for Nerve Conduction Velocity [13]. It has been also observed that subjects who are more active have been found to have a low impact of age in their Nerve conduction velocity. Even there is low declination rate of Upper Limb nerves in compare to the Lower Limb nerves [24]. In the current research this these factor have not taken for the consideration but in future they can be consider for the research.

CONCLUSION

As increasing age of adults, there is a significant decrease in the conduction velocity of the motor and sensory fibers of the ulnar nerve. This finding may be useful to confirm the diagnosis of peripheral neurological diseases or any entrapment neuropathy with clinical correlation.





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Tables of Analysis

DECLINATION RATE OF ULNAR NERVE ANALYSIS

GROUP 1 WITH OTHER GROUPS						
	MNC CV MEAN DIFFERENCE	SNC CV MEAN DIFFERENCE	ULNAR MNC CV PER DECADE	ULNAR SNC CV PER DECADE	ULNAR MNC CV PER YEAR	ULNAR SNC CV PER YEAR
G1-G2	1.621	1.780	0.810	0.890	0.081	0.089
G1-G3	4.204	4.567	1.401	1.522	0.140	0.152
G1-G4	6.502	7.660	1.625	1.915	0.163	0.192

GROUP 2 WITH OTHER GROUPS						
	MNC CV MEAN DIFFERENCE	SNC CV MEAN DIFFERENCE	ULNAR MNC CV PER DECADE	ULNAR SNC CV PER DECADE	ULNAR MNC CV PER YEAR	ULNAR SNC CV PER YEAR
G2-G3	2.583	2.787	1.291	1.394	0.129	0.139
G2-G4	4.881	5.881	1.627	1.960	0.163	0.196

GROUP 3 WITH OTHER GROUP						
	MNC CV MEAN DIFFERENCE	SNC CV MEAN DIFFERENCE	ULNAR MNC CV PER DECADE	ULNAR SNC CV PER DECADE	ULNAR MNC CV PER YEAR	ULNAR SNC CV PER YEAR
G3-G4	2.298	3.094	1.149	1.547	0.115	0.155





The Effects of Myofascial Release Technique Combined with Lumbar Stabilization Exercise in Lumbar Strain Among College Students: Experimental Study.

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ABSTRACT

To evaluate the effects of Myofascial Release Technique (MRT) with combined with core stabilization exercises (CSE) in lumbar strain among college students. A total of forty-five participants were randomly divided into two groups (LSE and LSE+MRT). A lumbar stabilization exercise program was applied for the participants in the LSE group for 3 days per week for a total of 6 weeks. In addition to the lumbar stabilization exercises, myofascial relaxation technique performed for 3 days per week for 6 weeks for the participants in the LSE+MRT group. Participants were assessed in terms of pain, low back disability, lower body flexibility, lumbar stability endurance, spinal mobility, gait characteristics and quality of life both pre- and post-treatment. It was found that the improvement in lumbar stability endurance ($p=0.031$) and spinal mobility (in the sagittal plane) ($p=0.022$) was greater in the LSE+MRT group compared to the LSE group. There was no significant difference between the two groups in terms of pain, low back disability, lower body flexibility, gait characteristics and quality of life ($p>0.05$). The current study suggests that myofascial release technique with lumbar stabilization exercises can be a better choice in the treatment of lumbar strain among college students.

Keywords: myofascial release technique, Lumbar stabilization exercises, lumbar strain.





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INTRODUCTION

Back pain is one of the most common chief complaints among students also a leading cause of disability and high healthcare.. Mechanical back strain is a subtype of back pain where the etiology is the spine, intervertebral discs, or the surrounding soft tissues[1-3]. While the majority of cases of mechanical back strains that present to the emergency department can be managed conservatively, healthcare providers must be aware of the common red flags that signal more emergent causes of back pain and that are associated with high morbidity and mortality when the diagnosis is delayed[4-5]. Because of the changes in fascia structures, dysfunction of deep muscles of back and trunk is common in chronic LBP. Injuries of low back are mostly caused from the superficial back line (SBL). The SBL contains the plantar fascia, gastrocnemius muscles, hamstring muscles, sacrolumbar fascia, erector spinae muscles and epicranial fascia. The deep muscles of back and trunk are attached to the superficial back line via thoracolumbar fascia. These deep muscles and fascia of the trunk form a continuous musculofascial corset-like system[6-8]. LBP is caused by deep muscle dysfunctions and altered fascia structures. In this case, it can cause the continuous musculofascial corset-like system to fail. In addition, changes in fascia due to aging (increased fascial thickness and disruption of the fascial alignment) may further affect this system. Several studies have shown that decreased strength in the deep muscles (the transverse abdominis and multifidus) is accompanied by increased activation in the superficial muscles (such as the erector spinae) in patients with LBP[9].

The use of various non-pharmacological and non-invasive methods such as exercise, mobilization, and manipulation is well known in LBP treatment. Lumbar stability exercise is a common exercise modality in the treatment of LBP. To decrease pain and increase back specific functional status in patients with LBP, core stability exercise is more effective than general exercise[10]. Lumbar stability exercises improve the strength of deep muscles of trunk and low back disability in college students. Myofascial release technique is another method among the possible management options in the treatment of chronic musculoskeletal pain. It has been demonstrated that myofascial release technique produces a significant improvement in both pain and disability. However, there is no study in which myofascial release technique is performed along Lumbar stabilization exercises among college students with lumbar strain[11-12]. To the best of our knowledge, there is no randomized controlled study investigating the effects of the myofascial release technique and lumbar stabilization exercise among college students.. Therefore, the aim of the current study was to strengthen deep muscles through lumbar stability exercises and decrease SBL activation with myofascial release technique and investigate all the possible effects.

MATERIALS AND METHODS

Study Design

This study was designed as a single blind randomized controlled study. The patients were randomly (a matched randomization method based on gender and age) divided into two groups as the lumbar stability exercise (LSE) group and lumbar stability exercise and myofascial release technique (LSE+MRT) group. Before and after the treatment, all assessments were evaluated by a researcher who was blind to the groups.

Participants

Patients older than 65 years with LBP were included in this study. The inclusion criteria of the study were ongoing LBP for at least 3 months, having no neurological or orthopedic problems, and Standardized Mini-Mental State score equal to or greater than 24 points. Patients who had LBP originating from various pathologies, such as presence of cord compression, radiculopathy, osteoporosis or osteopenia (t score > -1), as well as those who received any treatment for their LBP using long-term anticoagulant or corticosteroid drugs were excluded from the study. All the participants provided written informed consent to participate in the study.



**Murali Sankar K S I et al.****Outcome Measures****Pain Severity**

The visual analog scale (VAS) was used to assess the severity of pain at rest and during activity. VAS provides a rapid (statistically measurable and reproducible) classification of pain severity. Patients marked the severity of their pain on a 10-cm-long line (0 = no pain, 10 = the worst pain possible).

Low Back Disability

Physical disability was assessed using the Oswestry Disability Index (ODI), which is a self-administered questionnaire to evaluate the limitations of various daily living activities. The ODI is one of the most common scoring systems used for patients with LBP. The total score ranges from 0 to 100, where a higher score indicates a higher level of disability.

Procedure

A total of forty-five participants who met the inclusion criteria and volunteered to participate were randomly divided into two groups (LSE and LSE+MRT). Heat modality (a hot pack for 15 mins) and electrotherapy (transcutaneous electrical nerve stimulation (TENS), a 50-Hz conventional TENS with a pulse duration <150 μ s), were applied to all participants. Lumbar Stability Exercise Group A lumbar stabilization exercise program was applied to the participants of the CLSE group for 3 days per week for 6 weeks with a total of 18 sessions. Each training session lasted for 60 mins, starting with a 10-min warm-up program and ending with a 5-min cool-down program. All patients began with learning to activate the abdominal wall. Exercises were designed from 1 set to 3 sets, from 8 to 15 repetitions and contractions from 5 s to 10 s. Rest intervals were set as 30s between the sets and 2–3 mins between the exercises.

All patients performed abdominal hollowing and bracing to facilitate activation of the transversus abdominis and internal oblique muscles. Once these activation techniques were learned and the transversus abdominis was “activated”, upper extremity exercises were added. Initial exercises were done in supine, hook-lying, or quadruped positions. Once a patient demonstrated good control (ie was able to complete 3 sets of each exercise, with 15 repetitions or 10-s-long contraction), he would gradually pass on into intermediate level exercises. Plank exercises were added in this level. At the advanced exercise level, unstable surfaces were used during the exercises.

Lumbar Stability Exercise and Myofascial Release Technique Group

In addition to the lumbar stabilization exercises, myofascial relaxation technique was performed with for 3 days per week for a total of 6 weeks. Myofascial relaxation was performed along the superficial back line (from plantar surface of toe phalanges to occiput) bilaterally. The roller massage application was carried out along four separate myofascial tracks (plantar fascia and short toe flexors, gastrocnemius/Achilles tendonham strings and sacrolumbar fascia/erector spinae) of the superficial back line. The applications were done in prone and standing position. The technique was repeated in 3 sets (1 min rest between sets) lasting for 30 s for each myofascial track. In accordance with the literature, the intensity of rolling massage was adjusted to ensure that 7/10 on the VAS was maintained.

Data Analysis

The data were analyzed using the SPS Statistics for Windows software (Version 20.0). Kolmogorov–Smirnov test was used to check normality. Values were expressed as mean \pm standard deviation and median (25–75 quartiles) for continuous variables, and frequencies were reported for categorical variables. Independent samples t-test (when samples met parametric conditions) and Mann–Whitney U-test (when samples did not meet parametric conditions) were used to compare the continuous variables between the two groups. Chi-square test was used to compare the categorical variables between the two groups. The paired sample t-test was used to determine the mean difference





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between before and after treatments. Changes over time within the groups and group-time interactions for continuous variables were assessed with mixed two-way repeated measures analysis of variance.

RESULTS

Out of 47 patients assessed at baseline, two patients were excluded because they did not meet the inclusion criteria. Participants (n=45) were randomized into two groups (the LEE group, n=23; the LSE+MRT group, n=22). Two participants in the CSE group and one participant in the LSE+MRT group dropped out. Finally, the study was completed with 21 patients in the LSE group, and 21 patients in the LSE+MRT group. Baseline characteristics of the participants in both groups were similar ($p>0.05$).

Pain

There was a statistically significant decrease in VAS at rest and VAS during activity values between pre- and post in both groups ($p<0.001$). Moreover, there was a statistically significant increase in PPT values between pre- and post-intervention in both groups ($p<0.001$). There was no statistically significant between groups (group \times time interactions) regarding VAS rest, VAS activity and PPT values ($p>0.05$).

Low Back Disability

There was a statistically significant decrease in ODI scores between pre- and post-intervention in both groups ($p<0.001$). However, there was no statistically significant difference between the groups regarding ODI scores ($p>0.05$).

DISCUSSION

This is the randomized controlled study investigating the effects of myofascial release technique combined with lumbar stabilization exercises in lumbar strain among college students. In addition, the present study is the first study in which myofascial release technique is performed along SBL in LBP subjects. The findings of our study revealed that there is reduce of pain and disability among lumbar strain college students. A previous study found that core stabilization exercise program over the period of six weeks is more effective in terms of pain reduction, compared to routine physical therapy exercises for a similar duration in patients with NSLBP. In addition, a systematic review showed that a lumbar stability exercise program is an effective method to alleviate chronic LBP.

Arguisuelas et al demonstrated that MRT displayed significant improvements in pain inpatients with NSLBP [13]. The findings of the present study are consistent with the results reported in the literature. Pain was found to decrease statistically in both groups. However, there was no statistically significant difference between the groups regarding pain. According to the literature as well as the present study, whether LSE and MRT are administered separately or together, both are effective in reducing pain in lumbar strain among college students. The Oswestry Disability Index (ODI) is one of the most commonly used scales that assess the disability related to LBP. A high pain level is associated with a high ODI score. A systematic review showed that a core stability exercise program is an effective method to improve ODI scores in patients with chronic LBP[14-15]. It is found that MRT can reduce disability in patients with NSLBP. The findings of the present study are consistent with the results reported in the literature. A statistically significant decrease was reported in terms of disability in both groups. However, there was no statistically significant between the groups regarding ODI scores.



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CONCLUSION

This is the first randomized controlled study investigating the impact of myofascial release technique with lumbar stabilization in lumbar strain among college students. LSE+MRT is more effective than LSE in terms of a greater increase in core stability endurance and spinal mobility also. These results suggest that myofascial release technique with lumbar stabilization can be a choice for treating lumbar strain among college students.

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**Murali Sankar K S I et al.****Table 1. Comparison of Outcome Measures**

Outcome Measures	LSE		LSE+MRT		P value
	Baseline	After	Baseline	After	
VAS rest (cm)	2.84±1.58	1.30±1.13	2.69±1.82	1.50±1.30	<0.001
VAS activity (cm)	5.68±1.26	3.37±1.01	6.19±1.79	3.73±1.51	<0.001
ODI Score	56.47±11.22	42.57±11.06	61.52±16.1	44.47±12.52	<0.001





Profenofos Induced Glycogen Level Alterations in Gill and Kidney of Freshwater Fish *Labeo rohita* (HAMILTON, 1882)

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ABSTRACT

The aquatic environment is ceaselessly being contaminated with harmful synthetics from modern, rural and homegrown exercises. The point of the current research was to evaluate the glycogen levels in gill and kidney of the fish *Labeo rohita* presented to sublethal groupings of profenofos low, medium and high of 15 and 45 days. The fish presented to profenofos showed a diminishing the glycogen levels in gill and kidney for 15 and 45 days. In any case, no data was on record concerning the two different sublethal convergences of pesticide profenofos on the glycogen level of fish. The goal of the current work was to assess the impact of pesticide profenofos on glycogen levels in gill and kidney of freshwater fish *Labeo rohita*.

Keywords: *Labeo rohita*, glycogen, pesticide, profenofos, gill, kidney.

INTRODUCTION

Contamination has been viewed as one of the most extreme threats to the climate, including heavy metals, pesticides, sewage, oil, and so on in both freshwater and marine climate (Clara Bindu *et al.*, 2021). Water tainting with colossal amounts of pesticides make fish mortality or starvation due crumbling of food organism. Moreover, different toxicants saw to impact the development boundaries, generation, and tissue harm (Srivastav *et al.*, 2002). Pesticides are helpful by giving solid, tenacious and somewhat unlimited oversight against destructive vermin with less expense and exertion and contributed impressively to human government assistance, however their unfriendly impacts on non-target life forms are critical. The utilization of agrochemicals in the field can possibly change the



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amphibian medium, influencing the resistance furthest reaches of oceanic fauna and verdure, as well as making risk to the environment. The evaluation of the eco toxicological dangers brought about by pesticides to environments depends on harmfulness information and the impacts of pesticide arrangements on non-target life forms (Vijayan and Thomas, 2018). India is basically an agro-based country with more than 60 - 70 % of its populace reliant upon agribusiness. Nonetheless, 30% of its agrarian produce is lost inferable from bug pervasion. Without even a trace of a superior other option, sending of pesticides becomes inescapable regardless of their known unsafe impacts (Velmurugan *et al.*, 2009). Use of pesticides in India is around 3% of the absolute world utilization and is expanding at the pace of 2-5 % per annum (Bhadbhade *et al.*, 2002).

Labeo rohita (rohu), the Indian significant carp (IMC), is one of the most favored cultivable fish species in carp polyculture practice. This Indo-Gangetic riverine species is broadly appropriated all through South Asia, South-East Asia, Sri Lanka, the previous USSR, Japan, China, Philippines, Malaysia, Nepal and a few nations of Africa (FAO, 2006). The conventional culture of rohu returns many years in the little lakes of the eastern Indian states; be that as it may, data on its way of life is accessible from the early piece of the twentieth century just (FAO, 2006). In India, it was brought into practically all riverine frameworks and presently possesses a focal situation in polyculture of fish in lakes (Majumder and Saikia, 2020). Fish is a significant wellspring of top caliber, adjusted and effectively edible food material. Fishes play critical part in nourishment, pay and business. Fishes are most significant wellspring of creature protein and other component. So it is vital for know the general biochemical constituents of the fish and their dietary structure for general wellbeing (Kadam and Patil, 2016). Fishes are especially exceptionally touchy to the water pollution. Consequently, poisons, for example, insect poisons, herbicides may essentially influence a few physiological and biochemical cycles when they go into the organs of fishes (Somaiah *et al.*, 2014). The present investigation was to assess the glycogen levels in gill and kidney of *Labeo rohita* exposed to three different sublethal concentrations of pesticide profenofos.

MATERIALS AND METHODS

Healthy *Labeo rohita* having mean weight 13-16 gm and length 10 – 14 cm were collected from PSP fish farm, at Puthur and acclimatized to laboratory conditions ($29 \pm 1^\circ\text{C}$). The fish were fed daily on oil-less groundnut cake. The unused food was renewed after 2 hours and water was changed daily. Prior to experimentation the fish were acclimatized to experimental tanks for at least one week.

Pesticide

Organophosphorus insecticide profenofos purchased from local agro chemist shop was used for the present study.

Experimental Design

A total of 40 fishes (10 fishes per aquarium) were separated as four groups. The following experimental groups were conducted in the freshwater fish *Labeo rohita* for the period of 45 days. Sub lethal doses preferred on previous references.

Group 1: *Labeo rohita*, without any pesticide exposure (control)

Group 2 *Labeo rohita*, on exposure to 0.50 ppb Profenofos for a period of 15 and 45 days

Group 3 *Labeo rohita*, on exposure to 1.00 ppb Profenofos for a period of 15 and 45 days

Group 4 *Labeo rohita*, on exposure to 1.50 ppb Profenofos for a period of 15 and 45 days

At the end of each exposure period, the fish were sacrificed and the required tissues were collected for glycogen estimation. The glycogen content of the tissues was estimated by the method of Kemp and Kits Van Heijninger (1954).

Statistical Analysis

The data so obtained were analyzed by applying analysis of variance DMRT one way ANOVA to test the level of significance (Duncan, 1957).





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RESULTS

The glycogen content declined substantially in gill and kidney of *Labeo rohita* exposed to low, medium and high sublethal concentration of pesticide profenofos for the periods of 15 and 45 days when compared to control. The maximum glycogen content reduction was observed in high sublethal concentration on 45th day exposure (Table 1 and 2).

Table 1: All the values mean SD of six observations; Values which are not sharing common superscript differ significantly at 5% ($p < 0.05$); Duncan multiple range test (DMRT)

Table 2: All the values mean SD of six observations; Values which are not sharing common superscript differ significantly at 5% ($p < 0.05$); Duncan multiple range test (DMRT)

DISCUSSION

The pesticidal effect on the liver and kidney of experimental fish, *Labeo rohita* to various periods revealed a significant decrease in glycogen content. The changes in biochemical parameters are important to indicate the susceptibility of organ system to pollutants (Barnes and Blackstock, 1973). In an organism toxic substances brings about a kind of stress and an organism responds to that by developing necessary potential (Hyalij, 2013). During stress, organism needs sufficient energy which is supplied from reserve food material i.e. protein, glycogen and lipid. Glycogen is a sub divisional polysaccharide and major storage of Glucose that serve as a form of energy in animals. It plays an important role in the glucose cycle that can be quickly mobilized to meet a sudden need for glucose (Somaiah *et al.*, 2014). The glycogen content in various tissues of *Labeo rohita* was decreased with increased toxicant concentration in the present experiment. Prakash and Verma, (2020) tended to that, *Heteropneustes fossilis* presented to sublethal grouping of chlorpyrifos shows various tissues glycogen was diminished when contrasted with control. The expanded glycogenolysis demonstrated an overall unsettling influence in carb digestion, which could adversely affect the existence of uncovered creatures. Tripathi *et al.* (2003) revealed that glycogen content was diminished in liver and kidney of new water teleost *Channa punctatus* because of poisonous impact of organophosphorus pesticides.

Padma Priya, (2013) detailed that the tissue glycogen content diminished on openness of fish *Channa punctatus* to imidacloprid. Diminished liver glycogen has been seen in fish *Oreochromis mossambicus* because of poisonous effect of dichlorvos (Lakshmanan *et al.*, 2013). Anitha susan *et al.* (2010), revealed that visualizes the varieties of glycogen and protein substance in significant tissues like liver, muscle, kidney, mind and gill of the two carps *Labeo rohita* and *Cirrhinus mrigala* presented to sublethal and deadly centralizations of pyrethroid, Fenvalerate. Satyavardhan, (2013) revealed that the glycogen in various tissues of fish *Ctenopharyngodon idella* was essentially diminished by the fenvalerate and malathion inebriation. Reason for the decrease in glycogen content may be the inhibition of the enzyme glycogen synthetase. Veeraiah *et al.* (2013) seen that openness to sub-deadly and deadly centralizations of cadmium chloride in the fish *Cirrhinus mrigala* for 96 h caused changes in the all out glycogen level which might be ascribed to poisonous pressure, bringing about the disturbance of proteins related with carbohydrate metabolism. A fall in glycogen levels in the fish *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* on openness to sublethal centralizations of chlorpyrifos (Rawat *et al.*, 2002). Fall in glycogen levels in the freshwater fish *Labeo rohita* under the fenvalerate openness (Tilak *et al.*, 2004). The exhaustion in glycogen levels under openness to kelthane an organochlorine insect poison in the freshwater fish *Channa punctatus* (David *et al.*, 2005).

CONCLUSION

Taking everything into account, accordingly the profenofos influence the typical capacity of fish in light of the fact that the poison upset the cells prompts decline the glycogen substance in gill and kidney of fish, *Labeo rohita* for defeating the profenofos toxicity. The impacted fish were not appropriate for human utilization.





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Table 1: Glycogen (mg/g) in Gill of *Labeo rohita* exposed to sublethal concentration of profenofos pesticide

	15 days	45 days
Control	38.50 ± 2.85	38.09 ± 2.86
Low	37.50 ± 1.95	30.96 ± 2.25
Medium	35.19 ± 2.65	25.80 ± 1.90
High	34.50 ± 2.60	17.58 ± 1.20

Table 2: Glycogen (mg/g) in Kidney of *Labeo rohita* exposed to sublethal concentration of profenofos pesticide

	15 days	45 days
Control	38.25 ± 2.45	32.80 ± 2.59
Low	30.86 ± 2.35	26.90 ± 1.60
Medium	29.66 ± 2.16	20.10 ± 1.50
High	23.40 ± 1.70	15.90 ± 1.10





Effect of Gong's Mobilization Versus Maitland Mobilization on Pain, Shoulder Abduction and External Rotation Mobility and Functional Ability in Subjects with Adhesive Capsulitis of Shoulder Joint- A Quasi-Experimental Study

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ABSTRACT

Adhesive capsulitis may be a common cause for shoulder pain and disability. There will be an unconstrained onset of shoulder pain along with progressive limitation of both active and passive Glenohumeral motion. This condition most frequently affects the people between 40-60 years old. Approximately 70% of the patients presenting with adhesive capsulitis are women, and 20-30% of these affected develop adhesive capsulitis in opposite shoulder. Adhesive capsulitis is usually occurring of 10-20% people with diabetes. It is significant on with the incidence of 2% generally population in India. To study and find out the effect of Gong's mobilization versus Maitland mobilization on pain, shoulder abduction, external rotation mobility and functional ability in subjects with adhesive capsulitis of shoulder over a period of two weeks. A Convenient study. 45 subjects were selected and assigned into 3 groups by simple random sampling method. Group A received Gong's mobilization together with conventional physiotherapy, Group B received Maitland mobilization together with conventional physiotherapy and Group C received only conventional physiotherapy exercises. Numerical Pain Rating Scale, Shoulder Pain and Disability Index scores, Goniometer were used as outcome measures to record pain, quality of life and shoulder abduction and external rotation of shoulder Range of Motion. This measurement was taken before and after the treatment over a period of two weeks. On analysis statistically using one-way Analysis of variance (ANOVA) test, it interpreted that all the 3 groups had





improvement in their outcome measures. The group A which received Gong's mobilization technique together with conventional physiotherapy showed more improvement than the group B which received Maitland mobilization together with conventional physiotherapy group.

Keywords: Adhesive capsulitis, Gong's mobilization, Maitland mobilization, Numerical Pain Rating Scale, Shoulder Pain and Disability Index.

INTRODUCTION

One of the common causes of shoulder pain and disability is adhesive capsulitis. It is characterized by spontaneous onset of shoulder pain accompanied by progressive limitation of both active and passive gleno-humeral movement. It is a disease that causes tissue degeneration, joint thickening and diminished glenoid cavity volume [1]. For diagnosis of shoulder capsulitis, the presence of capsular pattern is necessary. Although the range of motion varies depending upon which stage the patient presents, yet he/she still has limitations of passive range of motion in a capsular pattern [2]. "Frozen shoulder" is a term coined by Codman in 1934 is an orthopedic condition that is commonly encountered in general practice. In the pre-arthroscopic era, Neviaser was the first person to use the "Adhesive capsulitis" concept for chronic inflammation and fibrosis of the joint capsule. However, arthroscopic examination would prefer the term fibrotic capsulitis with absence of intra-articular adhesions [2,3]. Later Hannafin and Chiaia described four stages, including the arthroscopic stages described by Neviaser, clinical examination and historical findings [3].

Prevalence

This condition most often affects the people between 40-60 years old. Approximately 70% of the patients presenting with adhesive capsulitis are women, and 20-30% of those affected develop adhesive capsulitis in opposite shoulder. Adhesive capsulitis is commonly occurring of 10-20% people with diabetes. It is significant on with the incidence of 2% in general population in India [4].

Signs and Symptoms

The etiology of adhesive capsulitis of shoulder remains unclear. The antero-superior joint capsule and the coracohumeral ligament are particularly affected by the disease process. Arthroscopy shows a shoulder joint with loss of axillary fold and tight anterior capsule, mild or moderate synovitis and becomes adherent. Clinical symptoms include, dull or aching pain in the outer shoulder area, sometimes pain occurs in upper arm, limited range of motion, altered scapulohumeral rhythm, muscle weakness from disuse [4,5]. Physiotherapy offers various methods to address and manage pain and stiffness. To reduce pain there are different Strategies used are exercises and electrotherapy modalities. To correct the stiffness active and passive range of motion exercises, mobilization and manipulation techniques are used. For healing physical pain and distracting the mind from stress, Gong's mobilization technique is useful. It is a corrective antero-posterior or longitudinal caudal glide on the head of the humerus can be sustained while the patient actively abducts the arm. The reduction in the intensity of pain is the result of mechanoreceptor located within the joint. Reducing the intensity of pain along with improving the range of motion had a joint effect in the overall increase in functional activity [6,7,8].

Joints limited by pain are generally treated by Grade I and II of Maitland mobilization techniques. The oscillations repeatedly stimulate mechanoreceptors which block nociceptive pathways at the spinal cord or brain stem levels, resulting in an inhibitory effect on the perception of painful stimuli, these non-stretch motions help move synovial fluid to improve nutrition to the cartilage whereas Grade III and IV are primarily used as stretching maneuver [9,10,11].





In the current study, we focused on to improve the range of motion by breaking adhesion and stretching of capsules done with Maitland mobilization and to decrease pain and improve range of motion by Gong's mobilization. This study is to compare the effectiveness of Gong's mobilization versus Maitland mobilization assessing the patient with a frozen shoulder based on pain, range of motion and shoulder function. To study and find out the effect of Gong's mobilization versus Maitland mobilization on pain, shoulder abduction, external rotation mobility and functional ability in subjects with adhesive capsulitis of shoulder.

MATERIALS AND METHODS

The study design was a Quasi-Experimental study, it was conducted at the Sri Ramakrishna Hospital, SRIPMS, Coimbatore. The duration of the study was 6 months and the treatment duration was 2 weeks; 30 minutes in each session for 5 sessions per week. A total of 45 participants were randomly select and assigned in to three groups (an experimental group and a conventional group) with each group having 15 participants. A simple random sample technique was applied.

Criteria for Selection

Inclusion criteria

- AGE; 40-60 years.
- Unilateral adhesive capsulitis of shoulder joint.
- Type II diabetes mellitus.
- Both dominant and non-dominant hand.
- Both males and females.
- Pain more than 3 months [6].
- Phase II and III of adhesive capsulitis of shoulder joint.
- Subjectshaving limited ROM of shoulder abduction limited beyond 120° or less [7].
- Subjects having limited ROM of shoulder external rotation limited beyond 50 or less [8].

Exclusion criteria

- Patients suffering from thoracic outlet syndrome.
- Peripheral nerve injuries.
- Rheumatoid arthritis.
- Acute inflammation.
- Recent fractures in and around the shoulder [9,10].
- Recent shoulder dislocations
- History of any surgery on the affected side
- Rotator cuff rupture
- Tendon calcification.
- Patients who are all taking corticosteroids medications [12].

Measurement Tools

- Numerical pain rating scale [13].
- Shoulder pain and disability index assessment form [14].
- Universal goniometer of 0°-180°[15].





Method of Data Collection

Study procedure

All the subjects with Adhesive capsulitis of shoulder that reported to Sri Ramakrishna Hospital were screened. Their suitability was assessed as per the inclusion and exclusion criteria, after which they were requested to take part in the study. Informed consent form was obtained from their demographic data, pain intensity assessed with Numerical pain rating scale, range of motion assessed with Goniometer and functional disability assessed with Shoulder Pain and Disability Index (SPADI). Then the participants were randomly divided into 3 groups via Group A, Group B, Group C. Application of moist heat over the affected side shoulder for 5 minutes per session before mobilization for all 3 groups.

Treatment Techniques

GROUP A: Gong's mobilization and conventional physiotherapy

Treatment duration and frequency: 3 sets of 10 repetitions with 1 minute rest between sets.

Intervention duration: 5 sessions in a week for 2 weeks.

Gong's mobilization

The Gong's mobilization can be done either in high sitting or in side lying position with the affected shoulder upward.

Position of the patient: High sitting

The subjects sat on knee- high chairs with no back support with the spine in neutral position and comfortably extend both their arms.

Therapist position: stride standing

The subjects sat on high knee chair with no back support with the spine in a neutral position and comfortably extended both their arms. A physical therapist stood on the side opposite to the affected side shoulder. The therapist pushes the scapula of the affected side in posterior to anterior direction with one hand, and pushed the humeral head in the anterior to posterior direction parallel to the joint plane with the other hand. This restored the humeral head, which has been pushed forward, to its normal position. Simultaneously the subject was asked to quickly and powerfully perform shoulder abduction with no external rotation, with elbow flexion in the coronal plane, and with the back of the hand facing outside, and the palm facing inside. During this time, the hands of the therapist kept pressing the humeral head with the long axis of the palm align with the long axis of the humerus. Gong's mobilization was repeated for 10-15 times per session. When the subjects were performing shoulder abduction, the therapist followed them at the same speed, continuing with a little distraction, and adding acceleration in the end range.

Conventional therapy

The subjects were asked to perform Inferior Capsular stretch, Pendular exercise, Isometric scapular retraction, Strengthening rotator cuff, Horizontal abduction exercise, Scapular stabilization exercise. Then asked them to hold it for 10 seconds and perform all the above exercises for 3 sets with 10 repetitions.

GROUP B: Maitland mobilization and conventional physiotherapy

Treatment duration and frequency: 2-3 glides per second for 30 seconds for each glide and every glide is given for 5 sets with 1 minute rest between sets. Intervention duration: 5 sessions in a week for 2 weeks.

Maitland mobilization

Glenohumeral caudal glide; Subject lies supine with side to mobilize towards the edge of the table.

Therapist stands in lunge position superiorly to the subject's shoulder, one hand grasps around subject's distal humerus (holding the elbow in 90° flexion), and the web space of the other hand is around the proximal humerus. Therapist then provides a force to the proximal humerus in an inferior direction, while simultaneously flexing further starting with grade 1 and working up to grade 4 as per subject's tolerance.

Glenohumeral postero-anterior glide; Subject lies prone with arm to be mobilized towards the edge of the table in 90° of abduction, elbow flexed with some glenohumeral internal rotation. Therapist grasps the subject's distal humerus,





allowing the forearm to hang downward the ground. Heel of proximal hand is placed against the posterior humeral head with elbow locked. Therapist then provides a postero-anterior force moving their body/ trunk down through their locked-out elbow, starting with grade 1 and working up to grade 4 as per subject's tolerance.

Conventional therapy

The subjects were asked to perform Inferior Capsular stretch, Pendular exercise, Isometric scapular retraction, Strengthening rotator cuff, Horizontal abduction exercise, Scapular stabilization exercise. Then asked them to hold it for 10 seconds and perform all the above exercises for 3 sets with 10 repetitions.

GROUP C: Conventional physiotherapy group

The subjects were asked to perform Inferior Capsular stretch, Pendular exercise, Isometric scapular retraction, Strengthening rotator cuff, Horizontal abduction exercise, Scapular stabilization exercise. Then asked them to hold it for 10 seconds and perform all the above exercises for 3 sets with 10 repetitions.

DISCUSSION

The study was conducted to analyze the pain reduction, increase in shoulder abduction, external rotation and improvement in functional ability in subjects with adhesive capsulitis of shoulder joint. 45 patients were selected using simple random technique. Among which 15 patients were discontinued from the study, 4 from Group A (Gong's mobilization along with conventional physiotherapy) and 5 from Group B (Maitland mobilization) and 6 from Group C (conventional physiotherapy). The parameters used were NPRS, ROM, and SPADI. The treatment duration was 2 weeks with 5 sessions every week.

The results of the study demonstrated that Group A showed better improvement on pain reduction with the mean value of 3 than Group B 3.1 and Group C 3.7. In addition to that there was statistically significant improvement of range of motion in Group A compared to Group B and Group C. There was a substantial improvement observed in shoulder abduction mean value of 129.9 in Group A than Group B 128.5 and Group C 120.8 respectively. For shoulder external rotation Group, A showed better improvement in mean value of 60.4 than Group B 59.6 and Group C 56.8. In SPADI pain levels along with disability were reduced in Group A showed better improvement in mean value of 31.574 compared to Group B 34.711 and Group C 44.412 respectively.

In Group A (Gong's mobilization along with conventional physiotherapy), the analysis of pain and shoulder mobility within the group shows that there is a statistically significant change in means of NPRS, ROM and SPADI when analyzed from pre-intervention to post-intervention. In this study the increase in shoulder external rotation range of motion, reduction in pain and improvement in functional ability occurred because with Gong's Mobilization. Hence Gong's Mobilization can be considered as a useful manual therapy tool in the management of adhesive capsulitis of shoulder.

In Group B (Maitland mobilization with conventional physiotherapy), the analysis of pain and shoulder mobility within the group shows that there is a statistically significant change in means of NPRS, ROM and SPADI when analyzed from pre-intervention to post-intervention. In this study the increase in shoulder abduction range of motion, reduction in pain and improvement in functional ability occurred because with Maitland Mobilization.

In Group C (Control group), noticeable improvement may be due to beneficial effect of supervised exercise protocol. All 3 groups improved significantly in range of motion of external rotation, abduction, reduction in pain and improvement in functional ability on subjects with adhesive capsulitis of shoulder.

Both the groups received conventional physiotherapy consisting mobility and strengthening exercises that includes pendular exercise, isometric scapular retraction, horizontal abduction exercise, scapular stabilization exercise, strengthening rotator cuff exercise. This is the first study in which both Gong's mobilization versus Maitland





mobilization on pain, shoulder abduction, external rotation mobility and functional ability in subjects with adhesive capsulitis of shoulder. The scope is limited to make a direct comparison with other studies due to the lack of literature in this area. The results of this study can be compared to other studies in a general way only due to differences in treatment protocols, subject population, measures taken, and duration of treatment. In this study both the groups showed a marked improvement in NPRS, SPADI scores, and goniometer scores, and there was a significant difference in the scores observed between all the 3 groups.

Limitations

- ❖ This study was very short term.
- ❖ Since the study has been done with smaller number of subjects.
- ❖ Variation in personal habits, side of involvement, gender, age could not be controlled.

Recommendations

- ❖ To make it more valid long term is necessary.
- ❖ This study can be done for long duration.
- ❖ Immediate effects of these techniques can be analyzed.
- ❖ Further studies should be conducted with large group of population.

CONCLUSION

On the basis of present study, both the techniques were able to reduce pain and to improve shoulder abduction, external rotation ROM, and improved functional ability in subjects with adhesive capsulitis of shoulder joint. Statistically, there was a significant difference between the effect of two techniques to reduce pain and to improve shoulder abduction, external rotation ROM and improved functional ability in subjects with Adhesive capsulitis of shoulder joint. So that the study concluded that Gong's mobilization is effective in reducing pain and to improve functional ability among subjects with Adhesive capsulitis of shoulder over a period of 2 weeks. There is a significant difference in pain, shoulder external rotation and functional ability in subjects with Adhesive capsulitis of shoulder in Group A is greater than Group B and Group C. But the reported range of shoulder abduction was greater in Group B compared to the Group A and Group C.

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COVID-19 Prevention by Blocking 6MO3 from SARS CoV-2 of using *Ocimum sanctum* Extract: an *In silico* Analysis

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ABSTRACT

An infectious disease COVID-19 also known as “Corona virus disease 2019” which is caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2). This global epidemic has been affecting millions of people within a short period of time. Epidemic researchers are putting lot of efforts to discover a preventive traditional vaccine. The main objective of this article is to identify the phytochemicals as ligands which has the capability to deactivate the tyrosine lyase protein from SARS CoV-2(6MO3) that help for the growth of the virus. Molecular docking of phytochemicals with the viral proteins can be studied by the Biovia Discovery studio. The interaction of molecule was determined by the energy of -CDocker and-CDocker interaction respectively. More is the negative energy means high positive scores of these two parameters indicate that Luteolin can effectively inhibit the viral metabolic activity than other phytochemicals present in *Ocimum*. It can be predicted that these molecules can interfere with the infection phase of SARS-CoV-2 virus.

Keywords: *Ocimum* , Phytochemicals, COVID-19, Biovia, SARS-CoV-2

INTRODUCTION

Globally, COVID-19 is considered as an infectious communicable serious pandemic disease. It is imparting life risk consequences for aged people in various countries. On 30th January,2020 The World Health Organization(WHO) has declared that coronavirus outbreak is a Public Health Emergency of International Concern [1,2] This epidemic were first preliminary reported at Wuhan city of China in December 2019 and gradually spread its tentacles worldwide. [3][4]. According to WHO a confirmatory case is that “if a person has undergone with laboratory confirmation of COVID-19 infection”[5]. Unfortunately till today no vaccine and preventive molecular drugs has developed to fight against this disease [6].



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Nature has been a source of many medicines in the form of chemicals, enzymes and proteins present in the plants through which modern drugs have been derived [7]. The chemical substances produce by plant has some medicinal that can regulate or affect the human physiology are called as phytochemicals. These chemicals can be used as therapeutic purposes and these phytochemicals can extract from seed, barks, leaves, fruits and flowers etc. [8]. These phytochemicals are have various medicinal properties like anti-oxidant, anti diabetic, anti-cancer and anti inflammatory. These molecules can serve as the initiators of production of pharmaceuticals which are Safe and cost effective [9].

About 25% of pharmaceutical drugs available in the market are of botanical origin and the beneficial effects are evaluated by invitro bioassay or experiments using animal models [10]. Tulsi (*Ocimum sanctum* L.) is a valuable spice and a popular remedy for various ailments and physiological disorders. Garlic oils are used in case hypercholesterolemia also reduces the systolic blood pressure. *Ocimum sanctum* belonging to family *Allidaceae* is known to cure many fungal, viral, bacterial and parasitic infection. It is one of the earliest known medicinal plant which is used for health benefits that has sulphur containing compounds in the form of phytochemicals. The main objective of this study to identify phytochemicals present in *Ocimum sanctum* to cure the corona virus disease COVID-19 by inhibiting its metabolism.

MATERIALS AND METHODS

Biovia software (Dassault Systems of France) having Discovery Studio tool was analysed the molecular level interaction between the phytochemicals and enzymes. This software helps in prediction of molecular interaction through machine learning. Plant produces phytochemicals as secondary metabolites to protect them from predators and plants usually fights against the micro organisms. The plant is known to contain phytochemicals like Luteolin, eugenol, apigenin, gallic acid etc. The lyase protein from SARS CoV-2 (PDB:- 6MO3) is an important protein or enzyme play a very crucial for the survival and multiplication of this virus.

Molecular interaction between the viral protein and phytochemicals which form a covalent bond to inhibit the growth of the virus. Biovia discovery studio was used for analyzing the molecular interaction to identify the phytochemicals. Phytochemicals were selected from the plant and sdf file downloaded from the website. And PDB code of protein was downloaded from RCSB website. Active site as receptor cavity of this enzyme was selected as "receptor cavity" protocol found under tool "receptor-ligand interaction". For C-Docking in Biovia Discovery studio, enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. Both "-CDOCKER_ENERGY" and "-CDOCKER_INTERACTION_ENERGY" were determined the strength of molecular docking. High C-Docker score considered as the efficient interaction between the phytochemicals and the viral enzyme. Therefore, high positive values predict the effect of phytochemicals on treatment of COVID-19.

RESULTS AND DISCUSSION

Active site of the 6MO3 play a major role during this *in silico* analysis. and It is an *in silico*-based molecular docking method and optimized for accuracy. -CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b) small difference between -CDOCKER energy and -CDOCKER interaction energy. Lyase protein and Luteolin interaction value is represented in Table-1 and has the highest positive score of -CDOCKER energy 21.2264 kcal/mole and small value of the difference 0.6316 kcal/mole between -CDOCKER interaction energy and -CDOCKER energy. Thus, the results indicated that Luteolin can effectively deactivate 6MO3 thereby interrupting the hydrolase activity which process the amino-terminal end of the replicase polyprotein to generate two or three replicase products of the virus. Higher positive values for Luteolin indicated that it was the





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most active ingredient against SARS-CoV virus. Thus, the key phytochemicals Luteolin preventing COVID-19 caused by SARS-CoV2 virus,

CONCLUSIONS

From this it is concluded that *Ocimum sanctum* has anti-viral properties against COVID-19. It is found that Luteolin of *Ocimum sanctum*, which can have an interaction with the viral protein 6MO3 significantly to prevent COVID-19. From the molecular docking analysis, It is found that Luteolin can effectively interact with viral protein to deactivate the viral function. Other phytochemicals do not much influence on viral protein molecule to stop their life cycle. Therefore, it can be concluded Luteolin present in *Ocimum sanctum* has the medicinal values which will be help for curing COVID-19.

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Table 1. C-Docker results of phytochemicals of *Ocimum sanctum* with 6MO3

SL NO	Phytochemicals	- C Docker energy	- C Docker interaction energy	Difference between – C Docker interaction energy and – C Docker energy	Remark
1	Luteolin	34.6002	38.8782	4.278	Shows more interaction
2	Eugenol	-2.96986	6.13701	-9.10687	
3	Apigenin	31.433	36.6071	5.1741	
4	Carnosic acid	-16.2431	27.5532	11.3101	





A Review on Nutritional Dietary Bioactive Compounds used in Type II Diabetes Mellitus

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ABSTRACT

Type 2 diabetes mellitus is the most common type of diabetes, and around 90% of all diabetes cases with hyperglycemia and glucose intolerance defect. Therapeutic and preventative medicines that take into account the common characteristics of diabetes mellitus may be beneficial. Bioactive chemicals present in vegetables and fruits, such as polyphenols, vitamins, and carotenoids, can have anti-inflammatory properties. These properties make them better candidates for diabetes prevention and treatment. Bioactive substances, according to growing evidence from cell and animal models, may have direct effects on lowering hyperglycemia and increasing insulin production. This review discusses the probable molecular mechanisms at work.

Keywords: Type II diabetes mellitus, dietary, nutritional, bioactive compounds

INTRODUCTION

According to the International Diabetes Federation, more than 415 million individuals worldwide are affected with diabetes mellitus, and this number is expected to increase to 642 million or more by 2030. Type 2 diabetes mellitus affects approximately 90% of diabetic people around the world (T2DM). The expense of health care, including diabetes and its secondary problems, continues to rise, posing a significant financial burden on diabetic individuals and, in particular, developing countries (Diabetes Atlas, 7th edition, International Diabetes Federation, 2015). T2DM is a serious global health issue, the incidence of illnesses rises with age, obesity, stress, nutrition, lack of exercise, and inflammation, among other variables. Type 2 diabetes is a serious disease with a lot of complexities [1]. In the modern scenario, natural products and their bioactive compounds may be employed as a non-complicated



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alternative for the treatment of type 2 diabetes. A wide variety of potent medicinal plants and their natural bioactive compounds have already been demonstrated to have anti-diabetic properties [2]. Since ancient times, a variety of medicinal herbs have been used to treat and prevent diabetes and its complications [3].

Defining Dietary Bioactive Compounds

A bioactive compound is a type of chemical that can be found in small amounts in plants and some foods (like fruits, vegetables, nuts, oils and whole grains). These chemicals could have health-promoting properties in the body. These compounds have therapeutic potential by lowering pro-inflammatory, oxidative stress and metabolic diseases while also influencing calorie intake. They have the capacity to alter metabolic processes and exhibit beneficial features such as antioxidant activity, receptor inhibition, and so on [4]. Examples of some bioactive compounds: polyphenols, flavonoids, lycopene, tannins, lignan and indoles.

Defining Type II Diabetes Mellitus (T2DM)

Type 2 diabetes mellitus, also known as insulin resistance, is a type of diabetes in which the pancreas fails to produce enough insulin, a hormone that regulates the movement of sugar into cells, and the cells do not respond to insulin properly, resulting in less sugar intake [5]. Bioactive chemicals could be a viable therapy option for type 2 diabetes and its consequences that has no side effects. A large number of active medicinal plants and their bioactive molecules have already been reported to have anti-diabetic properties [6]. Since ancient times, a variety of medicinal plants have been used to treat and prevent diabetes and its complications [3].

Factors responsible for the maintenance of normal glucose tolerance in healthy subjects

- A. Insulin secretion
- B. Tissue glucose uptake
 - 1. Peripheral (primarily muscle)
 - 2. Splanchnic (liver plus gut)
- C. Suppression of hepatic glucose production
 - 1. Decreased free fatty acid
 - 2. Decreased glucagon
- D. Route of glucose administration

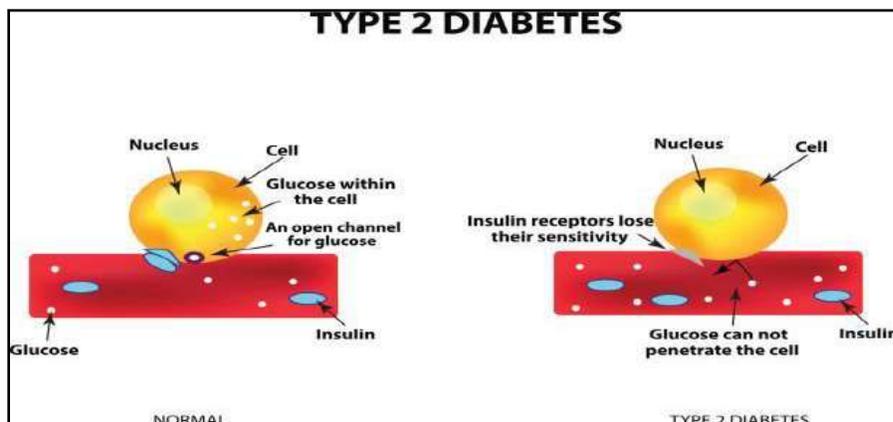
Pathophysiology of T2DM

Multiple disruptions in glucose homeostasis are seen in type 2 diabetics, including (i) impaired insulin secretion (ii) insulin resistance in muscle, liver, and adipocytes (iii) abnormalities in splanchnic glucose uptake (7-10).

Insulin secretion in type 2 Diabetes Mellitus

Type 2 diabetic patients with impaired insulin secretion are prevalent in all ethnic groups (11). Insulin resistance is a condition in which cells do not respond to normal insulin levels and develops primarily in the liver, muscle, and adipose tissues [12]. Insulin normally inhibits hepatic glucose production in both the post-meal and fasting stages, while in the case of hepatic insulin resistance, glucose production may increase after a meal [13]. Increased hepatic glucose production may be caused by increased lipid breakdown within fat [14]. Insulin resistance stimulates compensatory-cell proliferation and improves insulin secretion in the short term. However, long-term exposure to hyperglycaemia-induced oxidative stress, endoplasmic reticulum (ER) stress, and various cytokines may contribute to cell failure through apoptosis, autophagy, and impaired proliferation [14, 15]. Reduced insulin secretion and disruption of glucose homeostasis result from the progressive degeneration of beta-cell function [16].



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New trends in the treatment of T2DM: dietary bioactive compound

According to World Health Organisation (WHO) estimates, 50 percent of T2DM patients do not adhere to expert advice regarding their lifestyle and eating habits. As a result of this situation, nutrition research faces a challenge: finding a large number of nutritional dietary bioactive substances that are helpful against T2DM [17,18]. The dish has to meet three-dimensional requirements in order to be considered important.

1. It should be consumed as a part of the daily diet.
2. It should be made of natural ingredients.
3. The prevention or treatment of specific diseases
4. Maintaining physical and mental health.
5. Delay in the ageing process [19,20]

Anti-Inflammatory Based Therapeutics for Diabetes

Several therapeutic interventions are very effective in reducing acute and chronic inflammation and improving diabetes and its complications via indirect or pleiotropic mechanisms. Issues that reduce the inflammation (particularly, key inflammatory markers such as pro-inflammatory mediators $TNF\alpha$, IL-6, IL- 1β and CRP) could offer a vital public health tool to reduce the burden of diabetes and associated complications including cardiovascular diseases in the general population. The probability of regulating innate immunity-related inflammation as an important experimental approach for the management/prevention of T2DM is based on findings that investigated the therapeutic efficacy of anti-inflammatory agents [21].

Nowadays, the key therapeutic agents to treat T2DM and its complications, sulfonylureas, metformin, and insulin-sensitising glitazones all improve metabolic control and lead to control of various circulating inflammation mediators through innate immunity-related signalling pathways. Sulfonylureas and metformin are main drugs to prevent the T2DM, and sulfonylureas increase insulin production from pancreatic β -cells, while metformin suppresses glucose production in the liver and meanwhile increases insulin sensitivity in peripheral tissues [22]. Glitazones, another anti-diabetic drug, binds to peroxisome proliferator-activated receptors (PPARs), beginning a transcriptional activity that leads to improved insulin action through reducing the secretion of inflammatory markers. Consequently, glitazones reduced levels of CRP, PAI-1, $TNF-\alpha$ and other inflammatory markers. These drugs showed better anti-diabetic nature and also have the comparable anti-inflammatory potential [23-25]. Other therapeutic approaches for T2DM that would act as principals in the inflammatory system have been proposed in the form of salicylates, an anti-inflammatory therapeutic that inhibits I κ B kinase (IKK), and also lowering the glucose level through improvement of beta cell function [26]. Various well established non-steroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase inhibitors (e.g., ibuprofen, naproxen) are able to improve glucose-mediated insulin release, glucose tolerance, and reduce the insulin resistance in diabetic patients [27-28]. In clinical studies, treatment with NSAIDs enhanced many biochemical indices such as blood glucose level, glucose uptake, insulin clearance,





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CRP, lipid profile associated with obesity, and T2DM [29]. Though these findings support the notion that inflammation plays a key role in T2DM and its complications, attenuating inflammation as a strategy for disease prevention in a public health setting will demand a markedly different perspective. In this case, an approach that can be introduced into the population with the minimal side effects and the maximal therapeutic result should be adopted. In this connection, natural products based therapeutic approach would be a better opportunity to treat inflammatory associated chronic diseases like T2DM and its complications, since natural products derived agents are generally safer, lower cost and more highly available in the world.

Role of Natural Phyto Bioactive Compounds in Anti-Diabetics

Various natural phyto bioactive compounds are currently more in demand as compared to the synthetic medicines worldwide for the treatment of diabetes. They are owning the rich availability, efficacy and least side effects as compared to the synthetic medicines [30-31] The higher amount of these phyto bioactive compounds rich dietary compounds could be consumed on the daily basis in food items, that might increase the anti-diabetic activities. Not only in diabetes, It also performed well in multiple traditional medicines including Indian Ayurvedic and Chinese traditional medicines and showed enhanced bioactivity of those phyto compounds [32-33]. Different bioactive compounds are the rich extracts either as a single or the combination of the multiple extracts which mainly shows the enhanced anti-diabetic activities. These multiple combination extract therapy aids in the multiple actions of those bioactive compounds by single therapies thereby enhancing the beneficiary activities, which in the response reduces the drug load to the ones who are consuming. Among the multiple approved medications for the anti-diabetic activities from the last 10 years, 49% originated from the plant extract. Multiple phyto components, mainly 1200 phyto bioactive compounds, those plants were reported for their better anti-diabetic activities, among them 400 phyto bioactive rich plant extracts showed the type 2 anti-diabetic activities [33–35].

Phyto bioactive compounds mainly include the saponin, myrcelin, flavonoids, pectin, and glucosides which are rich and found in the multiple parts of the plants which mainly showed the enhanced antidiabetic activities. The antidiabetic activities of these phytocompounds can be varied on the basis of their mechanisms of actions for the lowering of the glucose including glucose absorption, target insulin resistance and pancreatic functions. In the another research work, Inulin, the soluble fiber showed regulation of GLP-1 homeostasis [36] and the further regulation of insulin resistance was noticed by various phyto bioactive compounds rich plants such as Dioscorea polysaccharides, blueberry anthocyanins, cinnamon and fenugreek seeds [37-39] the multiple phyto extracts express the combinational effect of these bioactivities which mainly includes the chili peppers, bitter melon, ginseng, turmeric and tea extracts [40–45]. Even though these phyto compounds showed multiple beneficial effects in the multiple in vitro studies of Type-2 Diabetes.

Effects of dietary bioactive compounds with application in the treatment of T2DM

Tomato

Tomato (*Solanum lycopersicum*), one of the world's most widely consumed vegetables, and the effects of its bioactive components on diabetes, particularly type 2 diabetes, have been studied. Tomatoes are high in potential antioxidants such as lycopene, ascorbic acid, flavonoids, and other tiny bioactive compounds but low in carbs and energy [46-50].

Lycopene

Lycopene is a carotenoid that occurs naturally in tomatoes and gives them their red colour [51]. Despite the lack of evidence for lycopene's probable anti-diabetic properties, some research has demonstrated the beneficial effects of lycopene on diabetes and its complications [52-54]. Some study was conducted with STZ-induced diabetic rats in which lycopene supplementation resulted in a dose-dependent reduction in H₂O₂, NO, and lipid peroxidation, as well as high antioxidant enzyme activity, which resulted in lower glucose levels, increased insulin levels, and improved serum lipid) [52]. Lycopene's antioxidant effects have also been shown to help STZ-induced diabetic rats recover from endothelial dysfunction [53]. Li *et al.* [54] used STZ-induced diabetic mice to evaluate the specific



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therapeutic impact of lycopene on diabetic nephropathy. Lycopene protects kidneys from morphological damage and function deficiencies caused by diabetes mellitus by improving oxidative state, increasing Akt phosphorylation, and managing connective tissue growth factor, according to the findings. Another investigation found that lycopene improved renal function by disrupting the AGE-RAGE axis [55]. Furthermore, lycopene has been studied for its ability to prevent diabetes-related cognitive impairment. In STZ-induced diabetic rats, Kuhad et al. [56] found a dose-dependent response to chronic lycopene administration that reduced cognitive impairment and cholinergic dysfunction, lowered NO and TNF, and enhanced acetylcholinesterase activity. The malfunction of endothelial progenitor cells (EPCs) has been compared to diabetes-related vascular problems [57][58] found that lycopene alleviated AGE-induced EPC apoptosis and oxidative autophagy, causing EPC quantity and function to be further harmed. As a result, lycopene may be able to help with T2DM vascular problems. When taken jointly, Lycopene's anti-diabetic qualities can be compared to its antioxidant and anti-inflammatory characteristics.

Soy

Soybean (*Glycine max*) belongs to a leguminous family cultivated for its seeds, which have medium oil and high protein content. Genistein is mainly present in soybean [59].

Genistein

Genistein is an isoflavone that can be found in a variety of plants, such as chickpeas and soybeans [60]. Soybeans offer various health benefits that are related to isoflavones, and recent evidence suggests that genistein have a potential to prevent and treat diabetes [61–63]. T2DM is characterized by the loss of functional cell mass, which reduces insulin secretion. The equilibrium between neogenesis, transdifferentiation, proliferation, and apoptosis regulates cell mass [61]. According to Fu et al. [62], the activation of the cAMP/PKA-dependent ERK1/2 signalling pathway by genistein incubation increased both INS-1 and human islet-cell proliferation. Genistein has also been shown to have an anti-diabetic effect in animal experiments. Ae Park et al. [64] investigated genistein's anti-diabetic effects in C57BL/KsJ-db/db mice, which have metabolic characteristics similar to human T2DM. When compared to the control group, blood glucose and HbA1c were significantly lower in the genistein group, while glucose tolerance and the insulin/glucagon ratio were similarly improved [63].

Furthermore, genistein supplementation improved plasma triglyceride, HDL cholesterol, free fatty acid, and total cholesterol levels in these mice. Increased hepatic glucokinase activity, as well as decreased hepatic fatty acid synthase, -oxidation, and G6Pase activities, may be linked to these effects [63]. Furthermore, genistein supplementation improved plasma triglyceride, HDL cholesterol, free fatty acid, and total cholesterol levels in these mice. Increased hepatic glucokinase activity, as well as decreased hepatic fatty acid synthase, -oxidation, and G6Pase activities, may be linked to these effects [63]. As a result, genistein may help people with T2DM by improving their lipid and glucose metabolism. In addition found that genistein decreased fasting glucose, inhibited cytosolic phosphoenolpyruvate carboxykinase (PEPCK), and activated the AMPK and ERK1/2 pathways in alloxan-induced diabetic mice, suggesting that it may alleviate hepatic gluconeogenesis failure in T2DM [64]. In addition, new research suggests that genistein could be a promising therapeutic option for the management of T2DM problems [66–67]. By regulating acetylcholinesterase, antioxidant levels, and neuroinflammation, Rajput et al. [66] found that genistein therapy reversed cognitive deterioration in diabetic rats. Another interesting study found that pretreatment with genistein alleviated obsessive-compulsive disorder in diabetic mice generated by STZ by enhancing serotonergic neurotransmission [66].

Oats

The plant family includes oats, which is an annual herbaceous plant. *Avena sativa* and *Avenabyzantica* are the most important species. It is high in biologically valuable protein, lipids, and a variety of vitamins and minerals. It contains vitamins as well as a significant amount of fibre, which is less vital than nutrients but is necessary for proper digestive function. [68]. Several studies over the last 30 years have shown that dietary fibres can lower blood glucose levels in patients with type 1 and type 2 diabetes. [68]



**Aarti Sati et al.****Vitamins****Vitamin A**

Vitamin A, also known as retinol, is an essential component for eyesight, reproduction, and normal growth. Retinol can be converted to retinal all-trans-retinoic acid (RA) or 9-cis-retinoic acid intracellularly [69]. Vitamin A may influence T2DM through a variety of pathways, including chelation of oxygen radicals, increased insulin sensitivity, cell regeneration, and modulation of obesity and adipose biology [69]. For example, all-trans-RA, for example, has been proposed to improve insulin signalling by decreasing protein kinase C (PKC) activity by binding to PKC isozymes. PKC levels were found to be higher in people with diabetes, and they inhibited insulin signalling [69]. RA also boosted insulin secretion and insulin mRNA levels in cultured islets by activating the glucokinase promoter, which increased pancreatic glucokinase [70]. Furthermore, retinol and RA are positive regulators of uncoupling protein 1 (UCP-1), and overexpression of UCP-1 may enhance glucose transport and insulin resistance in skeletal muscle [70]. Berry and Noy [200] also discovered that all-trans-RA reduced obesity and insulin resistance by increasing the expression of the PPAR and retinoid acid receptor (RAR) genes. According to a recent study [71], vitamin A-deficient diet-fed rats had lower levels of stearoyl-CoA desaturase 1 (SCD1) and monounsaturated fatty acids, which increased ER stress-mediated apoptosis and altered pancreas structure and function. The effects of vitamin A on the therapy of T2DM, on the other hand, are disputed. Retinoids are said to be able to reverse the metabolic availability of retinoid. [72]; therefore, vitamin A may not be an effective intervention for diabetic individuals with altered retinoid biology. Additionally, large-dose intakes of vitamin A interfere with bone metabolism and are associated with osteoporosis [69].

Citrus Fruits**Polyphenols****Hesperidin**

Citrus fruits like lemons and oranges contain hesperidin, a flavonoid glycoside. In vitro and in vivo investigations have recently been proven to be effective for the prevention and treatment of T2DM [70-73]. Hesperidin protected rat pancreatic islet cells from oxidative stress generated by IL-1, therefore enhancing islet cell activity and restoring insulin production and secretion [108]. Hesperidin treatment of diabetic rats generated by a high-fat diet (HFD)/STZ reduced hyperglycemia by improving peripheral glucose absorption, which could be linked to the increase of GLUT4 mRNA expression [73]. In HFD/STZ-induced diabetic mice, oral administration of hesperidin lowered glucose and HbA1c levels while increasing serum insulin, vitamin C, and vitamin E levels [74]. These advantages may be attributed to a reduction in oxidants and proinflammatory cytokines such as TNF- and IL-6 [74]. In addition, a recent study suggested that hesperidin may protect diabetic nephropathy patients by inhibiting transforming growth factor-1 (TGF-1) integrin-linked kinase (ILK-) Akt signalling [75].

Naringenin

Naringenin is a flavonoid abundantly found in citrus fruits such as oranges, lemons, grapefruits, and tomatoes [76]. In recent years, there has been increased attention on the benefits of naringenin on T2DM and its complications. In STZ-induced diabetic rats, oral administration of naringenin decreased the blood glucose level, normalized LDL, and VLDL concentrations and also normalized oxidative stress parameters in both the liver and pancreas; these effects may be attributed to the increased expression of mRNA and protein levels of GLUT4 and PPAR γ by naringenin [77].

Many studies have been designed to evaluate the role of naringenin in diabetes-associated complications, such as nephropathy, cardiac hypertrophy, vascular disease, hepatotoxicity, and neuropathy [78-80]. For instance, Kapoor et al. [78] demonstrated that the altered activity of liver and kidney enzymes, altered antioxidant status, increased generation of ROS, mitochondria dysfunction, and increased expression of apoptotic proteins could induce liver damage and diabetic hepatopathy in diabetic rats; all these effects were rescued after naringenin treatment; therefore, naringenin has potential for the management of diabetic hepatopathy. Roy et al. [79] showed that naringenin alleviated renal impairment and structural changes such as glomerulosclerosis in STZ-induced diabetic rats, possibly through downregulation of TGF- β 1 and IL-1 by reducing oxidative stress, modulating proinflammatory cytokine production and apoptotic events. Moreover, researchers found that naringenin ameliorated high glucose-induced



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endothelial dysfunction by decreasing oxidative stress and apoptosis via the ROS/caspase-3 and NO pathway in endothelial cells [80]. Furthermore, naringenin acted as an antioxidant and cholinesterase inhibitor and ameliorated diabetes-induced memory dysfunction in rats [81-87]. Moreover, in a recent study, naringenin has been shown to improve cardiac hypertrophy in diabetic mice; these effects may be related to the upregulation of cytochrome P450 2J3 and the activation of PPARs [88]. Overall, the beneficial effects of naringenin on diabetes and its complications have been investigated, partly through its antioxidant, anti-inflammatory, and antiapoptotic properties.

Whole Grain

Whole-grain cereals have received considerable attention in the last several decades due to the presence of unique blend of bioactive components like phytochemicals and antioxidants. Phytochemicals and antioxidants in whole-grains have not received as much attention as the phytochemicals in fruits and vegetables, although the increased consumption of whole-grains and whole-grain products has been associated with reduced risk of developing chronic diseases such as cardiovascular diseases, type 2 diabetes, some cancers and allcause mortality. These unique bioactive compounds in whole-grains are proposed to be responsible for the health benefits of whole-grain consumption. The additive and synergistic effects of the biologically active compounds may be responsible for the health benefits of diets rich in fruits, vegetables and whole-grains as the reduced risk of chronic diseases [89]. The recent evidence suggests that the complex mixture of bioactive components in wholegrain foods may be more health beneficial than individual isolated component. Whole-grains are a good source of dietary fibre, vitamins, minerals and bioactive compounds, which have been suggested to contribute to their protective effects as compared to refined grains. The outer layer of grain has been shown to contain much higher levels of bioactive compounds such as phenolic compounds, phytosterols, tocopherols and carotenoids than the inner parts [90- 92]. The phenolic compounds of whole-grains including lignans, alkylresorcinols and phenolic acids have been shown to be metabolized and absorbed in humans and are among the major compounds inducing physiological changes underlying the protective effects [93].

Various Bioactive Compounds Present in Whole-Grain Cereals

Whole-grains contain unique bioactive compounds that complement those in fruits and vegetables when consumed together. The major bioactive compounds in whole-grain cereals are phenolic compounds, phytosterols, tocopherols, dietary fibers (mainly beta-glucan), lignans, alkylresorcinols, phytic acid, γ -oryzanol, avenanthramides, cinnamic acid, ferulic acid, inositols and betaine. Some bioactive compounds are quite specific to certain cereals; γ -oryzanol in rice, avenanthramide and saponins in oats, beta glucans in oats and barley and alkylresorcinol in rye, although these are also present in other cereals like wheat but relatively in fewer amounts [94-98].

Olive Oil

The primary prevention of the diabetes is the regulated lifestyle especially in the type 2 diabetes. Nutritional intake of the olive oil, the key Mediterranean diet component has been associated with the management and the prevention of the multiple chronic diseases including T2D. Olive oil multiple bioactive compounds, especially in its extra-virgin form is mainly the distinct macronutrient lipid and the key culinary ingredients, it could be important to review the T2D preventative bioactive ingredients of the olive oil from the molecular to whole body level. Multiple bioactive ingredients at a molecular level within the olive oil have been repeatedly linked with anti-inflammatory and antioxidant preventive functional properties mainly those from monounsaturated fatty acids (MUFA) and the key biophenols such as oleuropein and hydroxytyrosol (HT). The health benefits of the OO in T2D prevention and management is the centre of attraction for the growing research interest [99-102].

CONCLUSION

This article has outlined the novel ways to treat type 2 diabetic patients and their consequences. According to preclinical and clinical studies, most anti-diabetic medications have the ability to reduce glucose levels and enhance insulin production through anti-inflammatory mechanisms, yet generally have few side effects. Current research





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suggests that bioactive chemicals obtained from natural sources can be used to treat chronic inflammatory disorders. Polyphenols appear to be important metabolic modulators because of their ability to alter a variety of cellular and molecular pathway targets that have been identified as possible polyphenolic targets. Nonetheless, the clinical application of active molecules derived from natural products has not yet been thoroughly studied using intracellular signalling pathways. Additional carefully designed clinical trials are needed to provide better evidence for the potential therapeutic application of bioactive compounds in the treatment of T2DM.

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Conflict Of Interest

There is no conflict of interest.

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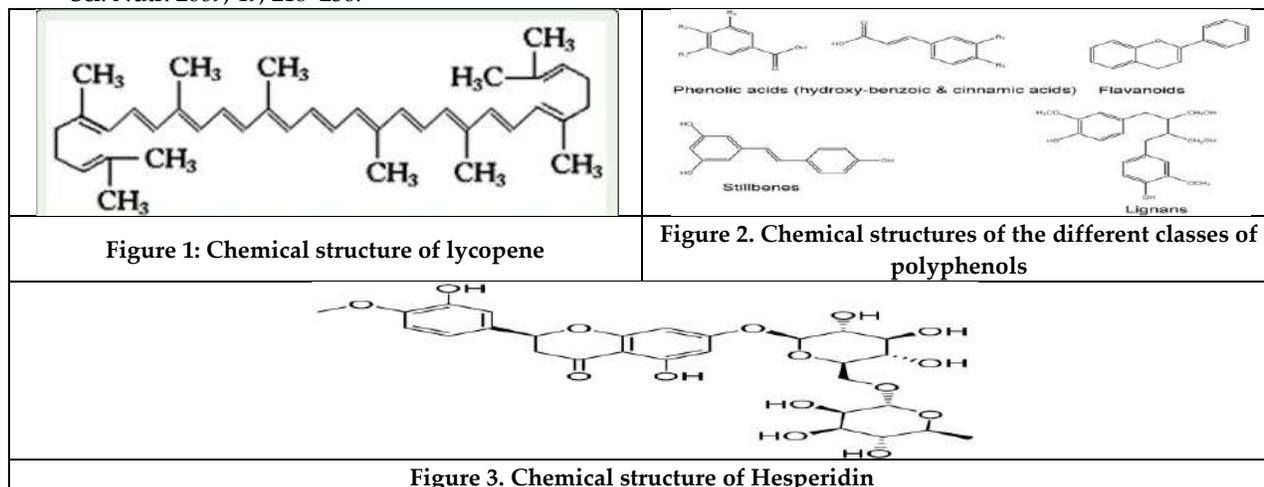
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Acute and Subacute Toxicity Studies of Ethanolic Extract of Leaves of *Thespesia lampas* Dalz & Gibs

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ABSTRACT

Thespesia lampas is an evergreen shrub commonly called as Ban Kapas. Traditionally, the roots and fruits are employed as a remedy for gonorrhoea and syphilis. The present study was carried out to evaluate the safety of an ethanolic extract of leaves of *Thespesia lampas* Dalz & Gibs. The acute and subacute toxicity studies were performed as per OECD guidelines (Organisation for Economic Co-operation and Development) – Guidelines 423 and 407. For the acute toxicity study, the female mice were treated with a single oral dose of ethanolic extracts of leaves of *Thespesia lampas* at 5,50,300 & 2000 mg /kg and observed for general toxicity and mortality for 14 days. In subacute studies, both male and female rats were treated with 100,200 & 400mg/kg orally for 28 days continuously. The animals were observed weekly for any changes in general behaviour, body weights, food intake, water intake, and signs of morbidity and mortality. The results of the present study demonstrated that the oral administration of this extract does not show any toxicity in both acute and subacute toxicity studies. Hence, it was concluded that the ethanolic extracts of leaves of *Thespesia lampas* was safer and non-toxic to rats and further chronic studies are required to confirm its therapeutic efficacy in animals and humans.

Keywords: *Thespesia lampas*, Acute toxicity, Sub-acute toxicity, Bio-chemical parameters, Haematological parameters.



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INTRODUCTION

Plants have been used for medicinal purposes long before the prehistoric period. For thousands of years, medicinal plants have been used to treat health disorders, add flavour and conserve food, and prevent disease epidemics. *Thespesia lampas* Dalz & Gibs is one such plant. *Thespesia lampas* Dalz & Gibs belong to the Malvaceae family, commonly known as "Ranbhendi" found as a wild herb growing during monsoon on the hills throughout India and also in Eastern Tropical Africa. *Thespesia lampas* is an erect, slightly branched, evergreen shrub growing to 2 - 3 metres tall. The plant is harvested from the wild for local use as a medicine and source of fibre and dyes. It belongs to the family of malvaceae. Ban Kapas is an erect, slightly branched shrub 2-3 m in height. The leaves are ovate, 10-20 cm long, somewhat 3-lobed or nearly entire, green and nearly smooth on the upper surface, somewhat hairy beneath, broad and heart-shaped at the base and pointed at the tip. The flowers are large, and borne in threes in the apex of the branches or at the axils of the leaves. The calyx is green, with 5 pointed lobes united below the middle. The corolla is bell-shaped, 6-8 cm long, yellow, and dark-purple at the centre. The capsules are ovoid, and about 3 cm long, with 4-5 valves. A weak rope is made from the bark of this plant [1,2]. Traditionally, the roots and fruits are traditionally used to treat Gonorrhoea, Syphilis, Jaundice, Inflammation, Hyperacidity, Epistaxis, Bronchitis, Cough, Dysentery, Fever, Sun stroke, Carbuncles. In the folk medicine, root paste used to cure jaundice in Korku tribe of Amravati district of Maharashtra and also in Nepal [3,4]. The roots of this plant are reported for anti-diabetic [5], anti-hyperlipidaemic [6], hepatoprotective [7], antioxidant [8,9] and anthelmintic activity [10]. The stems of the plant are used as a folk remedy and it is traditionally used in the treatment of inflammation, acidity, bleeding nose, bronchitis, cough, dysentery, fever, sun stroke, urinary complaints, anthelmintic, carbuncle [11]. *T. lampas* stems showed antimicrobial activity and reported for presence of gossypol [10,12, 13]. It was reported that flowers contain quercetin and some protocatechuic acid [14]. Despite these studies and the widespread use of this plant in traditional medicine, no works on the toxicological profile of extract from leaves have been reported. Thus, this study aims to evaluate the toxicological profile of the ethanolic extract of leaves of *Thespesia lampas*.

MATERIALS AND METHODS

Plant material

The leaves of the plant, *Thespesia lampas* was collected from the forest area of Nallamala near Nandyala region of Andhra Pradesh in the month of July 2020. The collected plants (leaves) were identified and authenticated by the Botanical Survey of India, Agri University, Coimbatore, Tamilnadu. The herbarium specimen of the plant (KSTL-1) was maintained in the college museum. The plant parts were shade dried at room temperature for 10 days and coarsely powdered and passed through sieve No.60.

Preparation of extracts

About 500 g of dried leaves were coarsely powdered and subjected to continuous hot percolation with different solvents of increasing order of polarity such as pet ether, chloroform, acetone, ethanol, and aqueous [15,16]. The extracts were dried under the rotary evaporator and then tested for various phytochemical constituents like alkaloids, flavonoids, glycosides, phenols, saponins, sterols, tannins, proteins, and carbohydrates.

Animals

Healthy adult female Swiss albino mice and Wistar rats were used for the acute and subacute toxicity studies respectively. The animals were procured from CPCSEA listed suppliers of Srivenkateshwara Enterprises, Bangalore, India. Animals should be nulliparous and non-pregnant. The animals were kept in well-ventilated polypropylene cages at 12h light and 12h dark schedule at 25°C and 55–65% humidity levels. The rats had been given a normal diet of pellets and free access to water. Each animal, at the commencement of the experiment, should be between 8 and 12 weeks old.



**Shaik Abdul Saleem et al.****Preparation of animal**

Healthy animals were randomly selected for the study and kept in their cages for at least 1week prior to dosing to allow for acclimatization to the laboratory conditions. Before each test, the animals were fasted for at least 12h; the experimental protocols were subjected to the scrutinization of the Institutional Animals Ethical Committee (P. col/37/2021/IAEC/VMCP) and were cleared by the same. All experiments were performed during the morning according to CPCSEA guidelines for the care of laboratory animals and the ethical guideline for investigations of experimental pain in conscious animals. The standard orogastric cannula was used for oral drug administration in experimental animals.

Toxicity Studies

Acute and subacute toxicity studies were performed as per OECD (Organisation for Economic Co-operation and Development) – Guidelines 423 and 407[17,18,19].

Acute oral toxicity studies

The acute toxicity studies were performed as per OECD guidelines 423. A total of 12 mice weighing between 25-30g were randomly divided into four groups of 3mice each. Animals were fasted prior to dosing (food but not water was withheld over-night). Following the period of fasting, the bodyweight of the animals was measured and the ethanolic extract of leaves of *Thespesia lampas* was administered orally to each group at single doses of 5, 50,300, and 2000 mg/kg, respectively. The control groups received the same volume of distilled water. All the animals were individually observed periodically during the first 24h after administering the extracts and then once a day for 14 days. All the animals were then allowed free access to food and water and observed for signs of acute toxicity. It includes changes in body weight, food and water intake, skin and fur, eyes and mucous membranes, respiratory and circulatory systems, autonomic and central nervous systems, somatomotor activity, and behaviour pattern. The number of deaths within this period was recorded. The urine analysis was performed to investigate any abnormalities in the excretion pattern after the exposure to the test drug for 14 days.

Subacute toxicity studies

The subacute toxicity studies were performed as per OECD guidelines 407. Rats weighing between 150-170g were divided into 3 groups of 10 rats each (5 male+ 5 female). All the animals were fasted over-night and after fasting, the bodyweight of the animals was measured. The ethanolic extract of leaves of *Thespesia lampas*(EETL) at the doses of 100,200 and 400 mg/kg was administered orally to each group respectively. The control groups were treated with the same volume of distilled water. All the animals were individually observed periodically during the first 24h after administering the extracts and then once a day for 28 days. During the 28days study, the body weights of all groups were measured once a week. Animals were also visually observed for mortality, changes in behavioural patterns, changes in physical appearance, and symptoms of illness.

Bodyweight

The individual weights of animals were determined shortly before the test substance was administered and at least weekly thereafter. Weight changes were calculated and recorded. At the end of the test surviving animals were again weighed.

Food and water intake

The food and water intake of each animal of both control and test groups was measured once per week throughout the study.



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Urine analysis

The urine analysis was performed to investigate any abnormalities in the excretion pattern after the exposure with the test drug for 28 days. On the 28th day, the animals were anesthetized with ether and the blood samples were collected by cardiac puncture for haematological and biochemical studies. After euthanasia, all the animals were sacrificed and the vital organs were removed. The weight of the organs was measured and subjected to necropsy and histopathological examination.

Statistical Analysis

The results were expressed as the mean \pm SEM and analyzed statistically by one-way ANOVA followed by Dunnett's t-test by using SPSS version 16. $P < 0.05$ compared to control was considered to be statistically significant.

RESULTS AND DISCUSSION

The purpose of this research is to give scientific validation to the plants by collect and extract plant materials and then to screen them for potential phytochemical and Toxicological aspects. several pharmacological studies have been reported with the leaves of this plant, there is no experimental evidence on its toxicity studies. Hence, the current research work focused on the evaluation of phytochemical and toxic effects of ethanolic extracts of leaves of *Thespesia lampas*.

Phytochemical screening

The leaves of the plant *Thespesia lampas* was collected from the forest area of nallamala near Nandyala region of Andhra Pradesh. The collected plants were identified and authenticated by a botanist. The leaves were shade dried at room temperature and coarsely powdered. The active principles present in the leaves were extracted by a continuous hot percolation method using various solvents and aqueous extract by cold maceration method. The active principles were identified by chemical tests, which showed the presence of various active principles such as Alkaloids, carbohydrates, gums and mucilages, phenolic compounds, proteins, and amino acids, flavonoids, phytosterols, tannins, and saponins.

Acute toxicity studies

Acute toxicity studies are performed to determine the short-term adverse effects of the drug when administered in a single dose orally. It also indicates the safety of the drug in-vivo. Acute toxicity study is generally carried out for the determination of LD50 value in experimental animals. The LD50 determination was done in mice as per OECD guidelines 423 and LD50 of the ethanolic extracts of leaves of *Thespesia lampas* was found to be 2000 mg/kg and the ED50 values were 200 mg/kg, respectively.

Subacute toxicity studies

During the 28 days study, there were no significant changes in the body weight, food, and water intake in all test groups animals were observed when compared to the control group. The body weight and daily food and water intake were not altered by the treatment with the test drug at various dose levels (low, medium, and high). The consequence of urine analysis does not show any abnormalities in the excretion pattern. The organs isolated from various groups did not reveal any abnormalities on gross examination. The weights of the important organs were listed in Table No.1 No statistically significant differences were observed in the weight of the liver, kidney, and heart, of all test groups when compared to the control group. At the end of the study period, no statistically significant differences were seen in the mean haemoglobin content, WBC, RBC, and differential cell counts of all test groups,



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when compared to the control group, as shown in table no.2. At the end of the study period, no statistically significant differences were seen in the mean biochemical parameters were depicted in table no.3.

The histopathological studies with liver, stomach, spleen, kidney, heart, and lungs did not reveal any pathological changes and they were found to be normal as shown in Fig no.1-6. There were no significant changes in any liver function parameters, such as SGOT, SGPT, and ALP compared to the control. The normal levels of blood urea and serum creatinine indicate that the extracts did not interfere with renal function and that renal integrity was preserved [20]. Several researchers have reported that plant drugs are safe and effective in the treatment of incurable diseases [21]. The present findings suggest that the tested extracts are non-toxic since no marked changes in haematological, biochemical, and histopathological parameters were observed.

DISCUSSION

Natural products play a major role in medicine because of their minimal side effects. Despite these, there is still a lack of scientific validation regarding the toxicological aspects of natural compounds. Hence, Scientific knowledge of toxicity studies is much needed. This will help us to identify the safe dose levels of the drug and also the therapeutic index of drugs [22]. In the present study, phytochemical screening of the ethanolic extracts of leaves of *Thespesia lampas* showed the presence of various active principles such as Alkaloids, carbohydrates, gums, and mucilages, phenolic compounds, proteins, and amino acids, flavonoids, phytosterols, tannins, and saponins.

In acute toxicity studies, the animals showed no significant changes in behaviour, breathing, cutaneous effects, sensory nervous system responses, or gastrointestinal effects during the observation period. No mortality or any toxic reaction was recorded in any of the four groups. Hence, it was safe up to 2000mg/kg. In subacute toxicity studies, the treatment of EETL showed no significant changes in the weight of the body and organs. All the animals showed a gradual rise in body weight without much difference between both control and EETL treated groups.

The haematological parameters showed that the extract was not toxic to RBC and not altered its production and platelets. The haemopoietic pathway is one of the most vulnerable sites for toxic compounds and is a major physiological and pathological status measure in humans and animals [23]. Similarly, no changes were observed with WBC count and other factors. In addition, most of the biochemical parameters were not also altered by the EETL treatment. It maintained the normal levels of liver enzymes, glucose, creatinine levels, which indicates the normal functioning of the liver and kidney. Thus, it also indicates that 28 days of treatment does not alter the physiology of the liver, kidney, and metabolism of animals. The above results were further confirmed by the histopathological studies of the vital organs.

CONCLUSION

In conclusion, the oral administration of ethanolic extracts of leaves of *Thespesia lampas* at the doses of 5,50,300,2000 mg/kg for a period of 14 days did not produce any short-term toxicological effects. Further, the oral administration of EETL at the doses of 100, 200, and 400 mg/kg for a period of 28 days was found to be safe in both male and female rats. It didn't show any severe EETL treatment-related toxicity. In the future, detailed chronic studies are essential to confirm the safety of this plant.

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Fig.1. Effects of ethanolic extracts of leaves of *Thespesia lampas* on liver in sub-acute toxicity.



Fig.2. Effects of ethanolic extracts of leaves of *Thespesia lampas* on stomach in sub-acute toxicity.

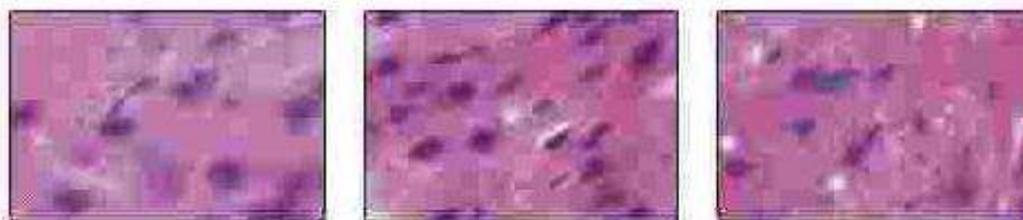


Fig.3. Effects of ethanolic extracts of leaves of *Thespesia lampas* on spleen in sub-acute toxicity.

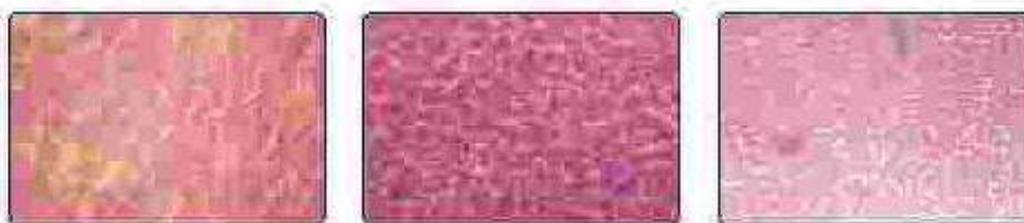


Fig.4. Effects of ethanolic extracts of leaves of *Thespesia lampas* on kidneys in sub-acute toxicity.



Fig.5. Effects of ethanolic extracts of leaves of *Thespesia lampason* heart in sub-acute toxicity.





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Fig.6. Effects of ethanolic extracts of leaves of *Thespesia lampas* on lungs in sub-acute toxicity.

Table 1. Weight of isolated organs of rats after 28 days exposure to test and control groups

S. No.	Group	Treatment	Dose mg/kg	Liver (g)	Kidney (g)	Heart (g)
1	I	Control	-	7.500±0.041	1.200±0.037	0.615±0.006
2	II	EETL	100	7.540±0.084 ^{ns}	0.152±0.035 ^{ns}	0.60±0.004 ^{ns}
3	III	EETL	200	7.597±0.046 ^{ns}	1.160±0.009 ^{ns}	0.591±0.006 ^{ns}
4	IV	EETL	400	7.484±0.029 ^{ns}	1.148±0.006 ^{ns}	0.609±0.006 ^{ns}

Values were expressed as Mean ± SEM of 6 rats in each group. The differences in mean weight of organs in extract treated groups were not significantly different from control group at the end of study (28 days).

Table 2. Effect of ethanolic extracts of leaves of *Thespesia lampas* on haematological parameters in sub-acute toxicity studies.

Groups	Dose (mg/kg)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total Protein (g/dl)	Total cholesterol (mg/dl)	Total bilirubin (mg/dl)
Control	-	191.83±1.28	80.33±0.67	230.50±0.56	7.23±0.03	121.33±3.42	0.50±0.004
ELDC	100	191.10±0.26 ^{ns}	80.08±0.13 ^{ns}	234.27±0.22 ^{ns}	7.86±0.01 ^{ns}	123.47±2.12 ^{ns}	0.49±0.012 ^{ns}
ELDC	200	190.13±0.40 ^{ns}	81.67±0.28 ^{ns}	235.24±0.14 ^{ns}	7.46±0.01 ^{ns}	120.22±3.13 ^{ns}	0.43±0.013 ^{ns}
ELDC	400	190.98±0.12 ^{ns}	81.13±0.19 ^{ns}	232.03±0.10 ^{ns}	7.14±0.02 ^{ns}	121.17±1.23 ^{ns}	0.44±0.021 ^{ns}

Values were expressed as Mean ± SEM of 6 rats in each group. The mean values observed in Hb, RBC, WBC and Differential cell count of extract treated groups were not significantly different from control group at the end of study (28 days).

Table 3. Effect of ethanolic extracts of leaves of *Thespesia lampas* on biochemical parameters in sub-acute toxicity studies.

Groups	Dose (mg/kg)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total Protein (g/dl)	Total cholesterol (mg/dl)	Total bilirubin (mg/dl)
Control	-	191.83±1.28	80.33±0.67	230.50±0.56	7.23±0.03	121.33±3.42	0.50±0.004
ELDC	100	191.10±0.26 ^{ns}	80.08±0.13 ^{ns}	234.27±0.22 ^{ns}	7.86±0.01 ^{ns}	123.47±2.12 ^{ns}	0.49±0.012 ^{ns}
ELDC	200	190.13±0.40 ^{ns}	81.67±0.28 ^{ns}	235.24±0.14 ^{ns}	7.46±0.01 ^{ns}	120.22±3.13 ^{ns}	0.43±0.013 ^{ns}
ELDC	400	190.98±0.12 ^{ns}	81.13±0.19 ^{ns}	232.03±0.10 ^{ns}	7.14±0.02 ^{ns}	121.17±1.23 ^{ns}	0.44±0.021 ^{ns}

Values were expressed as Mean ± SEM of 6 rats in each group. The mean values observed in biochemical parameters of extract treated groups were not significantly different from control group at the end of study (28 days).





Preparation Synthesis Characterization and Antimicrobial Activity of Silver Nanoparticles

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ABSTRACT

Nanoscience has been emergized as a new interdisciplinary science. It is widely recognized in the context of nanoscience and nanotechnologies. The recent studies included the chemical reduction of silver ions through trisodium citrate and testing for their antimicrobial activity. The scope of the present study is to synthesize silver Nanoparticles and evaluate its antibacterial activity. The present study to investigate To synthesize silver Nanoparticles and characterization of synthesized silver Nanoparticles confirmed by UV-visible spectrophotometer ,FTIR analysis and SEM analysis and also to analysis the antimicrobial activity of silver nanoparticles.

Keywords: Antimicrobial, FTIR , Nanoparticals- Silver , SEM, UV-spectrometer.

INTRODUCTION

Nanoscience emergized recently as a new interdisciplinary science. It can be predicted as a whole knowledge on fundamental properties of nano-size objects. The prefix “nano” indicates one billionth or 10⁻⁹ units. The nature of this unit being determined by the word that follows. It is widely accepted in the context of nanoscience and nanotechnologies, . It has been world wide accepted that nanoparticles are clusters of atoms in the size range of 1–100 nm. At present time nanochemistry is one of the unique direction of nanoscience. Metallic nanoparticles shows size and shape-dependent properties that are of interest for applications ranging from catalysts and sensing to optics, antibacterial activity and data storage . For instance, the antibacterial activity of different metal nanoparticles such as





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silver colloids have higher bacterial activity is closely related to their size:(ie),the smaller the silver nuclei. Organic and inorganic reducing agents are used to synthesis of silver nanoparticles is the common approach..

Scope and Objectives

The objective of the present study is to synthesize silver Nanoparticles and evaluate its antibacterial activity. The motive of the present study to investigate synthesise of silver Nanoparticles and to characterization of synthesized silver Nanoparticles confirmed by UV-visible spectra , FTIR and SEM analysis.

MATERIALS AND METHODS

Experimental

Ag nanoparticles are synthesized by AgNO_3 and $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ have been used in the synthesis of silver nanoparticles. To synthesize different-sized silver nanoparticles, the spherical silver nanoparticles were prepared according to the literature procedure by Fang et al.(2005), by reducing aqueous AgNO_3 with sodium citrate at boiling temperature. In typical procedure, 50ml of 0.001M AgNO_3 was heated to boiling. To this solution, 5ml of 1% trisodium citrate was added drop by drop. The solution was heated at boiling point under continuous stirring. The reaction was allowed to take place until the color changed to a yellow solution. The solution was then cooled to room temperature. The silver nanoparticles in this solution were called citrate silver nanoparticles.

UV-Visible Spectrum Analysis

The reduction of pure silver ions was monitored by measuring the UV-Visible spectra of the reaction media and the absorption spectra were recorded over the range of 300-700 nm using UV-Vis spectrophotometer .

FTIR Analysis

To determine fourier transform infra-red (FTIR) pattern of the AgNO_3 nanoparticles was freeze-dried and the dried powder was diluted with potassium bromide in the ratio of 1:100 and recorded the spectrum in perkin elmer FTIR spectrum.

SEM Analysis

The frozen and dried sample was taken to the analysis of SEM then the Nps are coated with double-sided adhesive tape and the specimen coated with platinum in a sputter coater and examined under JEOL 63861 SEM (Japan) at 10 Kv.

Preparation of Media

2.8 grams of nutrient agar is suspend in 100ml distilled water. The medium of solution is completely dissolve by heating to boiling. That solution is sterilize by autoclaving at 15 lbs pressure (121°C) for 15minutes. The sterilized solution is Mixed well and poured into the sterile petri plates. Bacteria were collected from the microbiology laboratory of bose laboratory, Madurai such as escherichia coli and staphylococcus aureus.

RESULTS AND DISCUSSION

The synthesis of silver nitrate nanoparticles through trisodium citrate were carried out. Silver is used as reducing agent as silver nitrate has distinctive properties such eco-friendly nanoparticles in bactericidal, wound healing and other medicinal and spectroelectronic application, makes this method potentially exciting for the large-scale synthesis of other inorganic nanomaterials. Silver nitrate and trisodium citrate were used as starting material for the preparation of silver nanoparticles. chemical reduction method was used to synthesis the silver colloid . The mechanism of reaction could be expressed as follows.



**Philip Arockiaraj and Kousalya****UV/VIS Spectroscopy**

The absorption band in the 350 nm to 450nm region is typical for the silver nanoparticles. The Plasmon absorption shifts towards red with increasing particles size,.

Fouier Transform Infrared Spectroscopy (FTIR) Analysis

In the case of spectrum of nanoparticles this may be the reason for the reduction of the transmittance at this region. The band of carboxyl or carbonyl group at 1660 to 1500 and 1390 to 1260 cm^{-1} region The shift of the band from 1656 to 1586 indicates the formation of metal carbonyl groups. It is due to the stabilization of silver nanoparticles by the acid group of trisodium citrate. This asymmetric shift can be comparable with the data presented by previous works .

SEM Analysis

SEM analysis is out to understand the topology and the size of the silver nanoparticles which exhibits the synthesis of higher density polydispersed spherical silver nanoparticles of various sizes. The SEM analysis showed the particle size 84.90 nm as well the spherical structure of the nanoparticles.

CONCLUSION

The following conclusion obtained from the study The aqueous Ag ions exposed to the $\text{Na}_2\text{C}_6\text{H}_5\text{O}_7$ e results in the preparation of silver nanoparticles, it was confirmed by the formation of brown colour. Synthesized silver nanoparticles further confirmed in UV Visible spectrum and FTIR. These synthesized silver nanoparticles were further confirmed by using SEM. The SEM analysis exhibited the particle size between 84.90nm as well the spherical structure of the nanoparticles. Antibacterial activity against different microorganisms such as *Escherichia coli* and *Staphylococcus aureus* established. It is confirmed the Ag NPs are capable of rendering high antibacterial efficiency and hence has a great efforts in the preparation of drugs used against bacterial diseases. Efficiency of Ag nanoparticles based on these findings may lead to valuable discoveries in various fields such as medical devices and antimicrobial systems. The present study exhibit a simple method of synthesis of silver nanoparticles from a novel primitive chemical source. This method can be further used for industrial production of nanoparticles at room temperature and with a of latest and more effective antimicrobial agents.

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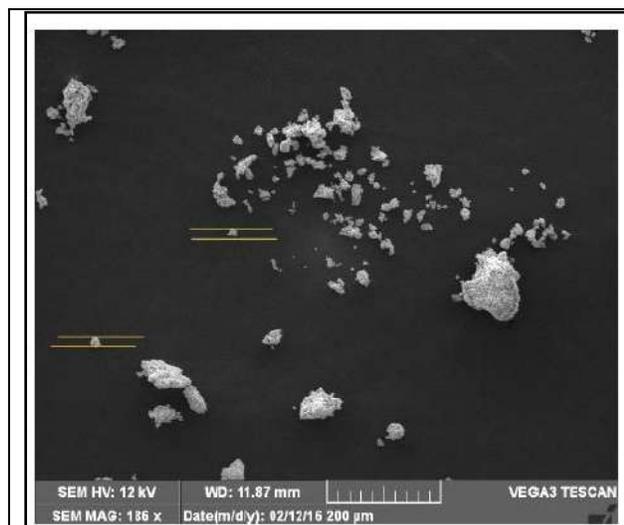


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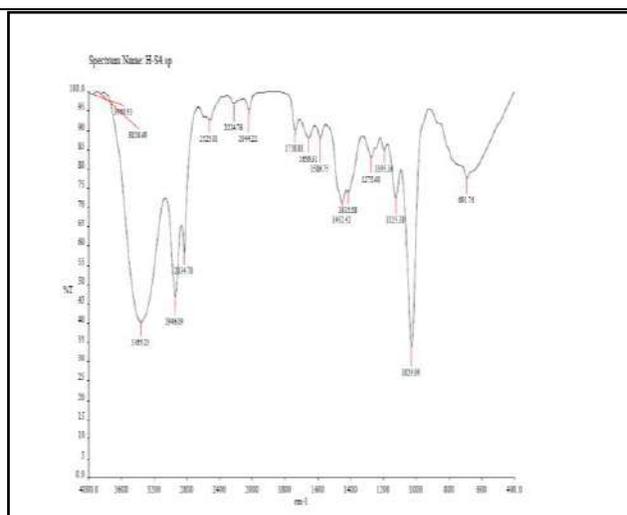
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Antimicrobial Activity Table

Microorganism	Silver Nitrate (30µl)	Silver Nano Particles (30µl)	Std (Chloromphenical) (30µl)	Con (30µl)
Escherichia coli (mm)	8	10	18	0
Staphylococcus auerus (mm)	7	10	19	0



SEM ANALYSIS



FTIR ANALYSIS





Effect of Phosphorus on Growth and Yield of Summer Maize (*Zea mays* L.)

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ABSTRACT

Response of Maize (*Zea mays* L.) on growth and yield to different levels of Phosphorus field trial was conducted during summer season of 2020 at Experimental Farm of Centurion University of Technology and Management, Odisha. This experiment laid out in split plot design comprising of three levels of phosphorus (0, 40, 80 P₂O₅/ha), in three replication. The variety of maize is hybrid SHARP. The results said that the application of 80 kg P/ha registered significantly more growth attributes, namely, plant height (189 cm), and leaf area index (1.46) than other treatments. Similarly, the treatment being statistically at par with 40 kg P/ha recorded superior yield attributes over other treatments. The grain yield (6344 kg/ha), Stover yield (6835 kg/ha), of summer maize was more with 80 kg P/ha, but it was statistically at par with 40 kg P/ha.

Keywords: Maize, phosphorus, yield attributes, yield

INTRODUCTION

Maize emerges as the world's most important crop after wheat and rice, which is considered the "Queen of Grain", due to its high production, easy processing, low cost than other cereals (Jaliya *et al.* 2008). Maize is using as staple food with nutritive values like 100 g of maize contains 89.1% moisture, 1.9 g protein, 0.2 g fat and 0.06 g ash, 8.2 mg carbohydrates, 28 mg calcium, 11 mg ascorbic acid and 86 mg phosphorus (Kumar *et al.*, 2018). Maize can be grown in a wide range of tropical and subtropical climates. Depending on the different regions and socio-economic conditions of the population, the maize-grain is used for various purposes including food, feed, fodder, green cobs, sweet corn, baby corn, popcorn, starch and several industrial products (Maitra *et al.*, 2019). The maize cultivation area in India was 9.18 million hectares, a production of 27.23 million tons and the production rate was 2965 kg/ha in 2018-19 (ICAR-IIMR, 2019-2020). In Odisha, maize is grown mainly in rainy season from June–October in an area of 2.62 lakh ha with an average productivity of 2368 kg ha⁻¹ (GOI 2018-19). Nitrogen and Phosphorus are two important nutrients required for maize production. Maize yield increased with an increase in the quantity of nitrogen applied (Prasanna



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et al., 2019). The combination of N and P fertilizer applications could enhance maize grain yield and nutrient uptake by stimulating root growth, leading to reduced accumulation of potentially leachable NO^{-3}N in soil (Zhihui *et al.*, 2016). Among several functions nitrogen plays important role on plant metabolism (Sangoiet *et al.*, 2008). Maize response to nitrogen differs due to environment and genotype. It has maximum nitrogen use efficiency (50 per cent), but under poor management, its efficiency varies from 30-40 per cent (Prakash *et al.*, 2020). Phosphorus has a better function in root development, stem and stalk strength, flower and seed development, and resist over several plant diseases are the prominent features associated with phosphorus nutrition (Ahmad *et al.*, 2019). Increased levels of phosphorus significantly affected maize plant height, number of cobs plant⁻¹, number of grains cob⁻¹ and grain yield (Humtsoe *et al.*, 2018).

MATERIALS AND METHODS

The field experiment was conducted at Experimental Farm, Bagusala, M.S. Swaminathan School of Agriculture, Centurion University of Technology and Management, Odisha during the *summer* season of 2020. The Experimental site was located at 18.80° N latitude, 84.20° E longitude and at an altitude of 145 meters above mean sea level under the sub-humid and sub-tropical climatic conditions of the North-Eastern ghats of Odisha state, India. The experiment was conducted by adopting split plot design comprising of 4 levels of nitrogen and 3 levels of phosphorus which were replicated thrice. The net plot size was 5 m x 4 m. The hybrid taken for the experiment is 'Sharp' which was sown in February 2021 with a spacing of 60 x 25 cm. The recommended dose of potassium was applied at the rate of 60 kg K₂O per ha, where the sources of fertilizers were muriate of potash. Nitrogen and Phosphorus were applied as per the treatments by using urea and single super-phosphate. The soil was sandy clay loam in texture having organic carbon 0.50%, pH 6.6, available soil nitrogen, phosphorus and potassium values were 177.5, 13.2 and 126.6 kg/ha, respectively. To control weeds pre-emergence herbicide atrazine was applied 2kg/ha was applied followed by two hand weeding done at 20 DAS (days after sowing) and 40 DAS. Gap filling was also done with the first weeding at 20 DAS. Three irrigations were provided to the crop at pre-flowering, flowering and silking stages. The yield and growth were measured as per the schedule and finally, the yield (grain and Stover) was recorded at harvest. The data were analyzed statistically by the analysis of variance method and the significance of different sources of variations was tested by error mean square by Fisher Snedecor's 'F' test at a probability level of 0.05 (Panse and Sukhatme 1978).

RESULTS AND DISCUSSION**Effect of Phosphorus levels on Growth Attributes**

The growth parameters such as plant height (cm), dry matter accumulation (g m⁻²), and leaf area index of the flowering stage (60 DAS) were represented in Table 1. In the phosphorus fertilization, the application of 80 kg P₂O₅ per ha recorded the maximum plant height (179 cm) and it is significantly at par with the application of 40 kg P₂O₅ per ha (173 cm). The minimum plant height was obtained with the control plot (169 cm). Regarding dry matter accumulation, the maximum was seen due to 80 kg P₂O₅ per ha (10412g m⁻²) at the harvest period, and minimum dry matter can be obtained with no phosphorus level applied (9035 g m⁻²). The leaf area index (3.15) at 60 DAS was recorded significantly with applied 80 kg P₂O₅ per ha. The interaction of plant height and leaf area index was significantly influenced whereas dry matter was influenced non-significant.

Effect of Phosphorus levels on Yield

The height production in presence of phosphorus levels lead to an increase in grain yield and the application of 80 kg /ha P₂O₅ produced the highest grain yield (4938 kg/ha) and stover yield (5412 kg/ha) and the treatment remained significantly higher level than 40 kg P₂O₅/ha and control in both grain and stover yield of maize. Further, 40 kg P₂O₅/ha also recorded significantly more grain (4736 kg/ha) and stover yield (5212 kg/ha) than the control plot treatment. The maximum biological yield (10350 kg/ha) and harvest index (47.55%) were recorded significantly with the application of 180 kg N/ha and the minimum was seen in the control plot i.e., at no nitrogen level. However, the





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interaction effect between N and P was also significant in grain yield where the application of 180 kg N/ha and 80 kg P₂O₅/ha showed its dominance over the other treatment combinations (Table 2).

CONCLUSION

The implication of this research concludes life on land that the application of different levels of phosphorus during the growth and production of maize in *summer* season showed a significant impact on both growth attributes and yield. The maximum growth attributes and yield like highest grain yield (4938 kg/ha) and stover yield (5412 kg/ha) and can be seen due to 80 kg P/ha and the minimum was obtained with the control plot treatment.

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Table 1: Yield attributes of maize as influenced by Nitrogen and Phosphorus levels

Treatments	Growth attributes		
	Plant height(cm) at harvest	Dry matter (g m ⁻²) at harvest	Leaf area index at 60 DAS
Phosphorus levels (kg ha⁻¹)			
P ₀	169	9035	2.67
P ₄₀	173	9932	2.80
P ₈₀	179	10412	3.15
S.Em. ±	0.42	218	0.03
CD at 5 %	1.23	636	0.09



**Devalaraju Janakinadh Varma and Rahul Adhikary****Table 2: Effect of nitrogen and phosphorus levels on grain yield, Stover yield, biological yield and harvest index.**

Treatments	Grain yield (kg/ha)	Stover yield (kg/ha)	Biological yield(kg/ha)	Harvest yield (%)
Phosphorus levels (kg ha⁻¹)				
P ₀	4405	4920	9325	46.97
P ₄₀	4736	5212	9949	47.32
P ₈₀	4938	5412	10350	47.55
S.Em. ±	93	106	197	0.17
CD at 5 %	273	310	574	-





Acute and Subacute Toxicity Studies of Ethanolic Leaf Extract of *Carissa macrocarpa* (Eckl.) A.DC

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ABSTRACT

Carissa macrocarpa is a shrub with a thorny appearance. It is often referred to as the Natal plum native of tropical and southern Africa. It is a spiny, evergreen latex containing shrub with yellowish white flowers and delicious red fruits. In traditional medicine *Carissa* species used in the treatment of diarrhea and venereal diseases. Also, used in ayurvedic and Unani systems of medicines. However, the toxicological profile of the plant was not yet scientifically validated. The present research aims to assess the toxicological profile of *Carissa macrocarpa* (Eckl.) A. DC. in Wistar rats. The acute and subacute toxicity studies were performed as per OECD guidelines (Organisation for Economic Co-operation and Development) – Guidelines 423 and 407. For the acute toxicity study, the female mice were treated with a single oral dose of ethanolic leaf extracts of *Carissa macrocarpa* at 5, 50, 300 & 2000 mg/kg and observed for general toxicity and mortality for 14 days. In subacute studies, both male and female rats were treated with 100, 200 & 400 mg/kg orally for 28 days continuously. The animals were observed weekly for any changes in general behaviour, body weights, food intake, water intake, and signs of morbidity and mortality. The results of the present study demonstrated that the oral administration of this extract does not show any toxicity in subacute toxicity studies. Hence, it was concluded that the ethanolic leaf extracts of *Carissa macrocarpa* was safer and non-toxic to rats and further chronic studies are required to confirm its therapeutic efficacy in animals and humans.

Keywords: *Carissa macrocarpa*, Acute toxicity, Sub-acute toxicity, Bio-chemical parameters, Haematological parameters.





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INTRODUCTION

Plants play a vital part in the creation of novel medications. The usage of plants based remedies for addressing various ailments is as old as human civilisation and is employed in all societies throughout history. The primitive man started to differentiate between useful and harmful are poisonous plants through trial and error. Natural products continue to play a major part in drug discovery programmes of the pharmaceutical industry and other research groups [1,2]. Plants are the most potential food sources, and they have been extensively studied in the search for new natural and safer bioactive compounds to use in medicine. Many studies report that numerous active molecules were derived from plant products. Due to their diverse medicinal properties, it has created the belief that all plant products are safe [3]. Hence, a systematic study of medicinal plants for potential toxicity is a necessary step for the evaluation of their therapeutic effect. In this connection, many toxicological studies were conducted to evaluate the toxicity of medicinal plants and their products.

Carissa macrocarpa is a fast-growing, evergreen tree commonly known for its attractive foliage, flowers, and fruits belongs to the family of **Apocynaceae**[4]. This dense, spreading plant will reach a height of only 12 to 18 inches. The natal plum has small, leathery, ovoid leaves that are dark green in color accompanied by sharp, bifurcate (forked) spines about 1 ½ inches long. White, star-shaped flowers that are 2 inches wide appear throughout the plant in the spring. The fragrant flowers are solitary and have overlapping petals. Bright red fruits are about 2 inches long and ripen throughout the year. They are plum-shaped berries occasionally used for jellies and preserves. Twigs bleed a milky sap when they are injured. In traditional medicine *Carissa* species used in the treatment of diarrhea and venereal diseases [5]. Different morphological parts of *C. macrocarpa* are used in south Africa folk medicine to treat coughs and venereal diseases, also their leaves are used against diarrhea in livestock and the fruits have some effects against the human immunodeficiency virus (HIV) and hepatitis. It also presents hepatoprotective, hypoglycemic, anti-lipidemic and anti-proliferative effects [6,7]. Despite these studies and the widespread use of this plant in traditional medicine, no works on the toxicological profile of extract from leaves have been reported. Thus, this study aims to evaluate the toxicological profile of the ethanolic leaf extract of *Carissa macrocarpa*.

MATERIALS AND METHODS

Plant material

The leaves of the plant, *Carissa macrocarpa* was collected from the hill station of Singarayakonda region of Andhra Pradesh in the month of May 2020. The collected plants (leaves) were identified and authenticated by the Botanical Survey of India, Tamilnadu, Agri University, Coimbatore, Tamilnadu. The herbarium specimen of the plant (SKCM-1) was maintained in the college museum. The plant parts were shade dried at room temperature for 10 days and coarsely powdered and passed through sieve No.60.

Preparation of extracts

About 500 g of dried leaves were coarsely powdered and subjected to continuous hot percolation with different solvents of increasing order of polarity such as pet ether, chloroform, acetone, ethanol, and aqueous [8,9]. The extracts were dried under the rotary evaporator and then tested for various phytochemical constituents like alkaloids, flavonoids, glycosides, phenols, saponins, sterols, tannins, proteins, and carbohydrates.

Animals

Healthy adult female Swiss albino mice and Wistar rats were used for the acute and subacute toxicity studies respectively. The animals were procured from CPCSEA listed suppliers of Srivenkateshwara Enterprises, Bangalore, India. Animals should be nulliparous and non-pregnant. The animals were kept in well-ventilated polypropylene cages at 12h light and 12 h dark schedule at 25°C and 55–65% humidity levels. The rats had been given a normal diet



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of pellets and free access to water. Each animal, at the commencement of the experiment, should be between 8 and 12 weeks old.

Preparation of animal

Healthy animals were randomly selected for the study and kept in their cages for at least 1 week prior to dosing to allow for acclimatization to the laboratory conditions. Before each test, the animals were fasted for at least 12h; the experimental protocols were subjected to the scrutinization of the Institutional Animals Ethical Committee (P.col/37/2021/IAEC/VMCP) and were cleared by the same. All experiments were performed during the morning according to CPCSEA guidelines for the care of laboratory animals and the ethical guideline for investigations of experimental pain in conscious animals. The standard orogastric cannula was used for oral drug administration in experimental animals.

Toxicity Studies

Acute and subacute toxicity studies were performed as per OECD (Organisation for Economic Co-operation and Development) – Guidelines 423 and 407 [10-12].

Acute oral toxicity studies

The acute toxicity studies were performed as per OECD guidelines 423. A total of 12 mice weighing between 25-30g were randomly divided into four groups of 3 mice each. Animals were fasted prior to dosing (food but not water was withheld over-night). Following the period of fasting, the bodyweight of the animals was measured and the ethanolic extract of leaves of *Carissa macrocarpa* was administered orally to each group at single doses of 5, 50, 300, and 2000 mg/kg, respectively. The control groups received the same volume of distilled water. All the animals were individually observed periodically during the first 24h after administering the extracts and then once a day for 14 days. All the animals were then allowed free access to food and water and observed for signs of acute toxicity. It includes changes in body weight, food and water intake, skin and fur, eyes and mucous membranes, respiratory and circulatory systems, autonomic and central nervous systems, somatomotor activity, and behavior pattern. The number of deaths within this period was recorded. The urine analysis was performed to investigate any abnormalities in the excretion pattern after the exposure to the test drug for 14 days.

Subacute toxicity studies

The subacute toxicity studies were performed as per OECD guidelines 407. Rats weighing between 150-170g were divided into 3 groups of 10 rats each (5 male + 5 female). All the animals were fasted over-night and after fasting, the bodyweight of the animals was measured. The ethanolic leaf extract of *Carissa macrocarpa* (EECM) at the doses of 100, 200 and 400 mg/kg was administered orally to each group respectively. The control groups were treated with the same volume of distilled water. All the animals were individually observed periodically during the first 24h after administering the extracts and then once a day for 28 days. During the 28 days study, the body weights of all groups were measured once a week. Animals were also visually observed for mortality, changes in behavioural patterns, changes in physical appearance, and symptoms of illness.

Body weight

The individual weights of animals were determined shortly before the test substance was administered and at least weekly thereafter. Weight changes were calculated and recorded. At the end of the test surviving animals were again weighed.

Food and water intake

The food and water intake of each animal of both control and test groups was measured once per week throughout the study.



**Shaik Abdul Saleem, R et al.****Urine analysis**

The urine analysis was performed to investigate any abnormalities in the excretion pattern after the exposure with the test drug for 28 days. On the 28th day, the animals were anesthetized with ether and the blood samples were collected by cardiac puncture for haematological and biochemical studies. After euthanasia, all the animals were sacrificed and the vital organs were removed. The weight of the organs was measured and subjected to necropsy and histopathological examination.

Statistical Analysis

The results were expressed as the mean \pm SEM and analyzed statistically by one-way ANOVA followed by Dunnett's t-test by using SPSS version 16. $P < 0.05$ compared to control was considered to be statistically significant.

RESULTS AND DISCUSSION

The purpose of this research is to give scientific validation to the plants by collect and extract plant materials and then to screen them for potential phytochemical and Toxicological aspects. several pharmacological studies have been reported with the leaves of this plant, there is no experimental evidence on its toxicity studies. Hence, the current research work focused on the evaluation of phytochemical and toxic effects of ethanolic leaf extract of *Carissa macrocarpa*.

Phytochemical screening

The leaves of the plant *Carissa macrocarpa* was collected from the hill station of Singarayakonda region of Andhra Pradesh. The collected plants were identified and authenticated by a botanist. The leaves were shade dried at room temperature and coarsely powdered. The active principles present in the leaves were extracted by a continuous hot percolation method using various solvents and aqueous extract by cold maceration method. The active principles were identified by chemical tests, which showed the presence of various active principles such as Alkaloids, carbohydrates, gums and mucilages, phenolic compounds, proteins, and amino acids, flavonoids, phytosterols, tannins, and saponins.

Acute toxicity studies

Acute toxicity studies are performed to determine the short-term adverse effects of the drug when administered in a single dose orally. It also indicates the safety of the drug in-vivo. Acute toxicity study is generally carried out for the determination of LD50 value in experimental animals. The LD50 determination was done in mice as per OECD guidelines 423 and LD50 of the ethanolic leaf extracts of *Carissa macrocarpa* was found to be 2000 mg/kg and the ED50 values were 200 mg/kg, respectively.

Subacute toxicity studies

During the 28 days study, there were no significant changes in the body weight, food, and water intake in all test groups animals were observed when compared to the control group. The body weight and daily food and water intake were not altered by the treatment with the test drug at various dose levels (low, medium, and high). The consequence of urine analysis does not show any abnormalities in the excretion pattern. The organs isolated from various groups did not reveal any abnormalities on gross examination. The weights of the important organs were listed in Table No.1 No statistically significant differences were observed in the weight of the liver, kidney, and heart, of all test groups when compared to the control group. At the end of the study period, no statistically significant differences were seen in the mean haemoglobin content, WBC, RBC, and differential cell counts of all test groups, when compared to the control group, as shown in table no.2. At the end of the study period, no statistically significant differences were seen in the mean biochemical parameters were depicted in table no.3.

The histopathological studies with liver, stomach, spleen, kidney, heart, and lungs did not reveal any pathological changes and they were found to be normal as shown in Fig no.1-6. There were no significant changes in any liver



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function parameters, such as SGOT, SGPT, and ALP compared to the control. The normal levels of blood urea and serum creatinine indicate that the extracts did not interfere with renal function and that renal integrity was preserved [13]. Several researchers have reported that plant drugs are safe and effective in the treatment of incurable diseases [14]. The present findings suggest that the tested extracts are non-toxic since no marked changes in hematological, biochemical, and histopathological parameters were observed.

DISCUSSION

Natural products play a major role in medicine because of their minimal side effects. Despite these, there is still a lack of scientific validation regarding the toxicological aspects of natural compounds. Hence, Scientific knowledge of toxicity studies is much needed. This will help us to identify the safe dose levels of the drug and also the therapeutic index of drugs [15]. In the present study, phytochemical screening of the ethanolic leaf extracts of *Carissa macrocarpa* showed the presence of various active principles such as Alkaloids, carbohydrates, gums, and mucilages, phenolic compounds, proteins, and amino acids, flavonoids, phytosterols, tannins, and saponins.

In acute toxicity studies, the animals showed no significant changes in behaviour, breathing, cutaneous effects, sensory nervous system responses, or gastrointestinal effects during the observation period. No mortality or any toxic reaction was recorded in any of the four groups. Hence, it was safe up to 2000mg/kg. In subacute toxicity studies, the treatment of EECM showed no significant changes in the weight of the body and organs. All the animals showed a gradual rise in body weight without much difference between both control and EECM treated groups. The haematological parameters showed that the extract was not toxic to RBC and not altered its production and platelets. The haemopoietic pathway is one of the most vulnerable sites for toxic compounds and is a major physiological and pathological status measure in humans and animals [16]. Similarly, no changes were observed with WBC count and other factors. In addition, most of the biochemical parameters were not also altered by the EECM treatment. It maintained the normal levels of liver enzymes, glucose, creatinine levels, which indicates the normal functioning of the liver and kidney. Thus, it also indicates that 28 days of treatment does not alter the physiology of the liver, kidney, and metabolism of animals. The above results were further confirmed by the histopathological studies of the vital organs.

CONCLUSION

In conclusion, the oral administration of ethanolic leaf extracts of *Carissa macrocarpa* at the doses of 5,50,300,2000 mg/kg for a period of 14 days did not produce any short-term toxicological effects. Further, the oral administration of EECM at the doses of 100, 200, and 400 mg/kg for a period of 28 days was found to be safe in both male and female rats. It didn't show any severe EECM treatment-related toxicity. In the future, detailed chronic studies are essential to confirm the safety of this plant.

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Fig.1. Effects of ethanolic leaf extract of *Carissa macrocarpa* on liver in sub-acute toxicity.



Fig.2. Effects of ethanolic leaf extract of *Carissa macrocarpa* on stomach in sub-acute toxicity.





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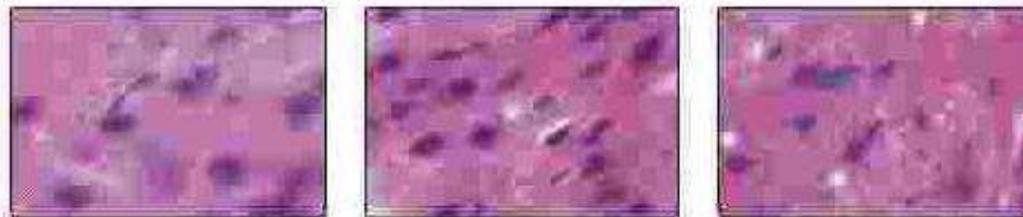


Fig.3. Effects of ethanolic leaf extract of *Carissa macrocarpa* on spleen in sub-acute toxicity.

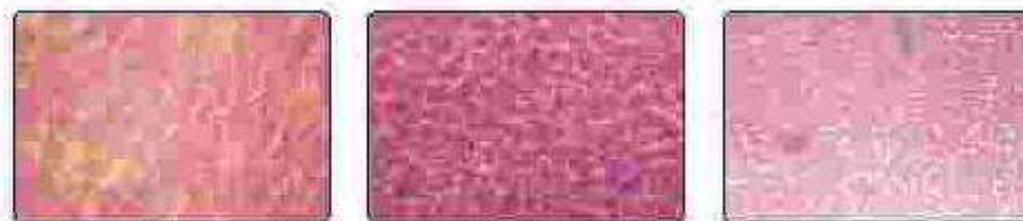


Fig.4. Effects of ethanolic leaf extract of *Carissa macrocarpa* on kidneys in sub-acute toxicity.

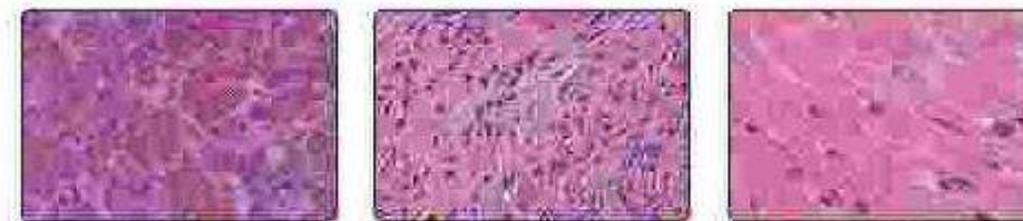


Fig. 5. Effects of ethanolic leaf extract of *Carissa macrocarpa* on heart in sub-acute toxicity.

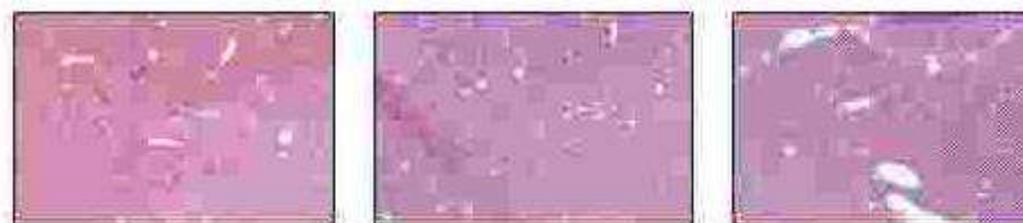


Fig. 6. Effects of ethanolic leaf extract of *Carissa macrocarpa* on lungs in sub-acute toxicity.

Table 1. Weight of isolated organs of rats after 28days exposure to test and control groups

S. No	Group	Treatment	Dose mg/kg	Liver (g)	Kidney (g)	Heart (g)
1	I	Control	-	7.300±0.052	1.100±0.037	0.555±0.006
2	II	EECM	100	7.320±0.093 ^{ns}	0.992±0.035 ^{ns}	0.558±0.004 ^{ns}
3	III	EECM	200	7.217±0.048 ^{ns}	1.020±0.009 ^{ns}	0.588±0.006 ^{ns}
4	IV	EECM	400	7.267±0.049 ^{ns}	1.027±0.006 ^{ns}	0.602±0.006 ^{ns}

Values were expressed as Mean ± SEM of 6 rats in each group. The differences in mean weight of organs in extract treated groups were not significantly different from control group at the end of study (28 days).





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Table 2. Effect of ethanolic leaf extracts of *Carissa macrocarpa* on haematological parameters in sub-acute toxicity studies.

Groups	Dose mg/kg	Hb (g/dl)	RBC (million/mm ³)	WBC (1000/mm ³)	Differential count				
					Neutrophils %	Eosinophils%	Basophils %	Lymphocytes%	Monocytes %
Control	-	13.17±0.31	9.32±0.21	10.33±0.50	26.33±0.33	2.73±0.08	0.17±0.005	76.33±0.49	2.67±0.21
EECM	100	14.00±0.37 ^{ns}	8.73±0.39 ^{ns}	9.33±0.42 ^{ns}	25.00±0.26 ^{ns}	2.12±0.06 ^{ns}	0.16±0.009 ^{ns}	76.17±0.40 ^{ns}	3.00±0.45 ^{ns}
EECM	200	14.17±0.31 ^{ns}	8.58±0.27 ^{ns}	9.83±0.40 ^{ns}	26.33±0.21 ^{ns}	2.32±0.09 ^{ns}	0.17±0.006 ^{ns}	76.67±0.33 ^{ns}	2.83±0.31 ^{ns}
EECM	400	14.33±0.33 ^{ns}	8.85±0.35 ^{ns}	9.50±0.22 ^{ns}	25.33±0.67 ^{ns}	2.50±0.05 ^{ns}	0.17±0.006 ^{ns}	74.50±0.34 ^{ns}	2.67±0.33 ^{ns}

Values were expressed as Mean ± SEM of 6 rats in each group.

The mean values observed in Hb, RBC, WBC and Differential cell count of extract treated groups were not significantly different from control group at the end of study (28 days).

Table 3. Effect of ethanolic leaf extract of *Carissa macrocarpa* on biochemical parameters in sub-acute toxicity studies.

Group	Dose (mg/kg)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total Protein (g/dl)	Total cholesterol (mg/dl)	Total bilirubin (mg/dl)
Control	-	191.83±1.28	80.33±0.67	230.50±0.56	7.23±0.03	121.33±0.42	0.50±0.004
EECM	100	190.50±0.76 ^{ns}	79.83±0.70 ^{ns}	235.67±0.42 ^{ns}	7.04±0.04 ^{ns}	122.67±0.42 ^{ns}	0.45±0.019 ^{ns}
EECM	200	190.33±0.80 ^{ns}	81.00±0.37 ^{ns}	236.17±0.48 ^{ns}	7.10±0.07 ^{ns}	122.67±0.33 ^{ns}	0.45±0.015 ^{ns}
EECM	400	190.33±0.67 ^{ns}	81.33±0.33 ^{ns}	233.33±0.80 ^{ns}	7.13±0.03 ^{ns}	121.50±0.43 ^{ns}	0.44±0.011 ^{ns}

Values were expressed as Mean ± SEM of 6 rats in each group.

The mean values observed in biochemical parameters of extract treated groups were not significantly different from control group at the end of study (28 days).





Impact of Nickel Stress on Germination, Seedling Growth of Mustard [*Brassica campestris*]

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ABSTRACT

Pollution of Environment due to man made activity like uncontrolled discharge of contaminated industrial and mining effluents has become an issue of major concern. Seed is the developmental stage that is highly protective against different external stress in plant life cycle. However soon after imbibitions and subsequent vegetative developmental processes, they become stress sensitive. So seeds are thought to carefully monitored against such external parameters as light, temperature and nutrient in order to maintain the protective state although such critical regulatory mechanisms are likely to operate in seeds at the onset of imbibitions. In this study, germination was conducted in Mustard [*Brassica campestris*] in order to find out the effect of nickel toxicity on germination and growth parameters of seedlings. The seeds of mustard were germinated in six different concentrations of nickel salt solution having 0-50 mg/l of nickel. It was found that there were very less number of seed found to be germinated in petriplates which was treated with 100 ppm of iron solution. Germination percentage, Radicle length and Seedling vigour index, Metal tolerance index were reduced and the percentage of phytotoxicity was increased. The soil culture experiment revealed that, shoot length, root length were found to be increased upto 20 mg/Kg of soil and above that concentration it was found to be decreased with increase in concentration of nickel. It was also observed that seeds of Mustard showed better result in terms of growth upto 20mg/kg of nickel in soil thereby indicating that nickel within 20mg/kg could facilitate the plants growth.

Keywords: Nickel, Pollution, Germination, Growth, Mustard, Sustainable Agriculture



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INTRODUCTION

Environmental pollution has increased dramatically due to rapid increase of mining and industrialization. Heavy metals are significant environmental pollutants, and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. The term “heavy metals” refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration. There are 35 metals that concern us because of occupational and residential exposure, out of which 23 are the heavy elements or “heavy metals”. Heavy metal can include elements lighter than carbon and can include some of the heaviest metals [1]. Metals such as aluminium, arsenic, cadmium, cobalt, chromium, copper, lead, manganese, mercury, nickel, selenium and zinc have been considered as the major environmental pollutants and their phytotoxicity has been established [2, 3, 4, 5]. It has been reported that metals such as cobalt, copper, chromium, iron, magnesium, manganese, molybdenum, nickel, selenium and zinc are essential nutrients that are required for various biochemical and physiological functions. Inadequate supply of these micronutrients results in a variety of deficiency diseases or syndromes. Elevated concentrations of both essential and nonessential heavy metals in soil and water can lead to toxicity symptoms and growth inhibition in most plants [6, 7, 8]. Absorption, translocation and accumulation of heavy metal ions of Hg, Pb, Cr and Cd by plants, reduce qualitative and quantitative productivity of the species and cause serious health hazards through the food chain to other life forms [9, 10, 11, 12, 13, 14, 15]. Different heavy metals at supra-optimal concentrations have been shown to inhibit various metabolic processes in plants resulting in their reduced growth and development [16, 17, 18, 19, 20, 21]. As nickel is one of the serious metal pollutants, this work was undertaken with an objective to determine its effect on the growth and development of Mustard seedlings in presence of nickel toxicity.

MATERIALS AND METHODOLOGIES

The present study was undertaken with nickel chloride solutions at 10, 20, 30, 40 and 50 ppm along with control (untreated). Seeds of Mustard each of were surface sterilized with 0.1% mercuric chloride and washed thoroughly with tap water and then with distilled water. Fifty uniform sized seeds were placed in petri-dishes of 10 cm diameter with different concentrations of Nickel chloride solution. The seeds of mustard were germinated in six different concentrations of nickel salt solution having 0-50 mg/l of nickel and one with control at a constant temperature of 26 °C. The seeds were sown in soil culture medium (with nickel treated and untreated). Each treatment was replicated five times. The number of seeds germinated in each treatment was counted on 5 days after sowing and the total germination percentage was calculated. Tolerance index and Vigour index of seedlings were calculated. This experiment was done in triplicates and the data was statistically analyzed and standard errors of mean (SEM) was calculated.

RESULT AND DISCUSSION

Germination study

Significant changes were found in the germination in different concentration of nickel. The germination percentage was decreased with increased concentration of nickel. Observations on the germination study of Mustard seedling depicted in Table 1. It was clearly indicated that the Seedling vigour index and Metal tolerance index were decreased with increase of concentration of nickel while the phytotoxicity was increased with increase of nickel concentration. The significant decreases in radical length of Mustard seedling suggest that low concentration of nickel was beneficial for seed germination as well as seedling growth.





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CONCLUSION

Toxicity of heavy metals has received considerable attention partly due to its occurrence in nature and by mining activities. The data on growth parameter study showed that, with the increase in nickel concentration, the growth rate decreased progressively. Nickel at higher levels may inhibit the growth and development directly by inhibition of cell division or cell elongation or combination of both, resulting in the limited uptake and translocation of nutrients as well as water which causes mineral deficiency. At higher concentrations it acts as a toxic metal. From the result of this investigation, it can be concluded that iron at lower concentration has a stimulating effect on the germination process and seedling growth and will inhibit the same at higher concentrations. This research work reflects the impacts of heavy metals on plant growth and its impacts which ultimately reaches us through food chain. It is the need of the hour to know about the way of toxicants passes through it and to promote the sustainable agriculture that links to SDG 2.

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Table 1: Effect of Ni on Mustard seed germination

Concentration of Fe	Germination (%)	Radicle length (in cm)	Seedling Vigour Index	Metal Tolerance Index	Pytotoxicity (%)
0.0 ppm (control)	95±2.5	4.8±0.35	456	100	0
10 ppm	80±1.5	4.5±0.25	453	93	6.25
20 ppm	80±2.5	4.6±0.15	368	95.83	4.16
30 ppm	65±1.5	3.4±0.15	221	70.83	29.16
40 ppm	50±1.5	2.6±0.25	130	54.16	45.83
50 ppm	35±2.5	1.2±0.15	42	25	75

Values of 5 replicate ±SD

Table 2: Effect of Ni on growth parameters of Mustard seedling in soil culture

Concentration of Fe(mg/kg of soil)	Root length (cm)	Shoot length (cm)	No. of seed sown	Number of seedlings survives	%ge of survival
0.0	3.6±1.5	2.4±0.55	20	18±1.5	90
10	3.8±1.25	2.5±0.25	20	19±0.25	95
20	3.9±0.55	2.8±1.5	20	18±0.5	90
30	2.9±0.25	2.2±1.5	20	14±1.5	70
40	2.2±1.5	1.8±0.5	20	12±0.25	60
50	1.8±0.55	0.8±0.25	20	10±1.5	50

Values of 5 replicate ±SD





Road Safety Audit Confirming the Highway Safety Standards; Case Studies Kalahandi, Odisha

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ABSTRACT

India standing at around 1.38 billion population by 2020 is under anthropogenic stress like urbanisation, industrialization and modernization. Over population have surged up the road sector & transportation graph for better connectivity. The present burgeon in automobile and vehicular movements has harshly raised the number of accidents above 450k and the fatalities 1.51k specifically affecting the productive juvenile group of range 15-35. India, a signatory in 2015 to Brasilia meet, declared to halve its road accident by 2022 but not complied. The frequent road accidents and rate of fatalities necessitate the road safety audit in 21st century is warranted for the Indian roads. Present work envisages the public health concerned with road safety in national highway (NH- 26) over hill terrain connecting Bhawanipatna to Koksara in Kalahandi district of 60.2km length lacking any road safety appraisal. The number of road accidents have surged since last 20years particularly in NH-26. Field visit of the stretch of road and identifying the ground profile that fail all the modern geometric norms of the highway. The inferences are that road accidents are the result of several repeated mistakes and casually repeated errors made by the drivers, night sleepiness and the road users, or mechanical faults. Disobeying traffic rules, bizarre speed, debilitate road design, and network are the most common causes of road crashes and fatalities in NH 026. Therefore, it is too alarming and the need of the hour to prepare the road safety audit of the road under investigation and proceed effectively in implementing the right measures for road safety.

Keywords: Road safety audit, road accidents, Traffic rules, Geometric design, Road safety Inspections, Road signals





INTRODUCTION

Road mishaps have been a most significant source of concern in India and globally. In the year 2019, India faced over 151K fatalities due to street accidents. India invests 3 to 5% percent of the nation's GDP to avert these road accidents. World banks reported that India houses $\approx 1\%$ of the globe's number of vehicle and have 6% of the population, but it is of great concern that 6% of road incidents. The worst vulnerable group road mishap's about 70% are the young and juvenile age group, the India's future. The RSA, (Road Safety Audit), practices the official safety recitals, inspection of a standing/future road or intersection/roundabouts by a self-governing, multidisciplinary group of officials or road safety managers, <https://safety.fhwa.dot.gov/tsa/>.

The road network of India is cumulatively equal to a 6.2million km., which is the 2nd largest in the world. However, the National highways of our country contribute only 3% of the total road network but it carries around half the total traffic volume of the entire country. Technological revolutions and easy finance have upgraded the lifestyle of lower and middle-class families thereby letting them opt for personal automobiles. Industrial developments have also given rise to the use of more heavy vehicles on road. Despite all these developments, the road structure of India is too slow in the process of up-gradation. Moreover, the new roads constructed come under the sheer pressure of corruption and forgery resulting in its immediate debilitation and damage. Damaged roads including potholes often catalyse the rate of road accidents. Around 1.5 lakh people die in road accidents in 2018, which is a comparatively much higher rate as compared to death, caused by diseases like malaria, TB, etc. UN has also declared the decade of 2011-2020 as the road safety decade intending to enhance the priority of the Road Safety Audit (RSA). The RSA is a combined approach that includes analysing and inspecting new or existing road networks by a special team thereby focusing on planning, designing, constructing, operating, and maintaining these roads as per the requirements. RSA have the role of well judging the existing road safety, and recommending the inevitable investment, which is on enactment, will surely help in identifying and nullifying the flaws in the road architecture and traffic management. RSA shall plummet the redundant loss of life, mostly the young/juvenile group caused by road accidents in India.

Statistics Accidents In India

As per the annual reports of the National Crime Records Bureau (NCRB) – a total of 3, 54,796 road accidents were registered during the year 2020 out of which 1,33,201 were the number of total deaths and 3,35,201 injured cases. Moreover, the number of lives lost per 100 road crashes was 35 in 2019, 34 in 2018, and 32 in 2017. This depicts the severity of accidents and their increasing curve. On average, the annual count of road accidents in India is 450000 out of which 150000 people die. More scintillatingly, records reveal that there are 53 road accidents in our country per hour. In 2015 after becoming a signatory to Brasilia's declaration on road safety, India had pledged to reduce traffic fatalities and road accidents by 50% by 2022 but is way behind the mark today (MoRTH, 2020[1]). The main cause of road accidents can attribute to the enormous increase in the use of vehicles on roads in the last decade. The road demography and the network has been under upgrading and grown by 40% in our country whereas the newly registered vehicle has grown by 160%. More precisely, highways both state and national comprise about 5% of the total road network but witness 52% of total accidents. This clearly shows that although there is a continuous growth of vehicular use on roads, particularly on NH, the capacity and quality of roads are still constant and are not increasing at the same pace thereby resulting in unavoidable regular congestions, old road degradation, and informal driving leading to consecutive accidents and other fatalities. Even considering the accident fatality index for the year 2019. The state Tamil Nadu held the first rank with several 57228 numbers of road accidents followed by Madhya Pradesh and Karnataka in the year 2019. Accidents are very common during peak hours. About 67% of accidents occur between 9 am to 9 pm. As per NCRB, the Odisha state is accident-prone and an average of 33.1% of deaths occur against the total accidents in India, <https://ncrb.gov.in/sites/default/files/accidental-deaths-11.pdf>. Road accidents are globally killing more than 1.25 million people and injuring 50 million people per annum. It is worth noting that the major public health problem by WHO. About 90% of the above casualties occur in developing countries only.





LITERATURE REVIEW

India houses 1% of the vehicles on earth but 11% of the universal demises are from road mishaps that transpire in India, as per the World Bank, (ShyriahNiazi, 2021[2]). High speed, traffic-less streets, lacking law enforcement, alcohol, drug abuse, non-use of safety gear (helmet or seat belts), red-light jump, distraction to drivers, defying traffic rules, and faulty overtaking *etc.* are responsible for road accidents in India, Gopikrishnan *et al.*, 2012[3], Sharma *et al.*, 2018[4], Pal *et al* 2019[5], Gutierrez, *et al.*, 2022[6]. COVID -19 has reduced both the frequency of street clashes and resulting deaths due to lockdown, shutdown, quarantine, closed industries, and blocked roads, Yasin *et al*, 2021[7], Peralta *et al*, 2022[8]. Among the most vulnerable age group with high risk are the youths, Raina *et al.*, 2016[9]. Vehicle standard, maintenance, traffic culture defiance plays vital role in RTI's in India, Biswas 2015[10], Maheswari 2017[11], Paul, 2019[5]. Road safety audit has become inevitable in the Indian context to reduce the rising numbers of transport vehicles, frequency of accidents and fatalities, Vardaki *et al.*, 2014[12]. World Health Organisation (WHO) has reported that road traffic injuries are one among 10 global killers are 20% due to RTI (Road Traffic Injuries) with DALY up to 15%, Paul *et al.*, 2019[5]. Number of road accidents and related deaths reported in Odisha state were 11064, and 5333 during the year 2019, Morth report 2019[1]. The necessity implementing on of road safety audit (RSA) is well felt recently in India as an aftermath effect of continuous road accidents and safety threats and especially for roads in Hilly terrain. (Mishra *et al*, 2021). The qualification to become a road safety auditor /inspector not yet assigned at present except few particulate training programs aimed at imparting sufficient knowledge on road safety audits. Enhancement of road safety by Highway Engineers is achievable during planning, design, construction and repair and maintenance stage, Velmurgan, 2016[13].

RSA in India

The RSA aims at reducing the life cycle price, risk and vulnerability of road crashes, health issues of the road scheme. The regular safety audit shall develop a safe road consensus and lower the over all, annual repair and maintenance cost along with decrease of road accidents, related trauma and deaths. CRRRI conducted the first of such audits, and started training stakeholders during the year 2000 on the Indore Bypass. The severity of road accidents in India was increasing rigorously. Based on the facts, the International Road Federation (IRF) advised India to prioritize the requirement for such regular audits. As a result, to this, the ministry of road finalized to implement such audits as mandatory for every new construction and as an inspection tool for existing ones. Later in the year 2014, the Ministry of road transport and Highway (MoRTH -2018[14]) decided to prepare a procedure to inspect the existing road section through such audits. MoRTH also mandated and issued guidelines to its subordinate agencies that all road projects costing more than \$800000 will have to undergo road safety audits.

RSA in Odisha

In the Bhubaneswar city Odisha, during Jan-Dec 2012 the numbers of road accidents as per the Regional Transportation Authority (RTA) were 625, and reported deaths are 30% of the accidents. About 84% of all accidents befell in towns and particularly on National Highways (NH), (46.7%), 18% of RTA happened during heavy rainfall, though no significant association simulated. Rather as per the reported data, the frequency of accidents attributed to motor cars (37%) and trucks (19.1%) was predominately involved. The majority of the victims were in the productive age group, 18–24, years and mainly constituted males (68%).

METHODOLOGY

The RSA objectives is to minimally optimizing total cost, severity of road clashes, (R/M), repair and maintenance post completion stage of a road project. RSA also create awareness towards road safety measures during planning, design, ongoing and post completion of the project. RSA is an organized process for inspecting the road safety implications used for highway up-gradation and new road schemes. The main objective of the process is to diminish future road crashes and their fatality once the scheme completed that make the road into use. The prominent work of a road safety auditor is to account for the cases of all road users and particularly the vulnerable groups like pedestrians and pedal cyclists. After identifying and collecting the possible potential road safety problems, the





Jyotiprakash Bagh and Siba Prasad Mishra

auditor then makes advisory recommendations for improvement. The requisite client then proceeds by studying the report and the recommendations provided further keeping in mind the best possible viable solutions to the identified lacunas. In India, the entire RSA process consists of 5 stages that are linearly arranged to ensure that the needs of all road users are considered during each phase of road development for smooth traffic, reduced VOC (Vehicle Operating Cost) and reducing travel time, safe movement of traffic and finally the GDP and economic growth of the state.

Feasibility or planning stage

Detailed designing stage

Construction stage

Pre-opening stage

Existing/maintenance stage

Feasibility or planning stage

During reconnaissance survey for road projects the road safety are to be foreseen by considering Land use and land cover (LU/LC) of the terrain, provision of more bypasses in congested roads, and self-contained zones to exclude traffic in the neighbourhoods. The concept of hierarchical system of road network planning should consider the forgiving highways, safe horizontal alignment and vertical profile, Geometric design and design parameters along with the safety needs of all and different user groups. This stage involves route choice, design standard, impacts on the next-door road network, intersection types etc.

Detailed designing stage:

The design stage should consider the Principles of Safe Design. Determining the norms and standards during design stage considering speed, cross section, geometry (both vertical and horizontal), separate lane for fast uninterrupted traffics, and provision of various road/traffic controlling fixtures (lighting, signals, sight distances etc.) so that after implementation the road shall be well self-explaining, lean roadside to optimise least accidents. Along with proper vertical and horizontal design, the designer should give importance to facts like driver's behaviour and errors, roadside services, rest places, truck Lay-by/ terminals etc.

Construction stage:

Safety at construction site should be in strict compliance with IRC: SP-55 [2013] (I), and provision of protection should confirm time-to-time specification in various road congresses later on. The task demands segregation of construction zone by barricading or diversion roads, proper signals, safety, and atmospheric pollution control from dust, sound and water logging. During construction stage, the road safety manager should emphasize on speed Management considering the road hierarchy, signing, speed zoning, markings including those to create stereoscopic illusion. Main safety considerations need considering while designing two//four/Six lane national highways, expressways and main urban roads that common mistakes need to avoid.

Preopening stage:

Preopening stage of a newly constructed road have the road ready to use but the road and pavement safety architectures are sometime incomplete. Necessary action plans, signals, and traffic arrangements are necessary to avoid unnecessary collisions. The road safety team should inspect and pass the road and its safety aspectlike traffic Signs, road markings and delineators: before inauguration for regular use of any road.

Operation stage:

To avoid delay, accidents and fatigue, it is essential that the highways and urban traffic system. The roads should have provisions of ATMS (Automated Traffic Management System). They can be electrified roads (Auto freight charging roads), Smart wireless (digital traffic signs) in road, V2X; CV-2X; V2X, I2X, &P2X communications &VANETs (Talking car) with smart street lights, (Park et al, 2021[15], Dey et al., 2022[16], Miao et al, 2022[17])



**Jyotiprakash Bagh and Siba Prasad Mishra****History RSA/ RSI:**

Safety engineering in newly built roads in England during 1980's became black spots for accidents and deaths, emanated as Road safety audit (RSA). Road safety manual introduced during many countries in USA, s Australia, New Zealand, Denmark, later Malaysia, South Africa, and Singapore, in 1990's. Road safety inspection focusses on the provision, expansion, renovation, upgradations and maintenance of boulevards against prevention of accidents and trauma. The manual was for the planners, architects, designers, engineers, technocrats, consultants, contractors, managers and project workforce, irrespective of category of road and the work place. The road safety auditors empowered to be vigilant and apt in design standards, no compromise between load carrying capacity and road's operational safety factors (IRC 88 – 2019 (II)). The road connecting Koksora to Bhawanipatna, the Widening and strengthening to 2 lane with paved shoulder is under construction @ 290million INR (MoRTH, 2018[20]).

Case study of NH-26 (Bhawanipatna-Koksora)

This road safety audit focusses on the findings emergence for the road section from Bhawanipatna- to Koksora (NH 26) spanning a total length of 60.2 KM. Bhawanipatna is the district headquarter of the district of Kalahandi. It is also a prime location in the Kalahandi district having a population of about 85 thousand. Koksora is one of the Towns in the Dharamgarh sub-division in Kalahandi District. NH-26 leads to Vijanagaram of Andhra on the south side and leads to Raipur in Chhattisgarh. The safety audit has performed from the place Bhawanipatna to Koksora that is in Kalahandi District, India, where the road conditions are studied and safety measures reported. The accident-prone NH-26 is our present case study. The new Indian express poised concern that the segment between Koksora and Bhawanipatna have many black spots and need immediate RSA, The New Indian express, 23rd July 2021. As per the news agency there are 8 numbers of black spot expanses within Ampani and Kuadala within the stretch of road considered for investigation (Table 1). In spite of empty roads due to the influence of the Pandemic COVID 19, there is alarming rise in the major and grievous injuries by 35.8% and 20.65% in number of accidents. The number of mortalities may be due to active health care services available in hospital due to actively availability of hospital attendance during the pandemic. There is few regular road mishaps and fatalities over the 60.2km long road and few cases, (Table-2) High Embankment/ Water body locating adjacent to the road consider, as potential safety is the concern. MBCB need installation at the edge of the formation along the span of water body with pasting of delineating Reflector to increase visibility at night and enhance safety of the user as per IRC: 73-2015 (III), Fig 3 (a), (b) & (c)

Objects Hazard Markers

Some very risk zones are reported during risk audit

Lacking of and vandalized object hazard markers at bridge are potential safety hazard both during night hours but also in day. Hazard marker needs installations at every parapet of CD/bridge on faces of traffic direction with conforming IRC-67:2012-15.64(IV). (Fig 4 (a), (b), (c) & (d).

Deficiency at Curves

Lack of informatory sign (speed limit, Left/Right turn), Chevron signboard, solid centerline & raised pavement markers major safety concern. All curve shall have warning and signs and warning sign board with conforming IRC: 67-2012 and curve less than 450m radii are no overtaking zone, need markings solid line at center as per IRC: 35(V) Fig 5(a), (b), (c), (d):

Vandalized and Missing Sign Boards

Lack of safety measures on School/ Hospital zone. Transverse Bar Marking and Pedestrian Crossing conforming to IRC 35-1997 need have provision along with speed limit sign, No horn Sign, and rumble sign near all schools and hospitals located near project road. School Ahead sign need to be provided only on one side of the project and both sides are required





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Minor Junction

At Atrial, road major road ahead sign, stop sign, and rumble signboards are not provided leads to confusion In the mind of the user and the potential for a head-on collision. At every approach road, a Major Road ahead sign, stop sign, and rumble signboard shall need installation to convey a clear message to the users. The regulatory, warning and informal signs need displaying as per requirement Fig 7 (a), 7(b), 7(c), 7(d) .

Requirement of Road Delineators

Retro Reflective Sheet were not pasted on a tree near to carriageway/ shoulder is hazardous or the user at night. Tree/ Object near to shoulder shall be pasted Retro Reflective Sheet to increase visibility at night and enhance the safety of the user Fig 8 (a), 8 (b), 8 (c), 8 (d) .

DISCUSSION

The RSA is an informal check and part to traffic management are personnel with apposite prerequisite, qualification, skill and experience beyond the design segment. The safety audit team should be vigilant in maintaining pavement standards, accident investigations, regular design check and suggest for innovations in road safety that should be comprehensive, reasonable and practicable. The Road safety audit report has been prepared and given in IRC: Road dev. Plan [21] and NHAI Safety Manual[VII], Table 2 and Table 3 and Fig 9(a) & ((b) This project road starts from CH-181.000Km of Bhawanipatna, and ends at Koksora at CH-239.280 Km of length 58.28 Km.. The stretch CH-227.550 to CH-227.800, CH-233.150 to CH-234.510, and CH-235.650 to CH-236.600 is double lane (7.0 meters) and CH-181.000 to CH-227.550, CH-227.800 to CH-233.150, CH-234.510 to CH-235.650, and CH-236.300 to CH-239.280 is Double Lane + Paved Shoulder (7.0 m + 2 x 1.5 m). Though eight stretches of road detected, major damage has occurred from Ch 181/000 to Ch 239.80. Fig10,& Table-3

Recommended action plans after RSA:

- The NHAI/R&B authorities' needs to be swap substitute or renovate the dented signage, solar blinkers, speed limit boarding, rambler and speed breakers.
- Constant police watch and ward on the heavy traffic roads during peak hours. The patrolling vehicles should be alert at night for immediate health care attendance immediately after the trauma caused due to clashes.
- Reflective Sheets were need pasted on tree near to carriageway for avoiding accidents.
- The road in patches should be under CCTV's surveillance at the black spot areas
- For effective use of the roads, the specialist's team should conduct RSA as independent entity to the design process.
- The Projects need RSA, that are major four-laneroads, reconstruction, realignment projects, roundabout, Intersection projects both signalized and non-signalized.
- Pedestrian and bicycle routes should be separate from the NH/SH roads.
- The Traffic management, need organised and disciplined in the accident drop schemes
- Old culverts need to be wider and renovated to accommodate the 21st century increased traffic specifically in hilly portion (Mishra *et al*, 2021)
- In addition towards Surface defects to be checked biannually by the NSV (Network Survey Vehicles)
- To check Roughness of pavement, to check loss of grip of tyre biannually by laser profile-meter.
- To safety of bridges to be checked biannually by MBIU team (mobile bridge inspection unit).
- To check the road strength, to be checked once annually by falling weight reflectometer (FWD)
- Road signs and signals are to be inspected by RSA team biannually using Retro-reflectometer Road signs Retro-reflectometer At least twice a year





CONCLUSION

In this study, the main objective of RSA was to account all possible safety lacunas and deficiencies in accident-prone zones across the two-lane highways and to prescribe appropriate practical recommendations and solutions to tackle them. During the inspection, various risk factors were observed and identified and it was found conclusive that elements of risk in the case of two-lane highway were different from that of 4 lane highway. Following were the findings of the safety assessments conducted based on RSA format and procedure:

Sight distance: The main requirement for a lucid and smooth flow of traffic on NHS and high-speed corridors is proper. But throughout the study, it was found that there was a lack of adequate sight distance across horizontal curves and intersection points. Proving healthy sight distance can surely accentuate the smooth movement of traffics in many cases.

Focussing the roadside buildings and locations: A specified judicious speed limit for riders fixed and maintained across vulnerable buildings like medical, schools, and other crowded market places to maintain the smooth flow of traffic. Certain illegal encroachment of roads by vender and builders prohibited immediately to reduce the narrowing of highways and other roads.

Visual alert signals: Signboards depicting speed limits and various traffic signs including pedestrian safety must have light display to make the junction places. Different types of roads and areas require different speed adjustments. Hence, steps should be clearly visible and made to the riders get aware of this with the help of signboards installed within regular road intervals.

Road safety awareness: In most cases, there is lack of basic and common driving tactics sensed among many riders and drivers. They were not accustomed to dense roads. Hence, proper awareness programs carried on from the schooling level to enhance their skill in driving and maintaining road safety. Further intermediate small awareness programs and workshops for heavy vehicle drivers must be done within intervals to upgrade their skills in driving and make them familiar with new safety standards, traffic signs, the various vehicle acts and driving rules, and the penalties for breaking such rules.

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IRC: 73-2015
IRC-67:2012-15.64
IRC-35 :1997 (1st revision)
IRC-67:2012-15.64
NHAI Safety Manual.





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Table 1 : the accident status in various roads (NH 26 as major) in Kalahandi district of Odisha (The New Indian express published dated 23rd July 2021.

The type of trauma	January to June 2021	January to June 2020	% of rise/ fall
Number of Accidents	187	155	20.64516129
Number of Mortalities	75	83	-9.638554217
Number of Major Trauma	110	81	35.80246914
Number of Minor Trauma	52	56	-7.142857143

Table 2: Various available fatal accidents over NH-26 in Kalahandi district from 2014 to 2021

Date	Place	Cause of mishap Kalahandi dist.	Causalities/ death	Source
July 14, 2021	NH -26	Running over on a road bridge	1 dead ; 2 injured	Odisha Post
Jul 27, 2021	NH-26	Struck roadside palm tree	3dead; 2injured	Sambad
Feb 17, 2020	NH -26	Dashed against a vehicle	1dead	OMMCOM News
Jan 28, 2019	NH -26	Cannot negotiate sharp curve	2dead, 30injured	Times of India
Sept 1, 2019	NH- 26	3 traumatised bus hit bike	2dead, 1 hurt	Kalinga TV
May 22, 2019	NH-26	Careless driving	5dead 2injured	The Siasat daily
Apr 13, 2018	NH- 26	Fall to a 10m gorge	2dead, 30 injured	Times of India
Dec 13, 2016	NH-26	Death by hitting tree at night	2dead, 2 injured	Times of India
Jan 12, 2015	NH- 26	Hit motor cyclist, then a tree	7dead, 2 injured	Times of India
Feb. 20, 2014	NH-26	Hit roadside tree (night)	8 dead	The States man

Table 3: The RSA report of the NH 26 from Bhawanipatna Ch.181/000 to Koksora ch.239/280

Findings	CH	Risk	Description	Priority	Recommendation
Metal Beam Crash Barriers: High Embankment	CH-188+350 RHS CH-234+850 LHSCCH-185+330	Very High	High Embankment/Water body locating adjacent to the road are considered as potential safety Concern.	Essential	MBCB need installation at the edge of the formation along the span of water body with pasting of delineating Reflector to increase visibility at night and enhance safety of the user as per IRC:73-2015(III).
Objects Hazard Markers	CH-216/500 CH-237/850 CH-223/200 CH-118/400	High	Lacking of and vandalized object hazard markers at bridge are potential safety hazard not only during night hours but also in daytime.	Highly Essential	Object hazard marker need to be installed at every parapet of CD/bridge on faces of traffic direction with conforming IRC-67:2012-15.64 (VI)
Deficiency at	CH-188/950	High	Lack of informative sign (speed limit, Left/Right turn), Chevrons sign board, solid center line &	Essential	All curve shall have warning and informative and warning sign board with conforming IRC: 67-2012 and





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Curves	CH-201/675 CH-202/750 CH-189/420		Raised Pavement Markers major safety concern.		Curve having less than 450m radii are no overtaking zone, shall be marked solid line at center as per IRC:35; Lianzo <i>et al</i> , 2019 ^[22]
Vandalized and Missing Sign Boards	CH-224/560 Hospital area CH-226/950 School area CH-200/900 School A area CH-185/250 School A area	High	Lack of safety measures School /Hospital zone.	Desirable	Transverse Bar Marking and Pedestrian Crossing confirming to IRC 35-1997, need to be provided along with speed limit sign, no horn Sign and rumble sign near all School and hospital located adjacent to project road. School A head sign need to be provided only in one side of the project and both side required.
Minor Junction	CH-228/500 CH-216/100 CH-210/000 CH-205/600	High	At Atrial Road major road a head sign, stop sign and rumble sign boards are not provided lead confusion in the mind of user and potential for head on collision.	Desirable	At every approach road, Major Road ahead sign, stop sign and rumble sign board need installation to convey a clear message to the users.
Requirement of Road Delineators	CH-222/350 CH-223/900 CH-214/200 CH-224/930	High	Retro Reflective Sheet were not pasted on tree near to carriageway/shoulder is hazardous For user at night.	Desirable	Tree/ Object near to shoulder shall be pasted Retro Reflective Sheet to increase visibility at night and enhance safety of the user.





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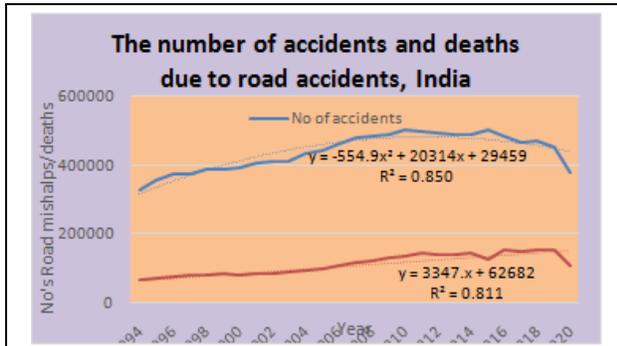


Fig 1: Numbers of road accidents and related fatalities in India (1994-2020)

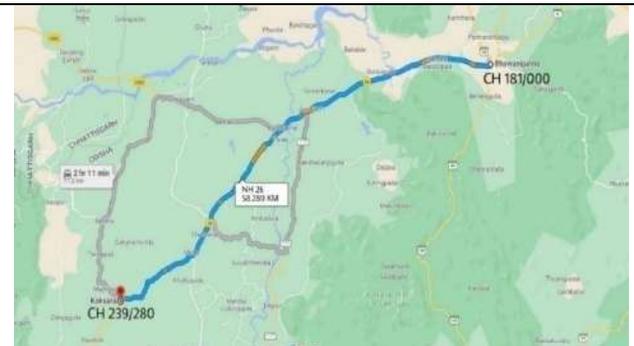


Figure 2: Stretch of NH 26, Bhawanipatna to Koksara (CH 181.000 to CH 239.280)



Fig 3 (a), (b)& (c): high risk zones of unprotected banks need metal beam crash barriers



Fig 4 (a), (b), (c) & (d): Risk zones due to want of hazard marking at risk zones of NH-26





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Fig 5(a), (b), (c), (d): High-risk zone due to lack of informatory sign (speed limit, Left/Right turn), Chevron signboard, solid centerline etc.



Fig 6(a), 6(b), 6(c), 6(d): High Risk areas with lack of safety signs in the school/Hospital





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Fig 7(a) CH-228/500



Fig 7(b) CH-216/100



Fig 7 (c) CH-210/000



Fig 7(d) CH-205/600

Fig 7 (a), 7(b), 7(c), 7(d): Minor junctions not taken care of the traffic signals in NH-26



Fig 8 (a) CH-222/350



Fig 8(b) CH-223/900



Fig 8 (c) CH-214/200



Fig 8 (d) CH-224/930

Fig 8 (a), 8 (b), 8 (c), 8 (d): The noncompliance of road delineators in NH -16





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Fig 9(a) & (b) : Some accident photographs in NH 26 within the Kalahandi district

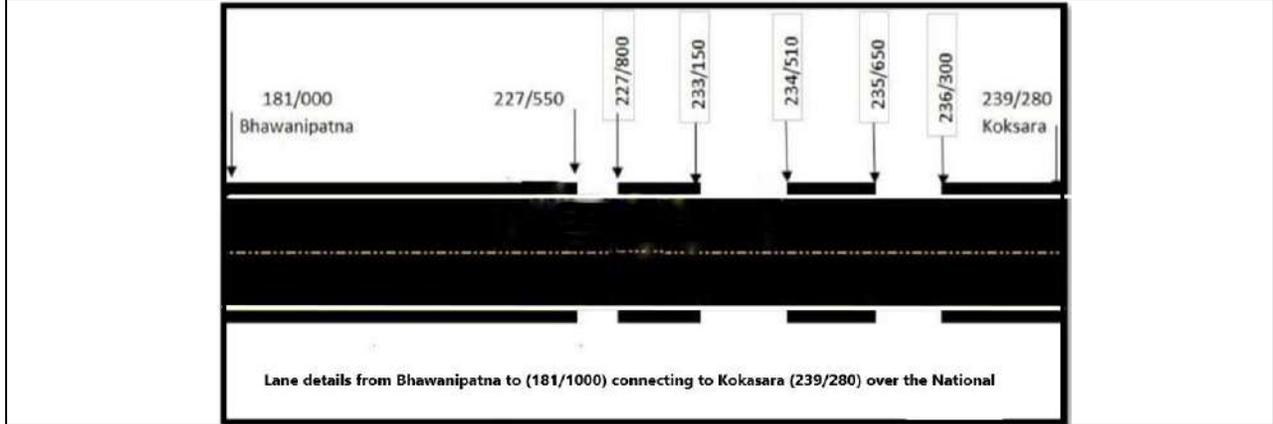


Fig 10: The lane details for NH -26 from Bhawanipatna to Koksora (CH 181/000 to CH 239.80)





Pulsatile Drug Delivery System- Recent Technologies: A Mini Review

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ABSTRACT

Pulsatile drug delivery systems (PDDS) provide many advantages over conventional dosage forms. They provide the drug at the right time, place, and dose, providing greater benefit than standard dosages and increasing patient compliance. The drug is released fast and completely as a pulse after a lag time. These products have a sigmoid release profile. These systems help medications with chronopharmacological behaviour, requiring nocturnal dosage, and pharmaceuticals with a first-pass impact. This review covers pulsatile delivery methods and technology. Marketed inventions including Pulsincap™, Diffucaps®, CODAS®, OROS®, and PULSYSTM use the same mechanism. For example, PDDS shows promise in treating asthma, peptic ulcers, cardiovascular diseases, arthritis, ADHD in children, and hypercholesterolemia. Pulsatile medication delivery devices may revolutionise the treatment of numerous diseases.

Keywords: Pulsatile drug delivery; Advantages; Classification; Marketed technologies.

INTRODUCTION

Oral drug delivery has been the largest market segment. It is the favoured drug delivery method. Oral controlled-release systems have a consistent pattern of drug release that keeps the medication concentration in the therapeutic window for a long time. Certain conditions necessitate a drug's release after a lag time. That is, they need pulsatile drug delivery (PDDS). Pulsatile systems are gaining popularity as they allow for controlled medication release. Pulsatile medication delivery provides spatial and temporal delivery, boosting patient compliance. A pulsatile drug delivery system either delivers the medication fast and completely after a specified off-release interval (lag time), or it has a novel mechanism of rapidly and totally delivering the drug after a lag time (lag time). Pulsatile release describes such a pattern. [1].



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The suprachiasmatic nucleus, the body's main circadian clock, regulates endogenous circadian rhythms. In order to treat disorders (asthma, myocardial infarction, angina pectoris, rheumatoid arthritis, ulcer, and hypertension) that have circadian rhythms in their pathophysiology, pulsatile medication delivery devices are required [2]. There are many other conditions that require pulsatile release, such as hormone secretion [including follicle stimulating hormone (FSH), LH, LHRH, oestrogen, and progesterone], acid secretion in the stomach, gastric emptying, and gastrointestinal blood transfusion. Tolerant drugs require a system that prevents their constant presence in the biophase, which reduces their therapeutic effect. The lag period is critical for medications that degrade in stomach acid and irritate the mucosa or cause nausea and vomiting (peptide drugs). To target a drug to a distal organ like the colon, the drug must be stopped in the second third of the GIT. First-pass metabolism drugs (like β -blockers or estradiol) require delayed release to minimise bioavailability, altered steady-state levels of drug and metabolite, and potential food drug interactions [3]. All of the above factors can be used to design a delivery system that releases the drug in a controlled manner at a specific site.

Advantages

Pulsatile drug delivery methods have many advantages. Listed below are a few:

- These systems can be used day or night.
- They minimise dose frequency, size, and cost, lowering adverse effects and enhancing patient compliance.
- Suit Circadian rhythms of physiological functioning or disorders.
- Drugs can be targeted to a specific place, like the colon.
- They protect mucosa from irritants.
- Prevents drug loss due to first pass metabolism.
- They maintain steady medication levels and prevent peak-valley swings[4].

Disadvantages:

- Poor drug loading capacity and incomplete drug release
- Multiple production steps

CLASSIFICATION OF PULSATILE DRUG DELIVERY SYSTEMS

Pulsatile drug delivery systems can be classified in to three categories:

Time-controlled pulsatile release systems**Delivery systems containing erodible coating layer****Bulk-Eroding system**

Bulk erosion occurs when water ingress exceeds deterioration. A critical molecular weight is reached when degradation occurs across the polymer sample. At this stage, breakdown products are small enough to be dissolved, and the structure becomes porous and hydrated. As a result, the medicine is not released until the critical molecular weight is attained. Several research groups have used this approach to study purposed formulation [5].

Surface eroding system

In a chronotropic system, the medicine is imprisoned in the core layer of hydroxyl propyl methyl cellulose (HPMC) and an extra layer of enteric-coated film outside it. It is made up of solid dosage forms coated with lipid barriers such carnauba wax and beeswax, as well as surfactants. When these systems are exposed to aqueous media, the coat emulsifies or erodes. Irrespective of gastric residence and gastrointestinal motility. The lag time and action start are governed by the polymer's thickness and viscosity grade [6].



**Syed Gouse Firoz et al.****Rupturable coating delivery system**

The medication is released when the covering layer disintegrates. Excipients with effervescent properties, swelling agents, or osmotic pressure can cause coating rupture. A tablet core covered with ethyl cellulose included an effervescent citric acid and sodium bicarbonate combination. The carbon dioxide created by water entering the core caused a pulsatile release of the medication once the coating ruptured. Dependent on the coating's mechanical qualities. Compared to more flexible films, the weak and non-flexible ethyl cellulose film ruptured adequately. The lag time increases with coating thickness and core table hardness [6]. A capsule-based system with a medication, swelling agent, and rupturable polymer layer was designed using superdisintegrants[7].

Bai et al. created a pulsatile drug delivery system made up of many particles separated into several delivery units, each with its own makeup. The individual particles were the same as the interior core, but the external coating layer thickness varied. The membrane rupture controlled drug distribution. The coating thickness and amount of water-soluble polymer required to accomplish pulsed release regulated the timing [8].

Capsule-shaped system with release plug

The pulsincap method was invented by R.P. Scherer International Corporation in Michigan. A plug is forced away by swelling or erosion, releasing the medicine as a pulse from the insoluble capsule body. A water-insoluble capsule encloses the drug reservoir. The medication was sealed within the capsule body with a swellable hydrogel stopper. When the capsule is exposed to dissolution fluid, the plug grows and eventually forces itself out of the capsule, releasing the medication. Lagging was controlled by the plug's length and insertion position [9].

Stimuli-induced pulsatile release system

Stimuli-based drug delivery systems release drugs in response to biological stimuli. The medication is released from the gels or micelles in response to stimuli that cause them to deswell, swell, or erode. In these systems, the medicine is released after a biological or chemical stimulus[10]. The drug is released from the gel by the fluid phase syneresis, by diffusion along a concentration gradient, by electrophoresis of charged drugs toward an oppositely charged electrode, and by eroding the gel or micelle complex. In the presence of a certain enzyme or protein, a stimuli-sensitive delivery system releases therapeutic drugs. These systems are ideal delivery candidates because they can be tailored to the task at hand. They are divided into:

Thermosensitive pulsatile release

Thermosensitive gels are hydrogels that change volume in response to temperature. Thermosensitive hydrogels have been studied as stimuli-responsive drug carriers. Biopolymer hydrogels are crosslinked networks of polymers. These gels shrink at a temperature related to the linear polymer's lower critical solution temperature (LCST). Hydrophobic groups like methyl, ethyl, and propyl are found in many temperature-sensitive polymers. Poly(N-isopropylacrylamide) (PNIPAm) is arguably the most widely utilised temperature-sensitive polymer. Swelling at temperatures below 32°C and shrinking at temperatures over 32°C have been seen in PNIPAm cross-linked gels. Krezoski et al. describe the use of a polyol polymer, such as Pluronic®, in reverse thermal gelation. This form of polymer gel has low viscosity at room temperature and rapidly increases viscosity with temperature. They used a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer (F-68) and polyvinyl alcohol to create temperature-sensitive drug delivery devices (PVA) [11]. The pulsatile release of acetaminophen occurred between 35°C and 40°C[12].



**Syed Gouse Firoz et al.****Chemical stimuli induced pulsatile release**

The newest focus has been on stimuli-sensitive delivery devices. These systems release therapeutic agents in response to enzyme, pH, or other chemical stimuli. This technology has been used to develop a system that can automatically release insulin in response to high blood glucose levels. Kazunori et al.[13] created a PNIPAAm gel with phenylboronic acid moieties that reduced glucose-induced swelling. This type of glyco-sensitive gel may be used in self-regulating drug release systems as well as actuators, regulators, and separation systems.

In a glucose-rich environment, such as the bloodstream after a meal, glucose oxidase catalyses the oxidation reaction of glucose to gluconic acid, which lowers pH to approximately 5.8. This reaction can cause a pH-dependent membrane to swell. The result was a dual membrane system with glucose oxidase immobilised on cross-linked polyacrylamide. As an intermediary between the insulin store and the detecting membrane, The barrier membrane was made of DEA-HPMA and N,N-diethylaminoethyl methacrylate [14].

Externally regulated pulsatile release system**Electroresponsive pulsatile release**

An electric field as an external stimulus has advantages such as exact control of current magnitude, pulse duration, and pulse interval. Polyelectrolytes are used to make electrically and pH responsive delivery systems. Electroresponsive hydrogels deswell, swell, or degrade when exposed to an electric field. P(AMPS-co-BMA) hydrogels were employed for electrically induced drug delivery devices [15]. Kwon et al. used P(AMPS-co-BMA) hydrogels for electrically induced drug delivery [16]. For example, when the fluid phase syneresis out, a drug is expelled from the gel, drug diffusion along a concentration gradient, and drug electrophoresis toward an oppositely charged electrode, a drug is entrapped, and the gel complex erodes, releasing the drug. Polyelectrolytes are used to make electrically and pH responsive delivery systems. Electroresponsive hydrogels deswell, swell, or degrade when exposed to an electric field. P(AMPS-co-BMA) hydrogels were used for an electric-stimulated drug delivery method [17].

Ultrasonically induced

Skin, lungs, intestinal wall, and blood vascular permeability are all improved with ultrasound. In controlled medication delivery, ultrasonography has been described multiple times. Kost et al. showed an ultrasound-enhanced polymer. Miyazaki et al. employed ultrasound to boost 5-fluorouracil release from an EVAc matrix up to 27-fold. The amount of 5-fluorouracil released increased with the power of the ultrasound [18].

Pulsatile magnetic release

One of the first externally controlled drug delivery systems examined used an oscillating magnetic to govern drug distribution from a polymer matrix. Incorporated materials such as magnetite, iron, nickel, cobalt, etc. respond to a magnetic field. The magnetic carriers used in biomedical applications must be non-immunogenic and non-toxic. The technique works by slowing the passage of oral medicines in the gastrointestinal system by magnetic attraction. Adding a magnetic component to capsules or tablets allows this. An external magnet can then slow down the speed of transit through the stomach and intestines, affecting the time and/or extent of drug absorption [19].

Marketed Technologies of Pulsatile Drug Delivery

Pulsincap™, Diffucap®, three-dimensional printing®, CODAS®, OROS®, IPDAS®, GEOCLOCK®, Ritalina®, Uniphyll®, Opana®ER are some of the marketed PDDS [20].



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CONCLUSION

The pulsatile drug delivery system is a newer breakthrough in the field of drug delivery that is easy to construct and has considerable therapeutic effects. These systems provide the medicine at the proper time, place, and dose. Contrary to popular belief, pulsatile medication delivery systems are particularly effective in treating circadian disorders. With the development of pulsatile medication delivery, one can be guaranteed of achieving safe and effective therapy. A number of disorders necessitate particular drug/bioactive delivery methods. With normal dose forms, the same cannot be done or the effects are just partial. The time-controlled pulsatile release of bioactive chemicals is required in the treatment of such illnesses. The aetiology of terrible diseases can be connected to the release of certain medications through these systems, improving therapy. Despite these achievements, there are still certain undiscovered features of pulsatile drug delivery that can offer new vistas through better engineering.

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A Comparative Study of LQR and PID Controller for the Speed Control of a DC Motor

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ABSTRACT

In this paper Linear Quadratic Regulator(LQR) technique is used for the speed control of DC Motor. Controller is used in order to facilitate the motor shaft for maintaining its speed of rotation with respect to a particular step response. The controller has been modelled in MATLAB and from the results it is observed that it gives better performance and less settling time on using LQR as compared to PID controller.

Keywords: DC Motor, Speed Control, PID controller, LQR

INTRODUCTION

Many industries prefer DC motor due to its efficient speed control characteristics even after having higher maintenance costs as compared to induction motor. This brought the attention of many authors towards the position control of DC motor and resulted in various methods for the control speed of such motors. The Proportional-Integral-Derivative (PID) controllers are the most widely used controller for speed and position control of DC motors. A position controller for the DC motor was designed by selecting the various parameters of PID and also by using genetic algorithm (GA) and again the PID controller parameters were tuned by using the Ziegler and Nichols method[7]. The first method was found to give better results than the second one.[4], presented and compared two types of controllers which are PID controller and optimal controller. (GA) is used to design the PID compensator,





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whereas Kalman filter helps to make the other compensator optimal and integral state feedback controller. The various simulation results lead to the conclusion that the second controller gives less settling time, less overshoot and better performance encountering with noise and disturbance parameters variations as compared to other controller. Other authors like [1], used a particle swarm optimization (PSO) instead of (GA). They gave a PID controller where the controller parameters were tuned using (PSO) method. The PID-PSO controller was modelled in MATLAB and the results were compared with a fuzzy logic controller (FLC) using PSO. The results showed that PID-PSO controller gives better performance and minimal rise time than FLC-PSO controller. A novel PID dual loop controller for a solar photovoltaic (PV) powered industrial permanent magnet DC (PMDC) motor drive is presented in [5]. MATLAB/SIMULINK was used in the analysis for the GUI environment. The optimal strategies for speed control of permanent magnet synchronous motor (PMSM) was introduced by linear quadratic regulator (LQR) and linear quadratic Gaussian (LQG) methodologies [6]. It was observed from the simulation results that these proposed controllers gave better performance with respect to design criteria like overshoot and settling time of the step response. A novel optimal PID controller using (LQR) methodology in tuning the parameters of PID controller is presented in [3]. Speed control of brushless DC motor (BLDC) is done by the new PID controller. So from all the MATLAB simulations and experimental output results it is found that the better performance is observed in the proposed controller as compared to the traditional controller. This paper represents a detailed study of LQR controller for controlling the speed of a DC motor. The rest of the paper is presented as follows: at first the plant model is discussed. The next section is all about the study of the PID controller technique and the design mechanism of LQR. Then comparative analysis of various results are simulated. At the End, paper conclusion is presented.

PID Controllers

A Proportional Integral Derivative (PID) controller is a simple controller which has three principle effects. The proportional (P) controller function is to give a change in the input directly proportional to that of the control error. The integral (I) controller function is to give a change in the input proportional to the integrated error. The main aim of the integral is elimination of the offset. The less commonly used derivative (D) action is used in some cases to speed up the response or to stabilize the system, and it gives a change in the input proportional to the derivative of the controlled variable. PID controller is a commonly and widely used feedback controller. It is basically used to calculate an error value which is the difference between measured process variable and a desired response. The controller tries to minimize the error by adjusting the process control input.

Linear Quadratic Regulator

The Linear Quadratic Regulator (LQR) is an optimal control method used in modern control theory in which the system is analyzed using the state state approach. It uses a simple state space approach in which multiple input – multiple output system is used and hence can be utilized in many wide applications. The measure of performance is a quadratic function made up of state vector and control input. When the linear time-invariant system is controllable, then the optimal control law is obtained by solving the algebraic Ricci equation. The minimization of the speed of the motor is the main function of Linear Quadratic Regulator (LQR). The input voltage is the speed of the motor and the output will be compared with the input.

Plant Model

In a DC motor the speed is proportional to the applied voltage whereas the torque is proportional to the motor current. Speed can be controlled by varying the battery tapings, changing the supply voltage, or by changing the armature resistances. In Fig.1 shows the armature circuit of a simple dc motor model where armature resistance (R_a) is connected in series with an inductance (L_a), and a voltage source (e_b) representing the back emf (back electromotive force) induced in the armature when during rotation.

The relation between the motor Torque T_m and armature current, i_a , is given as;

$$T_m = K_i i_a \quad (1)$$

Where K_i is a torque constant

The back emf, V_{emf} , is related to angular velocity by;





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$$Vemf=kb \omega m =kb \frac{d\theta}{dt} \tag{2}$$

By applying KVL in fig.1 :

$$La \frac{di_a}{dt} +Raia=V-kb \frac{d\theta}{dt} \tag{3}$$

$$Jm \frac{d^2\theta}{dt^2} +Bm \frac{d\theta}{dt} =Kiia \tag{4}$$

The state-space representation of the plant model which is given by the equations:

$$\dot{x} = Ax + Bu \tag{5}$$

According to equations from (2) to (4), the state space model will be:

$$\dot{x}_1 = x_2 \tag{6}$$

$$\dot{x}_2 = -\frac{1}{L}x_1 - \frac{R}{L}x_2 + \frac{1}{L}V - \frac{kb}{L}x_2 \tag{7}$$

The DC motor parameters assumed for this research work are

Symbol	Value and unit
E	= 12volt
Jm	= 0.01kgm ²
Bm	= 0.00003kgm ² /s
Ki	= 0.023Nm/A
Kb	= 0.023V/rad/s
Ra	= 1Ω
La	= 0.5H

Block Diagram

MATLAB Script file

The simulation procedure may be summarized as follows:

- Let's take the data of the DC motor as input,
- State space representation of the plant model is written as in eq.(6)
- Open loop transfer function and the closed loop step response is found out
- Lastly system response of PID controller and LQR controller is studied and their results are compared.
- Then output will be taken as ωm(s) from Fig.4.

MATLAB script file is:

```
clear
clc
t = 0:0.001:10;
% DATA1=====
J = 0.01;
b = 0.00003;
K = 0.023;
R = 1;
L = 0.5;
A = [-b/J K/J
```





```

-K/L -R/L];
B = [0
     1/L];
C = [1 0];
D = 0;
% sys = ss(A,B,C,D);
num=K;
den=[(J*L) ((J*R)+(L*b)) ((b*R)+K^2)];
open=tf(num,den);
closed= feedback(open,1)
%=====PID=====
Kp = 150; %
Ki = 150; % took by try
Kd = 0.4; %
PID = tf([Kd Kp Ki],[1 0]);
PIDsys = feedback(PID*open,1);
% Linear Quadratic Regulator design LQR
% ++++++
Q=[.2 0;0 0.028];
R=[.2];
[KK,S,e]=lqr(A,B,Q,R)
ZZ=(A-B*KK);
LQR=ss(ZZ,B,C,D);
damp(LQR)
[num1,den1]=ss2tf(ZZ,B,C,D,1);
G=tf(num1,den1) %ALWAYS den=1 BECAUSE ONLY u
% ++++++
% Linear Quadratic Regulator design LQR Step
% ++++++
figure(1)
step(closed,t),title('Closed Loop step response')
xlabel('Time','FontSize',11);
ylabel('P.U. speed','FontSize',11);
figure(2)
step(PIDsys,t),title('PID step response')
xlabel('Time','FontSize',11);
ylabel('P.U. speed','FontSize',11);
figure(3)
step(LQR,t),title('LQR step response')
xlabel('Time','FontSize',11);
ylabel('P.U. speed','FontSize',11);
figure(4)
step(PIDsys,LQR,closed,t),title('step all')
xlabel('Time','FontSize',11);
ylabel('P.U. speed','FontSize',11);

```

Output Response

CONCLUSION





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Since controlling the Speed of a DC motor is a serious matter of concern for an electrical engineer, therefore in this paper authors present a comprehensive study of LQR Controller for the Speed control of DC Motor. The simulation results showed that by the use of the proposed controller result in reduction in settling time and also reduction in the overshoot than that of the traditional PID controller as shown in Table 1. The novelty of this research work is dedicated towards affordable and clean energy sources under sustainable development goals.

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7. https://www.researchgate.net/publication/336562984_Position_Control_Of_Robot_Arm_Using_Genetic_Algorithm_Based_PID_Controller

	Settling Time	Peak Amplitude	Overshoot
Closed Loop With Unity Feedback	3.83 sec	1.17	19.5%
PID Controller	2.76 sec	1.84	84.8%
LQR Controller	1.99 sec	1	0.525%

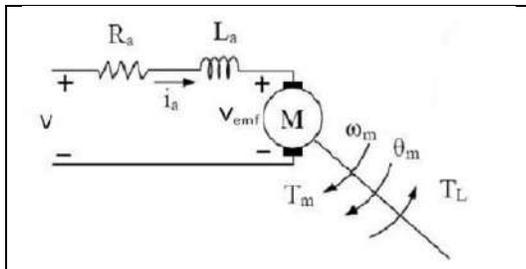


Fig.1 DC Motor

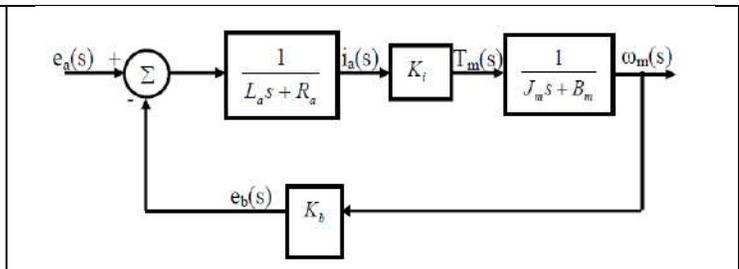


Fig. 2- DC-Motor System Block Diagram for speed (Plant System)



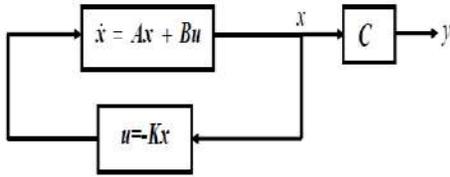


Fig. 3 Linear Quadratic Regulator Structure

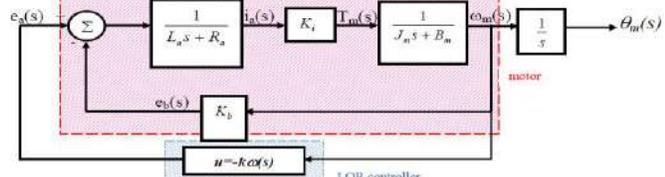


Fig. 4- DC-Motor System with LQR

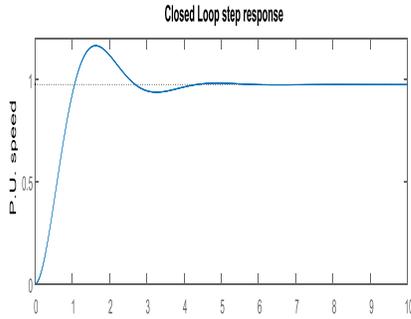


Fig.5 Closed loop step response

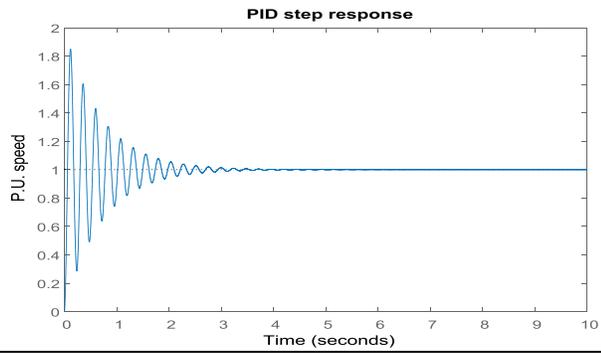


Fig.6 PID step response

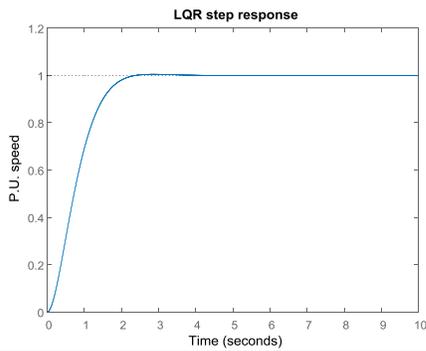


Fig. 7 LQR step response

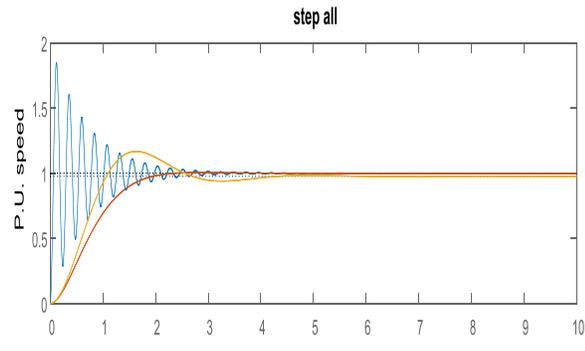


Fig.8 Closed loop, PID, LQR step response





Comparison between Two SCR Based Automatic Battery Charging Systems

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ABSTRACT

The battery has a vital role in the globally-focused electric vehicle technology and electrified automotive industries. Battery needs charging infrastructure to store energy for future use. A charging system or battery charger plays a crucial role in battery performance and its wide range of applications. In this paper, a comparison has been made between two silicon-controlled-rectifier (SCR) based automatic charging systems. An RC-triggering is proposed to operate the SCR instead of a conventional triggering method of SCR using a Zener diode as a voltage regulator. The superiority of SCR with RC-triggering is analyzed over the SCR with Zener-diode based battery charger. Mainly the triggering angle ranges of these two SCR based charging systems are highlighted. The advantages of SCR with RC-triggering are justified using the simulation results. This work is intended to be a part of the sustainable development of battery chargers for electric vehicles and industrial applications.

Keywords: SCR based battery chargers, triggering methods, battery charging systems, electric vehicles

INTRODUCTION

Due to the afraid of fossil fuel shortage and the greenhouse effect, the world is replacing internal combustion (IC) engines with electric vehicles (EVs) [1], [2]. This has been a trend for the last few years. The collection of electrical energy from renewable energy sources is everyone's interest now. The intermittent nature of renewable energy resources causes disrupted electric power which is undesirable for the electrical equipment operation and performance. This disrupted power supply issue must be addressed by enforcing an energy storage system (e.g., Battery). Battery technology is also the keystone of the electric vehicle revolution and automotive industries [3]. The battery is used to store the electrical energy for future use. The stored energy is considered as a source of direct current (DC) power supply which can be then converted into DC or alternating current (AC) for multidimensional domestic and industrial applications. Battery needs high-efficient, cost-effective, simple and reliable charging solutions to store the electrical energy[4]. The research and development of battery charging systems are mainly





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focused on circuit topology for charging and discharging, design and thermal management [5], [6]. A charging system is a combination of semiconductor devices, passive circuit elements and protection systems. Thus, it is important to propose an efficient circuit topology to ensure a better, safer and more reliable battery charging system.

The battery charging system consists of a silicon-controlled rectifier (SCR) to perform the automatic onboard battery charging. The SCR requires to be controlled precisely for the battery charging. Various triggering methods are available such as gate triggering (AC, DC and pulse), forward-voltage triggering, temperature triggering, light triggering, and dv/dt triggering. Resistive-Capacitive (RC)-triggering is a gate triggering method where the input signal and also gate controlling signal is AC[7]. This method has a better degree of freedom to control the output voltage. In this paper, an RC-triggering method is applied to SCR instead of a conventional resistance-Zener diode combination method to trigger the SCR. Both triggering methods are discussed and compared logically. The circuit topology of these two methods is explained in section-2. Simulation results and discussion of the proposed RC-triggering circuit of SCR are extensively presented in section-3. Finally, a summary of the simulation work is concluded in section-4.

Comparison of Circuit Topologies

A comparison has been made between SCR based two automatic battery charging circuit topologies. Turning ON and OFF of the SCR will decide the charging characteristics of a battery. Slow charging and fast charging of the battery are also completely dependent upon the SCR operation. As SCR is half controlled semiconductor switch which needs a triggering supply (i.e., firing angle) to start the charging process. A widely used resistive-Zener diode triggering circuit of SCR is shown in Figure 1. Similarly, an RC-triggering circuit of SCR is depicted in Figure 2.

Particularly, the firing angle ranges of these two triggering circuit topologies are discussed in this section. The two topologies have almost the same circuit components with minor differences. However, their operating ranges are different. Both circuits have an isolation step-down transformer converting the grid AC voltage (e.g., 230V, rms) to a low voltage level (say, 15V rms). The transformer is also meant for isolating the batteries from the input supply or vice-versa. Apart from the battery, Figure1 has three diodes, one SCR and one Zener diode of break down voltage 12V. The Zener diode is acting as a voltage regulator here which controls the charging of the battery. On the other hand, Figure2 has four diodes, one SCR, one variable resistance and two capacitors. One capacitor, C_f is connected across the two terminals of the battery to ensure a low ripple rectified current output. Otherwise, the ripple present in the current reduces the performance and longevity of the battery. In Figure 2, a variable resistor is used to allow the minimum gate current ($I_{g(min)}$) required for SCR triggering. The voltage across the capacitor, C (i.e., V_c) maintains the voltage between the gate-to-cathode terminal (V_g) of the SCR. That means the RC factor is responsible for turning on the SCR. A Li-ion battery having an internal resistance of 0.002Ω is considered for the circuit topologies. Details of battery parameters are listed in Table-1, section-3.

The triggering or firing angle (α) of SCR varies from 0° to 180° giving a chance to control the positive half cycle of the input AC signal. The designing value R and C for a time period of $T(=1/f)$ is given as:

$$RC \geq \frac{1.3T}{2} \quad (1)$$

A minimum gate-to-cathode voltage ($V_{g(min)}$) should be maintained to turn on the SCR. Considering a voltage across diode $D4$ (V_{D4}), a minimum value of R can be given in Eq. (2) to provide a minimum gate current to SCR.

$$R = \frac{V_s - V_{g(min)} - V_{D4}}{I_{g(min)}} \quad (2)$$

The average rectified output voltage (V_0) w.r.t firing angle (α) and input peak voltage (V_m) is given as:

$$V_0 = \frac{1}{\pi} \int_{\alpha}^{\pi} V_s dt = \frac{V_m}{\pi} (1 + \cos\alpha) \quad (3)$$

From Figure 1 and Figure 3, it is clear that the Zener diode restricts the triggering of SCR. When the input supply (V_s) voltage amplitude is greater than the breakdown voltage of the Zener diode (i.e., when $V_s > 12V$), the SCR conducts. Otherwise, the SCR will be in OFF condition as shown in Figure 3. The combination of resistance and Zener diode provides a little room for battery charging i.e., less charging option. The SCR operates within a firing angle range α_1





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to α_2 (i.e., $45^\circ < \alpha < 150^\circ$) if the input peak voltage is two times that of battery terminal voltage (i.e., $V_m \approx 2V_B$) or higher. It requires higher input voltage to increase its firing angle range which is costlier and not efficient. The average voltage value, refer eq. (3), will be too small if the input peak voltage is 15V (considering a 12V battery charging). Even this small voltage may not provide sufficient current to the battery for charging. This topology is suitable only for floating charging where an in-service battery will be charged to compensate for load necessity. A small charge is supplied to the battery to fulfill the losses during battery in-service. However, this charging system is not suitable for fast charging of new and highly discharged batteries. Thus, the proposed RC-triggering circuit of SCR is suitable for both fast-charging and floating charging purposes where the firing angle range is $0^\circ < \alpha < 180^\circ$ (i.e., high degrees of freedom). The RC-triggering circuit is also suitable when the input voltage peak has a low amplitude (example: $V_m=15$ and $V_B=12V$). Detail of simulation results of the proposed battery charging circuit is discussed in section-3.

RESULTS AND DISCUSSION

A MATLAB/Simulink model is assembled as per the circuit diagram shown in Figure 2. The MATLAB model is depicted in Figure 4. Considered battery model parameters for simulation are presented in Table-1. The corresponding circuit parameters taken for the simulation are listed in Table-2. MATLAB simulation results are presented and discussed in this section. The SCR is triggered at a wide range of firing angles ($0^\circ < \alpha < 180^\circ$) by designing the RC triggering circuit of the battery charging system as presented in Eq. (1) and (2). From e. (3), the range of average voltage ($V_m/\pi < V_0 < 2V_m/\pi$) is achieved that is used to charge the battery. The proposed circuit topology can be used for the fast charging and floating charging mode of the battery. In the RC-triggering circuit, a fixed capacitor value of $38\mu F$ is used to maintain the gate-to-cathode voltage of SCR. The value of resistance is varied from 50Ω to 9000Ω in order to achieve the firing angle range of $0^\circ < \alpha < 180^\circ$. The simulation results are shown at different firing angles (α) of the SCR in figures starting from Figure 5 to Figure 8. The figures show the primary side voltage (V_p) of the transformer, secondary side voltage (V_s) of the transformer, SCR voltage (V_T) and SCR current (I_T). The SCR firing angles of approximately $20^\circ, 45^\circ, 90^\circ$ and 150° are depicted in Figure 5, Figure 6, Figure 7 and Figure 8 respectively. Figure 9 represents the fully charged battery voltage (V_B), charging battery current (I_B), and state-of-charge (SOC in %) graph of the proposed battery charger. The battery is fully charged at around 14V with a nominal voltage of 12V. The battery can provide a different current of 2A, 3.5A, or 4.5A without affecting the battery performance. MATLAB/Simulink uses an inbuilt dynamic model of Li-ion battery. The model implements a modified Shepherd curve-fitting model for analysis. A discharge characteristic of the battery is shown in Figure 10. This characteristic graph confirms a better voltage polarization and battery current. It also represents the state-of-charge (SOC) effects on the battery. Finally, a comparison between Resistance-Zener diode (RZ) triggering and RC-triggering of SCR is illustrated in table-3.

CONCLUSION

A comparative analysis between the RZ-triggering circuit and the RC-triggering circuit of the battery charging system is performed. From the cost point of view, both circuits are almost the same. However, their operations have a great difference. It is found that the RC-triggering circuit has a larger scope in controlling the firing angle of SCR than that of RZ-triggering. The RC-triggering has the highest degree of freedom in terms of controlling the voltage required for battery charging than an RZ-triggering circuit. The firing angle range for RC-triggering is 0° to 180° whereas, the RZ-triggering circuit has a firing angle range between 60° to 150° for an input AC voltage of 15V (peak) and battery terminal voltage is 12V. Thus, it is concluded from the simulation analysis that RC-triggering of SCR for battery charging systems is superior to RZ-triggering-based circuit topology. It is expected that this work will provide a broader picture to develop an innovative industrial battery charging system. An infrastructure can be proposed further to execute the battery charger and battery management system.





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Table-1: Battery parameters for simulation

Input parameters and value
Nominal voltage = 12V
Rated current capacity = 49 Ah
Maximum current capacity = 49 Ah
Nominal discharge current = 21.30 A
Fully charged voltage = 13.96 V
Total internal resistance = 0.002 Ω
Capacity at nominal voltage = 44.31 Ah

Table-2: MATLAB simulation parameters of the proposed battery charging system

Input voltage, $V_s = 22$ V (peak) = V_m
Input frequency, $f = 50$ Hz
Resistance, $R = 50\Omega$ to 9000Ω
Capacitance, $C = 38\mu\text{F}$
Equivalent internal resistance connected at load = 0.1Ω

Table-3: Comparison of RZ-triggering circuit and RC-triggering circuit of SCR-based battery charging system. The assumed input peak voltage is 15V, and the nominal battery voltage is 12V.

RZ-triggering circuit	RC-triggering circuit
Battery charging when the input voltage is greater than the breakdown voltage of the Zener diode irrespective of battery voltage level.	It will follow the battery voltage level and charges the battery when necessary. It is the prominent feature of RC-triggering circuit.
SCR has a firing angle between 60° to 150° .	SCR has a firing angle between 0° to 180° .
Operates in constant voltage (CV) charging mode.	Operates in constant current and constant voltage (CC-CV) charging mode.
Floating (i.e., in-service) charging only.	Both Fast and floating charging.





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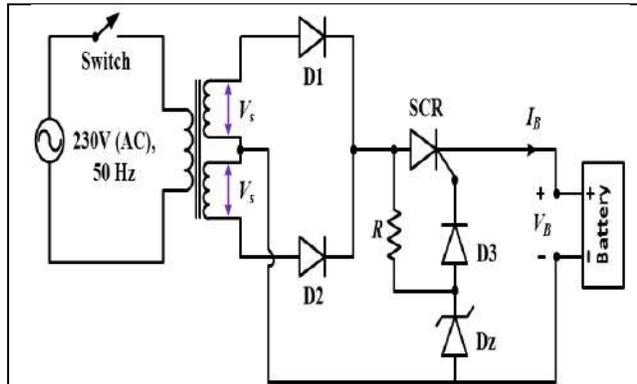


Figure 1: Battery charging system with resistive-Zener diode type triggering circuit.

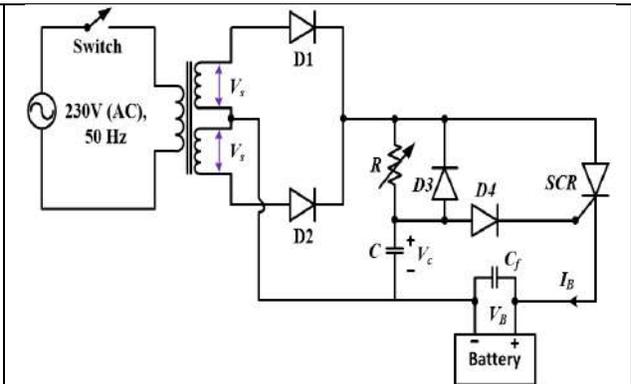


Figure 2: The proposed battery charging system with a resistive-capacitive (RC) type triggering circuit

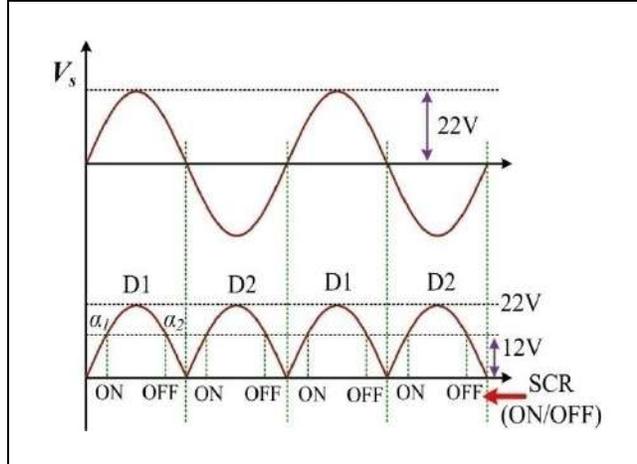


Figure 3: Triggering pattern of SCR using resistive-Zener diode circuit topology (Refer Figure 1).

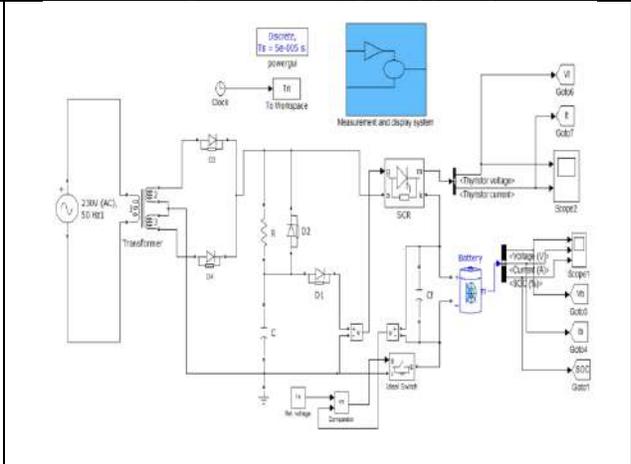


Figure 4: MATLAB Simulink model of the proposed battery charging system.

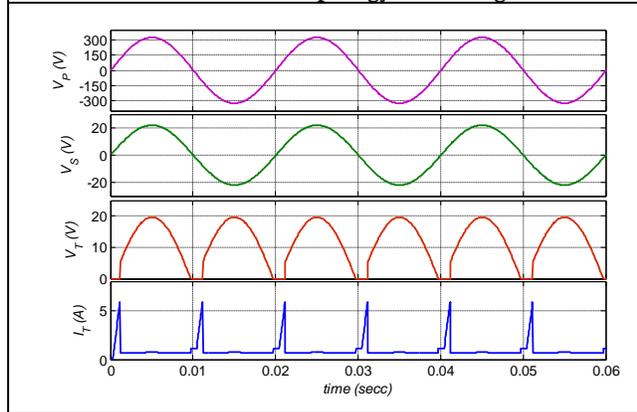


Figure 5: SCR operation at 20° w.r.t primary side voltage (V_P) of the transformer, secondary side voltage (V_S) of the transformer, SCR voltage (V_T) and SCR current (I_T).

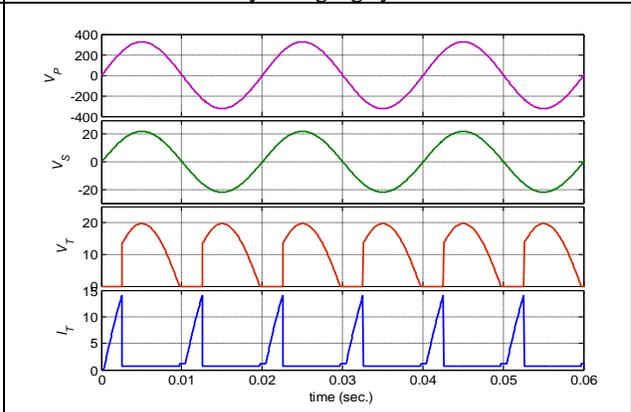


Figure 6: SCR operation at 20° w.r.t primary side voltage (V_P) of the transformer, secondary side voltage (V_S) of the transformer, SCR voltage (V_T) and SCR current (I_T).





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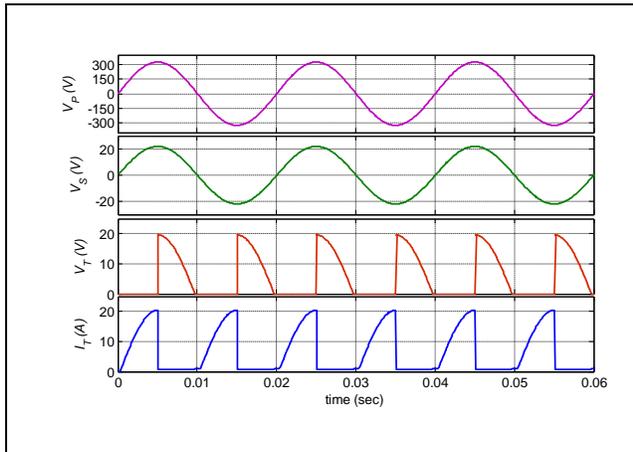


Figure 7: SCR operation at 90° w.r.t primary side voltage (V_P) of the transformer, secondary side voltage (V_S) of the transformer, SCR voltage (V_T) and SCR current (I_T).

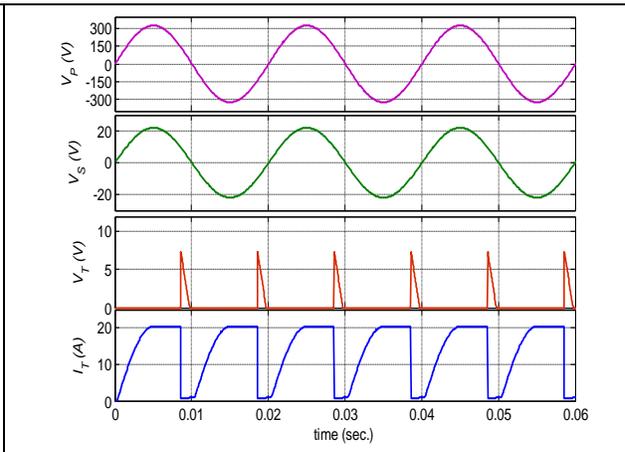


Figure 8: SCR operation at 150° w.r.t primary side voltage (V_P) of the transformer, secondary side voltage (V_S) of the transformer, SCR voltage (V_T) and SCR current (I_T).

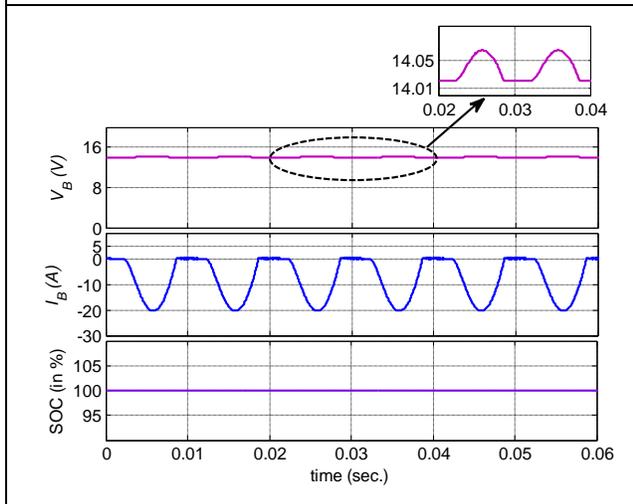


Figure 9: Represents fully charged battery voltage (V_B), charging battery current (I_B), and state-of-charge (SOC in %) graph.

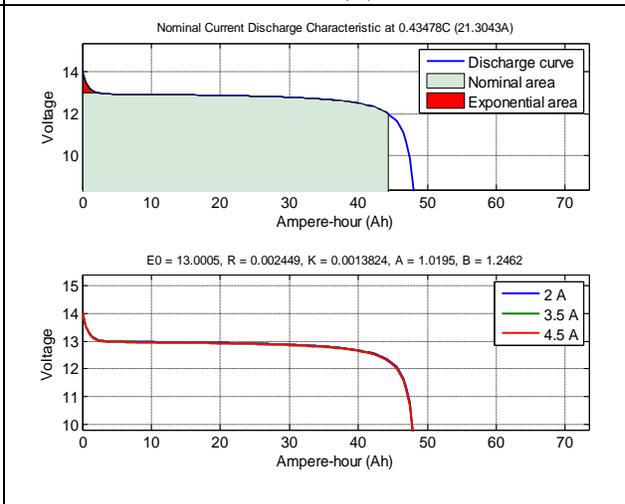


Figure 10: Battery discharge characteristics.





Cardioprotective Effect of Ethanolic Root Extracts of *Picrorhiza kurroa* Royle Ex Benth against Isoproterenol- Induced Myocardial Infarction in Rats

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ABSTRACT

The present study aims to evaluate the protective effect of ethanolic root extracts of *Picrorhiza kurroa* Royle ex Benth (ERPK) against Isoproterenol (ISO)- induced myocardial infarction in rats. The animals were exposed to isoproterenol (200 mg/kg. s.c) twice at an interval of 24 hrs. Cardio-protective effect was assessed by observing ECG parameters, serum marker enzymes and histopathology of the heart. Pre-treatment of ERPK at 200 and 400mg/kg resulted in a significant ($P < 0.001$) increase in P wave, QRS complex and R-R interval, whereas heart rate, QT interval and cardiac cycle were maintained near to normal values. ERPK showed significant ($P < 0.05$; $P < 0.001$) reduction in all the tested diagnostic markers compared to ISO treated group. Histological studies on the structural changes of heart tissue supported the protective activity of ERPK. These results suggested that treatment of ERPK prior to ISO has a significant role in protecting the animals from ISO induced myocardial infarction.

Keywords: *Picrorhiza kurroa* Royle ex Benth; Isoproterenol; ECG; Marker enzyme; Cardioprotective





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INTRODUCTION

Ischaemic heart diseases, especially acute myocardial infarction (MI), remain the leading cause of death in both developed and under developing countries as seen over the past quarter century [1,2]. Ischemic heart disease (IHD) is the leading cause of morbidity and mortality in worldwide, and according to the world health organization it will be the major cause of death in the world by the year 2020[3]. MI results from the prolonged myocardial ischemia with necrosis of myocytes due to interruption of blood supply to an area of heart [4]. Isoproterenol (ISO) induced myocardial necrosis is a well-known standard model to study the beneficial effect of many drugs on cardiac dysfunction [5]. ISO is a β -adrenergic agonist that causes severe stress in myocardium and necrotic lesions in the heart muscles. ISO induced myocardial injury involves membrane permeability alterations, which brings about the loss of functions and integrity of myocardial membranes [6]. MI induced by ISO in rats has been shown to be accompanied by hyperglycaemia, hyperlipidaemia, and increase in serum creatinine phosphokinase, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase activities [7,8]. The mechanism proposed to explain isoproterenol induced cardiac damage involves generation of highly cytotoxic free radicals through auto-oxidation of catecholamine and has been implicated as one of the causative factor. Reduction of mortality rate and prevention of myocardial infarction are of vital importance. Western drugs such as angiotensin-converting enzyme (ACE) inhibitors, calcium channel blockers, angiotensin II receptor antagonists, etc. have been proven to have cardioprotective effects in both preclinical and clinical studies. ACE inhibitors have been gradually introduced into the treatment of hypertension, congestive heart failure and myocardial infarction since the 1970s [9,10,11]. Experimental studies also showed that ACE inhibitors administered chronically before acute MI might limit myocardial infarct size, improve cardiac function and prevent cardiac hypertrophy [12] although modern drugs are effective in preventing the disorders, their use is often limited because of their side effects, and adverse reactions. However, there is a growing interest in the use of alternative medicine for long-term prevention of heart attack in high risk patients. A wide array of plants and its active principles, with minimal side effects, provide an alternate therapy for Ischemic heart diseases. Moreover, the plant kingdom represents a largely unexplored reservoir of biologically active compounds in cardiovascular diseases. The present study was designed to investigate the cardioprotective effects of ethanolic root extracts of *Picrorhiza kurroa* in ISO-induced electrocardiographic, serum marker enzymes and histopathological changes.

Picrorhiza kurroa Royle ex Benth. (Scrophulariaceae), is a small perennial herb found mainly in the Himalayan region growing at an elevation of 3,000-5,000 m [13,14]. The leaves of the plant are flat, oval and sharply serrated. The leaf, bark and the underground parts of the plant, mainly rhizomes are widely used in the traditional Indian systems of medicine (Ayurved) since ancient times. *Picrorhiza kurroa* is traditionally used to treat disorders of the liver, upper respiratory tract, fevers, dyspepsia, chronic diarrhea, scorpion sting and cancer [15]. The DNA protective ability of the plant has been reported [16]. Although it shows antioxidant, anti-inflammatory and immunomodulatory activities, it is most valued for its hepatoprotective effect. *Picrorhiza kurroa* rhizomes are widely used against indigestion problems since ancient times due to improper digestive secretions [17].

MATERIALS AND METHODS

Drugs and chemicals

Isoproterenol hydrochloride (ISO) purchased from Sigma Chemical Company, St louis MO USA, ECG Electrodes procured from Biopac Santa Barbara California. Sodium carboxy methyl cellulose (Na-CMC) from LobaChemie, Mumbai, India, Pentobarbitone and anaesthetic ether were also used. All chemicals used were of analytical grade.

Experimental animals

Male Wistar rats of 150 – 200 g were used for the study. The inbred colonies of rats were purchased from Venkateshwara Enterprises, Bangalore. They were acclimatized to controlled conditions of temperature (23 ± 2 °C), humidity ($50 \pm 5\%$) and 12-h light-dark cycles. The animals were randomized into different experimental and control groups and housed in sanitized polypropylene cages containing sterile paddy husk as a bedding. They had free



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access to standard pellets as basal diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) animals and the care of the laboratory was taken as per the CPCSEA regulation. We selected male rats for our studies, since females are shown to be protected from cardiovascular complications[18].

Plant material

The dried root powder *Picrorhiza kurroa* was obtained from Herbo Nutra, Uttar Pradesh, India, in the month of August 2021 was used for the study.

Preparation of extract

Dried powder of root was exhaustively extracted with Ethanol (95 in a Soxhlet apparatus. The extracts were concentrated by rotary flash evaporator, under reduced pressure and controlled temperature, followed by freeze drying and stored in a desiccator. EEPK suspensions were prepared in Sodium carboxy methyl cellulose (Sod. CMC) using distilled water, to assess the cardio protective effect of the plant.

Induction of Myocardial Ischemia

The rats were divided into six groups of six animals each.

Group I served as a control, Group II rats were administered with isoproterenol (20 mg/100g administered subcutaneously twice at an interval of 24 h) dissolved in normal saline, to induce Myocardial ischemia [19]. Group III, IV and V rats were pre-treated with *Picrorhiza kurroa* root extract (100,200 &400mg/kg) for a period of 30 days[20]and isoproterenol (20 mg/100g subcutaneously twice at an interval of 24 hrs) at the end of the treatment period on the 29th and 30th days.

Measurement of ECG

At the end of experimental period (after 24 h of second ISO injection i.e on 30th day of extract/vehicle treatment) the rats were anaesthetized with light anesthetic ether and ECGs were recorded using computerized data acquisition system (Biopac MP 35, Santa Barbara, California). Recordings were made on the bi-polar standard lead-I, lead-II and lead-III. In all cases of myocardial infarction, Lead II show the clear, distinct individual waves than Lead I & III. Therefore, ECG was monitored on Lead II only.

Biochemical analysis

After recording the ECG, blood samples were collected from retro-orbital plexus, serum was separated [21] for estimation of marker enzymes. The activities of AST, ALT, Lactate dehydrogenase (LDH), Creatine kinase (CK), glucose, triglycerides (TG) and total cholesterol were measured by using standard kits.

Histopathological Studies

Animals were sacrificed by injecting higher dose of thiopental sodium (CPCSEA annexure 6). The hearts were removed, washed immediately with saline and then fixed in 10% buffered formalin. The hearts were stored in 10% buffered formalin then embedded in paraffin, sections cut at 5 mim and stained with hematoxylin and eosin. These sections were then examined under a light microscope for histological changes



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Statistical Analysis

Results are expressed as means \pm SEM for six rats in each group and statistical significant differences between mean values were determined by one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparisons test.

RESULTS

ECG parameters

Effect of *Picrorhiza kurroa* on ECG parameters Fig. 1. represents the electrocardiographic pattern of control & experimental animals. Normal control and different doses of, ERPK at 100, 200 and 400mg/kg) treated rats showed a normal ECG pattern, whereas animals treated with ISO alone showed significant elevation in ST segment, reduction in P wave, QRS complex and R-R interval, in addition there was an increase in heart rate, prolongation of QT interval and cardiac cycles compared to normal control animals. Pre-treatment of ERPK at 100, 200 and 400mg/kg administered rats exhibited normal ECG pattern with a slight elevation in ST segment. Furthermore, treatments also resulted in significant ($P < 0.001$) alterations in P wave, QRS complex and R-R interval, whereas heart rate, QT interval and cardiac cycle were maintained near to normal values especially with high dose. The data of the experimental animals such as P wave, QRS complex, QT interval, R-R interval, heart rate & cardiac cycle are represented in Table 1.

Effect of *Picrorhiza kurroa* on serum marker enzymes

ISO treated rats exhibited significantly ($p < 0.001$) higher levels of serum myocardial injury marker enzymes such as AST, ALT, LDH, CK, glucose, triglycerides and total cholesterol compared to normal control rats (Table 2). Pre-treatment with ERPK at 100, 200 and 400mg/kg for 30 days and ISO (200 mg/kg, for two days) administration showed significant ($P < 0.05$; $P < 0.001$) reduction in all the tested diagnostic markers compare to ISO alone treated group. However, there were no changes in any of these marker enzyme levels in ERPK at 100, 200 and 400mg/kg treated groups as compared to normal control group.

Histopathological studies

Histopathological examination of myocardial tissue obtained from normal control animals and animals treated with *Picrorhiza kurroa* depicted clear integrity of myocardial membrane and an infiltration of inflammatory cells are not seen in these experimental groups (Fig. 2A). The histological sections obtained from the hearts of animals receiving ISO alone (Fig. 2B) shows various degrees of focal lesions in many sections consisting of molten staining, fragmentation of muscle fibers with confluent retrogressive lesions. In addition, marked sequestering mucoid edema and vacuolar changes along with hyaline necrosis were clearly visible in ISO treated rats. Pretreatment with ERPK at 100, 200 and 400mg/kg groups demonstrated marked improvement in ISO-induced alterations such as vacuolar changes, edema, capillary dilatation and leukocyte infiltration compared to ISO administered group (Fig. 2C, D&E) respectively.

DISCUSSION

Oxidative stress is one of the major concerns in the treatment of ischemic heart diseases. There is ample evidence for a detrimental role of ROS in cardiovascular disease[22,23]. Isoproterenol is well known cardio-toxic agent, destruct the myocardial cells, as a result of this, cytosolic enzymes such as lactate dehydrogenase (LDH), transaminases (ALT and AST) and creatine kinase (CK) were released into blood stream and serve as the diagnostic markers of myocardial tissue damage. The amount of these cellular enzymes present in blood reflects the alterations in plasma



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membrane integrity and/or permeability. Drug treatments such as naringin, silibinin, and squalene evidenced by a decline in lactate dehydrogenase, glutamic oxalacetic transaminase and creatine kinase levels indicated their membrane stabilizing action [24-27]. In the present study, ISO treated rats showed significant elevation in the levels of these diagnostic marker enzymes (AST, ALT, LDH and CK). Moreover, elevated levels of these enzymes are an indicator of the severity of ISO-induced myocardial membrane necrosis, which is in line with an earlier report. The prior administration of ERPK at 100, 200 and 400mg/kg showed significant reduction in the ISO-induced elevated serum marker enzymes except CK. This reduction in enzyme levels could be due to its action on maintaining membrane integrity thereby restricting the leakage of these enzymes. It is well known that isoproterenol-induced myocardial injury is mediated primarily via the β_1 -adrenergic receptor. Acute β -adrenergic receptor stimulation not only rapidly generates reactive oxygen species, but also depresses total cellular antioxidant capacity, down regulates copper-zinc superoxide dismutase enzyme activity, protein and mRNA, and reduces glutathione level, leading to the loss of membrane integrity and inducing heart contractile dysfunction and myocyte toxicity finally producing myocardial [28]. In the present study, we found that *Picrorhiza kurroa* extracts protected myocardium from isoproterenol induced myocardial functional and structural injury via normalization levels of diagnostic marker enzymes. Current epidemiological evidence suggest that inadequate intake of certain nutrients predispose humans to chronic degenerative diseases [29]. In particular, it was demonstrated that intake of an adequate diet rich in vegetable and fruit reduces the likelihood of cardiovascular diseases, but the exact mechanisms for this protective effect are inadequately understood. However, increased circulating antioxidants are believed to be important. This is supported by recent trials reporting that the intake of antioxidant flavonols predicts a reduced rate of coronary-heart disease mortality in elderly male, in particular, those epidemiological studies show that dietary intake of flavanoids (quercetin, catechin and epicatechin), notably present in red wine but also in fruits and vegetables is inversely associated with subsequent coronary heart disease[30,31]. This effect seems to be in part related to their antioxidant activity. Therefore, the observed myocardial protective effect of ERPK could be due to the flavanoids which are known free radical scavengers.

Electrocardiograph-abnormalities are the main criteria generally used for the definite diagnosis of myocardial infarction. ST-segment elevation was observed either in patient with acute myocardial ischemia or in isoproterenol induced myocardial infarction in rat[32,33].The study shows significant alterations of ECG patterns in ISO administered rats as compared to normal control rats. The characteristic findings were reductions in the P wave intensity, QRS complex, R-R intervals, QT interval and prolongation of cardiac cycle. We also observed a significant elevation in the ST segment and increase in heart rate. These alterations could be due to the consecutive loss of cell membrane in injured myocardium[34]. In the present study, we observed an elevation of ST-segments in isoproterenol induced rat, and pre-treatment with *Picrorhiza kurroa* fractions markedly inhibited isoproterenol-induced ST-segment elevation suggestive of its cell membrane protecting effects. The appearance of Q wave & ST segment elevation are some of the indicative signs of ischemia. In the present study we did not observe pathological Q wave due to conditions of ischemia. The prominent Q wave were seen only on severe ischemia, infarction and in patients with severe heart diseases[35].The consecutive loss of cellular membrane damage due to oxidative stress might be characterized by ST elevation[36,37].ERPK at 100, 200 and 400mg/kg administration showed a protective effect against ISO-induced altered ECG pattern and eliminated the acute fatal complications by protecting the cell membrane damage. Electrocardiograph and biochemical findings were further supported by histopathological studies. Histopathological examination of myocardial tissue in negative control depicted clear integrity of the myocardial cell membrane. No inflammatory cells infiltration was seen in the rat heart of normal control. In ISO administered group, focal lesions in many sections consisting of molted staining and fragmentation of muscle fibres with confluent retrogressive lesions, hyaline necrosis, sequestering mucoid edema were observed. Pre-treatment with ERPK at 100, 200 and 400mg/kg demonstrated inhibited focal lesions, fragmentation of muscle fibres and retrogressive lesions with hyaline necrosis seen with ISO treated group. Inflammatory cells were seen with reduced density in ERPK at 200 and 400mg/kg treated groups confirming the further cardio-protective activity exerted by *P. kurroa*. However,ERPK at 200 and 400mg/kg p.o) treated normal rats had no toxic effects on cardiac architecture. Higher dose of isoproterenol induced subendocardial ischemia, hypoxia, necrosis, and finally fibroblastic hyperplasia with decreased myocardial compliance and inhibition of diastolic and systolic function, which closely





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resembles local myocardial infarction-like pathological changes seen in human myocardial infarction [38]. In the present study, we found that *Picrorhiza kurroa* root fractions protected myocardium from isoproterenol-induced myocardial functional and structural injury. The phytochemical investigations of ERPK revealed the presence of flavanoids, tannins and phenolics. The observed cardio-protective activity of ERPK may be attributed to the presence of these bioactive principles and their synergetic properties. Further, the mode of cardio-protective action may be due to the prevention of stimulation of β -adrenergic receptor, thereby decreasing the generation of reactive oxygen species which in turn maintains the membrane integrity in myocardial tissue. The data of the present study clearly shows that these extracts modulated the most of the electrophysiological, biochemical and histopathological parameters to normal status in experimental rats treated with isoproterenol, suggesting that the beneficial effect of *Picrorhiza kurroa* as a cardio-protective agent.

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Fig.no.1. Effects of *Picrorhiza kurroa* on ECG pattern in Isoproterenol -induced Myocardial infarction in rats.

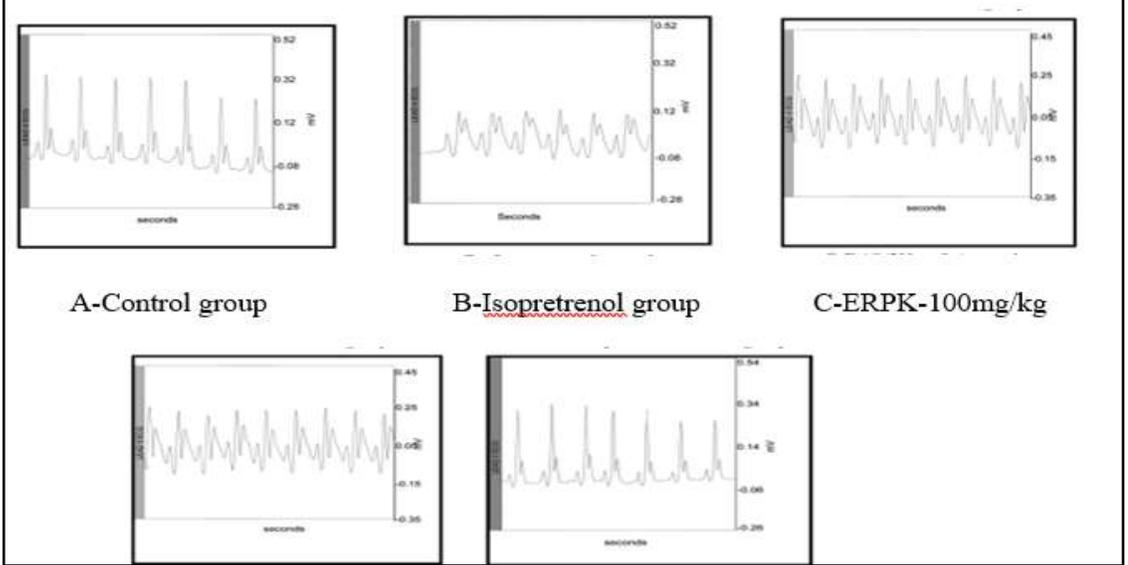
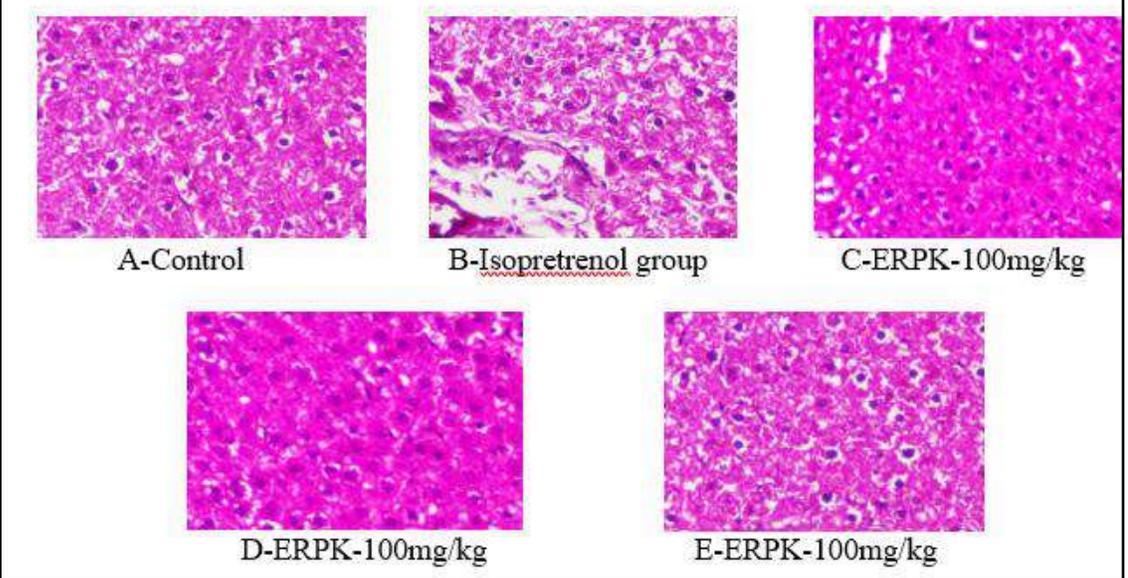


Fig.no.2. Effects of *Picrorhiza kurroa* on histopathological changes in rat myocardial tissue.





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Table 1. Effect of *Picrorhiza kurroa* root extracts on ECG parameters in Isoproterenol -induced Myocardial infarction in rats.

Groups	P Wave	QRS Complex	Q-T Interval	R-R Interval	Heart rate	Cardiac cycle
Control	0.03850±0.0014	0.04292±0.0009	0.08500±0.0033	0.1879±0.0033	321.2 ±5.87	0.1213±0.0037
Isoproterenol (200mg/kg)	0.03178±0.0007 ^a	0.03492±0.0001 ^a	0.09944±0.0019 ^a	0.1215±0.0021 ^a	417.9 ±6.90 ^a	0.1361±0.0033 ^a
ERPK-100mg/kg	0.03489±0.0011 ^b	0.03992±0.0005 ^b	0.08640±0.0012 ^b	0.1835±0.0013 ^b	372.0 ±9.35 ^b	0.1276±0.0031 ^b
ERPK-200mg/kg	0.03689±0.0011 ^b	0.04192±0.0004 ^b	0.08340±0.0026 ^b	0.1851±0.0027 ^b	355.0±10.35 ^b	0.1252±0.0037 ^b
ERPK-400mg/kg	0.03789±0.0011 ^b	0.04212±0.0004 ^b	0.08233±0.0016 ^b	0.1865±0.0011 ^b	328.3±12.25 ^b	0.1220±0.0037 ^b

The data were expressed as Mean ± S.E.M for six rats in each group. Statistical comparisons were performed by one-way

ANOVA followed by Tukey's post-test, The ECG parameters are expressed in seconds (sec) and the Heart rate as Beats per Minute

(BPM). a P < 0.001 compared with control, b P < 0.001 compared with ISO treated group.

Table 2. Effect of *Picrorhiza kurroa* root extracts on serum biochemical parameters in Isoproterenol-induced myocardial infarcted rats

Groups	ALT(IU/L)	AST(IU/L)	LDH(IU/L)	CK(IU/L)	Glucose mg/dl	TG mg/dl	TC mg/dl
Control	66.40±10.04	97.31±9.437	1021±27.34	389.8±11.74	88.75±2.955	42.25±4.76	37.25±4.51
ISO200mg/kg	120.6±8.24 ^a	195.2±7.04 ^a	1924±91.2 ^a	713.8±21.10 ^a	137.02±2.34 ^a	119.0±3.24 ^a	77.00±3.80 ^a
ERPK-100mg/kg	84.60±4.70 ^b	145.8±7.66 ^b	1341±87.26 ^b	564.9±28.59	112.04±5.78	52.26±1.21 ^b	66.60±2.20 ^b
ERPK-200mg/kg	77.60±4.70 ^b	122.8±7.66 ^b	1226±73.26 ^b	506.4±54.59	103.07±4.18	40.43±1.21 ^b	54.60±3.20 ^b
ERPK-400mg/kg	70.20±9.10 ^b	104.3±8.12 ^b	1109±94.8 ^b	418.7±16.93 ^b	95.51±2.75 ^b	31.12±1.96 ^b	43.60±1.26 ^b

The data were expressed as Mean ± S.E.M for six rats in each group.

Statistical comparisons were performed by one-way

ANOVA followed by Tukey's post-test.

a P < 0.001 compared with control, b P < 0.001 compared with ISO treated group.





Role of Melatonin in Plant Development and Abiotic Stress Control

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ABSTRACT

Melatonin (N-acetyl-5-methoxy tryptamine) is a naturally occurring compound in plants and has been detected in the roots, leaves, fruits, and seeds of a considerable variety of plant species. Melatonin is a ubiquitous and highly conserved molecule in plant and animal kingdoms and has been identified in insects, arthropods, planarians, mollusks, dinoflagellates, and brown algae. The known physiological functions of melatonin in animals include timing circadian rhythms, signalling environmental changes, cancer inhibition and detoxification of free radicals, and other reactive oxygen species (ROS) and related products. There are reports demonstrating the ability of melatonin in plants to alleviate the effects of abiotic stresses such as oxidative, metallic toxicity, low temperature, salinity and water stresses during seed germination. Zhang *et al.*, 2014 reported that seed treatment with melatonin has a positive role in overcoming water stress by regulating the genes involved in both ROS and plant hormone metabolism. Melatonin relieves the inhibitory effect of high salinity on germination of cucumber seeds by two mechanisms. One relies on the antioxidant properties of melatonin to alleviate ROS damages. In the other way, melatonin regulates seed germination through positively up-regulating GA biosynthesis and ABA catabolism. In another study, melatonin increases plant growth, seed production, and abiotic stress tolerance in soybean plants, possibly through enhancement of photosynthesis, carbohydrate metabolism, and antioxidative actions (Wei *et al.*, 2014). Melatonin treatment also improved the antioxidant levels, activities of the ROS scavenging enzymes (superoxide dismutase, peroxidase, and catalase), reduced chlorophyll degradation, stimulated root generation and vitality and increased the root: shoot ratio thus all these factors help in overcoming drought stress in cucumber (Zanget *al.*, 2012). So, seed treatment with melatonin helps in overcoming different abiotic stresses.



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INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) is a naturally occurring compound in plants and has been detected in the roots, leaves, fruits, and seeds of a considerable variety of plant species. Melatonin is a ubiquitous and highly conserved molecule in plant and animal kingdoms and has been identified in insects, arthropods, planarians, mollusks, dinoflagellates, and brown algae. The known physiological functions of melatonin in animals include timing circadian rhythms, signaling environmental changes, cancer inhibition and detoxification of free radicals, and other reactive oxygen species (ROS) and related products. There are reports demonstrating the ability of melatonin to alleviate the effects of abiotic stresses such as low temperature, copper stress, and light conditions during seed germination, but little is known about its comprehensive effects in plants. Evidence suggests the role of melatonin in seed germination, and plant survival may be related to melatonin-induced changes in membrane and protein peroxidation. In addition to its antioxidant and growth-regulating functions, melatonin may play a role in regulating photoperiod and circadian rhythms in plants. Furthermore, melatonin may play a role in protecting tissues during flower and seed development in *Daturametel*. Many investigations have examined the effects of melatonin on in vitro organogenesis, such as improving cotyledon expansion, promoting hypocotyl and coleoptile growth, and preventing apoptosis during cold-treatment in *Daucuscarota* cell suspensions. It is known that the deleterious effects resulting from the cellular oxidative state may be alleviated by the enzymatic and non-enzymatic antioxidant systems. Plants respond and adapt to water stress by altering their cellular metabolism and invoking various defense mechanisms. The addition of this indoleamine treatment enables plants to survive under environmental stresses by enhancing recovery potential.

Chemical structure of Melatonin

- Melatonin has the molecular formula ($C_{13}H_{16}N_2O_2$)
- It has many chemical names such as
- N-Acetyl-5- methoxytryptamine
- 73-31-4 Circadian
- 5-methoxy-nacetyltryptamine N-[2-(5-methoxy-1H-indol-3-yl)ethyl]acetamide (IUPAC name)

Biosynthesis of Melatonin

The biosynthetic pathway of melatonin (N-acetyl-5-methoxytryptamine) is well known in vertebrates. In these species, tryptophan (an amino acid) is converted to 5- hydroxytryptophan; tryptophan 5-hydroxylase (T5H) is involved in this conversion. Thereafter, 5- hydroxytryptophan is converted to serotonin (Falcón *et al.*, 2009). In animals, 5-hydroxytryptophan is the exclusive pathway for serotonin production in the pineal gland. In the plant *St. John's Wort (HyericumperforatumL.)*, 5 hydroxy tryptophan is also involved in serotonin synthesis. A recent study on rice, however, documents that the tryptamine pathway (tryptophan to tryptamine to serotonin) is more important in the production of serotonin; this pathway has subsequently been found to be common to many plant species. Serotonin, in both plants and animals, is converted to N-acetyl serotonin catalyzed by serotonin Nacetyltransferase (SNAT), which is then methylated by hydroxyindole-O-methyltransferase (HIOMT; also known as acetyl serotonin methyl transferase, ASMT) resulting in the formation of melatonin. In plants (rice), N-acetyl serotonin is also directly produced from tryptamine and N-acetyltryptamine serves as an intermediate product; this pathway is catalyzed by SNAT and tryptophan 5-hydroxylase.

Melatonin can be directly produced from serotonin with 5-methoxytryptamine serving as intermediate product in a process and catalyzed by HIOMT/ASMT and SNAT. Indole acetic acid (IAA) is also produced from tryptamine and indole-3- acetylaldehyde serves as an intermediate product. The concentration of melatonin in plants is much higher than levels in animals, and the biosynthesis of melatonin in plants also seems more complicated than in animals. Due to more limited availability of information on the biosynthesis of melatonin and related products in plants, the definitive pathways await final definition. Recently, transgenic rice has been shown to have genes for all the enzymes involved in the biosynthesis of melatonin and it is expected that soon the biosynthetic pathway will be described in





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detail. As far as the degradation of melatonin is concerned, N -acetyl-N -formyl-5-methoxykynuramine (AFMK) has been shown to be a secondary metabolite in vascular plants due to its enzymatic or non-enzymatic conversion from melatonin. Recent studies on rice document that other metabolites of melatonin include 2 hydroxymelatonin (99%) and, to a much lesser extent, 4-hydroxymelatonin (0.05%) (Nawaz *et al.*, 2016).

Role of Melatonin in plants

Melatonin has proven to be ubiquitously synthesized in plant organs. Pleiotropic roles ranging from enhancing germination to delaying senescence of plants have been reported. While melatonin's role have highlighted the modulation of circadian rhythms in mammals, this function has not been thoroughly examined in plants.

Propagation

In vitro germplasm storage via cryopreservation is an effective tool to ensure conservation of tree species, but plant cells and tissues are exposed to multiple stresses including osmotic injury, desiccation and low temperature injury during the cryopreservation process; this contributes to problems during the regrowth of cryopreserved materials. Supplementing both preculture and regrowth media with melatonin (0.1–0.5 μM melatonin for 24 h) significantly enhanced regrowth of frozen shoots compared with the untreated shoots. Similarly, 0.1 μM melatonin as pre-cryopreservation treatment to callus of *Rhodiolacrenulata* (endangered plant species) also improved their recovery (Zhao *et al.*, 2011). Seed treatment with 100 μM melatonin for 12 h significantly improved the percentage germination of cucumber seeds (Zhang *et al.*, 2013). Low concentrations of melatonin (1 μM) enhanced the germination rate of cucumber under salinity stress by regulating the biosynthesis and catabolism of abscisic acid (ABA) and gibberellic acid (GA) (Zhang *et al.*, 2014). Cuttings are also used as a means of propagation for many commercially important horticultural crops. The exogenous application of melatonin to roots of grape cuttings improved their growth by enhancing water stress tolerance.

Growth and development

Several studies have noted that melatonin regulates these physiological functions of plants; melatonin generally improves the growth of roots, shoots and explants (Hernández-Ruiz *et al.*, 2005). The initial report of the direct involvement of melatonin in stimulating plant growth was reported in 2005 by Hernández-Ruiz *et al.* (2005); they observed that melatonin extended the coleoptiles (10–55%) of canary grass, wheat, barley and oat (monocots). Later, it was found that 0.5–1 μM application of melatonin enhanced the initial seminal root length, growth and root biomass of transgenic rice plants. Melatonin is now known to alter many plant characteristics including germination (Zhang *et al.*, 2014), seedling growth, alteration of flowering time, grain yields, and senescence. A recent study provides direct evidence that seed coating with melatonin significantly increased the leaf area, plant height, pods per plant, seeds per plant, and fatty acid contents of soybean plants (Wei *et al.*, 2015). This study suggests new avenues to enhance crop yields. Seeds coated with melatonin could be potentially used for a large number of commercially important agronomic and horticultural crops. This has the potential to revolutionize the seed industry.

Stress tolerance

Salinity is a major environmental factor that limits crop growth and productivity; it leads to huge economic losses worldwide. Salinity not only induces water deficit caused by osmotic stress, it also disturbs key biochemical process (photosynthesis, protein synthesis, energy, and lipid metabolism) in plant cells. Plants use various strategies to cope with these stressors; these involve the exclusion of selective ions, ion compartmentalization, synthesis of compatible solutes, alterations in the photosynthetic pathway, changes in membrane structure, induction of antioxidant enzymes, stimulation of phytohormones and regulation of gene expression (Parida and Das, 2005). Exogenous application of melatonin (0.1 μM) significantly alleviated the growth inhibition caused by elevated salinity; this enabled the plants to maintain their photosynthetic capacity. The application of melatonin also decreased the oxidative damage caused by ROS by directly scavenging HO and enhancing the activities of antioxidant enzymes including ascorbate peroxidase, catalase, and peroxidase (Li *et al.*, 2012). Salinity exerts its negative impact irrespective of growth stage of the plants, and its effects range from seed germination to plant senescence, and occur





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throughout the life cycle. Seed germination and plant growth is severely affected by saline stress. In every case melatonin proved its importance by ameliorating the effects caused by salt stress and improved germination and plant growth. In cucumber (*Cucumis sativus* L.), pre-sowing seed treatment with melatonin (1 μ M) enhanced the rate of germination and subsequent growth under 150 mM NaCl stress; this increase was accompanied by approximately a 5-fold elevation in antioxidant enzyme activities (superoxide dismutase, catalase, peroxidase; Zhang *et al.*, 2014). Melatonin application enhanced tolerance to salt and drought stress in soybean, and up-regulated the expression of genes that were inhibited by salt stress (Wei *et al.*, 2015).

Cold

Low temperature stress leads to significant damage to agricultural crops; low temperature alters plant physiology, biochemistry and molecular biology (Bajwa *et al.*, 2014). Many scientists are working on the development of cold tolerant commercially-important crop cultivars. Recently, melatonin was shown to significantly alleviate cold stress in a number of plants. Melatonin treated (10– 30 μ M) *Arabidopsis thaliana* plants produced higher fresh weight, root length and plant height compared to untreated plants (Bajwa *et al.*, 2014). Like other plants, low temperature damages wheat plants by reducing leaf area, leaf water content, photosynthetic pigment content, and the accumulation of ROS caused lipid peroxidation of membranes. The application of melatonin (1 mM for 12 h) to wheat seedlings increased the activity of the antioxidant enzymes, superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase, and glutathione reductase leading to improved plant growth by reducing oxidative damage (Turk *et al.*, 2014). More recently, it has been found that the exogenous application of melatonin increased salt, drought and cold resistance in bermudagrass (*Cynodon dactylon* L. Pers.). In this study, melatonin activated not only several antioxidants but also induced higher concentration of 54 secondary metabolites including amino acids, organic acids, sugars, and sugar alcohols (Shi *et al.*, 2015).

Heat stress

Extremes temperatures affect membrane fluidity and enzyme activities leading to alterations in growth and development patterns and yield losses. In plants under stressful conditions, the genes responsible for melatonin biosynthesis are typically activated resulting in higher levels of melatonin. As an example, under high temperature conditions the level of melatonin is increased in rice (Byeon and Back, 2014) suggesting a role of melatonin in defense against heat stress. Melatonin application increased germination percentage of heat stressed *Arabidopsis thaliana* seeds up to 60% compared to control; this effect was likely due to powerful antioxidant activity of melatonin (Hernández *et al.*, 2015). Similarly in another recent study, Shi *et al.* (2015b) reported that application of melatonin activated stress responsive genes in Bermuda grass. *C-REPEATBINDING FACTORS/DEHYDRATION-responsive ELEMENT-BINDING PROTEIN (CBF/DREB)* genes and target genes, heat shock transcription factors (TFs), zinc finger TFs, *WRKY*, *MYB*, *bHLH* genes, and hormone-related genes exhibited a 16-fold over expression compared to levels in control plants.

Drought, ultraviolet radiations, heavy metals, and chemicals stress

Melatonin has also proven its protective role against drought, ultraviolet radiation, heavy metals and chemicals stress. Transgenic Micro-Tom tomato plants overexpressing the homologous ovine *AANAT* and *HIOMT* genes exhibited loss of apical dominance and enhanced drought tolerance (Wang *et al.*, 2014). Plant species sensitive to ozone damage have lower levels of melatonin compared to ozone resistant species (Dubbels *et al.*, 1995). Similarly, Alpine and Mediterranean plant species growing in high UV-exposed natural habitats have higher levels of melatonin compared to their counterparts growing under low UV exposure areas. Zhang *et al.* (2012) confirmed the protective role of melatonin against UV-B. When exposed to UV-B radiation, DNA damage was in transgenic *Nicotiana sylvestris* plants expressing melatonin synthesis genes. Melatonin is also useful to save plants from heavy metals stress, as presowing seed treatment of red cabbage seed (*Brassica oleracea* var. *rubrum*) eliminated the toxic effects of copper ions (0.5 and 1 mM) during germination and early seedling growth. Similarly it has been observed that application of zinc sulfate (1 mM) increased the concentration of melatonin up to 6-fold in barley (*Hordeum vulgare* L.) roots, suggesting the protective role of melatonin against chemical and other abiotic stressors.





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Phytoremediation

The water hyacinth grown under bright sunlight (10,000–15,000 $\mu\text{W}/\text{cm}^2$) produces extremely high concentrations of melatonin and N-acetyl-N-formyl-5-methoxykynuramine (AMFK) as compared to plants grown in artificial light (400–450 $\mu\text{W}/\text{cm}^2$) (Tan *et al.*, 2007). On the basis of these findings and others, the authors proposed that the presence of high concentrations of these molecules save these pollutant-resistant plants from the harsh environmental contaminants.

CONCLUSION

Schematic representation of two pathways that melatonin alleviated the inhibitory effects of NaCl stress on seed germination. Both reactive oxygen species (ROS) and plant hormones were regulated by melatonin. The arrows represent the positive role of melatonin. The genes or molecules in red and green are up and down-regulated by melatonin, respectively.

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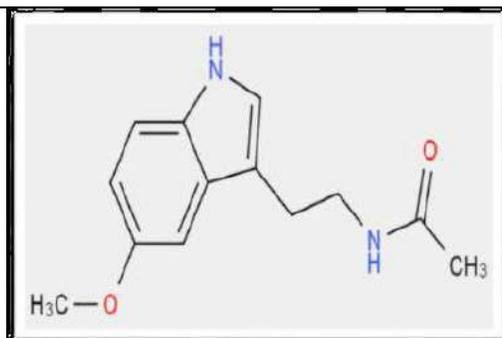
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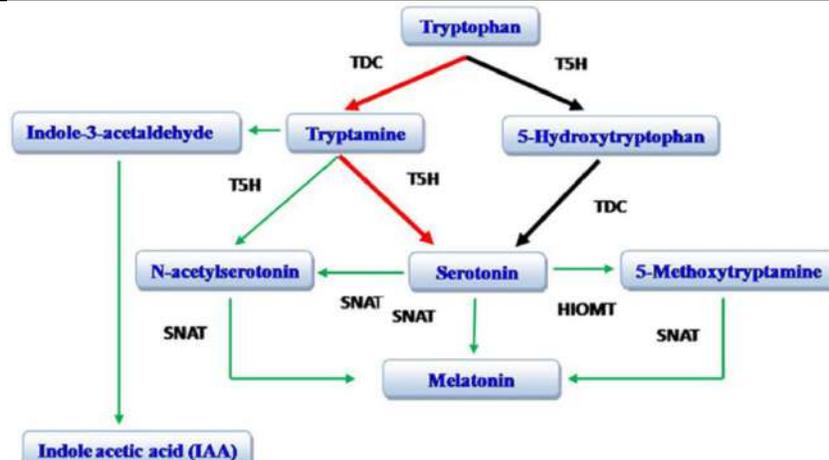


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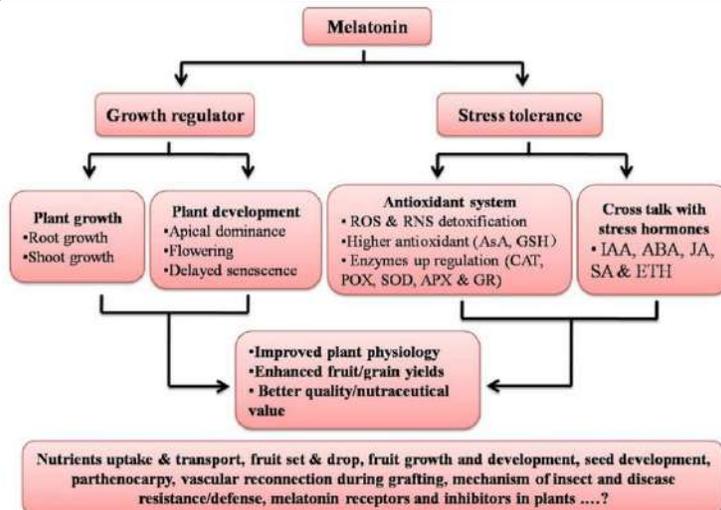
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Chemical structure of Melatonin



Biosynthesis of Melatonin

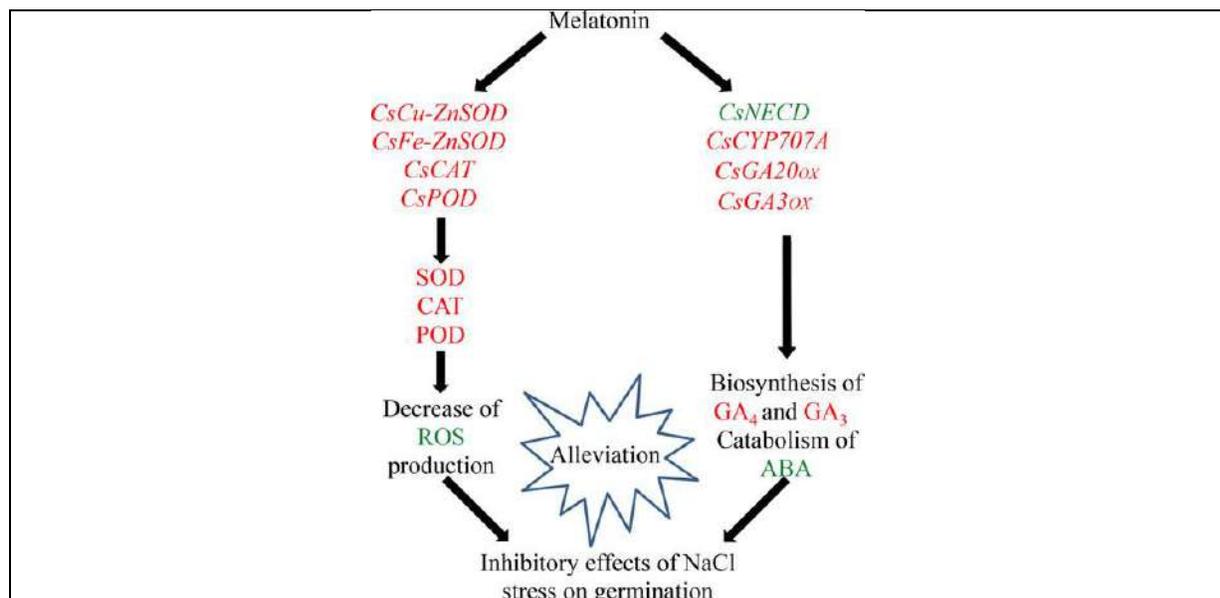


Phytohormonal





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Morpho-taxonomic Characterization of Water Apple (*Syzygium sps.*) Germplasm Under West Bengal Region.

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ABSTRACT

Water apple is a tropical evergreen tree with a bell shaped edible berry which is highly nutritive, crispy and juicy. Fruit being high in water content serves in quenching thirst and removing harmful effects of dehydration. The fruit is mostly consumed in the raw form but it is also used in the form of juice, jelly and for wine making. To safeguard the existing diversity of this fruit and to achieve sustainable development based on use of available genetic wealth, promotion and conservation of this species is of immense importance. *In-situ* characterization and preliminary evaluation are performed following Biodiversity International Tropical Fruit Descriptor. Total 16 numbers of accessions were studied from different locations of West Bengal. Tree age of water apple accessions varied from 8yrs to above 20yrs. Elliptic leaf blade shape, narrowly-broadly acuminate leaf apex, obtuse-oblique leaf base, opposite leaf orientation and arcuate leaf venation was observed in accessions. Maximum fruit length is recorded in ACC-11 (4.85 cm) and fruit breadth of 4.47 cm in ACC-11. Maximum fruit weight (29.42 g) in ACC-5 and maximum number of seeds (2.2) in ACC-1, ACC-3, ACC-3 and ACC-6 is recorded. TSS ranged from 2.04° Brix (ACC-7) to 7.05° Brix (ACC-2) and maximum total sugar 5.74 mg/100 gm pulp. Reducing sugar of 7.75 mg/100 gm (ACC-2), acidity 0.359 mg/100 gm (ACC-13) and Vit.C 28.08 mg/100 gm (ACC-5 and ACC-6) are been recorded. Average linkage technique clustering when applied on squared Euclidean distance matrix a total of 6 different clusters were formed for plant physical parameters, fruit physical parameters and for fruit biochemical parameters.

Keywords: Water apple, Conservation, Sustainable development, Linkage Technique, Physical and Biochemical Parameters.



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INTRODUCTION

Genus *Syzygium* is the largest, belonging to family Myrtaceae. The centres of diversity are the southern Asian, Southeast Asian, Malesian, Australian and New Caledonian regions, with decreasing species richness elsewhere [1] [2]. Water apple fruit is a bell shaped edible berry with white, pale green or red fruit colours. Texture of the fruit is crunchy and juicy with mild scented flavour and contains 93% of water, 0.60 g of protein, 0.30 g fats, 5.70 g carbohydrates and 25 kcal energy and is a good source of minerals and vitamins like calcium, potassium, phosphorus, magnesium, ascorbic acid and vitamin A. Twenty species of *Syzygium* has been documented occurring in Vanuatu region and identification keys are provided for flowering and vegetative material [3]. Morphological variation of a crop indicates the genetic diversity and effect from environment. Both environmental and genetic effects contribute to phenotypic variation within and among populations [4]. To safeguard the existing diversity of this fruit and to achieve sustainable development based on use of available genetic wealth, promotion and conservation of this species is of immense importance. Beside its importance for nutritional value and a source of income, diversity of these fruits also has a cultural, social value and contributes to the stability of ecosystems [5].

MATERIALS AND METHODS

Different agroclimatic zones of West Bengal were stratified depending on availability of fruit crop and on convenience of the study. The study has been carried out in Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal during the years 2013-2015. Data for this study has been generated through key informant survey and individual household survey from respondents by using semi-structured questionnaire. In-situ characterization and preliminary evaluation of under-utilised/minor fruit Water apple (*Syzygium* sps.) are performed following Biodiversity International Tropical Fruit Descriptor. The general information like age of the plant, behaviour of the plant was documented by making a questionnaire at the time of the survey. The genotypes were selected randomly with their varying age and location, which were further given accession numbers considering each accession as a treatment and replicating it for five times for all the quantitative parameters. The analysis has been carried in three parts according to the variation in age of the plants and observations to be recorded. Under the observation for characterization, the plant physical parameters has been analyzed and so for preliminary evaluation fruit physical parameters and fruit biochemical parameters has been analyzed. Multivariate analysis of characterization and evaluation parameters will be done following nearest neighbourhood method of hierarchical clusters analysis of squared Euclidean distance matrix on the basis of characters measured following Dillon and Goldstein 1984.

RESULT & DISCUSSION

Total 16 numbers of accessions were studied from different locations including institutional farm, local vicinity Fatepur and Chowgacha (Nadia) and Basirhat (North 24 parganas). Branching pattern was observed to be irregular, irregular to oblong crown shape and spreading type tree growth habit in all the accessions (**Table 1**). Elliptic leaf blade shape, narrowly-broadly acuminate leaf apex, obtuse-oblique leaf base, opposite leaf orientation and arcuate leaf venation was observed in water apple accessions (**Table 1**). A study has reported ovate leaf, symmetric leaf base, apex acute or short acuminate, acumen flat and margin flat to slightly undulate in *Syzygium samarangense* [3]. The proximity value 8.70 denoted the minimum value between ACC-1 and ACC-7 followed by 13.74 between ACC-14 and ACC-16. The maximum value 22305.39 between ACC-3 and ACC-15 denoted the minimum similarity (**Table 2**). Average linkage technique clustering when applied on squared Euclidean distance matrix a total of 6 clusters were formed for plant physical parameters. The clusters 1, 2, 5 and 6 comprised of homogeneous types having similarity in characterization attributes (**Table 3, Fig. 1**). Cluster 3 and 4 comprised of single member namely ACC-10 and ACC-13. ACC-1 and ACC-7 formed the first cluster at the distance of 8.70 and ACC-14 and ACC-16 formed the second cluster at 13.74. ACC-4 combines with the first cluster (ACC-1 and ACC-7) at the distance of 45.75.





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Two types of water apple (Pink type and white type) have been reported to be studied for different fruit physical and bio-chemical characters [6]. A study conducted on physico-chemical characters in water apple recorded data on length, diameter, weight, volume, TSS and chemical composition. Fruits of Type 2 (greenish white in colour and weighing 68-76 g) were studied to have the highest physico-chemical quality and those of Type 4 (completely purple and weighing 5-8 g) were judged to be poorest in quality [7]. At later stage ACC-16 and ACC-3 pairs with ACC-8 at the distance 2.50 and 2.82 respectively. ACC-5 and ACC-11 joins the first cluster when they paired with ACC-3 at the coefficient distance 5.21 and 6.81. In second cluster ACC-1 and ACC-12 paired at the distance 1.06 followed by ACC-4 and ACC-6 at 5.47 which later joins to form a cluster at coefficient distance 6.85. The sixth cluster was formed between ACC-10 and ACC-13 at the coefficient distance 7.63 respectively. Two cultivars of water apple (*Syzygium samarangense*) were evaluated, Semarang Rose and Kristal Taiwan and observed that the Kristal Taiwan cultivar was larger in size and weight but smaller in length compared to Semarang Rose [8].

The minimum value was 0.03 between ACC-5 and ACC-6 denoted maximum accessions similarity followed by 0.13 between ACC-11 and ACC-16. The maximum value 365.89 between ACC-5 and ACC-10 denoted minimum similarities with respect to fruit biochemical parameters (**Table 6**). Average linkage technique clustering when applied on squared Euclidean distance matrix a total of 6 clusters were formed for fruit biochemical parameters. Cluster 1, 4, 5 and 6 comprised of homogeneous types having similarity in characterization attributes (**Table 7, Fig. 3**). Cluster 2 and 3 comprised single member namely ACC-3 and ACC-2. The first cluster comprised of ACC-5, ACC-6 and ACC-7 where ACC-5 and ACC-6 paired at the coefficient distance 0.03. The fourth cluster comprised of ACC-13 and ACC-14 paired at the coefficient distance 7.27 respectively. In fifth cluster ACC-8 and ACC-9 pairs at the distance 0.26 and later ACC-10 at 0.63. ACC-11 and ACC-16 paired at the distance 0.13 to form the sixth cluster along with ACC-1, ACC-12, ACC-15 and ACC-4 at the maximum coefficient distance 14.28 respectively. A study conducted on Chalta, Rose apple and wood apple germplasm reported multiple numbers of clusters being formed for plant physical parameters, fruit physical parameters and for fruit biochemical parameters with average linkage technique clustering when applied on squared Euclidean distance matrix[9abc].

CONCLUSION

With dissimilarity to water apple tree age from 8yrs to above 20yrs, the tree height and trunk girth varied in different germplasm. Branching pattern was observed to be irregular, irregular to oblong crown shape and spreading type tree growth habit in all the accessions. Water apple have elliptic leaf blade shape, narrowly-broadly acuminate leaf apex, obtuse-oblique leaf base, opposite leaf orientation and arcuate leaf venation. Length of the leaf blade, breadth, fruit length, fruit breadth, fruit weight and numbers of seeds is recorded to have significant variation. The diversity in fruit can be supported with the findings those of Kristal Taiwan and Semarang Rose mentioned earlier.

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Table 1: General tree and leaf characters of water apple accessions

ACC no.	BP	TGH	CS	LBS	LAS	LB	LO	LV
ACC-1	1	2	1	4	5	7	9	10
ACC-2	1	2	1	4	5	7	9	10
ACC-3	1	2	1	4	5	8	9	10
ACC-4	1	2	1	4	5	8	9	10
ACC-5	1	2	1	4	5	8	9	10
ACC-6	1	2	1	4	5	7	9	10
ACC-7	1	2	1	4	5	7	9	10
ACC-8	1	2	1	4	5	8	9	10
ACC-9	1	2	1	4	5	8	9	10
ACC-10	1	2	1	4	5	8	9	10
ACC-11	1	2	1	4	6	7	9	10
ACC-12	1	2	1	4	5	7	9	10
ACC-13	1	2	3	4	5	7	9	10
ACC-14	1	2	1	4	6	7	9	10
ACC-15	1	2	1	4	5	7	9	10
ACC-16	1	2	1	4	5	7	9	10

ACC- Accession, BP- Branching Pattern, TGH- Tree Growth Habit, CS- Crown Shape, LBS- Leaf Blade Shape, LAS- Leaf Apex Shape, LB- Leaf Base Shape, LO- Leaf Orientation, LV- Leaf Venation, 1- Irregular, 2- Spreading, 3- Oblong, 4- Elliptic, 5- Broadly Acuminate, 6- Narrowly Acuminate, 7- Obtuse, 8- Oblique, 9- Opposite, 10- Arcuate





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Table 2: Proximity matrix for water apple plant physical attributes

	Squared Euclidean Distance														
	ACC-1	ACC-2	ACC-3	ACC-4	ACC-5	ACC-6	ACC-7	ACC-8	ACC-9	ACC-10	ACC-11	ACC-12	ACC-13	ACC-14	ACC-15
ACC-2	55.50														
ACC-3	441.04	656.08													
ACC-4	55.48	55.77	798.10												
ACC-5	103.31	234.26	123.56	295.88											
ACC-6	245.05	418.71	30.33	520.24	31.62										
ACC-7	8.70	46.55	551.30	36.03	166.23	333.09									
ACC-8	112.99	26.61	875.91	44.76	357.67	589.22	96.89								
ACC-9	77.63	19.32	684.69	58.33	244.41	435.66	82.69	15.33							
ACC-10	1503.37	1155.58	3495.46	1000.56	2322.17	2885.29	1357.53	879.56	1103.81						
ACC-11	10374.68	9498.73	15046.35	8951.92	12469.06	13746.92	9922.26	8726.73	9420.11	4087.22					
ACC-12	10962.22	10045.02	15752.45	9508.32	13120.75	14427.60	10489.34	9263.99	9985.21	4465.15	19.58				
ACC-13	6316.04	5670.79	10009.80	5198.59	7917.56	8943.70	6003.25	5023.18	5532.36	1727.55	637.16	831.67			
ACC-14	16385.52	15281.17	22153.40	14587.15	19002.07	20572.20	15807.71	14305.72	15192.55	8119.25	690.23	550.96	2568.27		
ACC-15	16509.81	15393.39	22305.39	14726.01	19159.34	20730.11	15910.66	14447.52	15355.66	8265.66	767.87	588.84	2746.75	44.85	
ACC-16	16498.57	15367.14	22280.29	14706.07	19131.27	20701.90	15909.45	14409.03	15302.72	8211.95	725.63	571.32	2681.76	13.74	28.16

This is a dissimilarity matrix

Table 3: Details of clusters for characterization variables using average linkage clustering methods on squared Euclidean distance matrix for plant physical characters

No. of clusters	Cluster members (Allowed distance co-efficient 77.59)
1	Acc-1, Acc-7, Acc-4, Acc-8, Acc-9, Acc-2
2	Acc-3, Acc-6, Acc-5
3	Acc-10
4	Acc-13
5	Acc-11, Acc-12
6	Acc-14, Acc- 16, Acc-15

Table 4: Proximity matrix for Water apple fruit physical attributes

	Squared Euclidean Distance														
	ACC-1	ACC-2	ACC-3	ACC-4	ACC-5	ACC-6	ACC-7	ACC-8	ACC-9	ACC-10	ACC-11	ACC-12	ACC-13	ACC-14	ACC-15
ACC-2	15.19														
ACC-3	12.57	50.73													
ACC-4	4.00	21.32	7.17												
ACC-5	27.74	80.61	3.64	20.84											
ACC-6	7.31	6.55	24.97	5.47	47.53										
ACC-7	12.55	47.29	.57	5.31	5.79	21.33									
ACC-8	14.25	57.64	1.99	12.62	3.45	33.10	3.80								
ACC-9	13.94	56.40	1.97	11.95	3.94	31.90	3.43	.08							
ACC-10	115.76	48.19	184.87	119.45	240.02	74.10	173.46	203.01	199.47						
ACC-11	9.55	47.93	7.53	14.68	11.47	31.66	10.68	3.44	3.97	190.52					
ACC-12	1.06	10.77	20.57	8.13	38.53	7.95	20.45	21.38	20.95	103.61	13.60				
ACC-13	164.14	79.52	252.27	175.10	315.68	119.12	240.79	270.66	267.22	7.63	249.49	146.88			
ACC-14	141.76	248.78	89.44	143.33	60.27	201.16	99.94	71.48	73.77	509.24	78.72	157.10	606.32		
ACC-15	133.59	236.07	73.13	125.30	46.40	180.49	82.22	65.01	67.16	483.21	80.96	153.99	586.51	20.71	
ACC-16	6.55	38.71	2.69	5.36	9.24	19.47	3.05	2.59	2.41	164.68	3.78	11.46	225.59	96.62	92.41

This is a dissimilarity matrix





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Table 5: Details of clusters for characterization variables using average linkage clustering methods on squared Euclidean distance matrix for fruit physical characters.

No. of clusters	Cluster members (Allowed distance co-efficient 7.63)
1	Acc-8, Acc-9, Acc-16, Acc-3, Acc-7, Acc-5, Acc-11
2	Acc-1, Acc-12, Acc-4, Acc-6
3	Acc-2
4	Acc-14
5	Acc-15
6	Acc-10, Acc-13

Table 6: Proximity matrix for Water apple fruit biochemical attributes

	Squared Euclidean Distance														
	ACC-1	ACC-2	ACC-3	ACC-4	ACC-5	ACC-6	ACC-7	ACC-8	ACC-9	ACC-10	ACC-11	ACC-12	ACC-13	ACC-14	ACC-15
ACC-2	216.30														
ACC-3	112.48	69.80													
ACC-4	14.67	287.20	125.51												
ACC-5	233.05	59.02	23.28	256.05											
ACC-6	232.56	57.44	23.13	256.00	.03										
ACC-7	167.31	59.19	5.83	184.96	5.81	5.76									
ACC-8	14.01	337.29	191.88	13.07	346.33	346.06	263.54								
ACC-9	13.41	334.65	194.79	16.00	349.30	349.03	266.53	.26							
ACC-10	16.75	349.61	207.77	19.91	365.89	365.72	281.33	.93	.34						
ACC-11	.20	205.01	104.84	15.92	221.45	221.01	157.68	16.97	16.34	19.91					
ACC-12	1.16	202.44	108.85	20.06	225.69	225.17	161.85	18.76	17.74	21.17	.93				
ACC-13	35.45	93.45	28.43	60.83	91.84	91.75	54.60	89.89	89.47	97.00	30.63	32.04			
ACC-14	13.02	131.63	53.59	32.22	141.05	140.63	91.77	50.25	50.27	56.65	10.18	11.36	7.27		
ACC-15	2.57	229.91	107.46	6.45	229.32	228.79	162.80	13.20	14.17	18.47	3.11	5.24	39.50	15.29	
ACC-16	.33	203.64	101.15	14.29	216.98	216.49	153.58	17.37	17.06	20.99	.13	1.31	29.87	9.49	2.16

This is a dissimilarity matrix

Table 7: Details of clusters for characterization variables using average linkage clustering methods on squared Euclidean distance matrix for fruit bio-chemical characters.

No. of clusters	Cluster members (Allowed distance co-efficient 14.28)
1	Acc-5, Acc-6, Acc-7
2	Acc-3
3	Acc-2
4	Acc-13, Acc-14
5	Acc-8, Acc-9, Acc-10
6	Acc-11, Acc-16, Acc-1, Acc-12, Acc-15, Acc-4





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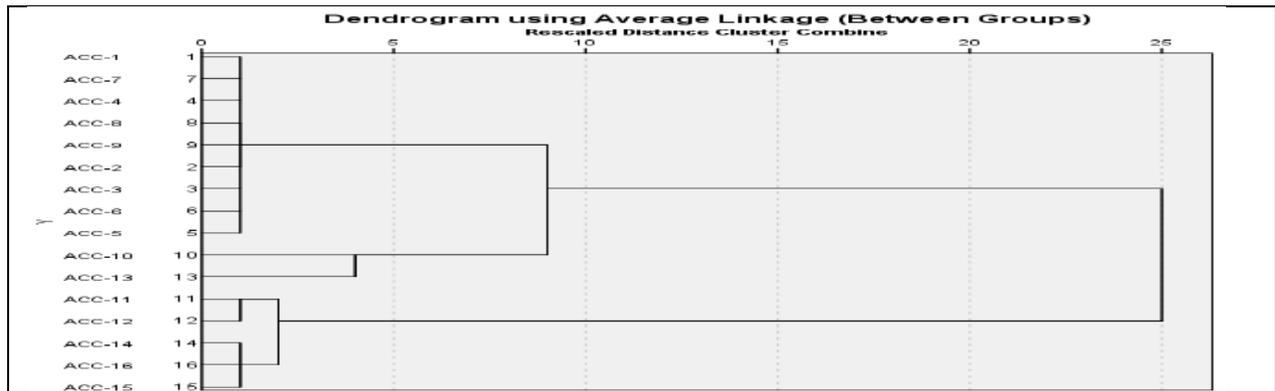


Figure 1: Dendrogram using average linkage hierarchical clustering of squared Euclidean distance matrix for water apple plant physical attributes.

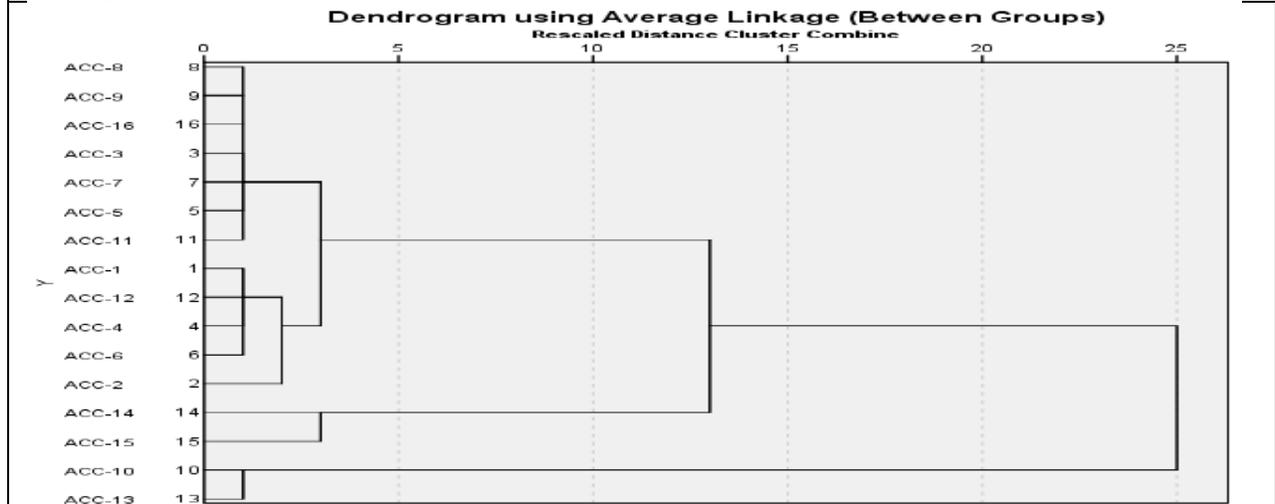


Figure 2: Dendrogram using average linkage hierarchical clustering of squared Euclidean distance matrix for Water apple fruit physical characters.

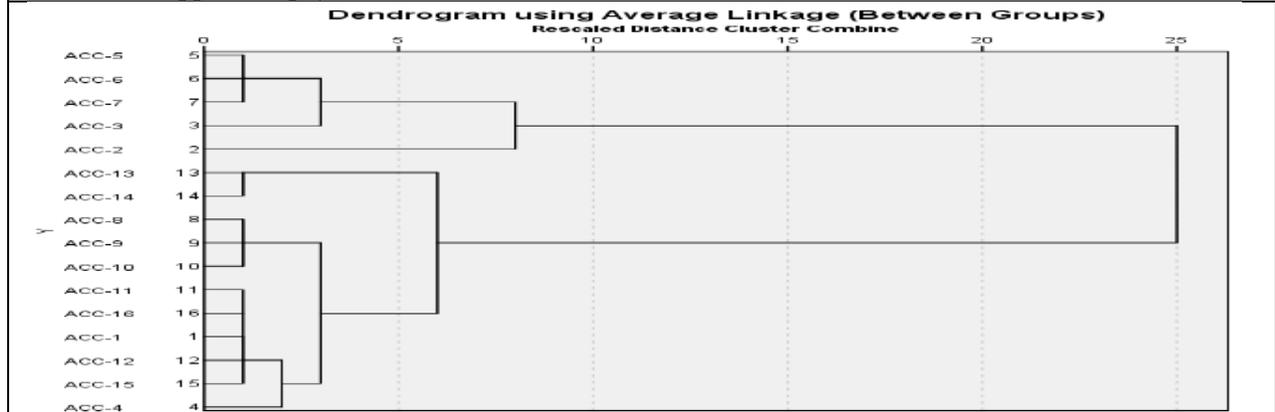


Figure 3: Dendrogram using average linkage hierarchical clustering of squared Euclidean distance matrix for Water apple fruit bio-chemical characters.





Young Female Athletes – A Cross Sectional Survey

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ABSTRACT

The main purpose of this cross-sectional survey is to understand the difference between male and female athlete. Hereby female athletes needs more attention and training to improve their performance skills, due to the variation in genetics and availability of resources. Female athlete is far beyond the male's capacity, the challenges faced by them like sports inequity, body image issues, eating disorders, increased mental distress, and internalization of emotions makes them fall behind in performance. The results of the survey makes us understand that the female athletes are not aware of the fitness components required for their sport and also about the latest performance enhancing training regime available to improve their performance. There is a need to identify the highly influential Exercise training regime to improve the performance of female athletes and it is the role of Sports physiotherapists to identify performance enhancing Exercise Training program.

Keywords: Plyometric exercises, performance, Skill related fitness, Agility, Speed, Power, Young Female athletes.

INTRODUCTION

Fitness is the key to healthy Life and skilled related fitness is the primary prerequisite to all athlete for their performance in sports. Physical fitness is asset of Physical attributes that can be acquired over a period of time. The degree to which an athlete acquires these attributes can be measured with specific tests [1]. Compared to male, a female athlete needs more attention and training to improve their performance skills due to the variation in genetics and availability of resources. Though the withstanding capacity of the training session of a female athlete is far beyond the male's capacity, the challenges faced by them like sports inequity, body image issues, eating disorders,





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increased mental distress, and internalization of emotions makes them fall behind in performance [2]. Women differ from men not only in physique, but also in body composition and hormonal milieu, and also differ from one another. Their monthly hormonal cycles, with fluctuations in estrogen and progesterone, have varying effects on metabolism and fluid retention [3] Women have higher amount of stored body fat than men due to presence of breast tissue and other sex tissues. They also have less lean muscle mass compared to men. Thus is the major reason why men excel in sports than women easily. But this can be overcome by nutritional plan and fitness strategies. It is estimated that the essential fat is around 10 to 12% in women whereas it is 7% in men [4]. Over the past century, there has been an exponential increase in the number of female athletes both for competitive and/or recreational facilities [5]. Hence the entire female athlete community is in need of a working model to improve their performance and skill. There is growing need to identify the recent trends in exercise prescription for athlete. Plyometric exercises uses the pre-stretch of the muscle-tendon unit physiological length-tension curve in order to enhance the ability of the muscle fibers to generate more tension and resultant force production [6]. Agility is defined as a rapid whole-body movement with change of velocity or direction in response to a stimulus" [7].

The aim of this survey is to find out the training pattern, training difficulties, training during menstrual cycle, nutrition value intake, frequency of training, intensity of the training, any rehabilitation or therapy taken by female athletes in Tamil Nadu between the age group of 15 & 22 years.

MATERIALS AND METHODS

A cross sectional survey study was done between January 2022 and April 2022 through online method to target more respondents. A set of 17 questions were designed from existing fitness related questionnaires. The responses to the questions were mostly Yes/No/Maybe. Certain questions allowed multiple answering. As the answers were nominal and subjective in character and did not involve any ranking or rating the need for validity and reliability were winnowed out. The survey mainly targeted the female athletes throughout Tamil Nadu between the age group of 15 and 22, to know about the awareness on the fitness needs of each sports and if their need matched with the expected sports performance. Assurance was given to maintain the confidentiality of the participants and the participation was purely on self-interest. A google form of the questionnaire was prepared and a link was created and the same was circulated through WhatsApp, Facebook and Instagram. The demographic data were recorded. The answers were inspected by a therapist who was not included in the study. The questionnaire is presented in Table 1.

Statistical Analysis

The results of the study were displayed as percentages.

RESULTS

247 respondents submitted their responses of which 37 were excluded as the answers were irrelevant with all markings either 'Yes' or 'No' and some were marked by male respondents. Hence a total of 210 were considered for analysis. The mean age of the participants was 18.8. The surveys showed higher participation of female athletes (71%) in the age group of 19-22 on the contrary to 29% in the age group of 15-18 years. 49% of respondents were between 51-60 kgs and 25% were between 4-50 and 61-70 kgs. The most desirable results was 80% of respondents were aware of Plyometric training and 50% of respondent answered that they were aware of the role of Plyometric in improving performance.

DISCUSSION

The study aimed at scrutinizing the knowledge and awareness of various fitness related components that are available in the field and which recent trends has gained popularity among female athletes. Out of 17 questions 5





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aimed at assessing the nutritional status and its relation in performance. There was a sufficient awareness among the athletes about the Plyometric training in improving the performance which supports Radegard et.al[8]. Some questions identified role of fluid intake and supplements on performance skills. The last question dealt with the sleeping duration which affects the performance of the athlete directly. Some of the answers are represented in charts below.

CONCLUSION

The results of the survey make us understand female athletes are not aware of the fitness components required for their sport and also about the latest performance enhancing training regime available to improve their performance.

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Conflict of Interest

The authors have none to declare.

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Table: 1 (questionnaire)

1. Age
 1. 15-18 years
 2. 19-22 years
2. Sex
 1. Female
 2. Other
3. Weight
 1. 40-50
 2. 51-60
 3. 61-70
 4. Above 70
4. Education
 1. School
 2. College
 3. Others
5. Sports
 1. Short distance runner
 2. Long distance runner
 3. Volley ball
 4. Shuttle
 5. Foot ball
 6. Others
6. Do you know that Exercise / Therapy can improve your performance?
 1. Yes
 2. No
7. Are you aware of ' Plyometric Exercises?
 1. Yes
 2. No
 3. Maybe
8. Do you know that plyometric exercises will improve your sports performances?
 1. Yes
 2. No
 3. Maybe
9. Which of the following factors do you believe will improve your performance level?
 1. Exercise
 2. Advanced skill training
 3. Diet and nutrition
 4. Therapy
10. Regarding your sport, which of the following components you are working for?
 1. Performance
 2. Speed
 3. Strength
 4. Balance
 5. Power
 6. Other





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11. Frequency of sports training?

1. 7 days a week
2. 4 - 5 days / week
3. 2 - 3 days / week

12. Are you consuming any of the following supplements?

1. Whey / organic protein
2. BCAA(branched chain amino acid)
3. Creatine
4. Other:

13. Anxiety level during or before your sports events?

1. Very mild
2. Mild
3. Moderate
4. High
5. Severe

14. Daily intake of water or fluids?

1. 3 liters
2. 4-5 liters
3. 5 liters and above

15. During menstrual cycle, your participation in sports training? (for females alone)

1. Daily
2. Alternate days
3. After the cycle
4. Other:

16. Which of the following diets you follow?

1. Mother's recipe for health
2. Vegan
3. Non vegan
4. Paleolithic
5. Ketogenic
6. Intermittent fasting
7. Other

17. Sleep duration?

1. 5 -6 hours
2. 6 & above
3. Less than 5 hours





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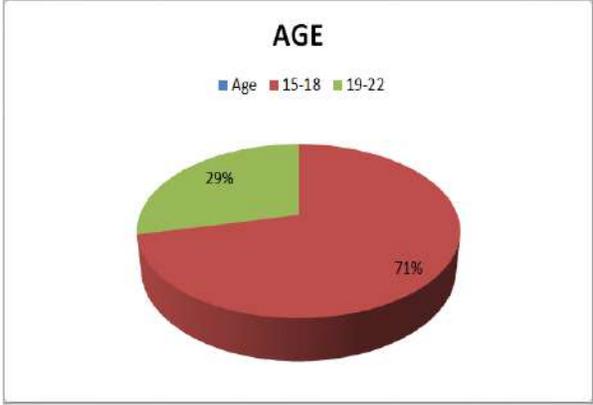


Figure 1: Age of Respondents



Figure 2: Weight of the respondents

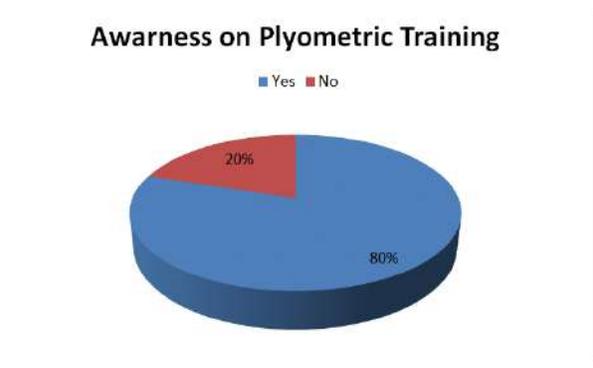


Figure 3: Plyometric Awareness

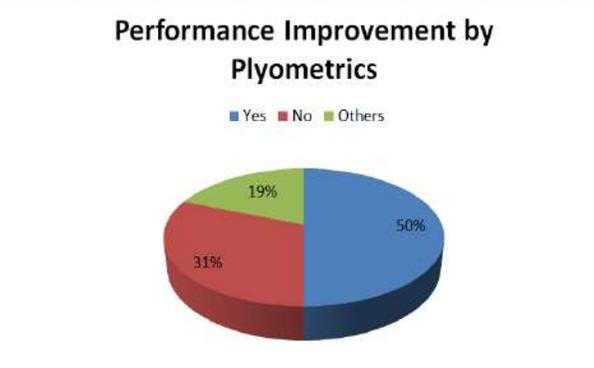


Figure 4: Will Plyometric improve Performance?





In vitro Propagation of Chrysanthemum through Petals

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ABSTRACT

Chrysanthemum (*Chrysanthemum grandiflora* Tzvelev) is one of the commercial crops in India. Studies were conducted for standardization of micro propagation protocol from ray florets of chrysanthemum. The experiments revealed that the highest percentage of aseptic culture and lowest percentage of contamination was observed in treatment with mercury chloride (0.1%) and ethanol (70%), the maximum callus percentage was obtained in treatment MS medium with BAP 4 mg l⁻¹ and NAA 1 mg l⁻¹, highest percentage of shoot proliferation and more number of micro shoots per clump were seen in MS medium with 5 mg l⁻¹ BAP and 1 mg l⁻¹ NAA. Number of days taken for rooting was less in half MS medium with IBA 1.5 mg l⁻¹. Number of roots per shoot were more in half MS medium with IBA 1.5 mg l⁻¹. Half MS medium with IBA 1 mg l⁻¹ resulted more root length in Poornima Red.

Keywords: Chrysanthemum cultivar Poornima Red, Petals, callus, MS medium

Abbreviation: MS: Murashige and Skoog; BAP: Benzylaminopurine; NAA- Naphthaleneacetic acid; IBA: Indole Butyric Acid

INTRODUCTION

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is one of the important commercial cut flower and pot plant, commonly called as 'Autumn Queen'. Chrysanthemum ranks second in the global cut flower market after rose (Datta and Gupta, 4). In floriculture industry, chrysanthemum has large demand and popularity because of its range of flower forms, colours and their growth habits. *In vitro* propagation will be useful for rapid plant multiplication, germplasm conservation, elimination of pathogens, genetic manipulations, and for secondary metabolite production. Plant tissue culture has emerged as an approaching tool and forms the backbone of plant biotechnology and the better quality planting material is a basic need of growers for accelerating the productivity. (Chebet *et al.*, 2) The success of the *in vitro* propagation depends on numerous factors like genotype, type of media, plant growth



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regulators and type of explants, which should be used during the process (Kim *et al.*, 7). The present article emphasizes the protocol standardization for rapid multiplication of chrysanthemum cultivars through *in vitro*.

MATERIAL AND METHODS

Standardization of protocol for organogenesis was carried out in the Tissue Culture Unit, Department of Biotechnology and Crop Improvement during 2018-19. For the study, chrysanthemum cultivar Poornima Red was selected and the ray florets were used as explants. Flowers were collected from healthy mother plants which were grown in well maintained poly house. Flowers were washed with running tap water thoroughly for 15 minutes followed by washing with sterile water for 2 to 3 times before culture under laminar air flow chamber served as control. Similarly flowers were treated with different chemicals sodium hypochlorite, mercuric chloride and ethanol in different concentrations with specific time period. Finally 3-4 times rinses with sterile water. After sterilization, ray florets were peeled off from the flower and cut horizontally. Basal portion was placed adaxially on Murashige and Skoog medium (Murashige and Skoog, 9) with 30g/l⁻¹ sucrose, 8g/l⁻¹ agar and Benzyl amino purine (BAP, at 2.0, 3.0, 4.0 mg l⁻¹), Kinetin (KN, at 2.0, 3.0, 4.0 mg l⁻¹) in combination with Naphthalene acetic acid (NAA, at 1.0 mg l⁻¹) for callus initiation.

The pH of the medium was adjusted to 5.7 before autoclaving at 121 °C for 20 minutes. After callus formation, callus was transferred to the MS medium with BAP (4.0, 5.0 and 6.0 mg l⁻¹) and NAA (1.0 mg l⁻¹). After formation of shoots, shoots were transferred to the half MS medium either with Indole butyric acid (0.5, 1.0, 1.5 mg l⁻¹) or NAA (0.5, 1.0 and 1.5 mg l⁻¹) for rooting. Media was supplemented with 30g l⁻¹ sucrose as a carbon source and 7g l⁻¹ agar-agar as a gelling agent. pH was maintained in between 5.7-6. Media was autoclaved in 121°C temperature and 15 lb pressure for sterilization. In rooting media activated charcoal has been added at 2g l⁻¹. All the media were kept and observed for contamination for minimum three days before using for culture. Baby jar bottles (250 ml) with autoclavable polypropylene caps were used as culture containers. Culture room maintained at a temperature of 25 ± 2°C with uniform light intensity (ca 1000 lux) was provided by fluorescent tubes (7200°K) over a light and dark cycle of 16 and 8 hours respectively. Each treatment had 6 replications arranged in a completely randomized design.

RESULTS AND DISCUSSION

The data on percentage of aseptic culture was recorded one week after culture initiation in all three selected chrysanthemum cultivars. The highest aseptic culture was noticed in mercury chloride and ethanol treatment with 86.60 percent aseptic culture, respectively. No aseptic culture was recorded in untreated control (Table 1). Significantly, the lowest contamination (5.00%) was recorded when treated with mercuric chloride and ethanol (Table 1). The highest percentage of callus (93.75%) induction was observed in MS medium with BAP 4 mg l⁻¹ and NAA 1 mg l⁻¹ in all varieties of chrysanthemum. Whereas, control (MS media without growth regulators) treatment showed zero percentage of callus induction (Table 2). These results shows that BAP combined with NAA may be important for callus induction. Obukosia *et al.* (11) also reported the effect of auxin in callus production. NAA acts as an auxin to induce cell development and enlargement at low concentration. Cell enlargement is associated with an increase in activity of enzymes which will affect the cell wall plasticity and new cell wall materials (Cleland, 3). Nahid *et al.* (10) in chrysanthemum reported that, the induction of callus formation in chrysanthemum was maximum in MS medium with 2 mg l⁻¹ BA and 0.1 mg l⁻¹ NAA. Verma *et al.* (14) described that the 81.67% callus formation was seen in MS medium with 4 mg/l BAP and 1 mg/l NAA in chrysanthemum.

The highest percentage of micro shoot proliferation 79.00% in Poornima Red observed in treatment MS medium with BAP 5 mg l⁻¹ and NAA 1 mg l⁻¹. The highest number of micro shoots per clump (22.30 in Poornima Red) was observed in treatment MS medium with BAP 5 mg l⁻¹ and NAA 1 mg l⁻¹ followed by treatment BAP 6 mg l⁻¹ and NAA 1 mg l⁻¹ (6.16). Whereas, there was no emergence of micro shoots in remaining treatments which were used in this experiment (Table 3). Successful shoot regeneration is important in organogenesis of any crop. Cytokinins are



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generally used for inducing shoot proliferation, combination of auxin and cytokinin was shown effective for shoot regeneration (Kumar *et al.*, 8). Highest percentage of shoot proliferation and a greater number of micro shoots per clump were seen in MS medium with 5 mg l⁻¹ BAP and 1 mg l⁻¹. These results were quite similar to the results obtained by Verma *et al.*, (14) and Chakrabarty *et al.*, (1) in chrysanthemum. Shoot regeneration might be due to the concentration of BAP (cytokinin) which will induce the axillary branching and production of multiple shoots. Poornima Red has shown good response for shoot proliferation in MS medium with 5 mg l⁻¹ BAP and 1 mg l⁻¹ NAA but, the percentage of response will be varied with variety, it might be due to the capacity of genotype for uptake of growth regulator concentration. Significant differences were found among treatments with respect to number of days taken for rooting, number of roots per shoot and root length. Number of days taken for rooting was less in half MS medium with IBA 1.5 mg l⁻¹. Number of roots per shoot was more in half MS medium with IBA 1.5 mg l⁻¹. These results were quite close to the results obtained by Swarna *et al.*, (13) in chrysanthemum. Gautheret (5) suggested first the importance of auxin in root induction, Hoque (6) was used IBA at different concentrations in MS medium for root induction on *in vitro* regenerated shoots in chrysanthemum.

Root length was varied with cultivar to cultivar and concentration of growth regulators (IBA and NAA). Half MS medium with IBA 1 mg l⁻¹ resulted more root length and half MS medium with IBA 1.5 mg l⁻¹ resulted more root length. Similarly Swarna *et al.*, (13) reported that, high mean root length was recorded in media containing 1.0 mg l⁻¹ IBA and 1.5 mg l⁻¹ IBA in chrysanthemum and Shatnawiet *al.* (12) reported that maximum root length was obtained by using IBA or NAA in chrysanthemum. Significant differences were found among treatments with respect to number of days taken for rooting, number of roots per shoot and root length. It might be due to the capacity of genotype for uptake of growth regulator concentration and difference in endogenous growth regulators.

CONCLUSION

Highest percentage of aseptic culture and lowest percentage of contamination was observed in treatment with mercury chloride (0.1%) and ethanol (70%), the maximum callus percentage was obtained in treatment MS medium with BAP 4 mg l⁻¹ and NAA 1 mg l⁻¹, highest percentage of shoot proliferation and more number of micro shoots per clump were seen in MS medium with 5 mg l⁻¹ BAP and 1 mg l⁻¹ NAA in all three chrysanthemum. Rooting was good in half MS medium with IBA 1.5 mg l⁻¹. In this study, we standardized an efficient protocol for plantlet regeneration from callus using petals as explants which will be helpful for commercial propagation of contaminant free plants, in other biotechnological techniques which will be helpful for production of new varieties.

AUTHORS' CONTRIBUTION

Conceptualization of research (Mukund S.); Designing of the experiments (Ramanagouda SH, Seetharamu GK); Contribution of experimental materials (Patil, BC); Execution of field/lab experiments and data collection (Anitha G.); Analysis of data and interpretation (Sandhayarani N); Preparation of the manuscript (Anitha G, Mukund S).

DECLARATION

We do not have any conflict of interest.

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Table 1. Effect of surface sterilants on aseptic culture and contamination percentage in *In vitro* culture of chrysanthemum

S.No	Treatments	Aseptic culture (%)	Contamination (%)
1	Control	0.00 (0.28)	100.00 (89.70)
2	Sodium hypochlorite and ethanol	25.00 (29.70)	55.00 (47.90)
3	Mercury chloride and ethanol	86.60 (72.40)	5.00 (9.36)
4	Ethanol (75%)	22.50 (28.1)	65.80 (54.30)
S. Em±		3.25	2.53
C. D. at 1%		13.09	10.2

*Figures in parenthesis indicates arcsin transformed values





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Table 2. Effect of different growth regulators on callus induction in *In vitro* culture of chrysanthemum cv. Poornima Red

S.No	Treatments	Callus induction (%)
1	Control	0.00 (0.28)
2	BAP 2 mg/l+ NAA 1 mg/l	71.25 (57.58)
3	BAP 3mg/l+ NAA 1 mg/l	76.25 (60.83)
4	BAP 4 mg/l+ NAA 1 mg/l	91.25 (72.79)
5	Kinetin 2 mg/l+ NAA 1 mg/l	51.25 (45.72)
6	Kinetin 3 mg/l+ NAA 1 mg/l	57.50 (49.31)
7	Kinetin 4 mg/l+ NAA 1 mg/l	82.50 (65.27)
S. Em±		1.64
C. D. at 1%		6.55

*Figures in parenthesis indicates arcsin transformed values

Table 3. Effect of different growth regulators on microshoot proliferation and number of microshoots from callus in *In vitro* culture of chrysanthemum cultivars

S.No	Treatments	Microshoot proliferation (%)	Number of microshoots
1	Control	0.00 (0.28)	0.00 (0.28)
2	BAP 4mg/l+ NAA 1 mg/l	43.00 (40.98)	6.03 (14.18)
3	BAP 5mg/l+ NAA 1 mg/l	79.00 (62.73)	22.30 (28.15)
4	BAP 6 mg/l+ NAA 1 mg/l	66.00 (54.33)	6.16 (14.28)
S. Em±		2.23	0.61
C. D. at 1%		8.58	2.45

*Figures in parenthesis indicates arcsin transformed values

Table 4. Effect of different growth regulators on days taken for rooting, number of roots and root length in *In vitro* culture of chrysanthemum cv. Poornima Red

S.No	Treatments	Number of days taken for rooting	Number of roots	Root length (cm)
1	Control	19.97	5.10	6.83
2	½ MS + NAA 0.5 mg/l	14.73	8.91	6.60
3	½ MS + NAA 1 mg/l	15.53	8.00	6.90
4	½ MS + NAA 1.5 mg/l	16.96	8.20	7.90
5	½ MS + IBA 0.5 mg/l	11.40	8.03	8.66
6	½MS + IBA 1 mg/l	12.23	12.10	8.90
7	½ MS + IBA 1.5 mg/l	10.96	12.60	7.33
S. Em±		0.97	0.22	0.28
C. D. at 1%		4.07	0.93	1.16

*Figures in parenthesis indicates arcsin transformed values





Cheuri (*Diploknema butyracea* (Roxb.) H. J. Lam): An Under-Utilized Fruit of Sikkim and Darjeeling Hills Need Domestication

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ABSTRACT

Cheuri is one of the highly valued multipurpose, lesser-known, underutilized and economically important fruit species. It has been using for timber, fodder, fuel wood, medicine and other purposes for long time. It is known for the seed oil which is rich source of chemical compounds *viz.*, steric acid, palmitic acid, oleic acid and linoleic acid. The fruit contains 4.36% protein, 1.02% fat, 81.63% carbohydrate, 8.2% total sugar, 0.090% phosphorus, 0.065% sodium, 0.816% potash, 0.817% calcium and 0.178% iron. The medicinal properties of tree are also well recognized for good health and used for the treatment of rheumatic pain, ulcers, itching, haemorrhage, inflammation of tonsils, leprosy and diabetes. In spite of tremendous potentiality for commercial exploitation, it is facing a great habitat loss and improvement did not receive too much attention so far. The conservation and efficient use of resources of such species can validate in the improvement of livelihoods and sustainable development of the people residing in the regions.

Keywords: *Diploknema butyrace*, Conservation, Resources, Livelihoods, Under-utilized.





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INTRODUCTION

Sikkim and Darjeeling Hills are bestowed with diverse biological habitats that anchorage various species of flora and fauna of sub-tropical to temperate regions. Land-use systems of the region under forestry, agro-forestry, agriculture, horticulture and animal husbandry play an important role in maintaining the economy of the regions. Indigenous people of this region have sound traditional knowledge about the uses of various plant resources. In this context, highly valued multipurpose and economically important fruit species Cheuri (*D. butyracea*) become very important. Tree belongs to Sapotaceae family and nicked named as 'Indian Butter tree' in English, 'Phulwa' in Kannada, 'Beene Mara' in Sanskrit and 'Cheuri' in Nepali language. It is a hardy plant species and its economic age is approximately 80 to 100 years with fleshy fruits traded for edible purpose and alcohol distillation. Many chemical compounds viz., palmitic acid (55%), oleic acid (35%), poor in steric acid and linoleic acid (each 3-4% each) were isolated from seed oil [1]. The applications of Cheuri butter on the German market as palmitic acid is a known emollient (i.e. moisturiser) and this function is useful in skin care. Trees are important in soil and water conservation when grown in the ravines of hills and used for block planting. Local inhabitants do fulfil their nutritional requirements by consuming these fruits available freely in their vicinity [2]. The Chepang community in Nepal have special relationship with this tree as they have custom of giving Cheuri tree to their daughters as gift during marriage. In spite of tremendous potentiality for commercial exploitation, it is facing a great habitat loss and are yet to be given due importance in the region although it has great utilization in day-to-day life as great social, cultural, nutritional, medicinal, environmental and commercial importance. Still there are no organized systematic orchards and improvement did not receive too much attention so far. The conservation and efficient use of resources of such species can validate in the improvement of livelihoods and sustainable development of the people residing in the regions. [3] [4]

Botanical Description

Leaves: The simple, alternate, thick leaves are dark green with 15 -30 cm length and 7-15 cm width. Usually clusters at apex of branchlets, sometimes scattered, base cuneate, margin crenulate, apex with acumen 2-9mm long.

Flowers: Onset in February- March with light grayish white solitary flowers which is sticky with fragrant substance. Flower consists of 1.5-2 cm long oblong to spatulate corolla, 4-6 ovate sepals, 2-4.5 cm pedicel, 18-40 stamen inserted at base of lobe, 2x5mm conoidal ovary and 1.5-5cm long terete or subangular style.

Fruits: Available from April and lasts up to September-October. Matured fruit is ellipsoid of 2-2.5cm x 1-1.5cm and generally pointed by a remaining portion of the style. The ripe fruit pulp is juicy and sweet with 1- 2 kernels/seed in a shell.

Kernel/Seeds: Seeds are pale yellow, cylindrical to obovoid, glossy light brown shell, hilum lanceolate and 2-3mm wide. Testa crustaceous is rich in fats and oils.

Distribution

Cheuri is native to East Asian Sub-Himalayan tract up to the altitude of 5000 feet above sea level. It is well distributed in the agro-climatic conditions of Himachal Pradesh, Uttarakhand, West Bengal, Sikkim and adjacent parts of North-East region in India. Pithoragarh district of Uttarakhand particularly the areas bordering Nepal and adjoining areas of Almora, Bageshwar and Champawat is reported for the species distribution Var. *andamanensis* with brown hairy calyx, ovate corolla lobes and only 18 stamens is reported from semi deciduous and evergreen forests of Andaman Islands [5] [6].

Traditional Uses

The seeds are used to extract fatty, edible, dense and white colour oil (Cheuri ghee). This oil is used in daily cooking, fuel for lamps, honey, gur, chocolate, soap, lip balm, body lotion, candle manufacture, fertilizers [7]. After the extraction of oil, the byproduct oilcakes are used as manure having pesticidal properties and also as fish poison used in fishing [8]. The other residual substance is used as leech repellent in the rainy season. Ripen fruit are often eaten fresh, the pulp are also used for alcohol distillation and pulp syrup is mixed with tobacco for Hookka. Resins of tree



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are mixed with resins of Khirra (*Sapium insigne*) and Katahar (*Artocarpus heterophyllus*) to produce glue used for trapping birds and houseflies. During dry months, leaves are very useful for cattle feeding and considered beneficial for lactating cattles. Leaves are also used for making Tapari (traditional plates) in various rituals and customs in gorkha culture of the region.

Medicinal Uses

The species are well recognized for its medicinal properties and used for the treatment of rheumatic pain, ulcers, itching, haemorrhage, inflammation of tonsils, leprosy and diabetes [9] [10]. The resin is used as lotion for healing cuts and wounds by some tribal people in the region. The nectar obtained from flowers is considered of great medicinal and nutritional use especially during famine. Juice of the corolla is boiled into a liquid which is used as a syrupy sugar. About 4 teaspoons of the bark juice is given to treat indigestion, rheumatic pain and boils. The cleaned crushed roots are soaked overnight or whole day in water and consumed as tonic after straining the water and the fruit juice is used for intoxicating properties and to keep the body warm. Seed oils are applied for curing headache, rheumatism, boils, pimples, wounds, chapped skin and burns.

Factors Leading To Depletion Of The Trees

- Landslides, forest fire and deforestation are the major threat, which cause immense damage to the plant population.
- Due to the lack of proper knowledge in training and pruning, trees grow with large foliage and fragile branches. Therefore, harvesting of fruits become bit difficult and are collected by cutting off whole branches.
- Again, the steep landscape does not permit for ground collection of fruits, which adds up to the cutting of whole branches. Thus, regular felling of branches every year has lead to drying of tree.
- Lack of standardized practice or package of cultivation, harvesting and post-harvest practices for the production of Cheuri.
- The seeds are recalcitrant and even after being stored with pulp at 3° C the seeds lose viability within 3 weeks [11].
- Seed propagation in wild has not been observed since last few decades [12].
- Viability of seed and regeneration were almost lost and there is a great threat of becoming extinction of the plant from Sikkim and Darjeeling Hills.

Suggestive Plan Of Action

- Strengthening research on detailed analysis of the Cheuri habitat distribution, threats to the habitat to be identified and estimation of plant population and documentation.
- Need to identify the suitable agro-climatic locations throughout the Sikkim and Darjeeling hills for the proper in-situ and ex-situ conservation strategies.
- Standardization of regional specific package of cultivation, harvesting and post-harvest practices for the production of Cheuri.
- Advanced propagation techniques (tissue culture) are urgently needed and establishment of well-equipped nurseries and supplying of healthy planting materials to the farmer.
- The conservation, multiplication, extension, utilization and promotion of Cheuri should be done involving the Krishi Vigyan Kendra (KVK), Reginal Research Station (RRS), Central and State Agriculture Universities, State Forest and Horticulture Departments. SHGs, NGOs and Farmer Welfare Organizations should be promoted to work for the new plantation of Cheuri throughout the Sikkim and Darjeeling hills.

CONCLUSION

In current 21st century of human race the critical juncture of global challenges for food security, land degradation, habited loss and rapid climate change, it is necessary to combat the problems by focusing on conservation,





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multiplication, extension, utilization and promotion of such multipurpose species of knows-no-bounds importance to our daily life.

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Table 1. Nutritional properties of Cheuri.

Protein	4.36%	Fibre	3.17 %
Fat	1.02%	Ash	4.18 %
Carbohydrate	87.04%	Vitamin A	385.33 (IU)
Total sugar	8.21%	Vitamin E	12.44 (IU)
Phosphorus	0.090%	Ascorbic acid	22.72 mg
Sodium	0.065%	Anthocyanin	0.64%
Potash	0.816%	Total phenol	34.33 mg
Calcium	0.817%	Total flavonoid	70.14g
Iron	0.178%		

Table 2. Commercial products of Cheuri found in modern market.

Sl. no.	Products	Brands
1	Body moisturizer	Diva Himalaya
2	Day Cream	Officina Naturae
3	Hair Conditioner	Officina Naturae
4	Hair Cream Duo	Tweak'd by Nature
5	Himalaya Cheuri Butter (Chamomile)	Prakriti
6	Cheuri Honey	The Bee Keeping Shop (TBS), Lalitpur, Kathmandu.
7	Essential oil	Annapurna Aroma (USDA Organic)





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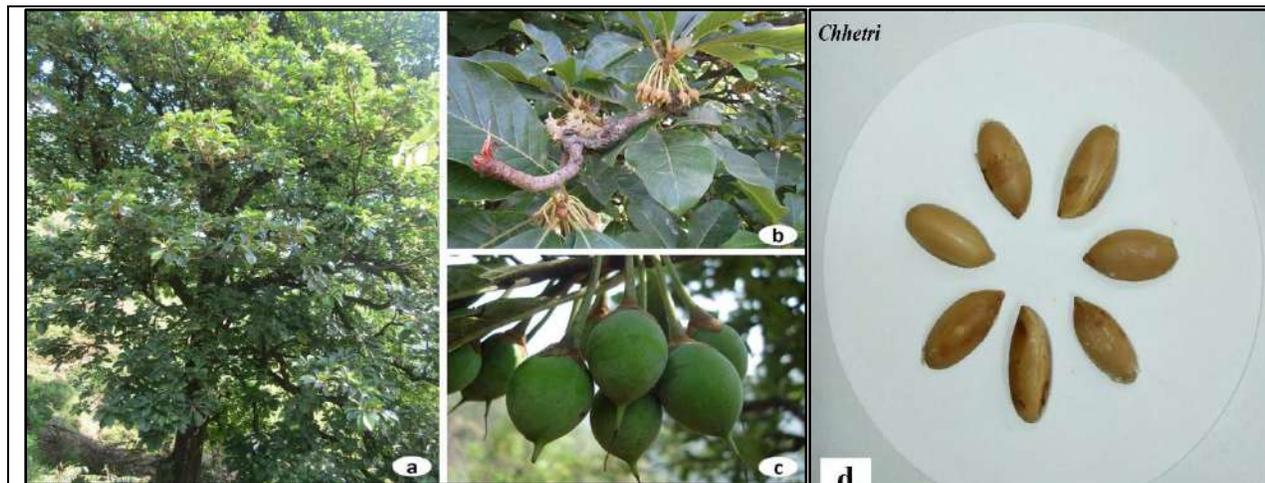


Figure 1. Cheuri tree (a), flowers (b), fruits (c) and seeds (d)

Photo Courtesy: (a and b) Dr. Khem Raj Joshi and (c)Mr. Kuber Jung Malla





Review on Neem: Development of Bio Pesticides and Biofertilizers

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ABSTRACT

Now a day's everyone are using synthetic pesticides and fertilizers for crop protection which has polluted environment. Most of the broad spectrum pesticides are biodegradable and toxic in nature and they are polluting soil environment and they also accumulate in food cycle and leads to biomagnifications. Instead of using synthetic pesticides and fertilizers we can use biopesticide and bio fertilizers as alternative. Neem extracts can be used to produce bio pesticides and bio fertilizers. They act as repellents and they can control leaf miners, whiteflies and fungi, aphids and also control storage pests in case of rice, wheat, etc. Neem can be preferred because it has pesticidal property of natural active ingredients and do not harm humans and beneficial insects and the pest therefore it cannot develop resistance against the bio pesticides. Using this can control pest efficiently, easily available, low cost and also farmers will not have any health hazards. It creates awareness about the side effects of pesticides and fertilizers. As we know that fertilizers also have negative impact on soil and environment. It remains in soil and reduces the fertility of the soil and leaching can cause eutrophication. It is very expensive so the farmers cannot afford them. Excessive use of fertilizer can damage crops like salt burn and dehydration of tissues. Also plants cannot take nutrients as they are easily washed away. So using of neem bio pesticides and bio fertilizers can be used to overcome above disadvantages and provide benefits.

Keywords:





INTRODUCTION

Neem belongs to family meliaceae order Rutales. It has many active components like azadirachtin, nortriterpinoids, etc. have insecticidal property. Based on the information in classical ayurvedic book neem is also known as “krimighna” resembling insecticidal property. For thousands of years the advantageous characteristics of neem have identified in Indian tradition have been recognized (Raizada, *et al.*, 2001). Both leaves and fruits are bitter in taste but having exemplary characters like fungicidal, insecticidal and nematocidal property (Schmutterer, 1995). Neem is used to control various insect pests in Agriculture by repelling or by inhibiting feeding and also by disrupting the growth of insect. It can alter behavior in significant way to decrease the pest damage by reducing fecundity and

kills insects indirectly. We already discussed that neem is easily available and low cost with high benefits. A lot of studies show IGR, antifeedant and reducing fecundity properties shown by Azadirachtin in many insects. Neem oil is extracted and used as pesticide and it is less harm to humans and beneficial organisms. Azadirachtin is used to reduce fecundity by inhibiting the prothoracicotropic hormone and allotropins from brain-corpus cardiacum complex. The use of neem is very efficient because it can be used as pesticide and fertilizer (Mossini and Kimmelneier, 2005; Sujarwoet *et al.*, 2016) and also after extracting neem oil the by-product is used as neem cake which is used as bio fertilizers, helps in providing nutrients to soil (Ramachandran *et al.*, 2007; Lokanadhan *et al.*, 2012) and also it enhances the quality of soil and the crop growth. Neem is used as denitrification by reducing the activity of bacteria, reduce the loss of urea (Musalia *et al.*, 2000; Mohanty *et al.*, 2008). Seed kernel is considered as important part in pesticidal property because it is having active compounds together called as triterpenes which are specifically known as liminoids; these are Azadirachtin, salannin, meliantriol and nimbin.

Neem importance as biopesticide

Using of organically produced bio pesticides are emerging techniques in pest control and mostly selected crop is neem among 2500 plant pesticides in world (Subbalakshmi, *et al.*, 2012). They are protecting crops against 12 species of nematode and more than 350 species of arthropods and 15 species of fungi, three viruses and 2 species of snails and 1 crustacean species (nigam, *et al.*, 1994). Neem is considered in between medium to broad spectrum pesticide (Vijayalakshmi, *et al.*, 1998) and it can work against household pest, storage pest and crop pests according to Subbalakshmi *et al.*, (2012). It is non-toxic and eco-friendly and also easily available. It is very hardy crop; it can be grown on waste lands where maintenance of other crops is very difficult. They increase efficiency of pesticide in combination with neem and it has pest fecundity control. It nurtures and maintains soil quality and replenishes the nutrients.

Neem formulation as pesticide

The neem has active compounds which are extracted in organic solvents like ethanol, hexane, methanol are used to have pesticidal property. Neem based pesticides are marketed in India as Margo, repelin, wellgro and neemark.

Neem seed kernel extract

Kernels of neem tree possess extraordinary gustatory repellent properties which are attributed to the active principles. As compared to all parts of neem tree, neem seeds have high amount of active compounds like azadirachtin, salannin and many. For preparation of NSKE, seeds from plants should be of 3-6 months old. The seeds have high content of azadirachtin among all which is efficient to kill pests (Vijayalakshmi, *et al.*, 1998). Some neem products like azatin, nim 80 are used as insecticide. Azatin at low concentration retard the growth of insects but in high concentration leads to mortality. Insect larvae unable to feed when exposed to azatin. It shows toxicity. Nim 80 restricts the larval development (mancebo, *et al.*; 2002). NSKE should be in milky white not brownish it doesn't work against sucking pests. NSKE is prepared by using followed steps:

- Neem seed kernel of 5 kg, grinded it to make powder.
- Soaked it overnight in 10 litres of water.





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- Stirred with wooden plank in morning until it turned to milky white.
- Filtered through double layer muslin cloth and made up to 100litres.
- Added 1% detergent and mixed it well.

We should not take kernels more than 8 months old and it should be prepared fresh. It is very effective when sprayed after 3:30pm. For spraying of NSKE is required 500-2000 ml per 10 liters of water tank for spraying.

Neem oil

Neem oil is very important in studying photochemical properties. It has vast uses as medicinal properties and as pesticide in organic farming. It is having offensive and pungent smell as it is containing peanut and garlic oil as carrier oil and also presence of sulphur containing volatile compounds (Dasarao and Seshadri, 1941). It has fatty acids as active compound. The quality of oil is described as SV, IV, and AV Values. We know that neem oil is comprised by many compounds but for pesticidal property of azadirachtin plays 90% role. Neem oil can be extracted from other parts but the quantity of azadirachtin content varies among them it is high in seed so it will show high efficiency as pesticide

Role of Azadirachtin and its mechanism

The chemical formula of azadirachtin is $C_{35}H_{44}O_{16}$. It has high pesticidal property and alters moulting, growth inhibition and malformation in insects which leads to mortality. It is a triterpenoid and it is first discovered by Rambold. Azadirachtin content in seed kernel (10gm/kg). The effect of azadirachtin varies from pest to pest. It disrupts the endocrine activity by reducing the haemolymph ecdysteroid level through ceasing the release of PTTH hormone from brain-corpora cardiaca complex and which directly inhibits moulting. It also alters juvenile hormone. It is predominant as antifeedant, repellents, growth inhibitors, ovipositional, larvicidal and sterility of insects. It is most favourable when used in IPM program as it decreases the use of conventional pesticides and non toxicant to non target organisms.

Mode of action and properties of neem as pesticide

Neem as repellents

Pure neem oil is repellent of aphids by using high concentration (Shannag, *et al.*, 2014). After 1 week of application it reduces aphid population but did not act as efficient when applied in low concentration.

Neem as antifeedant

According to jeyasankar *et al.*, (2010), *spodopteralitura*, a polyphagous pest controlled by many crude proteins. Neem extracts are responsible for antifeedant activity, because of azadirachtin, salannin and melandriol shows vomiting sensation in insects by creating anti peristaltic wave in alimentary canal which makes them not feed on neem treated crop and also block ability to swallow (vijyalakshmi *et al.*, 1998).

Neemas ovi positional deterrence

Neem based pesticides effect on fecundity of aphids and blocked neurosecretory cells by active ingredient azadirachtin and disrupted maturation and egg production (vimal, *et al.*, 2010). In one sentence, it restricts female to lay eggs.

Neemas growth regulator

It is a unique and very interesting property of neem based products. In neem products, mainly Azadirachtin gains the access over insect and suppress the ecdysone hormone so the larvae which feed on it, remains as larvae and dies. The abnormality of growth varies with the growth stage of insect on which the pest feeds on host (Shannag *et al.*, 2014). If concentration is low it kills larvae after emerging into pupae and when the concentration is very low then adult which is produced from pupae will be completely malformed and completely sterile (Vijyalakshmi *et al.*, 1998).



**Cherishma et al.,****Neem as biofertilizer**

Instead of using synthetic fertilizer, neem can be used as bio fertilizers as it helps in enriching the quality of soil as well as crop. The by products which are obtained after extraction of neem oil helps in preparing neem cake which is used to replenish the soil. We all know the importance of nitrogen to the crop and soil. But urea is volatile, it is not available to crops, in this case neem is used to retain urea and helps in denitrification by also reducing the activity of microorganisms like bacteria (Musalia et al., 2000 and Mohanty et al., 2008).

Advantages of using neem as bio fertiliser:

Neem cake is used as bio fertilizers which have two beneficial characters that are providing micro nutrients to soil and also has pesticidal property. It is cheap and there is no waste in production of neem oil and it does not have toxic effect on farmers.

Using neem as manure

The basic meaning of manure is using of any plant or animal material for soil replenishment (Tiwari et al., 2002 and Singh et al., 2006). It is biodegradable, non toxic and has soil conditioning properties, so it is widely accepted. In organic farming it is very useful. It is rich in nitrogen and phosphorous and it improves quantity of soil. It is also rich in calcium, potassium and sulphur (Adeoye et al., 2008). It is familiar that it doesn't have any side effects and also it is produced in large amount. It can be applied directly or blend with FYM or with sea weed or along with urea for denitrification.

Advantages

It shows compatibility with other manures and fertilizer combinations. It is eco-friendly with only positive characters. It helps in providing both micro and macro nutrients to the soil. It plays another role as pesticide by reducing the pest population by using a mechanism called antifeedant. Helps farmers in cost of crop production and reduce the side effect of fertilizer.

Neem as urea coating agent

As we learned about denitrification role of neem in urea, for this property of neem, it is used for urea coating agents (Bainset et al., 1971). It prevents the loss of urea and also act as antifeedant to pests along with fertilizing the soil so it plays a dual role.

Advantages

It helps not only in providing nutrients but also minimize the loss of urea by denitrification process. They increase the fertilizer use efficiency. Also reduce the cost pesticide as it play dual role as pesticide and fertilizer. They are more beneficial to farmers and also beneficial soil microorganisms and pest's natural enemies.

Neem as soil nurturing

Powdered seeds or neem seed granules are used as soil enrichment. It can be applied during sowing or sprinkling on soil. After sprinkling we must and should irrigate the field to make the product available to soil. It is a natural enhancer of soil quality and the fruit or crop quality (Smith et al., 2001).

Advantages

Neem is soil quality enricher and it is widely used for improving quality of soil. The fertility of soil is increased and also reduces the pest population. This is very famous and widely used because it is completely organic and cost of production is less than fertilizers and there are no side effects. In one word it is described as multifunctional soil conditioner.





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CONCLUSION

Main aim of this review is to create awareness in using of organic pesticides and fertilizers. It explains the advantages of using them and cost effects. This review describes merits over the using of synthetic products. Now a day's organic farming is followed by many farmers. This Review explained the benefits of organic farming economically in productive way and also it describes the properties of azadirachtin as pesticide and its mode of specificity as biopesticides.

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Health Benefits of Cinnamon

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ABSTRACT

Cinnamon has been utilized as a flavor and as customary home grown medication for quite a long time. The accessible and creature in vivo proof recommends that cinnamon has calming, antimicrobial, cell reinforcement, antitumor, cardiovascular, cholesterol-bringing down, and immunomodulatory impacts. In vitro investigations have exhibited that cinnamon might go about as an insulin mimetic, to potentiate insulin action or to invigorate cell glucose digestion. Moreover, creature studies have illustrated solid hypoglycemic properties. Nonetheless, there are truth be told, not many all around controlled clinical investigations, a reality that restricts the ends that can be made about the potential medical advantages of cinnamon for nothing living people. The utilization of cinnamon as an assistant to the treatment of type 2 diabetes mellitus is the most encouraging region, however further examination is required previously authoritative suggestions can be made.

Keywords: Cinnamomum, spice, insulin, diabetes, cinnamon

INTRODUCTION

Cinnamon has been utilized as a flavor in a few societies for hundreds of years. Notwithstanding its culinary purposes, cinnamon has been utilized as a stomachic and carminative for gastrointestinal grievances as well as different illnesses and is as yet utilized for these conditions in numerous nations (Teuscher, 2003). The German Commission E and the European Scientific Cooperative on therapy (ESCOP) endorsed two restorative spices of the sort *Cinnamomum*: *C. zeylanicum* (Blumenthal *et al.*, 1998a; European Scientific Cooperative on Phytotherapy, 2003) and *C. cassia* (Blumenthal *et al.*, 1998b). The bark is the main part of these plants that is utilized as a flavor or for





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clinical purposes (Cinnamomi cortex) (Blumenthal *et al.*, 1998a; 1998b) The unstable oils got from the bark, leaf, and root bark of *Cinnamomum zeylanicum* and *C. cassia* fluctuate significantly in substance organization which recommends that they differ in their pharmacological impacts too (Shen *et al.*, 2002; Wijesekera, 1978). These oils of three different plant parts possess a similar exhibit of monoterpene hydrocarbons in various extents. Notwithstanding, each oil has an alternate essential constituent: cinnamaldehyde (in the bark oil), eugenol (in the leaf oil), or camphor (in the root-bark oil). Three of the principle parts of the rejuvenating ointment acquired from the bark of *C. zeylanicum* are trans-cinnamaldehyde, eugenol, and linalool, which, as per Chericoni *et al.* (2005) address 82.5% of the absolute arrangement. Trans-cinnamaldehyde, the significant part of *C. zeylanicum* bark oil, represents roughly 49.9% (Singh *et al.* 2007) to 62.8% (Simic *et al.*, 2004) of the aggregate sum. Cinnamaldehyde and eugenol additionally are the significant parts of cinnamon extract (Usta *et al.*, 2002; 2003). The dried stem bark of *C. cassia* contains four trademark parts cinnamaldehyde, cinnamic corrosive, cinnamyl liquor, and coumarin. He *et al.* (2005) recognized high items in cinnamaldehyde (13.01-56.93 mg/g) in *C. cassia* bark. Friedman *et al.* (2000) found that eugenol also, linalool in food varieties are steady to warm, not at all like unadulterated cinnamaldehyde, which goes through a temperature-subordinate change to benzaldehyde affected by heat beginning at approximately 60°C. As per the U. S. Division of Agriculture (USDA) Financial Research Services, 1,797,000 pounds of cinnamon were brought into the U.S. for utilization in 2005.

History Of Cinnamon

Cinnamon has been known from distant artifact. It was imported to Egypt as soon as 2000 BC, yet the individuals who announced that it had come from China had mistaken it for *Cinnamomum cassia*, a connected animal groups. Cinnamon was so profoundly valued among old countries that it was viewed as a gift fit for rulers and in any event, for a divinity; a fine engraving records the endowment of cinnamon and cassia to the sanctuary of Apollo at Miletus.[10] Its source was maintained an exchange mystery the Mediterranean world for quite a long time by those in the flavor exchange, to safeguard their syndication as providers *Cinnamomum verum*, which deciphers from Latin as "genuine cinnamon", is local to India, Sri Lanka, Bangladesh and Myanmar. *Cinnamomum* (cassia) is local to China. Related species, all collected and sold in the advanced period as cinnamon, are local to Vietnam ("Saigon cinnamon"), Indonesia and other southeast Asian nations with warm environments.

In Ancient Egypt, cinnamon was utilized to preserve mummies. From the Ptolemaic Kingdom ahead, Ancient Egyptian plans for kyphi, a sweet-smelling utilized for consuming, included cinnamon and cassia. The gifts of Hellenistic rulers to sanctuaries here and there included cassia and cinnamon. The primary Greek reference to *κασία* : *kasía* is found in a sonnet by Sappho in the seventh century BC. As per Herodotus, both cinnamon and cassia filled in Arabia, along with incense, myrrh and labdanum, and were monitored by winged serpents.[14] Herodotus, Aristotle and different creators named Arabia as the wellspring of cinnamon; they related that goliath "cinnamon birds" gathered the cinnamon sticks from an obscure land where the cinnamon trees developed and utilized them to build their homes.

Pliny the Elder composed that cinnamon was brought around the Arabian landmass on "pontoons without rudders or sails or paddles", exploiting the colder time of year exchange winds. He additionally referenced cassia as an enhancing specialist for wine, and that the stories of cinnamon being gathered from the homes of cinnamon birds was a brokers' fiction made up to charge more. In any case, the story stayed current in Byzantium as late as 1310. As per Pliny the Elder, a Roman pound (327 grams [11.5 oz]) of cassia, cinnamon (*serichatum*), cost up to 1,500 denarii, the pay of fifty months' labour. Diocletian's Edict on Maximum Prices from 301 AD gives a cost of 125 denarii for a pound of cassia, while a rural worker acquired 25 denarii each day. Cinnamon was too costly to even think about being normally utilized on memorial service fires in Rome, yet the Emperor Nero is said to have consumed a year of the city's stock at the memorial service for his better half Poppaea Sabina in AD 65. Cente ages Through the Middle Ages, the wellspring of cinnamon stayed a secret toward the Western world. From perusing Latin scholars who cited Herodotus, Europeans had discovered that cinnamon came up the Red Sea to the exchanging ports of Egypt, yet where it came from was not exactly clear. Whenever the Sieur de Joinville went with his lord, Louis IX of France to



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Egypt on the Seventh Crusade in 1248, he announced and accepted everything he had been said: that cinnamon was fished up in nets at the wellspring of the Nile out at the edge of the world (i.e., Ethiopia). Marco Polo stayed away from accuracy on the point. The principal notice that the flavor filled in Sri Lanka was in Zakariya al-Qazwini's *Athar al-bilad wa-akhbar al-'ibad* ("Monument of Places and History of God's Bondsmen") around 1270. This was followed presently by John of Montecorvino in a letter of around 1292.

Indonesian pontoons shipped cinnamon straightforwardly from the Moluccas to East Africa (see additionally Rhapta), where nearby merchants then conveyed it north to Alexandria in Egypt. Venetian brokers from Italy held a restraining infrastructure on the zest exchange Europe, circulating cinnamon from Alexandria. The interruption of this exchange by the ascent of other Mediterranean powers, like the Mamluk kings and the Ottoman Empire, was one of many elements that drove Europeans to look through more broadly for different courses to Asia. Early current period Edit During the 1500s, Ferdinand Magellan was looking for flavors in the interest of Spain, and in the Philippines found *Cinnamomum mindanaense*, which was firmly connected with *C. zeylanicum*, the cinnamon found in Sri Lanka. This cinnamon in the end contended with Sri Lankan cinnamon, which was constrained by the Portuguese. In 1638, Dutch merchants laid out a general store in Sri Lanka, assumed command over the manufactories by 1640, and removed the leftover Portuguese by 1658. "The shores of the island are brimming with it," a Dutch skipper revealed, "and it is the most incredible in all the Orient. Whenever one is downwind of the island, one can in any case smell cinnamon eight associations out to the ocean." The Dutch East India Company kept on upgrading the techniques for reaping in the wild and in the long run started to develop its own trees In 1767, Lord Brown of the British East India Company laid out Anjarakkandy Cinnamon Estate close to Anjarakkandy in the Kannur region of Kerala, India. It later turned into Asia's biggest cinnamon bequest. The British assumed command over Ceylon from the Dutch in 1796.

Cinnamon

Cinnamon is a spice acquired from the internal bark of a few tree animal categories from the variety *Cinnamomum*. Cinnamon is utilized chiefly as a sweet-smelling topping and seasoning added substance in a wide assortment of cooking styles, sweet and appetizing dishes, breakfast grains, nibble food varieties, teas, and conventional food varieties. The smell and kind of cinnamon get from its medicinal balm and head part, cinnamaldehyde, as well as various different constituents including eugenol. Cinnamon is the name for a long time of trees and the business zest items that some of them produce. All are individuals from the variety *Cinnamomum* in the family Lauraceae. A couple of *Cinnamomum* animal varieties are developed industrially for flavor. *Cinnamomum verum* is now and again viewed as "genuine cinnamon", yet most cinnamon in worldwide trade is gotten from the connected species *Cinnamomum cassia*, additionally alluded to as "cassia". In 2018, Indonesia and China created 70% of the world's stockpile of cinnamon, Indonesia delivering almost 40% and China 30%.

Chemical Composition Of Cinnamon

Cinnamon comprises of an assortment of resinous mixtures, including cinnamaldehyde, cinnamate, cinnamic acid, and various natural oils. Singh *et al.* revealed that the hot taste and aroma are because of the presence of cinnamaldehyde and happen because of the retention of oxygen. As cinnamon ages, it obscures in variety, working on the resinous mixtures. Sangal detailed different physiochemical properties of cinnamon. The presence of a wide scope of rejuvenating oils, for example, trans-cinnamaldehyde, cinnamyl acetic acid derivation, eugenol, L-borneol, caryophyllene oxide, b-caryophyllene, L-bornyl acetic acid derivation, E-nerolidol, α -cubebene, α -terpineol, terpinolene, and α -thujene, has been reported.

Antioxidant Properties

In Flavors and vegetables have cancer prevention agent action that can diminish lipid peroxidation in organic frameworks (Shobana and Naidu, 2000). Receptive oxygen species have been involved in a scope of human sicknesses like atherosclerosis and certain malignant growths (Halliwell, 2007). Oxidative cycles by and large play a key job in fiery and invulnerable cycles. As oxidative stress has been embroiled in the pathogenesis of numerous





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human illnesses, the utilization of cancer prevention agents in pharmacology is generally examined (Clark, 2002). Dragland *et al.* (2003) saw as extremely high groupings of cancer prevention agents (i.e., >75 mmol/100 g) in the therapeutic spice Cinnamomi cortex. That's what the creators conjecture a few of the impacts of this spice are intervened by their antioxidant exercises. A water and alcoholic concentrate (1:1) of cinnamon showed significant restraint of lipoxygenase-subordinate enzymatic lipid peroxidation in an in vitro lipid peroxidation measure. Etheric, methanolic, and watery cinnamon removes, hindered in vitro oxidation in a beta-carotene/linoleic corrosive framework. Ethanol concentrates of dry bark of *C. cassia* displayed a more prominent hindrance of lipid peroxidation of rodent liver homogenate in vitro than alpha-tocopherol, high superoxide anion rummaging activity, solid enemy of superoxide development action ($P < 0.05$), and excellent cancer prevention agent action in enzymatic and nonenzymatic liver tissue oxidative frameworks. Cinnamon displayed higher level of hindrance of oxidation than butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate as tried by the lipid peroxidation measure. Cinnamomi cortex additionally inhibitorily affects lipid peroxidation and protein oxidative alteration by copper (Toda,2003).

Cinnamon oil showed superoxide dismutase (SOD)- like movement estimated by the hindrance of pyrogallol autoxidation that is catalyzed by the superoxide extremist. The unstable concentrates of cinnamon showed moderate antioxidant exercises in the aldehyde/carboxylic corrosive measure and in the formed diene test (Lee and Shibamoto, 2002).The rejuvenating ointment acquired from the bark of *C. zeylanicum* and three of its fundamental parts, eugenol, (E)- cinnamaldehyde, furthermore, linalool, were tried in two in vitro models of peroxynitrite prompted nitration and lipid peroxidation. The natural oil and eugenol showed extremely strong exercises. (E)-cinnamaldehyde furthermore, linalool were totally dormant. Notwithstanding, *C. cassia* bark-determined trans-cinnamaldehyde showed intense inhibitory consequences for NO creation in RAW 264.7 cells, still up in the air through the assessment of NO creation and expression of inducible nitric oxide. Practically zero action was noticed for cinnamic corrosive and eugenol. A few flavonoids got from cinnamon that were re-reported to display cell reinforcement and free extremist searching activities were tried for their 1,1-diphenyl-2-picrylhydrazyl (DPPH)revolutionary searching movement. As of late (2007) found solid free extremist rummaging activities in the bark of *C. zeylanicum* as demonstrated by an extremely low inhibitory focus esteem, productivity fixation esteem(DPPH), and diminishing power esteem (ascorbic corrosive reciprocals)as well as a sensibly high worth of against revolutionary power. These discoveries affirm prior outcomes which show extremely impressive movement for *C. zeylanicum* and a generally high action for *C. cassia*

Benefits Of Cinnamon

1.It has Anti-inflammatory property:

The cinnamon helps our body to fight infections and repair tissue damage. The antioxidants in cinnamon have anti-inflammatory effects which helps in lowering the risk of disease.

2. Cinnamon lowers blood sugar levels:

Cinnamon has been shown to reduce fasting blood sugar levels, having a potent anti-diabetic effect at 1–6 grams or 0.5–2 teaspoons per day.

3. Cinnamon Helps Fight Bacterial and Fungal Infections:

Cinnamaldehyde has antifungal and antibacterial properties, which may reduce infections and help fight tooth decay and bad breath.

4. Cinnamon is loaded with antioxidants:

Cinnamon contains large amounts of highly potent polyphenol antioxidants.

Role of cinnamon in several diseases:

It helps in preventing Alzheimer's disease:

A few creature studies have recommended that cinnamon might assist with forestalling Alzheimer's illness. As per researchers Trusted Source, a concentrate present in cinnamon bark, called CEppt, contains properties that might keep side effects from creating. Mice who got the concentrate encountered an abatement in highlights of Alzheimer's,



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like amyloid plaques, and enhancements in their capacity to think and reason. Assuming that further examination affirms its adequacy, this concentrate - however not really entire cinnamon - might be helpful in creating treatments for Alzheimer's.

Lowering the effects of high fat meals:

In 2011, analysts presumed that counts calories wealthy in "cancer prevention agent flavors," including cinnamon, may assist with lessening the body's negative reaction to eating high fat dinners. individuals consumed dishes containing 14 g of a flavor mix. Blood tests showed that cell reinforcement movement expanded by 13%, insulin reaction fell by 21%, and fatty oils fell by 31%.

Reducing the risk of cardiovascular disease:

Different mixtures in cinnamon might help the cardiovascular framework. Cinnemaldehyde, for instance, brought down circulatory strain in a creature study. In a 2014 study Trusted Source, rodents that got long haul treatment including cinnamon and vigorous preparation would be wise to heart work than those that didn't.

DISCUSSION

By and large, the restorative purposes of flavors were frequently indistinguishable from their culinary purposes. The worth phytochemicals corresponding to human wellbeing has been perceived for quite along time. The constituents of spices and flavors can have free what's more, covering activities, including decrease of irritation, cancer prevention agent impacts, of detoxification proteins, modulation of the invulnerable framework, and antibacterial and antiviral impacts. The accessible in vitro and creature in vivo preliminaries on the properties of *Cinnamomum zeylanicum* and *C. cassia* recommend that this flavor might have calming, antimicrobial, an-tioxidant, antitumor, cardioprotective, cholesterol-bringing down, hypoglycemic, and immunomodulatory impacts. Then again, The fact that cinnamon has gastroprotective prop makes there minimal properties. The lion's share of accessible in vitro and in vivo information recommend that cinnamon has medical advantages. In any case controlled human investigations will be important to decide if these impacts have general wellbeing suggestions. Most human examination on cinnamon has been led today out whether this flavor is reasonable for the treatment of type 1 as well as type 2 diabetes mellitus. These outcomes are clashing, be that as it may, some proof exists to help this theory.

The in vivo and in vitro investigations on the subject of insulin resistance and the insulin-mimetic activities of cinnamon, separately, have yielded reliably certain outcomes. Furthermore, four of the seven clinical investigations distributed to date didn't report statistically critical valuable impacts while the excess three investigations tracked down such impacts. The negative investigations utilized 1-1.5 g of cinnamon as a day to day portion, though the examinations with genuinely huge outcomes used up to 6 g. As no dose-ranging studies were conducted, it is possible to estimate from the available data that ≥ 3 g cinnamon daily may be effective. In addition, *C. zeylanicum* and *C. cassia* may demonstrate slight differences in their pharmacological effects so the source of cinnamon may be important. As noted further well-designed studies are needed before recommendations can be made that cinnamon is effective as treatment for type 2 diabetes mellitus. The available data do not provide support can be used to treat type 1 or type 2 diabetes or to reduce the risk of developing diabetes in healthy individual

CONCLUSION

From the findings of various studies, it can be concluded that cinnamon possess many specific functional properties such as antioxidant, anti-inflammatory, antimicrobial, and acaricidal activities, in addition to its medicinal values in relieving and treating a number of serious illnesses such as diabetes, etc.



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Review of Milk - (Functional Food for Humans)

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ABSTRACT

Milk is a widely consumed beverage that is essential to the diet of several millions of people worldwide because it provides important macro- and micronutrients. Milk is recognized as being useful during childhood and adolescence because of its composition; however, its relatively high saturated fat proportion raises issues of potential detrimental effects, namely on the cardiovascular system. This review evaluates the most recent literature on dairy and human health, framed within epidemiologic, experimental, and biochemical evidence. As an example, the effects of milk (notably skimmed milk) on body weight appear to be well documented, and the conclusions of the vast majority of published studies indicate that dairy consumption does not increase cardiovascular risk or the incidence of some cancers. Even though the available evidence is not conclusive, some studies suggest that milk and its derivatives might actually be beneficial to some population segments. Although future studies will help elucidate the role of milk and dairy products in human health, their use within a balanced diet should be considered in the absence of clear contraindications.

Keywords: Milk, functional food, essential food

INTRODUCTION

Milk is an essential component of the diet of ~6 billion people. The world production of milk reaches 730 million tons/y (1, 2). Even though mammals produce milk to feed their offspring, in many areas of the world humans continue to consume milk throughout their life. However, it must be emphasized that lactose intolerance is widespread throughout the world and that a large proportion of the world's population would not benefit from the



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putative benefits of milk. In addition to milk, several dairy products such as cream, butter, yogurt, kefir, and cheese have been produced and consumed worldwide for millennia. Therefore, the impact of milk and dairy products on human health is quantitatively relevant and has been the subject of several investigations, on both whole products and their isolated components. In particular, the fat portion of milk (largely composed of SFAs) and some of its minor components, notably calcium and oligosaccharides, are being actively researched for their potential health roles. This review summarizes the most recent studies on milk and human health and critically discusses the putative actions of milk and principal dairy constituents.

Effects on Body Weight

Of all the bioactive milk components, calcium and vitamin D have been chiefly studied for their effects on body weight and adipose tissue. Studies have been performed on these compounds as either isolated molecules (3–9) or as components of milk and dairy products (5, 7, 8, 10–12). Proposed targets include thermogenesis and lipid oxidation (which are enhanced by calcium and vitamin D) (13–15) and increased lipid fecal excretion (16–19). In the past few years, some studies have been published on other milk components and their potential effects on body weight (20, 21). For example, in addition to calcium and vitamin D, dairy proteins are being suggested as reducers of adipose mass (namely, visceral fat) and body weight (11, 14, 22, 23). These effects have been observed in healthy participants as well as in overweight, obese (21, 24–27), and diabetic (8, 28) patients. In addition to casein, whey protein appears to be particularly effective (29, 30), and their actions seem to be mediated by several mechanisms that include increased satiety and decreased appetite (29). In particular, inhibition of gastric secretion by cholecystokinin (31) and some branched amino acids, the abundance of leucine (32), increased secretion of glucagon-like peptide 1 (GLP-1) (33, 34) and glucose-dependent insulinotropic polypeptide (GIP) (35), the concomitant suppression of ghrelin secretion (36), and the potent satiating effects of α -lactalbumin (37) synergistically contribute to weight control.

The most recent studies in this area include randomized clinical trials and meta-analyses. A marked reduction in adipose tissue and an increase in lean mass were observed in 90 overweight and obese premenopausal women after 4 mo of a hypocaloric diet that included milk and dairy products. In particular, visceral adipose tissue was significantly affected (26). A study conducted in 903 healthy adolescents (15–16 y) that included at least 2 servings/d [1 serving = 200 mL of milk, 125 g of yogurt, or 28 g of cheese (38)] of dairy reported a significant weight loss and a reduction in body fat (39, 40). Male participants also witnessed a protective effect on abdominal obesity. From a mechanistic viewpoint, whey protein administered before a meal exerted insulinotropic effects and reduced postprandial insulinemic fluctuations in healthy participants (41) and in type 2 diabetic patients (42). In the latter, consumption of whey protein before a high-glycemic-load (white bread and potatoes) breakfast or lunch increased insulin response by 30–50% and reduced glycemia by ~20%, compared with controls (42). This effect is quantitatively comparable to that of sulfonylureas (43). In agreement with these studies, Dove et al. (44) reported that the intake of 600 mL of skimmed milk at breakfast (by 34 overweight men and women) had a stronger satiating effect (evaluated 4 h later) than that of an isocaloric intake of fruit juice. A significantly lower consumption of foods offered ad libitum at lunch (i.e., after 4 h) was also recorded (44). A recent meta-analysis (45) that reviewed the effect of 29 randomized clinical trials comprising 2101 cases confirmed the weight-loss effect of milk and dairy products when incorporated into hypocaloric diets. However, no beneficial effect of increasing dairy consumption on body weight and fat loss was seen in long-term studies or in studies without energy restriction, which calls for caution in attributing milk to slimming properties.

Blood Pressure

Whey proteins have long been studied for their potentially positive effects on blood pressure (60, 61). For example, Pal and Ellis (62) demonstrated that, in overweight and obese participants, the intake of 54 g/d of whey protein for 12 wk induced a significant reduction in both systolic and diastolic blood pressure, in agreement with Xu et al. (63) who published a meta-analysis of tripeptides and blood pressure. The former are bioactive peptides (64–66) that are formed from proteins via the actions of the microbiota and gastrointestinal enzymes and which are abundant in fermented dairy products (67). Tripeptides are being investigated because of their angiotensin-converting enzyme-



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inhibiting activities, which might have important clinical consequences. In particular, 2 tripeptides, namely isoleucine-proline-proline (Ile-Pro-Pro) and valine-proline-proline (Val-Pro-Pro) have been incorporated into functional foods because of their safety profile and purported beneficial activities, namely on blood pressure. It is noteworthy that, in addition to their activity on blood pressure, other peptides have been isolated and studied for their putative antithrombotic properties (68). Recently, McGrane et al. (69) reviewed the evidence of the hypotensive effects of milk tripeptides by updating a former 2010 review that 1) examined 223 articles published between 2004 and 2009 (which outlined the inverse association between milk tripeptide consumption and blood pressure) and 2) reviewed 163 studies published between July 2009 and December 2010 concerning vitamin D, calcium, phosphorus, and bioactive peptides in low-fat dairy as part of low-fat diets. A meta-analysis of 7 studies that included ~45,000 participants, of whom 11,500 were hypertensive (70), reported a significantly inverse association between low-fat dairy consumption and hypertension risk. Nine other cohort studies (57,256 participants followed for 2–15 y) confirmed this inverse correlation; furthermore, those who consumed the highest quantity of low-fat dairy products exhibited the lowest risk of hypertension (71). A prospective study recently published by Louie et al. (72), which analyzed 335 Australian children, their milk consumption at age 18 mo, and their blood pressure at 8 y of age, reported lower blood pressure values in those who consumed at least 2 servings/d.

Milk Components with Putative Functional Properties

Recently, several oligosaccharides have been categorized in milk and have been suggested as potentially bioactive ingredients. Even though bovine milk contains only trace amounts of these beneficial components (172), some researchers are working toward producing human milk oligosaccharides in transgenic animals (173). Due to the lack of suitable commercial standards for bovine oligosaccharides, we can only identify >70 fully annotated oligosaccharides in human milk and ~40 in bovine milk, of which 24 contain sialic acid (172). Oligosaccharides are composed of a lactose core bound to lactose-amine units via β 1–3 or β 1–6 links and carrying fucose or sialic acid in their terminal position (172, 174, 175). It is noteworthy that these molecules are abundant in human milk and have been proposed as important for child development. Neutral oligosaccharides—namely the monomer N-acetylglucosamine and fucose—are essential to the development of the microbiota of breast-fed neonates because of their immunomodulating actions (176).

Conversely, acidic oligosaccharides (where the monomer is sialic acid) help to prevent the adhesion of pathogens to the intestinal mucosa (177). Bovine milk also contains these oligosaccharides, which are abundant in colostrum (178, 179). Oligosaccharides are prebiotics and help to create a healthy microbiota (180–182). Even though a thorough discussion on the role of the microbiota in human health goes beyond the aim of this article, this is an important and dynamic field of research that is attracting considerable attention and is being addressed from several viewpoints (183–185). Even though many fruits and vegetables contain oligosaccharides and some of them have been synthesized, those from milk are remarkable in that they exhibit a branched rather than a linear structure. Moreover, they contain fucose and sialic acid, which are almost absent in other oligosaccharides. This structural difference might confirm that milk oligosaccharide activities are different than oligosaccharides of synthetic or vegetal origin. It must be underscored that the concentration of oligosaccharides in bovine milk decreases in a time-dependent fashion: ad hoc investigations are being carried out to formulate these compounds as nutraceuticals or as probiotic components of functional foods (172).

Finally, it is noteworthy that some kinds of cheeses, namely those infected with *Penicillium* such as Roquefort, Stilton, or Gorgonzola, exhibit high concentrations of andrastins A–D, which are potent inhibitors of farnesyltransferase, a key enzyme in cholesterol synthesis (186). Other peptides formed during ripening-induced proteolysis might further contribute healthful, albeit as yet unexplored, properties that would partially explain the relatively low incidence of CVD in high-cheese-consumption countries. Milk is also often fortified, e.g., with vitamin D or omega-3 FAs (187), because it provides an excellent vehicle for fat-soluble molecules (see below) and can be marketed to target population groups after appropriate regulatory evaluation (188). In summary, either fortified or



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“natural” milk and dairy products contain several compounds—even though they are often present in low concentrations—that might in the future be exploited for pharma-nutrition applications (189).

CONCLUSION

Milk and its derivatives are proposed as being useful foods throughout all life periods, in particular during childhood and adolescence, when their contents of calcium, protein, phosphorus, and other micronutrients might promote skeletal, muscular, and neurologic development. However, their relatively high saturated fat proportion [milk fat contains ~70% SFAs; myristic and palmitic acids combined account for ~50% (190), whereas the remainder are mostly short- and medium-chain FAs and oleic acid (191, 192)] has flagged them as potentially detrimental food items, especially in terms of cardiovascular health. The recent literature reviewed in this article helps shed some light on the role of milk in a balanced diet. The vast majority of epidemiologic and intervention studies performed during the past few years suggest that dairy products do not adversely affect surrogate markers of CVD and cardiovascular prognosis. Indeed, some studies suggest that SFAs, namely, shorter-chain SFAs, from milk and its derivatives are benign with regard to inflammation (193) and might actually be beneficial to some population segments (193). The available evidence suggests that calcium does not play a major role in coronary calcification (194) [at least from an epidemiologic viewpoint: randomized clinical trials are inconclusive (195)] and its intake is inversely associated with blood pressure, whereas its potential contribution to prostate cancer development is still controversial (196).

Therefore, the hypothesized association between calcium intake and cardiovascular risk is not currently supported by scientific evidence, and, in fact, the reverse might be true. In addition, some milk components such as trans fatty acids [which might have different physiologic actions than the industrial ones (197, 198)], butyric acid [which might be helpful for the intestinal mucosa's trophism (199)], conjugated linoleic acid [even though the jury is still out as far as its putative health effects are concerned, there are efforts to increase its concentrations in bovine milk (103)], phospholipids (200), tripeptides, calcium, phosphorus, lactoferrin (201), and oligosaccharides might exert useful, although as yet unproven, physiologic actions. Furthermore, milk has been shown to be an efficient vehicle for lipid-soluble nutrient absorption (187) because milk fat appears to be highly dispersed in very small micelles (202). In conclusion, whereas future studies will help to elucidate the role of milk and dairy products in human health, their use within a balanced diet should be considered in the absence of clear contraindications.

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Clove as an Immune Booster

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ABSTRACT

Clove (*Syzygium aromaticum*) has been used in herbal remedies to treat a variety of ailments. The purpose of this study was to look into the consequences of clove essential oil as eugenol and water-soluble ingredients on mouse splenocytes. Clove extracts were cultivated and applied to splenocytes at varying concentrations (0.001–1000 g/mL), as well as phytohemagglutinin (PHA = 5 g/mL) and lipopolysaccharide (LPS = 10 g/mL) activated splenocytes; splenocyte rapid spread was then measured using the MTT ([3-(4, 5-dimethylthiazole-2-yl) -2 Interferon (IFN)-, interleukin (IL)-4, IL-10, and reshaping growth factor (TGF)-cytokines were evaluated in culture supernatant. Clove extracts (100 g/mL and 1000 g/mL) inhibited PHA-stimulated splenocyte emergence while increasing LPS-stimulated cell elongation. According to the findings of this research, clove extracts may inhibit T cell cellular immunity while increasing humoral immune responses. Clove affection cytokine sequence moved toward modulatory and Th2 reactions, as well as cytokines that accelerate humoral immunity.

Keywords: Clove, Immunity, T-cell, cytokine



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INTRODUCTION

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Myrtales
Family: Myrtaceae
Genus: Syzygium
Species: *S. aromaticum*

In certain diversity, herbal remedies are used to treat specific diseases. Recognition of active preservatives and mechanisms of action of traditional current treatments at the immune device is highly desirable (solar et al.,2006). The immune system is divided into adaptive and innate structures, and the adaptive immune system is further subdivided into mobile and humeral responses. Macrophages are important players in inflammation and host defence. Inflammatory processes are mediated by a number of molecular mechanisms, including nitric oxide (NO), prostaglandins, leukocytes, reactive oxygen species, and a few stored cytokines (Moncada, 1999). Macrophage mediators facilitate a variety of physiological features, including non-specific host defence, anti-microbial defence, and anti-tumor operations, as well as pathophysiological functions, which include pathogens such as septic surprise and organ ruination in positive inflammatory and auto- immune responses. For centuries, ingredients such as cloves, oregano, mint, thyme, and cinnamon have been used as food preservatives and medicinal herbs, primarily for their antioxidant and antimicrobial properties. Many studies now verify spice plants' antibacterial, antifungal, antiviral, and anticancer properties. Clove, in specific, has captivated exposure due to its powerful antioxidant and antimicrobial effects, which set apart from the other spices. Folk remedies is often used to treat a variety of ailments in most cultures around the world. It is extremely coveted to identify the active components and underlying mechanisms of herbal remedies on the immune system [1]. The immune system is divided into two parts: adaptive and innate, with the adaptive immune system further subdivided into cellular and humoral responses. T and B lymphocytes are the primary cells involved in adaptive immune responses. Many of the stimulants and intermediaries used in diagnosis and treatment work by influencing immune cells, particularly lymphocytes. These representatives could be elements of nature, synthetic aspects, or body moderators [2], [3]. Cloves are dried flower buds deduced from the evergreen tree *Syzygium aromaticum* (L.) Merr. & Perry (i.e., *Eugenia aromaticum* or *Eugenia caryophyllata*), which is native to India, Indonesia, Zanzibar, Mauritius, and Iran [4]. It is frequently used in the formulation of traditional spicy rich dishes in Africa, Asia, and other parts of the world. It has a dark brown colour, a strong perfumery, and a bitter taste [5]. Clove has several healing potentials; it is a well-known food flavour and a popular cure in traditional Australian and Asian drug for dental and respiratory disorders, headache, and sore throat [6]. Clove has anti-diabetic, anti-inflammatory, antithrombotic, anaesthetic, anguish, and insect-repelling assets [7, 8]. The primary constituents of clove are eugenol (50–87%), eugenol acetate, tanene, thymol, and -caryophyllene [9]. These elements are responsible for the effect of clove extract under varying conditions. Furthermore, these elements have been shown to modify some immune function, including anti-inflammatory effects, though the processes underlying the effect are unknown [10]. Liquid ingredients, in addition to eugenol, which is extracted using an alcoholic method [11], play an important role. Clove has biological and pharmacological attributes, but its effect on the immune system has received little attention. Those who have researched the advantages of clove in herbal medicine suggest that it has an impact on the immune cells and immune system.

MATERIALS AND METHODS

Animals

The Pasteur Research center of Iran provided six-to-eight-week-old female inbred Balb/c mice (Tehran, Iran). All through the research, they were managed to keep in TarbiatModares University's animal house in Tehran, Iran, and



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were fed basic mouse chow and given sterilised water. The Ethical Committee of the Department of Veterinary Care and Use (TarbiatModares University, Tehran, Iran) authorised the research methodology.

Plant Material

The flower buds of clove (*Syzygium aromaticum*) were collected from plants cultivated in the Center of Medicinal Plants Research 25 km north of Tehran, Iran, and confirmed by the Center of Agricultural Research, Tehran, Iran.

Preparation of clove extracts The alcoholic extraction technique [11] was used to isolate eugenol (in the essential oil); in summary, powdered sample flower buds of clove (25 g) were drenched in 100 mL of ethanol to prepare the essential oil and in 100 mL of distilled water to gear up the aqueous extract. They were mixed on a rotary for 24 hours before being refracted through Whatman No. 1 filter paper. The filtrate extract was transferred to a lyophilizing vial. The vial was forced to evacuate using a vacuum filtration until it dried. The separates were retrieved, measured, and a noteworthy stock of 100 mg/mL was prepared in 5 mL of water or 50% ethanol for use in the various assay methods presented here. Yields for both components were calculated.

Analyses Of Extract Contents

Prior report stated that clove oil enclosed 75% eugenol, 5% -caryophyllene, 16% eugenol acetate, and 1% other elements [12]; evaluations of the aqueous extracts revealed that it consisted 49% eugenol [13]. The samples in this research were evaluated using that gas chromatography-mass spectrometry methodologies as those used by Lee et al [13], and the data revealed that eugenol composed 74% of the substance in the ethanolic extract and 43% in the aqueous extract. Although we did not specifically evaluate the levels of -caryophyllene and eugenyl acetate, we presume they are consistent with values observed by Chaieb et al [12] and Lee et al [13] and thus constitute the majority of the current materials in each extract.

Preparation And Treatment Of Splenocytes

Under mild diethyl ether anaesthesia, the mice were beheaded and their spleens were quickly expunged under aseptic condition. This tissue was then homogenised in a glass vortex mixer in 10 mL of cold RPMI 1640 full medium (Sigma Chemical Company, St. Louis, MO). To form a homogeneous cell suspension, homogenised spleen tissues were passed through a fine steel mesh. 0.75 percent NH₄Cl in Tris buffer (0.02 percent, pH = 7.2) was used to osmotically lyse the erythrocytes. Following centrifugation (360g at 4°C for 10 minutes), the powdered cells were rinsed 3 times with phosphate buffer solution (PBS) and solubilized in RPMI 1640 complete medium supplemented with 11 mM sodium bicarbonate, 2 mM L-glutamine, 100 U/mL penicillin, 100 g/mL streptomycin, and 10% foetal bovine serum (all reagents)

Cell Proliferation Assay

Cell proliferation was measured using the MTT [3-(4, 5-dimethylthiazole-2-yl) -2, 5-diphenyl tetrazolium bromide] reduction assay after 48 hours of incubation with various concentrations of clove extracts [14]. Following incubation, 20 L of MTT (5 mg/mL in PBS) were added to 200 L wells (one tenth of the total volume) and incubated for 4 hours at 37°C and 5% CO₂. The medium was then removed, and the formazan blue crystals formed in the living cells by reacting MTT with mitochondrial dehydrogenase were dissolved in 100 L of acidic isopropanol (0.04 M HCl in isopropanol). The plates were read at 540 nm with a Multiskan MS microplate reader (Thermo Scientific Vantaa, Finland).

Cytokine Enzyme-Linked Immunosorbent Assay

IFN-, IL-4, IL-10, and TGF- cytokines were measured in cell culture supernatants using a commercially available enzyme-linked immunosorbent assay kit (eBiosciences, Frankfurt, Germany) and the manufacturer's instructions. Every sample was measured at least twice.

Data Analysis

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SPSS version 15 for Windows was used for statistical analysis (SPSS Inc., Chicago, IL, USA). For multiple comparisons, data was analysed using one-way ANOVA, followed by a least significant difference test. A significant difference was defined as a p value of 0.05. The results are given as a mean standard deviation.

RESULTS**Lymphocyte Subtypes Proliferation**

LPS/PHA/unstimulated splenocytes were grown in the presence of various concentrations of clove essential oil and aqueous extracts. Clove essential oil at 100 g/mL and 1000 g/mL reduced PHA stimulated splenocytes (as T cells) proliferation (p 0.05) but increased proliferation of LPS stimulated (as B cells) or unstimulated splenocytes (p > 0.05). Aqueous extracts at 100 g/mL and 1000 g/mL reduced PHA stimulated splenocytes (as T cells) proliferation (p 0.05) but had no effect on the other two populations.

Cytokine Production

Cytokine assay on supernatant from treated splenocytes. Clove eugenol rich essential oil inhibited IFN- production at concentrations ranging from 1–1000 g/mL (62.17 5.93 ng/mL vs. 46.5 ng/mL in the control group vs. 1–1000 g/mL of clove oil). Water-soluble ingredients also inhibited IFN- production at concentrations ranging from 0.1 to 1000 g/mL (62.17 5.93 ng/mL vs. 48.2 ng/mL in the control group vs. 0.1–1000 g/mL in the aqueous extract). Both alcoholic and aqueous extracts in the 0.1–1000 g/mL range increased IL-4 release (6.06 0.98 ng/mL vs. >10.3 ng/mL of untreated splenocytes vs. 0.1–1000 g/mL ingredients affected by splenocytes). Both extracts stimulated IL-10 production (a roughly twofold increase in the range of 1–1000 g/mL for eugenol-rich essential oil and 0.1–1000 g/mL for water-soluble ingredients). The strongest stimulatory effect of clove components on IL-10 production was observed at concentrations of 10 g/mL (2.34-fold for essential oil and 2.22-fold for aqueous extract in comparison with untreated lymphocytes). Furthermore, 0.01–1000 g/mL essential oil increased TGF- release (32.11 7.44 ng/mL vs. >80 ng/mL of untreated splenocytes vs. 0.1–1000 g/mL affected splenocytes). Clove at higher concentrations of 10 ug/mL, 100 ug/mL, and 1000 ug/mL stimulated TGF-b release (>800 ng/mL vs. 32.11 7.44 ng/mL in control splenocytes). Significant differences in cytokines were defined as p 0.05.

DISCUSSION

Despite various studies on clove and its extensive use in traditional medicine, data on its effects on the immune system, particularly lymphocytes, is limited. The main component of clove is eugenol, which gives the spice its pungent, distinct aroma. Eugenol accounts for 70–90% of the essential oil and 15% of the dry weight of clove buds [15]. Cloves contain 14–21% volatile oil, 10–13% tannin, phenol, sesquiterpene ester, and alcohol [16]. These elements are in charge of clove's subsequent effects under various conditions. In this study, we looked at the effects of eugenol-rich essential oils as alcoholic extracts and aqueous extracts on the expansion of mitogen-stimulated lymphocytes (T and B cells), as well as changes in cytokine production. Higher concentrations (100 g/mL and 1000 g/mL) of clove essential oil reduced PHA stimulated splenocytes (as T cells) proliferation while increasing the expansion of LPS stimulated (as B cells) or unstimulated splenocytes. The same concentrations of aqueous extract only inhibited PHA-stimulated splenocyte proliferation (as T cells), with no effect on the other two lymphocyte populations. In comparison to these findings, another study found the same results using different methods. Halder et al [17] assessed humoral immunity using the hemagglutination titer to sheep red blood cells and delayed-type hypersensitivity (DTH) using foot-pad thickness, and their findings show that clove oil can modulate the immune response by augmenting humoral immunity and decreasing cell mediated immunity. Another study found some improvement in humoral and cellular responses, as well as some contradictory results. Carrasco et al [18] demonstrated that clove essential oil increased total white blood cell count and enhanced DTH responses in cyclophosphamide-immunosuppressed mice while restoring cellular and humoral immune responses. In this study, mice were immunocompromised, and improving T cell responses entails restoring function. As a result, these findings suggest that clove modulates T cell expansion and function; additionally, clove can restore cellular



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immunity, which could lead to beneficial applications. Similarly, to previous studies that found that clove ingredients increased antibody secretion [17] and restored B cells [18], our findings revealed that clove ingredients increased B cell expansion and humoral immune responses. Following the assessment of lymphocyte expansion responses, we examined the cytokine profile in the treated cells. Our findings revealed that effective concentrations of both ingredients (0.1–1000 g/mL) influenced cytokine production in splenocytes. Clove essential oil and aqueous extracts both inhibited IFN- production and stimulated the release of IL-4, IL-10, and TGF-. Grespan et al [19] also discovered that eugenol reduces IFN- secretion in mice, and Park et al [20] discovered that eugenol and its structural analogue, isoeugenol, inhibit IL-2 gene expression, which is another common T cell cytokine. Another study [19] found that eugenol increased IL-10 cytokine production. Another study looked at the effect of clove water extract on T helper 1 (Th1; IFN- and IL-2) and Th2 (IL-4 and IL-10) cytokine production in BALB/c mice over a short period of time. These findings indicated that clove treatment had no effect on the Th1/Th2 cytokine balance in mice [21]. Grespan et al [19] also stated that eugenol administration causes a significant decrease in TNF- and TGF- levels in mice. In addition to these findings, other research indicates that clove ingredients may influence immune responses. For example, a study on isoeugenol and its analogues (eugenol and allylbenzene) clove ingredients showed dose-dependent inhibition of nitric oxide production and inducible nitric oxide synthetase expression in LPS-stimulated RAW 264.7 murine macrophages [8]. Another study found that clove could reduce IL-1 and IL-6 production in macrophages after an LPS challenge [22]. Eugenol in essential oil was also found to reduce TNF- production from Kupffer cells [23].

In conclusion, our study found that clove ingredients could suppress T cell proliferation while increasing B cell expansion, which is consistent with previous findings of reduced cellular T cell responses and increased humoral functions. Cytokine release analysis revealed suppression of IFN- as a Th1 and proinflammatory mediator and an increase in IL-4, IL-10, and TGF- as Th2 and anti-inflammatory cytokines. The anti-inflammatory and modulatory properties of clove ingredients were demonstrated in studies on macrophages and other cells. As a result, clove may suppress T cells and their functions while enhancing B cell expansion, function, and humoral responses. The induction of cytokine patterns is geared toward Th2 responses, inflammation modulation, and the acceleration of humoral immunity.

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Clove plant



Clove





Garlic as Personalized Medicine - Review

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ABSTRACT

Throughout history, many different cultures have recognized the potential use of garlic for prevention and treatment of different diseases. Recent studies support the effects of garlic and its extracts in a wide range of applications. These studies raised the possibility of revival of garlic therapeutic values in different diseases. Different compounds in garlic are thought to reduce the risk for cardiovascular diseases, have anti-tumor and anti-microbial effects, and show benefit on high blood glucose concentration. However, the exact mechanism of all ingredients and their long-term effects are not fully understood. Further studies are needed to elucidate the pathophysiological mechanisms of action of garlic as well as its efficacy and safety in treatment of various diseases.

Keywords: Garlic, cardiovascular diseases, anti-tumor, anti-microbial, treatment

INTRODUCTION

Dietary factors play a key role in the development of various human diseases. Across cultures, there are many different dietary patterns which are believed to promote human health. Despite cultural differences, there are some shared characteristics of healthy dietary patterns. Perceiving plant foods as beneficial diet is advised by the folklore of many cultures over centuries. Garlic (*Allium sativum* L.) has acquired a reputation in different traditions as a prophylactic as well as therapeutic medicinal plant. Garlic has played important dietary and medicinal roles throughout the history. Some of the earliest references to this medicinal plant were found in Avesta, a collection of Zoroastrian holy writings that was probably compiled during the sixth century BC (Dannesteter, 2003). Garlic has





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also played as an important medicine to Sumerian and the ancient Egyptians. There is some evidence that during the earliest Olympics in Greece, garlic was fed to the athletes for increasing stamina (Lawson and Bauer, 1998).

Ancient Chinese and Indian medicine recommended garlic to aid respiration and digestion and to treat leprosy and parasitic infestation (Rivlrm, 1998). In the medieval period, garlic was also played an important role in the treatment of different diseases. Avicenna (1988), in his well-known book, *Al Qanoon Fil Tib* (The Canon of Medicine), recommended garlic as a useful compound in treatment of arthritis, toothache, chronic cough, constipation, parasitic infestation, snake and insect bites, gynaecologic diseases, as well as in infectious diseases (as antibiotic). With the onset of Renaissance, special attention was paid in Europe to the health benefits of garlic. Garlic has attracted particular attention of modern medicine because of widespread belief about its effects in maintaining good health. In some Western countries, the sale of garlic preparations ranks with those of leading prescription drugs. There is appreciable epidemiologic evidence that demonstrates therapeutic and preventive roles for garlic. Several experimental and clinical investigations suggest many favorable effects of garlic and its preparations. These effects have been largely attributed to *i*) reduction of risk factors for cardiovascular diseases, *ii*) reduction of cancer risk, *iii*) antioxidant effect, *iv*) antimicrobial effect, and *v*) enhancement of detoxification foreign compound and hepatoprotection (Colín-González, 2012; Aviello, 2009). In this review, a survey on current experimental as well as clinical state of knowledge about the preventive and therapeutic effects of garlic in different diseases is given.

Garlic is a bulbous plant; grows up to 1.2 m in height. Garlic is easy to grow and can be grown in mild climates (Figure). There are different types or subspecies of garlic, most notably hardneck garlic and softneck garlic. Allicin (allyl 2-propenethiosulfonate or diallyl thiosulfonate) is the principal bioactive compound present in the aqueous extract of garlic or raw garlic homogenate. When garlic is chopped or crushed, allinase enzyme is activated and produce allicin from alliin (present in intact garlic). Other important compounds present in garlic homogenate are 1-propenyl allyl thiosulfonate, allyl methyl thiosulfonate, (E,Z)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide (ajoene), and γ -L-glutamyl-S-alkyl-L-cysteine. The adenosine concentration increases several-fold as the homogenate is incubated at room temperature for several hours. Another widely studied garlic preparation is aged garlic extract. Sliced draw garlic stored in 15-20% ethanol for more than 1.5 year is refereed to aged garlic extract. This whole process is supposed to cause considerable loss of allicin and increased activity of certain newer compounds, such as S-allylcysteine, sallylmercaptocysteine, allixin, N-(1-Deoxy-D-fructos-1-yl)-L-arginine, and selenium which are stable and significantly antioxidant. Medicinally used, garlic oil is mostly prepared by steam-distillation process. Steam-distilled garlic oil consists of the diallyl, allylmethyl, and dimethyl mono to hexa sulfides (Lawson and Bauer, 1998). Botanically, *Allium sativum* is a member of the Lillaceae family, along with onions, chives, and shallots (Iciek et al., 2009; Lanzotti, 2006).

Kingdom	Plantae
Order	Asparagales
Family	Amaryllidaceae
Subfamily	Allioideae
Genus	Allium
Species	A.sativum

Effects Of Garlic On Cardiovascular Diseases

Garlic and its preparations have been widely recognized as agents for prevention and treatment of cardiovascular diseases. The wealth of scientific literature supports the proposal that garlic consumption have significant effects on lowering blood pressure, prevention of atherosclerosis, reduction of serum cholesterol and triglyceride, inhibition of platelet aggregation, and increasing fibrinolytic activity (Chan et al., 2013). Both experimental and clinical studies on



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different garlic preparations demonstrate these favourable cardiovascular effects. In *in vivo* animal experiments, intravenous administration of garlic extracts produced slight reductions in both systolic and diastolic pressures (Sial and Ahmed, 1982) and oral ingestion of garlic extract in hypertensive animals brought the blood pressure back to the normal level (Chandekar and Jain, 1973). Several clinical studies showed that garlic reduced blood pressure in more than 80% of patients suffering from high blood pressure (Auer *et al.*, 1989; Konig and Scneider, 1986; Petkov, 1979; Omar, 2013; Stabler *et al.*, 2012). In one trial, investigation on 47 hypertensive patients showed that garlic significantly decreased the mean systolic blood pressure by 12 mmHg and the mean supine diastolic blood pressure by 9 mmHg versus placebo. The authors stated that garlic was free from side effects and no serious complication was reported (Auer 1990). In another study, 200 mg of garlic powder was given three times daily, in addition to hydrochlorothiazide-triamterene baseline therapy, produced a mean reduction of systolic blood pressure by 10-11 mmHg and of diastolic blood pressure by 6-8 mmHg versus placebo (Kandziora 1988). However, these data are insufficient to determine if garlic provides a therapeutic advantage versus placebo in terms of reducing the risk of cardiovascular morbidity in patients diagnosed with hypertension (Stabler *et al.*, 2012). It has been suggested that the mechanism of antihypertensive activity of garlic is due to its prostaglandin-like effects, which decrease peripheral vascular resistance (Rashid and Khan, 1985). Aged garlic extract was superior to placebo in lowering systolic blood pressure in patients suffering from uncontrolled hypertension. A dosage of 240-960 mg of aged garlic extract containing 0.6-2.4 of S-allylcysteine significantly lowered blood pressure by about 12 mmHg over 12 weeks (Ried *et al.*, 2013a).

Garlic administration in rats suffering from hypercholesterolemia, induced by a high-cholesterol diet, significantly reduced serum cholesterol, triglyceride, and LDL, but there was no effect on serum HDL (Kamanna and Chandrasekhara, 1982). In *in vitro* experiments, garlic administration suppressed LDL oxidation and increased HDL, which may be one of the protective mechanisms of the beneficial effects of garlic in cardiovascular health (Rahman and Lowe, 2006). Long term application of garlic and its preparations on experimental atherosclerosis induced by a high cholesterol diet, showed 50% reduction in atheromatous lesions, particularly in the aorta (Jain, 1977). Most of human studies on lipid lowering effects of garlic and garlic preparations described significant decrease in serum cholesterol and triglyceride (Gardner *et al.*, 2001; Ziaei *et al.*, 2001). A meta-analysis including 39 primary trials of the effect of 2 months administration of garlic preparations on total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides was performed (Ried *et al.*, 2013b). The results suggest garlic is effective in reduction of total serum cholesterol by 17 ± 6 mg/dL and low-density lipoprotein cholesterol by 9 ± 6 mg/dL in subjects with elevated total cholesterol levels (>200 mg/dL). An 8% reduction in total serum cholesterol is of clinical relevance and is associated with a 38% reduction in risk of coronary events at 50 years of age. High-density lipoprotein cholesterol levels improved only slightly, and triglycerides were not influenced significantly. Garlic was highly tolerable in all trials and was associated with minimal side effects. This meta-analysis study concluded that garlic should be considered as an alternative option with a higher safety profile than conventional cholesterol-lowering medications in patients with slightly elevated cholesterol (Ried *et al.*, 2013b). However, a few studies using garlic powder, having low allicin yields, failed to show any lipid lowering effects (Lutomski, 1984; Luley *et al.*, 1986). It has been suggested that different people might have different responses to garlic, thus garlic may be more beneficial for some specific groups (Zeng *et al.*, 2013). Preventive effect of garlic on atherosclerosis has been attributed to its capacity to reduce lipid content in arterial membrane. Allicin, S-allyl cysteine, presented in aged garlic extract and diallyldi-sulfide, presented in garlic oil are the active compounds responsible for anti-atherosclerotic effect (Gebhardt and Beck, 1996; Yu-Yah and Liu, 2001). The plasma fibrinolytic activity in animals, which was decreased on cholesterol feeding, was considerably increased when this diet was supplemented with garlic (Mirhadi *et al.*, 1993).

Several human studies on plasma fibrinolytic activity have found that garlic increased fibrinolytic activity in healthy individuals as well as in acute myocardial infarction patients (Bordia *et al.*, 1998). It was shown that pre-treatment with garlic significantly inhibited intracellular Ca^{2+} mobilization, thromboxane- A_2 (a potent platelet aggregator) synthesis and protected against thrombocytopenia induced by collagen or arachidonate application in rabbits. These observations indicate that garlic may be beneficial in the prevention of thrombosis. Garlic has also been shown to



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inhibit platelet adhesion or aggregation in human investigations. It has been shown that the aged garlic extract inhibited the binding of ADP-activated platelets to immobilized fibrinogen. This suggested that aged garlic extract inhibited platelet aggregation via inhibition of the GPIIb/IIIa receptor and an increase in cAMP (Allison *et al.*, 2012). Furthermore, it was reported that garlic decreases the risk of peripheral arterial occlusive diseases, plasma viscosity, and unstable angina and increases elastic property of blood vessels and capillary perfusion (Sumiyoshi and Wargovich, 1990). Seventy-eight patients with peripheral arterial occlusive disease were randomized to receive garlic or a placebo medication. The dose of garlic was 400 mg oral standardized garlic powder twice daily. Both men and women aged 40 to 75 years were enrolled in the study. After twelve weeks of treatment, pain-free walking distance increased similarly whether receiving garlic or placebo. Similarly there was no difference in the changes in blood pressure, heart rate, and pressure differences between the ankle and brachial pressures. No severe side effects were observed although more people taking garlic (28%) than placebo (12%) complained of a noticeable garlic smell. This indicates that any improvements in symptoms of peripheral arterial occlusive disease with garlic may require longer-term treatment and follow up than in this study (Jepson *et al.*, 2000).

Anti-Tumor Effect Of Garlic

Many *in vitro* and *in vivo* studies have suggested possible cancer-preventive effects of garlic preparations and their respective constituents. Garlic has been found to contain a large number of potent bioactive compounds with anticancer properties, largely allylsulfide derivatives. Different garlic derivatives have been reported to modulate an increasing number of molecular mechanisms in carcinogenesis, such as DNA adduct formation, mutagenesis, scavenging of free radicals, cell proliferation and differentiation as well as angiogenesis. The growth rate of cancer cells is reduced by garlic, with cell cycle blockade that occurs in the G2/M phase (Capasso, 2013). In 1990, the U.S. National Cancer Institute initiated the Designer Food Program to determine which foods played an important role in cancer prevention (Dahanukar and Thatte, 1997). They concluded that garlic may be the most potent food having cancer preventive properties. Garlic has a variety of anti-tumor effects, including tumor cell growth inhibition and chemopreventive effects. In rodents, garlic and its constituents have been reported to inhibit the development of chemically induced tumors in the liver (Kweon *et al.*, 2003), colon (Knowles and Milner, 2003), prostate (Hsing *et al.*, 2002), bladder (Lau *et al.*, 1986), mammary gland (Amagase and Milner, 1993), esophagus (Wargovich *et al.*, 1988), lung (Sparnins *et al.*, 1986), skin (Nishino *et al.*, 1989), and stomach (Wattenberg *et al.*, 1989) in both rodent and human studies. Diallyl trisulfide (DATS), an organosulfur compound isolated from garlic, has been shown anticancer activity both in *in vitro* and *in vivo* investigations. The cytotoxicity of DATS toward prostate epithelial cells reduced as opposed to PC-3 cancer cells (Borkowska, 2013).

Possible anticarcinogenic mechanisms of garlic and its constituents may include the inhibition of carcinogen activation (Amagase and Milne, 1993), the enhancement of detoxification (Sumiyoshi and Wargovich, 1990), excretion (Tadi *et al.*, 1991a), and the protection of DNA from activated carcinogens (Tadi *et al.*, 1991b). Furthermore, DATS reduced tumor mass and number of mitotic cells within tumors. DATS reduced mitosis in tumors, decreased histone deacetylase activity, increased acetylation of H3 and H4, inhibited cell cycle progression, and decreased pro-tumor markers (survivin, Bcl-2, c-Myc, mTOR, EGFR, VEGF) (Wallace *et al.*, 2013). Garlic components have been found to block covalent binding of carcinogens to DNA, enhance degradation of carcinogens, have anti-oxidative and free radical scavenging properties, and regulate cell proliferation, apoptosis, and immune responses. Ajoene, a garlic stable oil soluble sulfur rich compound and garlic-derived natural compound, have been shown to induce apoptosis in leukemic cells in addition to the other blood cells of leukemic patients. Ajoene induced apoptosis in human leukemic cells via stimulation of peroxide production, activation of caspase-3-like and caspase-8 activity. Garlic synergizes the effect of eicosapentaenoic acid, a breast cancer suppressor, and antagonizes the effect of linoleic acid, a breast cancer enhancer (Tsubura *et al.*, 2011). Anti-proliferative activity of ajoene was demonstrated against a panel of human tumor cell lines (Li *et al.*, 2002). Furthermore, allicin inhibits proliferation of human mammary endometrial and colon cancer cells. Growth inhibition is accompanied by an accumulation of the cells in G1 and G2/M phase of the cell cycle. Thus allicin is also responsible for the anti-proliferative effect of garlic derivatives. Diallyl sulfide and diallyl disulfide, inhibit arylamine N-acetyltransferase activity and 2-aminofluorene-DNA in



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human promyelocytic leukemia cells (Lin *et al.*, 2002). Reduction of the risk of some malignancies by consumption of selenium-enriched plants, such as garlic was suggested (Finley, 2003). DATS inhibited cell growth of human melanoma A375 cells and basal cell carcinoma cells by enhancement of the levels of intracellular reactive oxygen species and DNA damage and by inducing endoplasmic reticulum stress and mitochondria-mediated apoptosis (Wang *et al.*, 2012).

Diabetes Mellitus

Although experimental studies demonstrated a clear hypoglycemic effect of garlic, the effect of garlic on human blood glucose is still controversial. Many studies showed that garlic can reduce blood glucose level in diabetic animals. Garlic was effective in reduction of blood glucose in streptozotocin- as well as alloxan-induced diabetes mellitus in rats and mice (Sheela *et al.*, 1995; Ohaeri, 2001). Short term benefits of garlic on dyslipidemia in diabetic patients were shown (Ashraf *et al.*, 2005). Garlic significantly reduced serum total cholesterol and LDL cholesterol and moderately raised HDL cholesterol as compared with placebo in diabetic patients (Ashraf *et al.*, 2005). S-allyl cysteine, a bioactive component derived from garlic, restored erectile function in diabetic rats by preventing reactive oxygen species formation through modulation of NADPH oxidase subunit expression (Yang *et al.*, 2013). Metformin and Garlic treatment in diabetic patients for 12 weeks reduced fasting blood glucose (FBG), but the percentage of change in FBG was more substantial with metformin supplemented with garlic than with metformin alone (Kumar *et al.*, 2013). Chronic feeding of garlic extracts showed significant decrease in blood glucose level. However, some other studies showed no change of blood glucose level after that in human. Therefore, the role of garlic in diabetic patients needs to be further investigated (Banejee and Maulik, 2002). The beneficial effect of garlic on diabetes mellitus is mainly attributed to the presence of volatile sulfur compounds, such as alliin, allicin, diallyl disulfide, diallyl trisulfide, diallyl sulfide, S-allyl cysteine, ajoene, and allyl mercaptan. Garlic extracts have been reported to be effective in reducing insulin resistance (Padiya and Banerjee, 2013).

Effect Of Garlic On Chemically-Induced Hepatotoxicity

Several studies showed that garlic can protect the liver cells from some toxic agents. Acetaminophen is a leading analgesic and antipyretic drug used in many countries. Overdose is known to cause hepatotoxicity and nephrotoxicity in humans and rodents. Although more than 90% of acetaminophen is converted into sulfate and glucouronide conjugates and excreted in the urine, a small portion is metabolized by different liver enzymes (Patten *et al.*, 1993). This can arylate critical cell proteins and cause toxicity. It is demonstrated that garlic protects against acetaminophen-induced hepatotoxicity. Gentamycin also induces hepatic damage as revealed by elevation of liver damage marker enzymes (aspartate transaminase and alanine aminotransferase) and reduction in plasma albumin level. Dietary inclusion of garlic powder protects rats against gentamycin-induced hepatotoxicity, improves antioxidant status, and modulates oxidative stress (Ademiluyi *et al.*, 2013). In addition, garlic attenuated hepatotoxicity effect of nitrate in rats. Garlic extract may reduce lipid peroxidation and enhance antioxidant defense system (El-Kott, 2012).

Anti-microbial effect of garlic

Garlic has been used for centuries in various societies to combat infectious disease. Historically, it is believed that Louis Pasteur described the antibacterial effect of garlic in 1858 for the first time, although no reference is available. More recently, garlic has been proven to be effective against a plethora of gram-positive, gram-negative, and acid-fast bacteria. These include Salmonella, Escherichia coli (Adler and Beuchat, 2002), Pseudomonas, Proteus, Staphylococcus aureus (Cavallito, 1944), Escherichia coli, Salmonella (Johnson and Vaughn, 1969), Klebsiella (Jezowa and Rafinski, 1966), Micrococcus, Bacillus subtilis (Sharma *et al.*, 1977), Clostridium (De Witt *et al.*, 1979), Mycobacterium (Delaha and Garagusi, 1985), and Helicobacter (O'Gara *et al.*, 2000). It has been documented that garlic exerts a differential inhibition between beneficial intestinal microflora and potentially harmful enterobacteria (Ress *et al.*, 1993).



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The antibacterial activity of garlic is widely attributed to allicin. It is known that allicin has sulfhydryl modifying activity (Wills, 1956) and is capable of inhibiting sulfhydryl enzymes. Cysteine and glutathione counteract the thiolation activity of allicin. Garlic extract and allicin have been shown to exert bacteriostatic effects on some vancomycin-resistant enterococci. An inhibitory synergism was observed when used in combination with vancomycin (Jonkers et al, 1999). It is thought that allicin modifies the sulfhydryl groups on the enzymes of the TN1546 transposon, which encodes vancomycin resistance, enhancing susceptibility to vancomycin. The antibacterial effect of different concentrations of garlic extract against human dental plaque microbiota has been shown in *in vitro* study (Houshmand et al., 2013). The synergism between ciprofloxacin with garlic extract has been shown, but not between ampicillin and the garlic extracts (Zain al-abdeen et al., 2013). The cloves of garlic and rhizomes of ginger, extracted with 95% ethanol, suggested to have anti-bacterial activity against multi-drug clinical pathogens and can be used for prevention of drug resistant microbial diseases. *Pseudomonas aeruginosa* was the most sensitive germ to the mixture (Karuppiah and Rajaram, 2013). Garlic also suggested as a treatment for multi-drug resistant tuberculosis (Dini et al., 2011).

Anti-Protozoal Properties

Several studies have shown that the extract was effective against a host of protozoa including *Candida albicans* (Lemar et al., 2002), *Scedosporium prolificans* (Davis et al., 2003), *tinea pedis* (Ledezma et al., 2000), *Opalina ranarum*, *Balantidium entozoon*, *Entamoeba histolytica*, *Trypanosomes*, *Leishmania*, *Leptomonas*, and *Crithidia* (Reuter et al., 1966). Due to the occurrence of unpleasant side effects and increasing resistance to the synthetic pharmaceuticals, garlic was recommended for the treatment of giardiasis. Inhibitory activity of garlic on giardia was noted with crude extract at 25 pg/mL and the lethal dosage was established as approximately 50 pg/mL. Encouraged by these results, a clinical trial was carried out on patients that had giardiasis (Soffar and Mokhtar, 1991). Garlic was established as an anti-giardial, removing the symptoms from all patients within 24 h and completely removing any indication of giardiasis from the stool within 72 h at a dosage of 1 mg/mL twice daily aqueous extract or 0.6 mg/mL commercially prepared garlic capsules. No *in vitro* calculations were possible, as the workers could not culture the protozoa *in vitro*. It was suggested that allicin, ajoene, and organosulfides from garlic are effective antiprotozoal compounds.

Antifungal Properties

Antifungal activity was first established in 1936 by Schmidt and Marquardt whilst working with epidermophyte cultures (Lemar et al., 2002). Many fungi are sensitive to garlic, including *Candida* (Yousuf, 2011), *Torulopsis*, *Trichophyton*, *Cryptococcus* (Fromtling and Bulmer, 1978), *Aspergillus* (Hitokoto et al., 1980), *Trichosporon*, and *Rhodotorula* (Tansey and Appleton, 1975). Garlic extracts have been shown to decrease the oxygen uptake (Szymona, 1952), reduce the growth of the organism, inhibit the synthesis of lipids, proteins, and nucleic acids (Adetumbi et al., 1986), and damage membranes (Ghannoum, 1988). A sample of pure allicin was shown to be antifungal. Removal of the allicin from the reaction by solvent extraction decreased the antifungal activity (Hughes and Lawson, 1991). Activity has also been observed with the garlic constituents, diallyl trisulfide, against cryptococcal meningitis (Cai, 1991), ajoene, and against *Aspergillus* (Yoshida et al., 1987). Thiol reduced the activity, suggesting the blocking of thiol oxidation by allicin. Inhibition of respiratory activity is thought to be due to inhibition of succinate dehydrogenase. The adhesion of *Candida* is also greatly reduced in the presence of garlic extract (Ghannoum, 1990). Again, this effect is diminished by the addition of thiol compounds. The addition of ajoene to some fungal growth mixtures, including *Aspergillus niger*, *C. albicans*, and *Paracoccidioides*, has resulted in inhibition at concentrations lower than that experienced with allicin. Studies with aged garlic extract (with no allicin or allicin-derived constituents) showed no *in vitro* antifungal activity. However, when given to infected mice, the number of organisms that were seen was reduced by up to 80% (Tadi et al., 1991a). It has been reported that garlic exhibited antifungal effects on two species, the air-borne pathogen *Botrytis cinerea* and *Trichoderma harzianum* (Lanzotti et al., 2012). Greater satisfaction with the use of garlic rather than nystatin was reported by the patients with denture stomatitis (Bakhshi et al., 2012).



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Antiviral Properties

In comparison with the antibacterial action of garlic, very little work has been done to investigate its antiviral properties. The few studies have reported that garlic extract showed *in vitro* activity against influenza A and B (Fenwick and Hanley, 1985), cytomegalovirus (Meng *et al.*, 1993; Nai-Lan *et al.*, 1993), rhinovirus, HIV, herpes simplex virus 1 (Tsai *et al.*, 1985), herpes simplex virus 2 (Weber *et al.*, 1992), viral pneumonia, and rotavirus. Allicin, diallyl trisulfide and ajoene have all been shown to be active (Hughes *et al.*, 1989; Weber., 1992). In the case of HIV, it is thought that ajoene acts by inhibiting the integrin dependent processes (Tatarintsev *et al.*, 1992). Allyl alcohol and diallyl disulfide have also proven effective against HIV-infected cells (Shoji *et al.*, 1993). No activity has been observed with allicin or S-allyl cysteine. It appears that only allicin and allicin-derived substances are active. Taken together, the beneficial effects of garlic extract make it useful in medicine. There are insufficient clinical trials regarding the effects of garlic in preventing or treating the common cold. A single trial suggested that garlic may prevent occurrences of the common cold, but more studies are needed to validate this finding. This trial randomly assigned 146 participants to either a daily garlic supplement (with 180 mg of allicin content) or a placebo for 12 weeks. The investigation revealed 24 occurrences of the common cold in the garlic group compared with 65 in the placebo group, resulting in fewer days of illness in the garlic group compared with the placebo group. However, claims of effectiveness of garlic on common cold appear to rely largely on poor quality evidence (Lissiman *et al.*, 2012). Many countries have used garlic extract for clinical treatments, but the untoward actions of garlic following long-term administration should be fully noted. Even though many studies on garlic and its derivatives have been performed, the exact biological mechanism of garlic extract still remains to be elucidated.

CONCLUSION

A recent increase in the popularity of alternative medicine and natural products has renewed interest in garlic and their derivatives as potential natural remedies. This review may be useful to increase our knowledge of garlic therapeutic effects and improve our future experimental and clinical research plans. Although it is shown that garlic may have a significant clinical potential either in their own right or as adjuvant therapy in different disorders, however, due to some issues, such as methodological inadequacies, small sample sizes, lack of information regarding dose rationale, variation between efficacy and effectiveness trials, the absence of a placebo comparator, or lack of control groups more standard experiments and researches are needed to confirm the beneficial effect of garlic in various diseases. Future trials on the effect of garlic should include information on the dosage of active ingredients of standardized garlic preparations for better comparison of trials. It would also be interesting to explore the effect of different forms of garlic extract on standard drug therapy, especially when used as adjuvant therapy. Although garlic is believed to be a safe substance, long-term trials of reasonable duration would provide insights into the possible side-effects of different garlic extracts. The safety of garlic should be tested especially in pregnant or breastfeeding women as well as in young children (Budzynska *et al.*, 2012; Dante *et al.*, 2013). Long-term and large trials are also needed to evaluate the differences in mortality, serious adverse events, and morbidity of cancer and cardiovascular diseases after garlic therapy.

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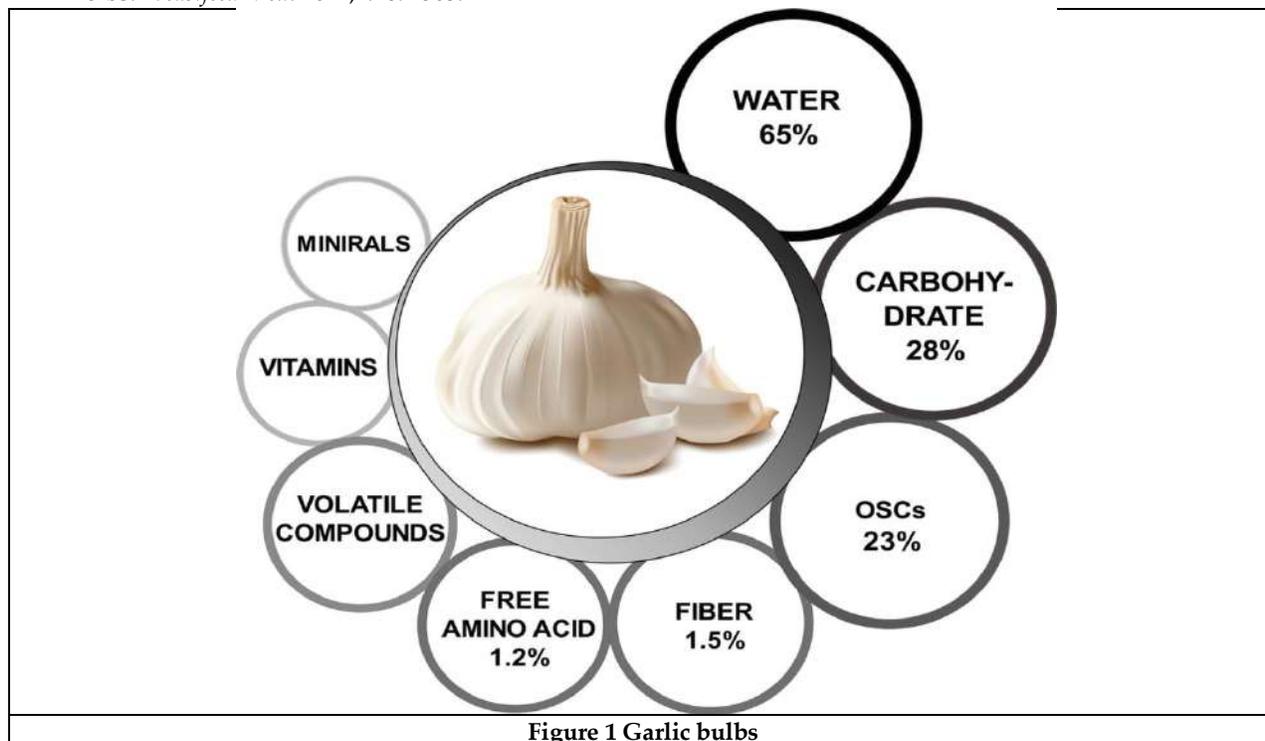
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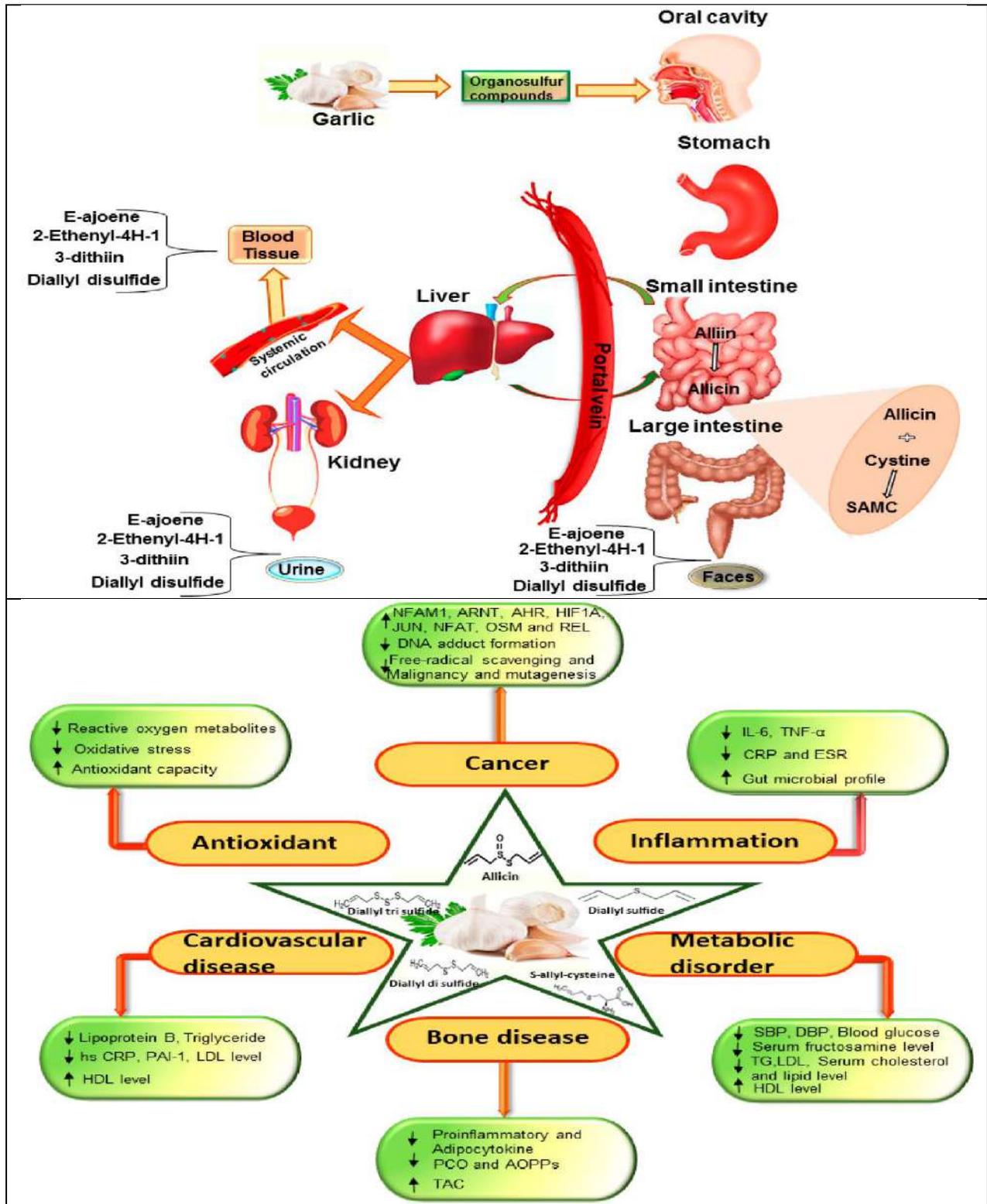
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Trichodiniasis

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ABSTRACT

The present study was aimed at screening for the presence of protozoan's among Cyprinid fishes collected from various fish ponds and farms in Jammu division of Jammu and Kashmir (J and K) state. Out of 75 fishes collected from local water bodies of Jammu division, only 35, (49.6 %) were infested with Trichodina. In light infestation Trichodina was usually present on gills, fins and skin of apparently healthy fish. Clinical signs of Trichodiniasis appeared on fish with heavy infections and in presence of one or more stress factors including, rough handling during transportation from ponds, over crowdedness, malnutrition, high concentration of free ammonia and low oxygen concentration.

Keywords: Fishes, Ectoparasite, Trichodinaheterodontata, Morphology, Taxonomy

INTRODUCTION

Trichodinids are ciliate protozoans widely known as ectocommensals and are probably the most commonly encountered protozoan parasites on wild and cultured fishes in marine as well as freshwater environments. Trichodina is a saucer-shaped parasite that attacks fish skin and gills. The typical signs of the disease include skin and gill damage, respiratory distress, loss of appetite and loss of scales. Skin and gill damage caused by this parasite may lead to the entry of other pathogens such as bacteria and fungus. To date, about 300 nominal Trichodina species have been reported from different environments in the world. Many species are morphologically variable and show low host specificity which make their determination difficult. Because of their direct transmission the trichodinid ciliates are able to invade their hosts within a short period, especially fish that are kept under less than optimal conditions. The taxonomy of Trichodinids is based on the structure and the appearance of the adhesive disc and number and size of its constituents. Today, ten genera are described within the family Trichodinidae. The genus



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(Trichodina Ehrenberg 1838) is the largest of this family with more than 200 species described from fish. trichodinids reproduce by binary fission and it has been the subject of study since the previous century. Most trichodinids are not pathogens, but when the relationship host/parasite/environment is broken by nutritional deficiency, poor water quality, infectious and/or parasitic diseases trichodinids may proliferate, being responsible for severe epidermal lesions and disease outbreaks.

Causative Agent and Disease

Trichodiniasis is caused by ciliated protozoans of the family Trichodinidae in which the most common of 6 genera is Trichodina represented by over 30 species. This protozoan is probably the most frequently encountered external obligate parasite in cultured freshwater fishes worldwide. Some species in this family also parasitize fish and shellfish in the marine environment. Trichodina (40-60 µm in diameter) is saucer-shaped and moves along the surface of the skin, fins and gills of fish by means of its cilia. It feeds on the detritus and other debris found on the surface of the fish using tooth-like structures called denticles. These denticles scrape the debris from the surface of the fish to the mouth of the parasite. When abundant, the scraping and movement of these organisms irritate the skin and gill surfaces causing hyperplasia of the epithelium. Extreme cases of hyperplasia can result in reduced gas exchange or reduced osmoregulation in the fish host. When environmental conditions are suboptimal or when fish tissues are mechanically damaged, more severe infestations may occur.

Host Species

Protozoa of this family are found parasitizing freshwater and marine fish species worldwide. Rainbow and steelhead trout, coho and Chinook salmon appear more susceptible than other species of salmonids. Young fish (yearlings or younger) are most susceptible. The parasite has also been reported from amphibian tadpoles.

Clinical Signs

Fish parasitized by Trichodina often have white patches and/or mottling of the skin and fins. Excessive mucus is produced causing a white to bluish sheen of the skin. Fins are generally frayed and fish exhibit flashing behaviour by scraping their bodies against hard surfaces. If the gills are heavily infested opercular movements may be labored.

Transmission

Fish are infested with Trichodina through direct transmission from fish to fish or from organisms in the water originating from a sub clinically infested reservoir host. The organisms reproduce by binary fission whereby daughter organisms either attach immediately to the original host or seek a new host in the water column.

Diagnosis

Diagnosis is easily made by microscopic observation of the highly motile spinning protozoan in a wet mount preparation of skin scrapes or gill tissues. When abundant, the organisms may be visible gliding on the skin surface with the naked eye. Genus and species identification require microscopic examination of the shape and arrangements of the denticles on the chitin disc surrounding. The current study is the first to report trichodinid fauna from the fish species (*Cyprinus carpio*, *Labeorohita*, *Catlacatla*, *Puntiusticto*.) captured from their natural environment.

MATERIALS AND METHODS

A number of 75 apparently healthy and naturally infected fishes of different species, (31 of *Cyprinus carpio*, 22 of *Labeorohita*, 15 of *Catlacatla*, 8 of *Puntiusticto*) were collected from November 2013 to February 2014 with the help of a drag net/hand net from different localities of Jammu division of J and K state. External examination on the gills, fins and body surfaces of the fish for ectoparasites was first carried out using hand lens for detection of parasitic manifestations. Later, skin smears were taken using scalpel blade. The procedure was performed using a spatula by which the skin scrapings (smears) from the head to the tail were obtained. Thereafter, the scraped samples of mucus together with the tissues were placed on a Petri-dish containing 3 ml of 0.9 % saline solution and stirred using a





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mounted pin. Some drops of the mixed solution were collected using dropper, placed on a clean slide and examined under microscope. Detection of parasites from the gills of the sampled fish was also made using the methods. Gills were cut by scissors, placed in a Petri-dish and gill filaments were dissected using anatomical needle and examined under the microscope. Gill scrapings were placed on few drops of water previously placed onto glass slides then covered with cover-slide and examined under the microscope. The stomach and the intestine of each of the fish were cut opened, and contents washed into the Petri-dish containing the saline solution. The lining of the gut lumen was also scrapped out and placed in the saline solution. One to two drops of the preparation were placed on slide covered with slips and observed using a light binocular microscope for endoparasites. The total numbers of trichodinids were determined by screening all body surfaces including the fins and gills using a light microscope at $10\times \times 100\times$ magnification. For species identification and determination of infestation site, following total counts.

CONCLUSION

Trichodinids are geographically a widely dispersed group of ectoparasites in freshwater, marine and euryhaline environments. Trichodinids are widely studied and well-documented parasites of fishes and their importance is reflected by the reported literature on several aspects of the biology of these parasites. About 70 species have been identified in marine fishes (and more than 112 from freshwater fishes worldwide. Some trichodinids including *T. domerguei* and *T. tenuidens* parasitising *Gasterosteus aculeatus* and *Pungitius pungitius* have been recorded in euryhaline waters. The morphological variations of denticle form and appearance of central circle in *Trichodina* parasite observed in this study are also in agreement with the statement of Lom and Stein. Host specificity in trichodinids was highly variable. In general, the severity of most ecto- and endoparasitic infections increases with the age of the host fish, possibly as a result of the greater accumulation period and/or the larger space for feeding and breeding of the parasite. noted a tendency to increase in the mean intensity of *Trichodina* spp in relation to the length of common carp. Our findings on the intensity levels of *Trichodina* agree with those reported by the above mentioned authors. Studies on the parasite fauna in farmed and wild fish in Jammu are quite rare.

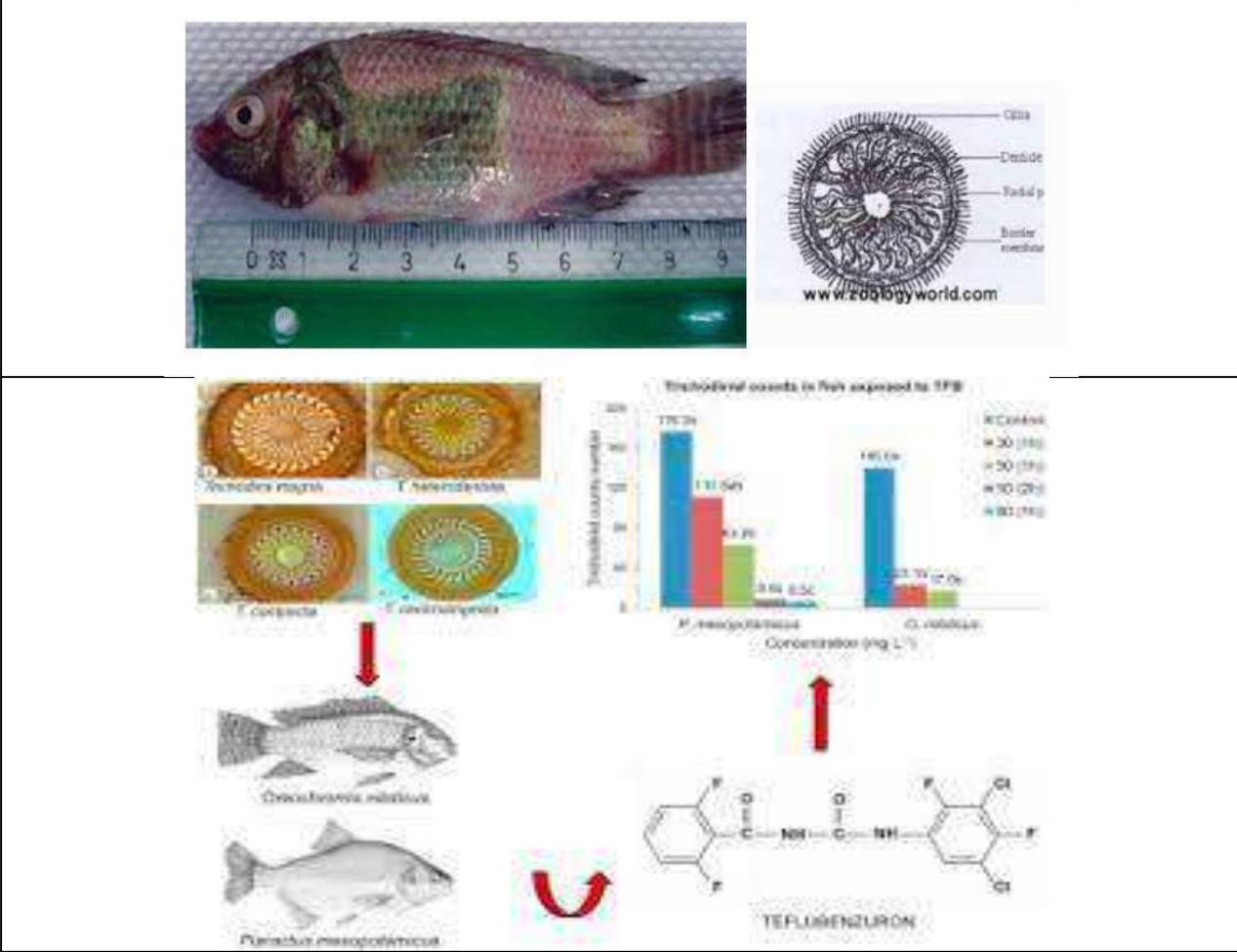
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Neem use as aImmune Booster for Eye Disorder

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ABSTRACT

Neem, (*Azadirachta indica*) is valuable plant in the world that have the solution of thousands of problems. The Neem tree (*Azadirachta indica*) has been known as the wonder tree for centuries in the Indian subcontinent. It has become important in the global context today because it offers answers to the major concerns facing mankind. Neem (*Azadirachta indica*) is considered harmless to humans, animals, birds, beneficial insects and earthworms, and has been approved by the US Environmental Protection Agency for use on food crops. The sanskrit name aristha of the neem tree meaning 'realieve of sicknesses. And hence is considered as 'sarbarogaribarini'. Due to its lots of ayurvedic properties it is the main source for the, so the tree is regarded as 'village dispensary' in India. Azadirachtin and other active ingredients in the neem leave have insecticidal properties that are effective against a broad spectrum of insects, many mites and nematodes, and even snails and fungi, and do not seem to generate resistance in the pests they affect. What's more, even neem contraceptives are available in the market these days. Neem extract which have Nimbinin, nimbandiol as active constituents, alcoholic extract of the leaves was found to possess a significant blood sugar lowering effect, which are very useful against different eye disorder. Neem also has shown antiviral, anti-fungal and anti-bacterial properties. Traditionally Neem has been used for skin and blood purifying conditions. Neem not only helps in curing diseases, but it also provides us with the strength of fighting diseases by enhancing our immunity.

Keywords: Neem importance, neem botany, origin and properties, plant description, chemical constituent.



**Chinmayananda Debata and Preetha Bhadra****INTRODUCTION**

Taxonomical description of Neem the neem has similar properties to its close, chinaberry. The taxonomic position of neem are as follows

Kingdom: Plantae
Division : Magnoliophyta
Order : Sapindales
Family : Meliaceae
Genus : Azadirachta

Commonly in India, Africa and America. It has been used in ayurvedic medicine for more than 4000 years due to its medicinal properties. Neem is called 'arista' in Sanskrit a word that means 'perfect, complete and imperishable' Arishtha is the sanskrit name of the neem tree meaning 'reliever of sickness' and hence is considered as 'Sarbarogaribarini'. The tree is regarded as 'village dispensary' in India. The importance of the neem tree has been recognised by the US National Academy of Sciences, which publish a report in 1992 entitled 'Neem- a tree for solving global problems'. The medical properties of Neem have been known to Indians since time immemorial. The earliest Sanskrit medical writings refer to the benefits of Neem's fruits, seeds, oil, leaves, roots and bark. Each has been used in the Indian Ayurvedic and Unani systems of medicines, and is now being used in the manufacture of modern day medicinal, cosmetics, toiletries and pharmaceuticals. The Neem tree has been known as the wonder tree for centuries in the Indian subcontinent. Neem has become important in the global context today for its variety of medicinal uses. Neem extract which have Nimbinin, nimbandiol as active constituents, alcoholic extract of the leaves was found to possess a significant blood sugar lowering effect, which are very useful against diabetes. Neem is used in Dermatitis Eczema, Acne, Bacterial, Fungal infections and other skin disorders. It has demonstrated its effectiveness as a powerful antibiotic.

It grows in much of Southeast Asia and West Africa; a few trees have recently been planted in the Caribbean and several Central American countries, including Mexico. The people of India have long revered the neem tree; for centuries, millions have cleaned their teeth with neem twigs, smiered skin disorders with neem-leaf juice, taken neem tea as a tonic, and placed neem leaves in their beds, books, grainbins, cupboard and closets to keep away troublesome bugs. The number of benefits of neem is listed in ancient documents like 'Charak-Samhita' and 'Susruta-Samhita'. It is commonly called 'Indian Lilic' or 'Margosa', belongs to the family Meliaceae, subfamily Meloideae and tribe Melieae. Neem is the most versatile, multifarious trees of tropics, with immense potential. It possesses maximum useful non-wood products (leaves, bark, flowers, fruits, seed, gum, oil and neem cake) than any other tree species. known to have antiallergenic, antidramatic, antifeedant, antifungal, anti-inflammatory, antipyorrhoeic, antiscabic, cardiac, diuretic, insecticidal, larvicidal, nematocidal, spermicidal and other biological activities.

Neem has become important in the global context today because it offers answers to the major concerns facing mankind. Neem is considered harmless to humans, animals, birds, beneficial insects and earthworm. Several studies of Neem extracts in suppressing malaria have been conducted, all supporting its use in treatment. Neem has broad applications to human and animal health, as well as organic farming. Neem is a powerful antiviral and antibacterial. But it has peculiarities that set it apart from other herbs in that class of broad antimicrobials. Neem oil is also commonly added to a variety of creams and salves. It is effective against a broad spectrum of skin diseases including eczema, psoriasis, dry skin, wrinkles, rashes and dandruff. A few drops can be added to hand healing salves and shampoo. Neem oil is highly effective as a mosquito repellent. Because of its unpleasant smell, it is best when it is added to a formula with other essential oils, such as citronella. Neem oil is an effective and environmentally safe pesticide when it is diluted and sprayed on crops through irrigation systems.

Plant description



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Neem is a fast-growing tree that can reach a height of 15–20 metres (49–66 ft), and rarely 35–40 metres (115–131 ft). It is evergreen, but in severe drought it may shed most or nearly all of its leaves. The branches are wide and spreading. The fairly dense crown is roundish and may reach a diameter of 20–25 metres (66–82 ft). The neem tree is very similar in appearance to its relative, the Chinaberry (*Meliazadarach*).

Origin and Distribution of Neem

Two species of *Azadirachta* have been reported, *Azadirachta indica* A. Juss –native to Indian subcontinent and *Azadirachta excels* Kack. – confined to Philippines and Indonesia. There are an estimated 25 million trees growing all over India (15) of which 5.5% are found in Karnataka and it is in the third place next to Uttar Pradesh (55.7%) and Tamilnadu (17.8%) occupying the first two places respectively. The other states of India where neem tree is found growing includes Andhra Pradesh, Assam, Bihar, Delhi, Gujarat, Haryana, Himachal Pradesh, Kerala, Madhya Pradesh, Maharashtra, Meghalaya, Orissa, Punjab, Rajasthan, West Bengal along with Andaman and Nicobar Islands, the Union territory. India stands first in neem seed production and about 4,42,300 tons of seeds are produced annually yielding 88,400 tons of neem oil and 3,53,800 tons of neem cake (Sharma Pankaj, et al., 2011).

Chemical constituents and properties

To give a brief background, chemical investigations of neem were undertaken by Indian pharmaceutical chemists in 1919, whereby they isolated acidic principle in neem oil, which they named as ‘margosic acid’. However, real chemical research originated in 1942 with isolation of three active constituents, viz, nimbin, nimbidin and nimbinene. To give a brief background, chemical investigations of neem were undertaken by Indian pharmaceutical chemists in 1919, whereby they isolated acidic principle in neem oil, which they named as ‘margosic acid’. However, real chemical research originated in 1942 with isolation of three active constituents, viz, nimbin, nimbidin and nimbinene. The principal constituents of neem leaves include protein (7.1%), carbohydrates (22.9%), minerals, calcium, phosphorus, vitamin C, carotene etc. But they also contain glutamic acid, tyrosine, aspartic acid, alanine, praline, glutamine and cystine like amino acids, and several fatty acids (dodecanoic, tetradecanoic, elcosanic, etc.).

Neem contains a bitter fixed oil, nimbidin, nimbin, nimbinin and nimbidol, tannin and uses are

- Anti-inflammatory (nimbidin, sodium nimbidate, gallic acid, catechin, polysaccharides).
- Antiarthritic, hypoglycaemic, antipyretic, hypoglycaemic, diuretic, anti-gastric ulcer (nimbidin)
- Antifungal (nimbidin, gedunin, cyclic trisulfide)
- Antibacterial (nimbidin, nimbolide, mahmoodin, margolone, margolonone, isomargolonone)
- Spermicidal (nimbin, nimbidin)
- Antimalarial (nimbolidfe, gedunin, azadirachtin)
- Antitumor (polysaccharides)
- Immunomodulatory (NB-II Pepto glycan, gallic acid, epicatechin, catechin)
- Hepatoprotective (aqueous extract of neem leaf)
- Antioxidant (neem seed extract)

Effect of neem on eye disorder

Constantly exposed to the elements, our eyes are very fragile and impacted by different aspects of modern life, such as the excessive use of computers and digital devices that can strain and dry our eyes, and cause infections. Our eyesight is one of our most important senses, with 80% of what we perceive coming from the sense of sight.

Problems Caused by Eye Drops

Millions of people today suffer from problems with their vision. Common eye problems can become a regular issue whenever our environment is detrimental to our eyes. Whether you are exposed to pollution, screens or stress, eye care is not optional, but a necessary part of overall health. While some of these problems can cause permanent vision loss and even blindness, others can be easily corrected with glasses or eye drops. As these chemicals are highly irritant, eye drops with preservatives should never be applied more than four times a day. A common go-to solution





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for most people are over the counter eye drops (OTC) like artificial tears, which often contain preservatives, for longer shelf life, and thickeners. If you are suffering from severe dryness, you might need more than four doses per day. In this case, you should carefully read the label and purchase preservative-free eye drops.

Benefits of neem for eyes

Neem's antibacterial and antiviral properties can help soothe a variety of eye problems. Known as the "village pharmacy" in India, neem leaves have been considered an essential part of Ayurvedic medicine for centuries, and are commonly used to treat infections and soothe irritated eyes. Naturally high in sulphur content, neem also acts as a building block for collagen, improving skin firmness around the eyes and reducing puffiness.

CONCLUSION

Plants are one of the most important sources of medicines. The role of medicinal plants in promoting the ability of human health to cope with the unpleasant and difficult situations is well documented from ancient times till date all over the world. One of the cardinal goals of millennium development goals (MDGs) is the quest to combat the incidence of diseases such as malaria, HIV/AIDS, eye disease and chronic diseases such as age-related degenerative diseases, cancer and cardiovascular diseases. Medicinal plants are rich in secondary metabolites which are potential sources of drugs and of therapeutic importance. There is increasing interest in the use of plant extracts as therapeutic agents. Neem belongs to use pharmacological potential as panacea. From the literature survey it is found that mango is a potential source of anticancer, anti-diabetic, anti-inflammatory, antimicrobial drugs as well as it also used as cardio protective, radio protective, recognition of memory and many others. The neem has very high beneficial properties which helps wellbeing of human. The neem use as the immune booster for the disease eye disease.

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Ichthyophthiriasis – A Review

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ABSTRACT

Ichthyophthiriasis (white spot disease) is an economically important protozoosis caused by *Ichthyophthirius multifiliis* in freshwater fish. Medication prevention and curation are the main methods to control this disease with vaccines in laboratory, but the efficacy of drugs practically acts on the freeliving (nonparasitic) stage of *I. multifiliis* and can be easily impaired by a variety of environmental factors. Thus, study on the biological properties of *I. multifiliis* and the complicated ecological relationships between *I. multifiliis* and other biotic or abiotic factors that influence epidemicity of *Ichthyophthiriasis* will contribute to integrated control of *Ichthyophthiriasis*. In this article, some *I. multifiliis* biological properties, such as systematic position and life cycle of *I. multifiliis*, are briefly reviewed, and the seemingly abnormal phenomenon associated with in vitro cultures is specially discussed; then, the epizootology of *Ichthyophthiriasis* is emphasized, which involves various biotic or abiotic factors that impact the life and action of *I. multifiliis*. The susceptibility and stress reaction of fish to *I. multifiliis* infection are stated. Also, the pathogenicity and diagnosis of *Ichthyophthiriasis* were covered, and an overall assessment is finally made on *Ichthyophthiriasis* control.

Keywords: Biotic or abiotic factors, epizootology, *Ichthyophthiriasis*, *Ichthyophthiriasis* control, *Ichthyophthirius multifiliis*, in vitro cultures, life cycle.





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INTRODUCTION

Ichthyophthirius multifiliis is a large, ciliated protozoan that causes "Ich" or "white spot disease." This disease is a major problem to freshwater aquarists and commercial fish producers worldwide. All species of freshwater fish are considered susceptible, and the parasite has been found in all areas of the world in both cultured and wild fish. These large parasites cause the characteristic white spots that are often seen on the skin and fins of infected fish. The disease is highly contagious and spreads rapidly from one fish to another without the need for additional hosts (direct life cycle). Although often considered a "warm water" disease, outbreaks often occur when water temperatures are changing, especially in the spring when water temperatures are increasing. The disease is particularly severe when fish are crowded. While many protozoans reproduce by simple division (one parasite "splits" into two), a single "Ich" organism can multiply into hundreds of new parasites in one generation, making early detection and treatment of this parasite crucial. The organism is unusual in that it is an obligate parasite, which means that it cannot survive unless live fish are present. "Ich" is capable of causing massive mortality within a short period of time. An outbreak of "Ich" is a true emergency situation and requires immediate treatment; if left untreated, this disease may result in 100% mortality.

Life Cycle

Although *Ichthyophthirius multifiliis* has a direct life cycle, it is fairly complex and has three distinct life stages:

- 1) the on-fish, feeding trophont;
- 2) the environmental, reproducing tomont; and
- 3) the infective, fish-seeking theront

The trophont invades and encysts between the thin outer layers of the fish host's skin and gills in order to feed on those tissues. Because of the covering by this epithelial tissue and mucus, the trophont stage is protected from chemical treatment. Once the trophont is mature, it stops feeding, leaves the fish, and becomes a tomont. The tomont quickly secretes a gelatinous-walled outer cyst that allows it to stick to surfaces in the environment. The tomont begins to divide quickly, forming hundreds of new "daughter" parasites (tomites) within a single cyst. This can occur in a day or less at warmer water temperatures. The gelatinous wall of the tomont cyst protects it and the daughter tomites from chemical treatment. The tomites begin to develop and become theronts within the tomont cyst. Following a period of days (warm water temperatures) or weeks (cool water temperatures), the theronts bore out of the tomont cyst and become free-swimming, infective parasites in search of a fish host. These infective theronts must find a live fish to complete the parasite's life cycle. This free-swimming phase is unprotected and, therefore, highly susceptible to chemicals. Treatment protocols should be designed to target this theront stage.

Disease Signs

The classic sign of an "Ich" infection is the presence of small white spots on the skin or fins (Figure 2). These spots are caused as the adult parasite (trophont) penetrates and creates a space in the outer layers of the fish's body surfaces (epithelium) in order to feed on the fish and move around. These lesions look like small white dots, blisters, or salt grains on the skin or fins of the fish. The white spots may not be as obvious on fish that are white or pale in color, or if the infection is limited to the gills. By the time the white spots are visible to the naked eye, the infected fish is very sick. Prior to the appearance of white spots, fish may have shown signs of irritation, flashing, weakness, loss of appetite, and decreased activity. A well-trained aquaculturist or aquarist will detect these changes before the fish's condition worsens and mortalities occur. If the parasite is only present in the gills, white spots may not be seen at all but fish will die in large numbers. In these fish, gills will often be pale and very swollen. White spots should never be used as the only means of diagnosis because other diseases may have a similar appearance. Gill and skin biopsies should be collected and examined with a light microscope when the first signs of illness are observed. If even a single "Ich" parasite is seen, fish should be medicated immediately because as the infection advances fish may not survive, even with treatment.





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Diagnosis Of "ICH"

While "Ich" can be suspected by the typical appearance of white spots on some fish, a diagnosis requires confirmation by identification of the parasite in infected tissue using a compound microscope. To do this testing, a glass coverslip can be lightly scraped down the slide of a fish (in the direction of head to tail) to remove some skin cells (preferably with some of the white spots) and mucus. A small area of gill or fin may also be carefully clipped using small, sharp scissors. Keep all tissue pieces small to minimize any harm to the fish. Further, it is very hard to view the parasites when examining thick tissue sections. Mount the skin, fin, and gill samples separately in drops of tank or other fresh water on a microscope slide and overlay gently with a glass coverslip. (Do not use chlorinated tap water, reverse osmosis water, or distilled water. Bottled spring water can be an acceptable clean, fresh water source.) The mature trophont is large, oval to round, dark in color (due to the thick cilia covering the entire cell), and measures 0.5 to 1.0 mm in size. This stage also has a horseshoe- or C-shaped nucleus that may be visible under 40x magnification (Figure 3). The parasite moves slowly in a rolling, sometimes amoeboid motion and, with practice, is easily recognized. The immature, free-swimming theronts are smaller, pear- or spindle-shaped, translucent, and move quickly, continuously spinning on its longest axis as it swims. Theronts can resemble other parasites (especially Tetrahymena), so if only this juvenile stage is seen, prepare a second slide and examine it closely for the trophont stage to confirm the diagnosis. Because one "Ich" organism produces hundreds of individuals in one generation, observation of a single "Ich" parasite is sufficient to make treatment necessary.

Prevention Of "ICH"

Prevention is always preferable to treating "Ich" (or any disease) after an outbreak is in progress. Preventing introduction of this parasite is one of the most important reasons all incoming fish should be quarantined. Transport and handling can cause newly arrived fish who may be asymptomatic carriers (those with no obvious clinical signs) to break with active disease, serving as a source of infection for other fish they may come in contact with. At the warm water temperatures required for many aquarium fish, active disease will often become evident 1–3 weeks after shipping. For this reason, a minimum 30-day quarantine period is recommended for new fish. The importance of this quarantine period for aquaculture or public aquarium facilities cannot be over-emphasized. Additionally, because the environmental tomont cyst is sticky, it can easily spread between systems. For this reason, nets, siphon hoses, and other equipment that have not been disinfected should not be shared between tanks, especially in a quarantine area. "Ich" may also be spread by aerosolization of water mist or spray so nearby systems should be watched carefully.

General Treatment Guidelines

Once an outbreak of "Ich" is in progress, it is important that a treatment protocol be started immediately. Control of this parasite can be difficult because of its complex life cycle and multiple protected stages. The disease outbreak will be controlled as more adult trophonts drop off the sick fish, encyst, and produce theronts that cannot survive the chemical treatment in the water. In a tank or vat, this process can be greatly enhanced if organic debris is removed following treatment. Because the sticky cyst of the tomonts may attach to organic material, cleaning this debris will help remove many cysts from the environment, further decreasing the number of emergent theronts. Any dead fish should be removed as soon as they are seen because mature trophonts will quickly abandon a fish once it has died and begin reproducing in the environment. Fish should be closely watched during recovery because the weakened fish may be susceptible to a secondary bacterial infection. Survivors of an "Ich" outbreak may also serve as reservoirs of infection. The immune system has been able to fight and control the parasite numbers enough that the fish will not show any clinical signs even if they have a lower level infection; however, they may be capable of spreading "Ich" to other fish which have not been previously exposed to the disease.

Chemical Treatment Options Copper Sulfate

The choice of chemical used to treat "Ich" will be based upon water quality conditions, species of fish to be treated, and the type of system the fish are housed in. In general, copper sulfate and formalin are both effective against "Ich" when applied at the correct dose in a repetitive manner as described above. Most aquacultured channel catfish in the





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southeast United States are reared in ponds. For these fish, the treatment of choice for "Ich" is often copper sulfate. The chemical is effective and relatively inexpensive, an important consideration when large volumes of water are treated. It is approved by the Environmental Protection Agency (EPA) as an algacide in aquaculture systems, and the Food and Drug Administration (FDA) currently classifies it as "action deferred" pending further study. The disadvantage of copper sulfate is that it is extremely toxic, particularly in water of low alkalinity. NEVER use copper sulfate without first testing the total alkalinity of the water, carefully measuring the dimensions of the pond to be treated, and weighing the amount of chemical to be applied.

The concentration of copper sulfate to apply in freshwater is calculated by determining the total alkalinity of the water and dividing that number by 100. For example, if the total alkalinity of the pond is 100 mg/L, then $100/100 = 1$ mg/L copper sulfate. Do not use copper sulfate if the total alkalinity is less than 50 mg/L. If you have never used copper sulfate, contact a UF/IFAS Extension aquaculture specialist for assistance. Because copper sulfate is an algacide, its use may lead to severe oxygen depletions; therefore, emergency aeration should always be available. Use of copper sulfate during hot weather or when algae (phytoplankton) blooms are dense is strongly discouraged. Remember, if you do not know the alkalinity of your water and cannot measure it, DO NOT USE COPPER SULFATE. For more detailed information on the use of this chemical, please refer to University of Florida EDIS fact sheet FA-13, Use of Copper in Freshwater Aquaculture and Farm Ponds.

Formalin

If fish are maintained in a tank system, formalin is often used to treat "Ich". Formalin is not the ideal treatment for ponds, but it works well in tanks. Vigorous aeration is required because every 5 mg/L of formalin removes 1 mg/L of oxygen from the water. Formalin-F (Natchex Animal Supply Company), Parasite-S (Western Chemical, Inc.) and Formacide-B (B.L. Mitchell, Inc.) are all formalin products approved by the Food and Drug Administration. Market availability changes, these products are approved to treat external parasites on all species of fish at all life stages. Formalin is usually applied at a concentration of 25 mg/L, which is equivalent to 1 ml of formalin per 10 gallons of water to be treated. For formalin-sensitive species, a "half-dose" of 12.5 mg/L (0.5 ml formalin per 10 gallons of water) may be used. In addition to chemical treatment, cleaning the tank will also decrease the number of parasites. Sick fish may be unable to tolerate a full treatment. If they appear stressed or try to jump out of the tank, flush the chemical from the system immediately with clean, well-oxygenated water. For more detailed information on the use of this chemical, please refer to UF/IFAS EDIS fact sheet VM-77, Use of Formalin to Control Fish Parasites. A slight increase in salinity can help decrease osmoregulatory stress caused by the damage to the external tissues of the fish. At warmer water temperatures (75–79°F), use of 4–5 g/L (= 4–5 ppt) salt (sodium chloride) in a prolonged bath for 7 to 10 days is another effective treatment in smaller systems, provided the fish species can handle the salt concentration. Because theronts are intolerant to increased salinity levels of 3–5 ppt, salt is often added to aquaria or tanks that are being treated with formalin to enhance the response to treatment. Most freshwater fish can tolerate 5 ppt salinity for several weeks and many can live in 3 ppt permanently; however, it is important to know the specific tolerances for each species to be treated.

Potassium Permanganate

Although potassium permanganate is a good choice for many external fish parasites, the repeated treatments necessary in a short period of time make it a more dangerous choice for control of Ich. Potassium permanganate is a strong oxidizer, and its use more than once a week is discouraged to prevent damage to the skin, gills, and eyes of the fish.

Special Considerations For Treatment Of Pet Fish

Pet fish can be treated with any of the chemicals discussed above to alleviate "Ich" infections. A number of commercial preparations are available from pet stores that contain one or more of these agents. In addition to chemical treatments, cleaning the tank every other day will help remove cysts attached to debris before the theronts emerge thereby helping prevent reinfection of the fish and completion of the life cycle.





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CONCLUSIONS

It is clear that *I. multifiliis* is obligate parasite with quite severe virulence. Nearly all freshwater becomes source of *I. multifiliis* infection owing to its wide distribution and its wide range hosts of virtually all species of freshwater fish, so the *Ichthyophthiriasis* is hard to be eradicated completely. The severity of *Ichthyophthiriasis* is not only due to *I. multifiliis* infection alone but also as a result of secondary infection or mutual enhancement of pathogenicity with other causative agents.

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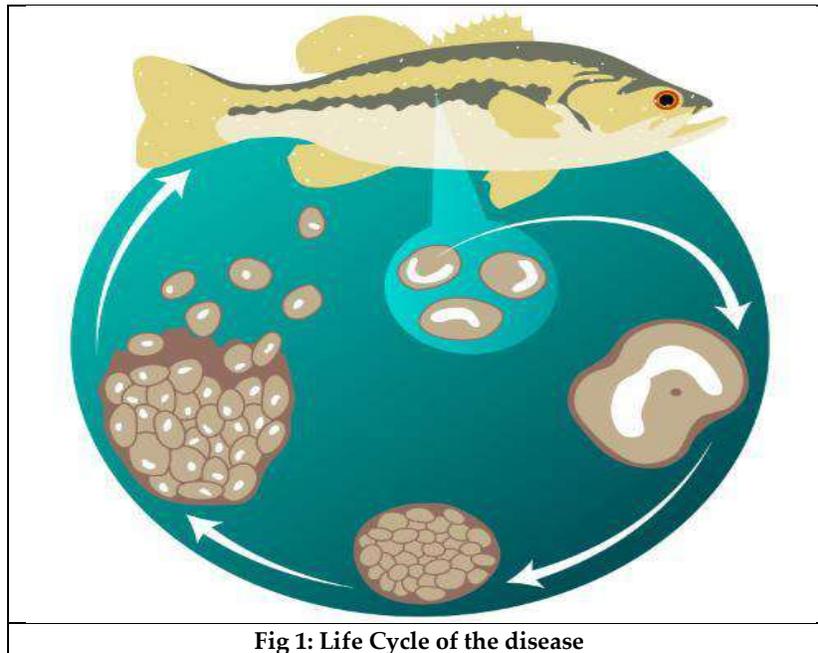


Fig 1: Life Cycle of the disease





Turmeric as a Personalized Medicine

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ABSTRACT

Treatment based on traditional medicine is very popular now a day, several researches are going on different types of medicinal plants which have been recognised for the treatment and prevention of different diseases. The effect of *Curcuma longa* and its chief constituent Curcumin has broad range of disease cure modulations over physiological and biochemical processes. Curcumin has a yellow polyphenolic pigment from the *Curcuma longa*. Turmeric is one of the nature's most powerful healers. The bioactive ingredient of turmeric is Curcumin. Over 2500 years in India, turmeric has been used. It has many medicinal properties which have been slowly revealing themselves over centuries. It is mainly known for its inflammatory properties which has been revealed as natural wonder and proving as beneficial treatment for health conditions and helps to cure diseases from cancer to Alzheimer. It is used as an antiseptic in India and act as active agent in curing *Staphylococcus aureus* which is a pus producing infections. It helps in decreasing Kapha and used to remove mucus in throat, watery discharges like leucorrhoea, and pus from eye, ears, and from wounds. Turmeric is also regarded as 'rasayana' herb which is a branch of Ayurvedic medicine. For desentry the roasted turmeric is used as a main ingredient. It also has a great effect in curing the aging. This review article mainly focuses on the pharmacological activities such as anti-diabetic, anti-microbial, hepato-protective activity, anti-inflammatory, anti-tumor activity and neurodegenerative diseases.

Keywords: Termeric, anti-diabetic, anti-microbial, hepato-protective , anti-inflammatory, anti-tumor





INTRODUCTION

Curcuma longa L. (Zingiberaceae), also known as turmeric, is a bright yellow spice native to Southwest India. Its rhizomes are used to make a brilliant yellow spice with a variety of medical uses. Turmeric is derived from the root of *Curcuma longa*, a ginger family flowering plant. It's frequently seen in spice jars. When purchased fresh, it resembles ginger root but has a more intense yellow to golden colour. Turmeric is being used to cure skin problems, digestive problems, other aches and pains in India. Turmeric is a plant native to southern India and Indonesia that is widely grown on the mainland and in the Indian Ocean islands. This was used as a perfume and a spice in ancient times. The rhizome has a peppery scent and a slightly bitter heated flavour, as well as a vivid orange-yellow staining colour. Turmeric, a spice and colour agent used in many cuisines, is used to flavour and colour yellow mustard. At a pH of 7.4, it changes colour from yellow (acidic form) to red (basic form). Tartaric acid is found in turmeric. *Curcuma* has been identified in 133 different species all over the world. The majority of them have popular names in their home countries and are utilised in a variety of medical compositions depicts some unique turmeric species. Temperatures between 20°C to 30°C are required for the turmeric plant to thrive, as well as a significant amount of annual rainfall. Turmeric, particularly its most active ingredient, curcumin, has numerous scientifically proved health advantages, including the ability to boost heart health and to prevent Alzheimer's disease and cancer. It has anti-inflammatory and antioxidant properties. It may also assist with depression and arthritic problems. India is the world's leading producer, consumer, and exporter of turmeric. Turmeric production is estimated to be over 11 lakh tonnes per year worldwide.

What Personalized Medicine?

A type of medication that prevents, diagnoses, or treats disease by using knowledge about a patient's own genes or proteins. In CF (Cystic Fibrosis) and many other diseases, personalised medicine is used to treat patients based on their symptoms. The use of digestive enzyme supplements in CF is a classic example. By enhancing the matching process between patients and medicines, as well as a patient's comprehension of the risk of major side effects, personalised medicine improves the health impact of existing treatments.

History

Turmeric's use stretches back approximately 4000 years to India's Vedic period, when it was employed as a culinary spice as well as having religious importance. By 700 AD, it had made its way to China, East Africa, West Africa, and Jamaica in the seventeenth century. Curcumin was discovered by Vogel and Pelletier roughly two centuries ago, when they isolated "yellow colouring-matter" from the rhizomes of *Curcuma longa* (turmeric) and termed it curcumin. This chemical was later discovered to be a combination of resin and turmeric oil. Vogel Jr. was born in 1842. The southern states of Telangana, Andhra Pradesh, Tamil Nadu, and Karnataka, as well as the eastern states of Orissa and West Bengal, and the western state of Maharashtra, are major turmeric producing states in India. Telangana is currently India's most important turmeric-producing state, accounting for 19.5% of total land, 33.3 percent production, and 6500 kg/ha productivity.

Origin and Distribution

Turmeric is a plant native to southern India and Indonesia that is widely grown on the mainland and in the Indian Ocean islands. It was used as a perfume and a spice in ancient times. The rhizome has a peppery scent and a slightly bitter heated flavour, as well as a vivid orange-yellow staining colour. Because the yellow dye is used to paint the robes of monks and priests, it migrated to Southeast Asia alongside Hinduism and Buddhism. Turmeric was also discovered before European contact in Tahiti, Hawaii, and Easter Island.



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Morphology of Turmeric

Turmeric, or *Curcuma longa*, is a tropical rhizomatous herbaceous perennial belonging to the ginger family. It grows to 3-4' tall with a leaf clump of ornamentally appealing, pleated, elliptic to lanceolate green leaves (each to 3 1/2' long). Turmeric plants grow to a height of around 1 metre (3.3 feet) and have long, simple leaves with long petioles (leaf stems). The leaves originate from rhizomes that branch just beneath the soil surface. Juvenile rhizomes typically pale yellow to brown-orange in colour, whereas older rhizomes are scaly and brown. The little yellow-orange blooms bloom inside the axils of waxy bracts that are typically pale green or purple-tinged.

Chemical Composition

Turmeric - Curcuminoids, a mix of curcumin, demethoxycurcumin, and bisdemethoxycurcumin, make up 3-6 percent of the polyphenolic chemicals in turmeric. Curcuminoids are important components that have a variety of biological effects. Curcumin [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6 heptadiene-3, 5-dione] is an orange-yellow component of turmeric (*Curcuma longa*), which is commonly used in curry powder. Leaves - A combination of high resolution GC and GC/MS was used to evaluate the chemical composition of *Curcuma domestica* L. leaf oil. More than 20 constituents have been discovered, with the monoterpenes -phellandrene (24.5%), 1,8-cineole (15.9%), p-cymene (13.2%), and -pinene (8.9%) being the most prominent.

Composition of Turmeric Powder

Turmeric powder is about 60–70% carbohydrates, 6–13% water, 6–8% protein, 5–10% fat, 3–7% dietary minerals, 3–7% essential oils, 2–7% dietary fiber, and 1–6% curcuminoids.

Phytochemicals

Curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerones, and turmeronols are only a few of the phytochemical found in turmeric. Turmeric's antimicrobial effect is indicated by the presence of phytochemical such as tannins, alkaloids, phenols, steroids, flavonoids, phlobatannin, cardiac glycosides, terpenoids, triterpenes, saponin, and others. Curcumin [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6 heptadiene-3, 5-dione] is an orange-yellow component of turmeric (*Curcuma longa*), which is commonly used in curry powder.

Turmeric Used As a Personalized Medicine

From many years awareness of turmeric and its use as medicine is continuously increasing. A flowering plant, Turmeric, in the ginger family, is commonly used as a food coloring and is one of the basic ingredients in curry powder. To heal many health disorders like liver problems, digestive disorders, treatment for skin diseases and wound healing turmeric has long been used in medicinal as an anti-inflammatory. Curcumin is the active ingredient in turmeric which has been shown to have a wide range of therapeutic effect. Turmeric is used to treat rheumatoid arthritis, chronic anterior uveitis, conjunctivitis, skin cancer, small pox, chicken pox, wound healing, urinary tract infections, and liver problems. The skin, heart, liver, and lungs are the key organs that turmeric helps. Turmeric is used to treat epilepsy and bleeding disorders, as well as skin illnesses, to purify the body-mind, and to aid in the expulsion of Kapha from the lungs. Alternative, analgesic, antibacterial, anti-inflammatory, anti-tumor, anti-allergic, antioxidant, antiseptic, antispasmodic, appetiser, astringent, cardiovascular, carminative, cholagogue, digestive, diuretic, stimulant, and vulnerary are just a few of Turmeric's properties.

Health Benefits of Turmeric

High Cholesterol levels - In overweight persons with high cholesterol, consuming turmeric extract twice daily for three months reduces total cholesterol, low-density lipoprotein (LDL or "bad") cholesterol, and triglycerides, according to research. Osteoarthritis- According to some studies, using turmeric extracts alone or in conjunction with other herbal ingredients can help people with osteoarthritis reduce pain and improve function. Turmeric was found to be about as effective as ibuprofen for reducing osteoarthritis pain in several studies. However, it does not appear to be as effective as diclofenac in relieving pain and function in osteoarthritis patients. Itching (pruritus) - According





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to research, consuming turmeric three times a day for eight weeks lowers itching in persons with chronic renal disease. Early research also reveals that using a special combination product (C3 Complex, Sami Labs LTD) combining curcumin plus black pepper or long pepper on a daily basis for four weeks reduces itching severity and improves quality of life in persons who have persistent itching caused by mustard gas. Ulcers in stomach - According to some studies, consuming turmeric three times a day for eight weeks does not help stomach ulcers. Furthermore, using powdered turmeric four times per day for six weeks appears to be less effective than taking a traditional antacid.

Another health benefit –

- It's an antiseptic and antibacterial substance that can be used to treat cuts and burns.
- It has been demonstrated to prevent prostate cancer and slow the progression of existing prostate cancer when mixed with cauliflower.
- In mice, it stopped breast cancer from spreading to the lungs.
- It has the potential to prevent melanoma and cause existing melanoma cells to self-destruct.
- Lowers the risk of leukaemia in children.
- It's a natural liver cleanser.
- By eliminating amyloid plaque build-up in the brain, it may help to prevent and reduce the progression of Alzheimer's disease.
- May help to prevent cancer metastases in a variety of cancers.
- It's a powerful natural anti-inflammatory that works just like anti-inflammatory medicines but without the negative side effects. Has shown to be promising.

CONCLUSION

Turmeric has long been used in India as a tasty, vibrant condiment as well as an Ayurvedic medication to enhance appetite, function as a carminative, and cure gallstones and other biliary issues, as well as dyspepsia. It's used as an ointment, paste, or poultice for scabies, boils, bruises, insect bites, and other skin lesions in India, China, and other Southeast Asian countries, and as an ointment, paste, or poultice for scabies, boils, bruises, insect bites, and other skin lesions. Turmeric is also used to treat menstruation issues, pain, epilepsy, respiratory tract infections, bleeding, diarrhoea, jaundice, and rheumatic illnesses when taken orally. It has recently earned a reputation as an anti-inflammatory agent, a hypercholesterolemia therapy, an antioxidant, and a cancer preventative, and it is believed to protect cardiovascular and other degenerative changes. Curcumin is used to prevent oxidation and improve the colour of foods like butter and margarine. Turmeric is a highly prized spicy condiment that has long been used to aid digestion and cure dyspepsia and inflammatory conditions. Turmeric and its main component, curcumin, are also marketed as antioxidants, cancer, HIV, and hypercholesterolemia therapies, and heart disease prevention. However, controlled clinical trials for these indications are either missing or have not produced clearly good outcomes. For peptic ulcer disease, no therapeutic benefit has been established, and one research for dyspepsia was inconclusive. Controlled trials for arthritis and inflammation have likewise failed to show that the treatments are effective. Other applications haven't been tested in a controlled clinical investigation.

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Diseases'

SL NO	DISEASES	TREATMENT
1	Liver disease	Turmeric is the best thing for liver because of its anti-inflammatory properties. Liver can be strengthened by increasing the amount of herbs and foods consumed in the spring. Turmeric contain liver protecting chemicals as found in milk thistle and artichoke plants. It reduces engorged hepatic ducts making it effective for curing the diseases like hepatitis, cirrhosis, and jaundice.
2	Cancer	Turmeric can treat a variety of disorders that can even slow the progression of cancer. This is a spice that is used to cure skin cancer and pre-cancerous disorders.
3	Atherosclerosis	Turmeric may aid avoid artery blockage which leads to heart attack or stroke. It lowers cholesterol levels and prevents LDL oxidation. Production of atherosclerotic plaque which accumulates in the walls of blood vessels. It also helps to inhibit platelet aggregation.
4	Osteoarthritis	Turmeric help to relieve the symptoms of osteoarthritis as it has the ability to reduce pain and disability.
5	Menstrual problems of women	Monthly cramps during menstruation can reduce if turmeric is taken twice a day for two weeks. Turmeric lowers digestion and menstrual cramps by acting as an antispasmodic to smooth muscles. Turmeric is a fantastic supplement for diet and menstrual cycle.
6	Bacterial infection and wounds	Turmeric is a useful an external antibiotic in preventing bacterial infections in wounds.
7	Eye disorder	Curcumin is effective as corticosteroids in the verities which is a type of eye disorder.

Anioxidant Activities

SI NO.	ACTIVITIES	OUTCOME AND FINDINGS
1	Antioxidant activity	Exerts powerful inhibitory effects against peroxide induced damage in human keratinocytes and fibroblasts, it shows herbal function in health management. Curcumin helps in detoxifying enzymes such as glutathione-S-transferase.
2	Anti-diabetic	Curcumin helps increase in gene expression such as insulin like growth factor-I, B-cell lymphoma, superoxide dismutase and GST. Activities like heme oxygenase-I gene expression and HO were significantly increased after the isolation in Islets of Langerhans.





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3	Anti-inflammatory	Curcumin shows vital effect in prevention of inflammatory process by inhibiting and modulating various molecular pathways. Its supplements linked lower plasma level tumor necrosis factor alpha, interleukin-6 and monocyte chemo attractant protein-I. Reduces infiltration in inflammatory condition.
4	Anti-microbial	Inhibits the growth of Helicobacter pylori strains in vitro which is isolated from patients suffering from gastrointestinal disorders. Possesses antibacterial property against Gram-positive and Gram-negative bacteria. Its exhibited inhibitory activity on methicillin-resistant Staphylococcus aureus strains having concentration value of 125-250 µg/ML.
5	Anti-ulcer	Antiulcer activity in indomethacin induced gastric ulceration associated with down regulation of MMP-9 and up regulation of MMP-2.
6	Anti-obesity	Curcumin therapy can reduce the inflammatory consequences of obesity. This also helps in animals to decrease NF-kB activity in liver tissue. These helps in potential health benefits for preventing obesity and associated metabolic disorders and up regulate the adipocyte energy metabolism.
7	Anti-cancer activity	Curcumin increased the activity of phase II enzymes, such as GSTs and down regulated VEGF through inhibition of PPAR in colon cancer cells.
8	Cardio preventive	Curcumin inhibits p300-HAT and finally prevent the development of heart failure.
9	Hypertension reducing	Curcumin helps in prevention of hypertension. Hypertension is increased by N-nitro-L-arginine-methyl ester which can partially be decreased by Curcumin.
10	Role in respiratory activity	Curcumin increases the expression of cathepsins K and L in lung which effect lung fibroblast cell behaviour. Its oral administration inhibits bleomycin-induced pulmonary fibrosis in rats. Its anti-inflammatory agent prevents release of TNF-α and protects against pulmonary and cardiovascular effects.
11	Anti-malarial activity	Turmeric shows cytotoxic effect in Giardia lamblia which inhibit the parasitic growth, induce morphological alterations and provoked apoptosis. Its oral administration showed reduced blood parasitemia by 80-90%.
12	Reduction in sperm mortality	Curcumin resulted in dose and time dependent loss of sperm motility by incubating normal human sperm.
13	Immunomodulatory activity	Curcumin imparted immunosuppression by mainly down regulating the expression CD28 and CD80 and up regulating CTLA-4. Also activate T cells, B cells, macrophages, dendritic cells, cell cycle protein, cell mediated and humoral mediated immunity.
14	Nephrotoxicity effect	Curcumin protects against diabetic nephropathy and oxidative stress against streptozotocin induced and showed protective effects against nephrotoxicity.
15	Neuro preventive	In vitro study confirmed that Curcumin improves the survival of cortical neurons induced OGD induced cell injury. In vitro levels of active oxygen decreased in chronic ischemic PC12 cells when treated with Curcumin.
16	Hepato protective activity	Carbon tetrachloride induced liver toxicity which proved that pre-treatment with picroliv, Curcumin, and ellagic acid which decrease the level of malondialdehyde to improve the antioxidant status and normalize the hepatic histo architecture.
17	Scavenger of reactive oxygen activity	Curcumin findings based on in vitro condition which is effective for scavengers of ROS and act as reactive nitrogen species.
18	Prevention of gastric lesions activity	Curcumin prevents from gastric lesions and helps in development in the gastric wall during the acute phase of gastric ulcer diseases.
	Radio sensitizer effect	PC3 is based on prostate cancer cell line and is confirmed as a major chemical





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19		component of turmeric which has radio sensitizing effects on cancer.
20	Anti-tumor activity	Curcumin seeds, leaves, flowers and stem plays major role in tumor prevention. Its chief constituent inhibit the activity of drug metabolizing enzymes i.e., cytochrome p450 and p450 reductive. It helps in the induction of apoptosis ,inhibit the proliferation of melanoma cells and is associated with down regulation of Notch-1 and NF-Kb.

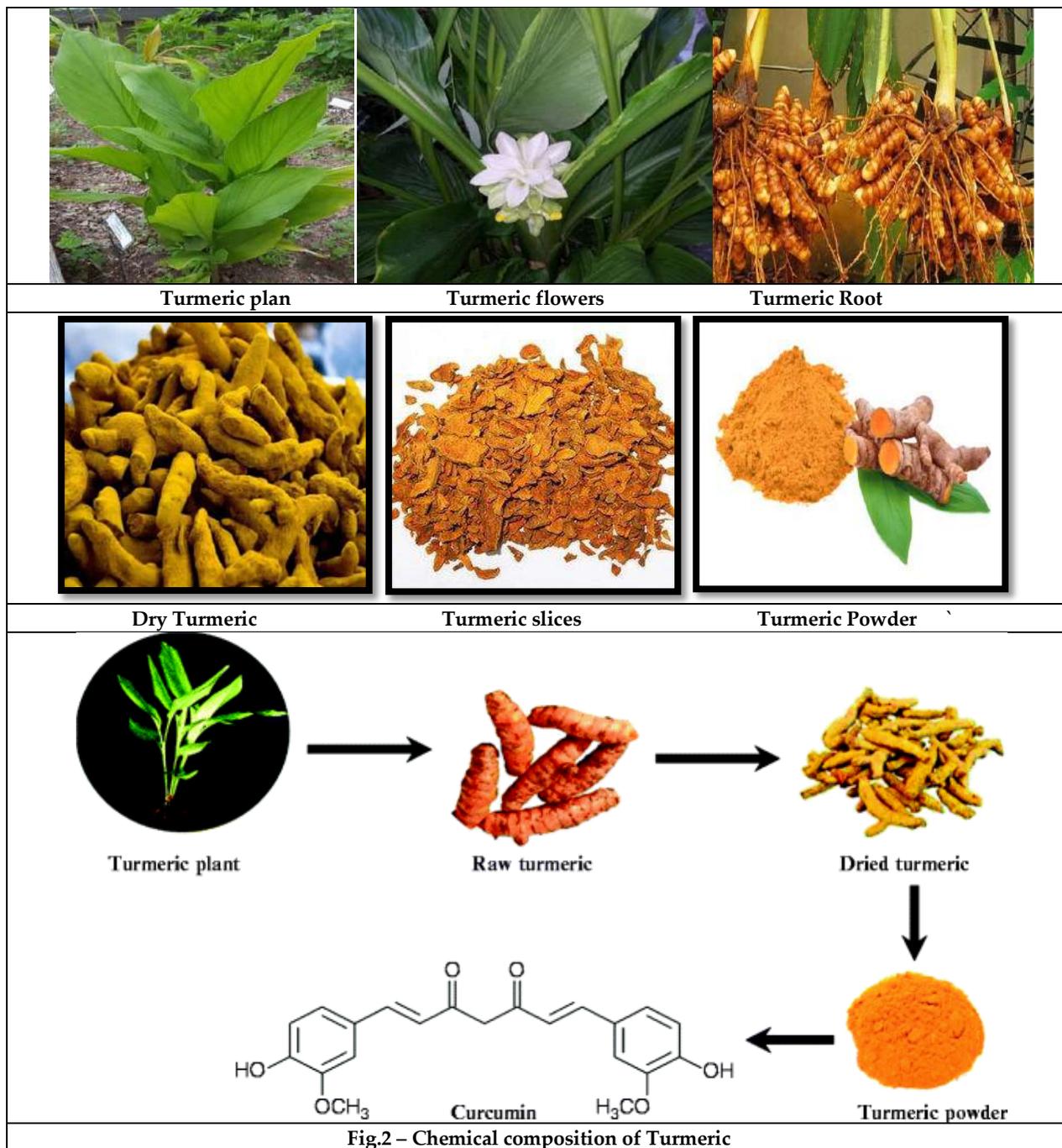
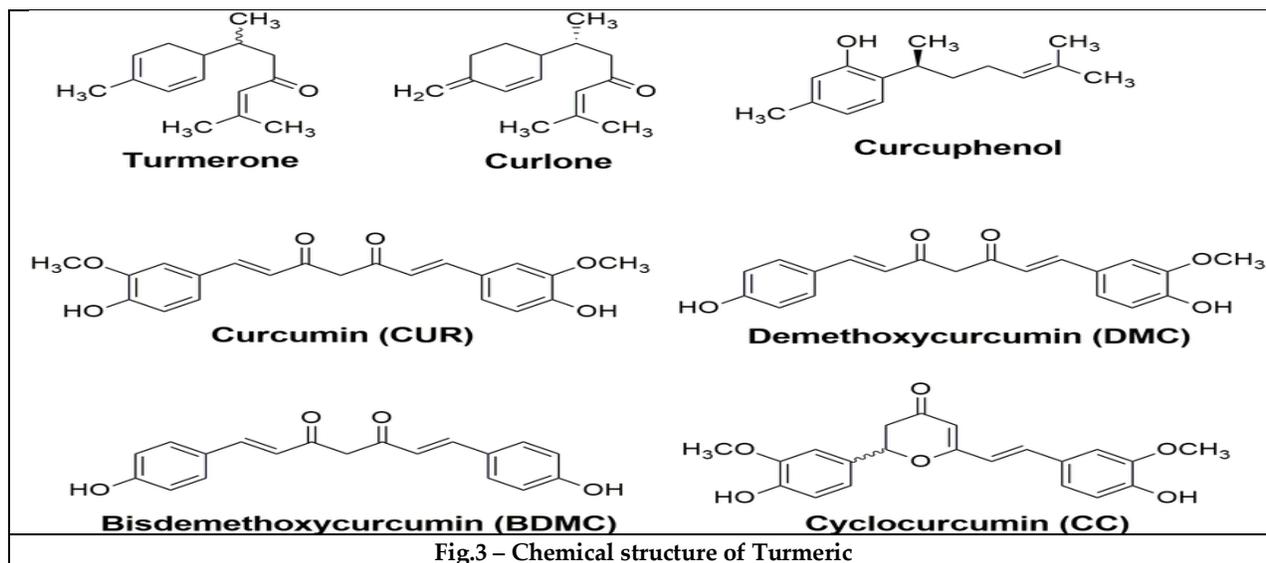


Fig.2 – Chemical composition of Turmeric





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Yersiniosis- A Review

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ABSTRACT

Yersinia ruckeri is the causative agent of yersiniosis or enteric redmouth disease leading to significant economic losses in salmonid aquaculture worldwide. Infection may result in a septicaemic condition with haemorrhages on the body surface and in the internal organs. Despite the significance of the disease, very little information is available on the pathogenesis, hampering the development of preventive measures to efficiently combat this bacterial agent. This review discusses the agent and the disease it causes. The possibility of the presence of similar virulence markers and/or pathogenic mechanisms between the *Yersinia* species which elicit disease in humans and *Y. ruckeri* is also examined.

Keywords: *Yersinia* , yersiniosis, haemorrhages , virulence , *Y. ruckeri*

INTRODUCTION

Enteric redmouth disease (ERM) is a serious septicemic bacterial disease of salmonid fish species. It is caused by *Yersinia ruckeri*, a Gram-negative rod-shaped enterobacterium. It has a wide host range, broad geographical distribution, and causes significant economic losses in the fish aquaculture industry. The disease gets its name from the subcutaneous hemorrhages, it can cause at the corners of the mouth and in gums and tongue. Other clinical signs include exophthalmia, darkening of the skin, splenomegaly and inflammation of the lower intestine with accumulation of thick yellow fluid. The bacterium enters the fish via the secondary gill lamellae and from there it spreads to the blood and internal organs. *Y. ruckeri* can be detected by conventional biochemical, serological and *Yersinia ruckeri* is the causative agent of enteric redmouth disease (ERM), which mainly affects salmonid fish. Isolates of *Y. ruckeri* from diseased salmonid fish were obtained over a 6-year period from eight fish farms in the State of Baden-Württemberg, Southwest Germany. The strains were characterized by biochemical methods and



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Fourier transform infrared spectroscopy (FT-IR) combined with artificial neural network analysis. These methods were complemented by 16S rDNA sequencing for several isolates. The set of strains from these fish farms included sorbitol-positive, gelatinase-positive and non-motile *Y. ruckeri*. These variants were differentiated with an advanced FT-IR module, which is part of a higher-ranking method including more than 200 well-defined *Yersinia* strains against a background of more than 1000 other Gram-negative isolates. Validation of the newly constructed method yielded 97.4% of *Y. ruckeri* identified correctly on the species level. Thus, the FT-IR analysis enables distinction of all *Y. ruckeri* from other *Yersinia* species (e.g. fish-borne *Y. enterocolitica*) and other Enterobacteriaceae typically misidentified because of similar biochemical reaction profiles, especially *Hafnia alvei*. The differentiation of sorbitol-positive variants of *Y. ruckeri* using FT-IR was demonstrated.

Host And Species Affected

The initial isolation and description of ERM was from farmed rainbow trout, *Oncorhynchus mykiss* (Ross *et al.*, 1966; Rucker, 1966), but has since been isolated from many species of fish, marine and freshwater, and from other animals. Environmental samples from river water, from sewage and from dairy situations have also been found to be positive (Pritchard *et al.*, 1995). The majority of clinical disease conditions occur in intensively cultured salmonids and it is considered primarily a disease of salmonids (Busch, 1982), but a number of other families of fish have also been found to be infected (Berc *et al.*, 1999; Carlson *et al.*, 2002; Danley *et al.*, 1999; Popovic *et al.*, 2001; Valtonen *et al.*, 1992; Xu *et al.*, 1991). In those species where isolations have been made without the presence of clinical signs, it is probable that such species would succumb to infection if sufficient husbandry stress were applied. Farmed species and young age were shown to be primary risk factors in ERM outbreaks in Canada (Good *et al.*, 2001). Brook trout (*Salvelinus fontinalis*) proved to be more susceptible than other species, and detection of the pathogen in the genus *Salvelinus* was generally more likely than in other genera included in the study (Good *et al.*, 2001). There was a significant increase in likelihood of detection in the 1-5 month age group compared with 6-19 month or in broodstock (>20 month).

Pathology

Internally, there is congestion of the blood-vessels throughout the peritoneum, and petechial haemorrhages, affecting liver, pancreas, swim-bladder, lateral muscles and adipose tissues associated with the pyloric caecae (Wobeser, 1973). The kidney and spleen may be swollen and there may be fluid in both the stomach and the intestine, where it has a yellowish, opaque, mucoid appearance (Busch, 1982). Light microscopy shows bacteria in virtually all tissues, particularly the kidney, heart, liver and gills. There may be severe necrosis of the haemopoietic tissues of the kidney. Wobeser (1973), Quentel and Aldrin (1986) and Lehman *et al.* (1987) observed acute anaemia, with an average haematocrit as low as 23% and total serum-protein values of 2.8 g 100 ml⁻¹. Miller (1983) attributed this to the effects of endotoxin on coagulation ultimately producing thrombosis in the capillaries and generalized haemorrhaging.

Transmission of the Disease

Early work and descriptions of the disease were made by those working in salmonid aquaculture, leading to the concept of yersiniosis as primarily a disease of salmonids. Confirmed clinical outbreaks were seen in both trout (rainbow, steelhead, cutthroat, brown and brook) and in salmon (coho, sockeye and Atlantic). Early epizootics in these species have been described in details. It has been recognized that the host range of *Y. ruckeri* is more diverse. Whether it is capable of a saprophytic existence as a part of the environmental flora outside a host is a matter of debate; Rucker (1966) suggested that this may be the case, but later authors, such as Klontz and Huddleston (1976), argued against this. Their findings reflected those seen for many other Gram-negative coliforms, including *E. coli* and *Aeromonas salmonicida*, where the survival in clean water in a laboratory experiment may be a matter of hours, but may be prolonged for 2-3 months in sediments or where there is organic matter present (Romalde *et al.*, 1994). This allows transmission from fish to fish under natural conditions without the need to propose that the organism has lived saprophytically outside an animal host.





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CONCLUSION

Yersinia ruckeri is not widely reported to cause significant wild fish kills. Treatment of diseased farm stock with antibiotics clearly has serious environmental implications. Use of vaccines and best practice can eliminate this impact. *Yersinia ruckeri* is not reported to be zoonotic and as any isolates are unable to initiate growth at 37°C (de Grandis *et al.*, 1988) this would appear to be an unlikely scenario. There have not been any reports of food safety issues related to *Y. ruckeri*. Despite the significance of the disease, very little information is available on the pathogenesis, hampering the development of preventive measures to efficiently combat this bacterial agent. This review discusses the agent and the disease it causes. The possibility of the presence of similar virulence markers and/or pathogenic mechanisms between the *Yersinia* species which elicit disease in humans and *Y. ruckeri* is also examined.

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Ginger in Prostate Cancer

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ABSTRACT

Prostate cancer is the second most commonly diagnosed cancer in men and the fifth highest cause of death globally. Early-stage prostate cancer is frequently asymptomatic and has an indolent course, requiring just active observation. According to GLOBOCAN 2018 estimates, 1,276,106 new cases of prostate cancer were reported worldwide in 2018, with developed nations having a greater prevalence. The usage of diagnostic testing varies around the world, resulting in variances in incidence rates. The incidence and mortality rates of prostate cancer are significantly linked to age, with the highest incidence reported in elderly men (> 65 years of age). Prostate cancer is a big health concern for women, and the global rate of the disease is increasing every day. Breast cancer is found in the cells of women's breasts. Prostate cancer is the most prevalent disease diagnosed in women in the United States, but it has already spread to every country and region. A lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, a newly inverted nipple, or a red or scaly patch of skin are all signs of prostate cancer. In the year 2018, 2.1 million people were impacted by prostate cancer. Being female, lack of physical activity, alcoholism, endocrine replacement medical care throughout menopause, ionised radiation associated early age at first menstruation, having children late in life or not at all, older age, having a prior history of Prostate cancer, and having a family history of Prostate cancer are all risk factors for developing carcinoma. For the treatment of prostate cancer, we must utilise ginger. Ginger is a common flavouring for a variety of meals and beverages. It comes from the rootstalk of *Zingiber officinalis*, one of the most widely used species of the ginger family. Ginger has a lengthy history of medicinal use that dates back over 2500 years. Ginger has been used for centuries in various parts of the world to aid digestion and alleviate stomach distress, diarrhoea, and nausea. Some of the pungent



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components found in ginger and other zingiberaceous plants have potent inhibitory and anti-inflammatory properties, and some of them have been shown to be cancer-preventive in animal studies.

Keywords: Prostate cancer, ginger, Cytotoxicity, Anti-Oxidant

INTRODUCTION

Prostate cancer can be asymptomatic in the early stages and has a slow progression, requiring little or no treatment. The most common complaint, however, is difficulty urinating, increased frequency, and nocturia, all of which are signs of prostatic hypertrophy. Because the axis skeleton is the most prevalent site of bone metastatic illness, more advanced stages of the disease may present with urine incontinence and back pain. Spices have become an astonishing detail in practically every life-style on the planet, including Asia, Africa, Europe, and the Americas. According to a new study, the use of non-obligatory therapy for chronic or severe illnesses, as well as minor diseases, has increased dramatically (Bernstein and Grasso, 2001), According to a study, 83 percent of 453 cancer patients had used at least one non-obligatory treatment, with many of the improvements being natural in nature (Elvin-Lewis, 2001). Zingiberene species are human people descended from a group of plants that have been used in cooking, medicine, and treatment for a long time, particularly in Asian regions. In animal model studies, many spices and flavours derived from humans from the Zingiberene family appeared to provide valuable protection against degenerative diseases such as tumours (Milner *et al.*, 2001; Surh, 2002). A few members of my family appeared to have the most effective cancer prevention, mitigation, and anticancer exercises (Rao *et al.*, 1995; Lee *et al.*, 1998, Surh *et al.*, 1999). Foods cultivated from the ground contain phytochemicals (carotenoids, polyphenolics, anthocyanins, alkaloids, N and S compounds) that have been shown to reduce the risk of disease in humans on a large scale. Garlic, ginger, turmeric, cruciferous vegetables (broccoli, Brussels sprouts, cabbage), and grape seed extracts are among the about 35 plant-based entirely fully dietary reassessments recognised by the NCI to be effective in cancer prevention. Ginger (*Zingiber officinale* Roscoe) is a widely used and preferred zest. It contains phenolic chemicals, terpenes, polysaccharides, lipids, natural acids, and crude filaments, among other synthetic ingredients. The medicinal properties of ginger are mostly attributed to its phenolic components, such as gingerols and shogaols. According to studies, ginger has a wide range of medicinal properties, including maximum cancer prevention, relaxing, antibacterial, anticancer, neuroprotective, cardiovascular, respiratory, anti-obesity, antidiabetic, antinausea, and antiemetic properties. In Iranian women, prostate cancer (PC) is a very common medical problem (Amirifard *et al.*, 2016). Malignancy treatment nowadays includes medical procedures, radiotherapy, chemotherapy, organic treatment, and many tactics, while chemotherapy, such as Trastuzumab treatment, is sometimes the only successful technique of malignancy treatment in the highly advanced stage (Payandeh *et al.*, 2015). Chemotherapy-induced nausea and vomiting (CINV) is a serious problem for patients. Prostate cancer is the most commonly occurring cancer in women, accounting for about one-third of all cancers in women. It is only second to cellular breakdown in the lungs as a cause of disease mortality, and it is the leading cause of death for American women under the age of 40, accounting for 55.1 percent of all deaths. A lady's lifetime risk of developing intrusive bosom disease is 12.6 percent. In the United States, one out of every eight females will get bosom malignant development at some point in her life. Carcinoma of the male breast is a relatively rare illness, accounting for less than 1% of all cases of malignant development in men. Because of the infection's rarity, the majority of evidence has come from small, single-institutional reviews or extrapolation from Prostate cancer studies in women. Despite scientific evidence indicating breast epithelial cell division rates are high during the luteal period of the monthly cycle when estradiol and progesterone levels are high, there is no evidence that high oestrogen levels in premenopausal women are linked to an increase in prostate cancer risk. Given the uncommonly perplexing methodological issues that must be addressed in these studies, the lack of absolute consistency among studies that have evaluated oestrogen contrasts, whether in breast disease patients versus controls or in subgroups of the population portrayed by various risk profiles for Prostate cancer, isn't surprising.



**Shubhashree samal and Preetha Bhadra****History of Ginger**

Ginger originated in Maritime Southeast Asia and was likely domesticated first by the Austronesian peoples. It was transported with them throughout the Indo-Pacific during the Austronesian expansion, reaching as far as Hawaii. Ginger is one of the first spices to have been exported from Asia, arriving Europe with the spice trade, and was used by ancient Greeks and Romans. Ginger is a notable herbaceous plant, has been generally utilized as an enhancing specialist and natural medication for quite a long time. Besides, the utilization of the ginger rhizome is an ordinary conventional solution for soothe regular medical issues, including torment, sickness, and heaving. Quiet, an unmistakable number of randomized clinical preliminaries (RCTs) have been directed to analyse ginger's antiemetic impact in different conditions, for example, movement disorder, pregnancy, and post-sedation. More than around 100 mixtures have supposedly been confined from ginger. In particular, the significant classes of ginger mixtures are gingerol, shogaols, zingiberene, and zingerone, just as other more uncommon mixtures, as well as terpenes, nutrients, and minerals. Among them, gingerols are thought-about because the essential parts, answered to possess many bioactivities. Accordingly, several connected organic exercises are investigated like those of cancer bar agent, antimicrobial, and against neuroinflammation, simply to provide some examples. In addition, as of late, the work of ginger has been stretched to anticancer, chemotherapy-prompted sickness and retching (CINV), and exhaustion, even as enhancements within the personal satisfaction in day-by-day human work. Ginger antecedently showed up in the southern items of the antediluvian China. From that point, it unfold to India, Maluku Islands (purported Flavour Islands), remainder of the Asia and West Africa. Europe saw ginger while not precedent for the primary century once the previous Romans changed with the Asian nation. At the purpose when the Rome fell, Europe forgotten ginger till Marco Polo brought it once more from his movement toward the East. within the Medieval times, a value of an outsized portion of a weight unit of ginger was appreciate of 1 sheep. within the fifteenth century, with the discovery of the New World, Ginger was dropped at the Caribbean wherever it began to develop effortlessly. Today, India is that the best maker of ginger in the world. Name "ginger" made considerable progress; however, its root is in Sanskrit word "srngaveram" which signifies "horn body" and depicts its root. While it develops, it has white and pink buds which sprout into yellow blossoms. At the point when the tail wilts, the rhizome is collected and quickly singed (which executes it) to forestall growing. Ginger is utilized from multiple points of view and for different reasons. Its essential use resembles a kitchen flavour. At the point when it is youthful, it is delicious and meaty and frequently cured in vinegar or sherry and eaten as a bite. Tea will be made victimisation the bits of the basis that are saturated with the effervescent water and mingling in with nectar. It tends to be even made into wine within the event that it's matured with raisins and blended (sustained) with cognac. At the purpose once left to develop, ginger is dry and might be made into a powder which is then utilised as a flavour or as a fixing in gingerbread, treats, saltines and cakes, soda, and ginger brew. Ginger has been targeted as an antiaging specialist that secures against aerobic pressure and aggravation within the pathologic process of chronic illness and maturing. Ginger (*Z. officinale* Roscoe) is viewed as a customary spice; it's utilised not even as a zest or flavour in preparation nonetheless in addition as a traditional medication to treat differing types of medical issues, for example, diabetes, queasiness, headache, and others. Normally, ginger will be found in subtropic and tropical Asia, Africa, region Asia, China, and India. Ginger is created out of many bioactive mixtures, as well as 6-gingerol, 6-shogaol, 10-gingerol, gingerdiones, ginger diols, paradols, 6-dehydrogingerols, 5-acetoxy-6-gingerol, 3,5-diacetoxy-6-gingerdioal, and 12-gingerol, that increase various organic exercises of ginger. Be that as it may, the essential dynamic mixtures in ginger are chemical irritant and shogaol. Figure a pair of shows the substance constructions of many dynamic mixtures in ginger.

Ginger

Ginger is burned-thru worldwide as zest, seasoning specialist, embellish, medication, and food additive and is carried out each new, in a brand-new glue, or dry, in a dry powder. New ginger can be fill in for dried ground ginger, albeit the forms of new and dried ginger are quite unique. The heady perfume of ginger is entering into and sweet-smelling. Ginger is called as "Adrak" (neighborhoods name) with inside the subcontinent like India and Pakistan and is a crucial element of numerous dishes.



**Shubhashree samal and Preetha Bhadra****Nutritional Composition of Ginger****Chemical Composition**

Ginger includes greater or much less half of of carbs, 9% protein and loose amino acids, 6-8 % unsaturated fats and fatty oils, 3-6 bris, and 3-6% tough fibre (on dry remember quantity premise) contingent upon assortment, topography, and climatic conditions (Leung, 1984, Tang, 1992). Some African ginger assortments encompass 5. ninety-8 and 3.72g/100 proteins and fats (Shrin Adel, 2010). Dissolvable and insoluble strands are likewise decided in ginger. Ginger is a first rate wellspring of crucial micronutrients like potassium, magnesium, copper, manganese and silicon. Potassium and manganese help to bring together protection from infection and ensure the coating of heart, veins and urinary entries. Silicon advances solid skin, hair, teeth, and nails and assists with absorbing calcium. Limited quantity of nutrients A, E and a few measures of B-nutrients and Nutrient C are further more decided in ginger rhizome (Adel and Prakash, 2010).

Phytochemical Composition

Ginger is an intricate substance comprising of in greater of 60 combinations (Srivastava *et al*, 2000). The ginger rhizome includes a crucial oil and gum said all subjects considered as oleoresin. The form of the crucial oil differs as indicated thru manner of way of the topographical beginning, however the essential components are sesquiterpene hydrocarbons, which may be chargeable for the trademark smell. Gingerol is the crucial phenolic compound and as quickly as corrupted gives shogaols, zingerone, and paradol. Zingerone and shogaols are decided in little sums in new ginger and in large sums in dried or extricated items. Ginger includes an brilliant accumulating of combinations called diasyleheptanoids, which includes gingerenone. A restricted quantity of curcumin is further more decided in ginger. Notwithstanding that it likewise includes little measures of alkaloids, saponins, carotenoids, flavonoids, steroids, and cardenolides (Shrin Adel, 2010).

Bioactive Compound of Ginger

Ginger is abundant in dynamic parts, for example, phenolic and terpene compounds [13]. The phenolic compounds in ginger are predominantly gingerols, shogaols, and paradols. In new ginger, gingerols are the primary polyphenols, like 6-gingerol, 8-gingerol, and 10-gingerol. With warmth remedy or long-time period stockpiling, gingerols may be modified into concerning shogaols. After hydrogenation, shogaols may be modified into paradols [2]. There are likewise numerous opportunity artificial resin compounds in ginger, for example, quercetin, zingerone, gingerenone-A, and 6-dehydrogingerdione [14,15]. In addition, there are some terpene segments in ginger, for example, β -bisabolene, α -curcumene, zingiberene, α -farnesene, and β -sesquiphellandrene, which can be regarded because the essential parts of ginger essential oils [16]. Other than these, polysaccharides, lipids, herbal acids, and crude strands are likewise found in ginger.

Ginger as an Anti-Oxidant

It has been located out that overproduction of loose extremists, like receptive oxygen species (ROS), has a sizeable impact with inside the improvement of numerous chronic illnesses. It has been accounted for that a set of characteristic devices have molecular reinforcement potential, like vegetables, herbal products, palatable blossoms, cereal grains, recovery plants, and natural mixtures. A few investigations have decided that ginger likewise has immoderate maximum cancers prevention agent movement. The molecular reinforcement movement of ginger has been assessed in vitro with the resource of ferric-decreasing maximum cancers prevention agent power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic corrosive) (ABTS) strategies. The consequences uncovered that dried ginger showed the most grounded maximum cancers prevention agent movement, in slight of the reality that the variety of phenolic compounds have become 5.2-, 1.1-, and 2.4-overlay higher than that of new, sautéed, and carbonized ginger, in my view. The maximum cancers prevention agent movement of severa gingers tended to be the accompanying: dried ginger > pan-seared ginger > carbonized ginger > new ginger. This have become basically related with their polyphenolic substance. At the element even as new ginger have become warmed, dried ginger with higher maximum cancers prevention agent movement have become acquired, due to the fact new ginger contains a higher dampness content. Nonetheless, even as dried ginger have



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become furthermore warmed to accumulate sautéed ginger and carbonized ginger, the molecular reinforcement movement dwindled, due to the fact the getting geared up need to extrade gingerols into shogaols. Also, a small a part of the dried ginger powder bountiful in polyphenols showed immoderate maximum cancers prevention agent movement relying on information from FRAP, oxygen extremist absorbance restrict, and molecular reinforcement movement measures. Also, the form of extraction dissolvable need to have an impact at the molecular reinforcement movement of ginger. An ethanolic concentrate of ginger showed immoderate Trolox-identical maximum cancers prevention agent restricts and ferric-diminishing capacity, and a watery concentrate of ginger displayed robust loose revolutionary searching movement and chelating capacity. Also, ethanolic, methanolic, ethyl acetic acid derivation, hexane, and water concentrates of ginger in my view restrained 71%, 76%, 67%, 67%, and 43% of human low-thickness lipoprotein (LDL) oxidation actuated thru manner of Cu^{2+} . Results from a xanthine/xanthine oxidase framework showed that an ethyl acetic acid derivation extricate and a fluid concentrate had higher maximum cancers prevention agent homes than ethanol, diethyl ether, and n-butanol separates did. Ginger concentrate need to decrease the arrival of ROS in human fibrosarcoma cells with H_2O_2 -instigated oxidative pressure. In cantered on rodent coronary coronary heart homogenates, ginger concentrate dwindled the substance of malondialdehyde (MDA), which have become identified with lipid peroxidation. Ginger and its bioactive mixtures, (for example, 6-shogaol) showed molecular reinforcement movement with the resource of the atomic element erythroid 2-related element 2 (Nrf2) flagging pathway. By and large, in vitro and in vivo examines have exhibited that ginger and its bioactive mixtures, for example, 6-shogaol, 6-gingerol, and oleoresin, have robust molecular reinforcement movement.

Cytotoxicity

Malignant growth is said to be a critical cause for demise, and there were sort of 9.6 million instances of demise in 2018. A few examination works have exhibited that ordinary items, for example, products of the soil flora have anticancer action. As of late, ginger has been normally explored for its anticancer homes in competition to severa malignant growth types, like bosom, cervical, colorectal, and prostate disorder. The possible systems of hobby include the restraint of multiplication and the recognition of apoptosis in malignant growth. The cytotoxic impacts and hidden systems of ginger in prostate malignancy were assessed every in vivo and in vitro. The anticancer components at the complete include the enlistment of apoptosis and the restraint of the expansion of malignancy cells.

Prostate Cancer

Like other solid tumours, prostate cancer has cell populations known as carcinoma stem cells (BCSCs) that are tumorigenic, pluripotent, and self-renewing. The presence of BCSCs may also be to blame for the high rate of metastasis, medical treatment resistance, and, as a result, the recurrence of prostate cancer. Prostate cancer growth and progression are influenced by diet and physical exercise. Dietary variables are primarily responsible for the reported global and ethnic disparities in prostate cancer incidence rates.

Isolation Of Components From Ginger**Gingerol**

Gingerol, accurately as [6]-gingerol, may be a phenol phytochemical compound located in new ginger that enacts zest receptors at the tongue. Atomically, chemical irritant is a relative of chemical irritant associated piperine, the combinations that are alkaloids, but the bioactive pathways are detached. it's normally located as an impactful yellow oil with inside the ginger rhizome, but will likewise body a low-liquefying semitransparent strong. This substance compound is located taking all matters collectively people from the Zingiberene family.

Biological Activity of Gingerol

In a pre-medical meta-analysis, gingerol's anticancer, calming, anti-contagious, most cancers preventive agent, neuroprotective, and gastroprotective properties were accounted for, as well as reads in vitro and in vivo. Gingerols





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have been shown in a couple of in-vivo studies to be a good glucose guiding principle for diabetics. Numerous studies have been conducted on the effects of gingerols on a wide range of malignant tumours, including leukaemia, prostate cancer, breast cancer, skin cancer, ovarian cancer, lung cancer, pancreatic cancer, and others. There haven't been any medical studies focused on gingerols physiological effects on humans in a long time. While a wide range of material additives relevant to the effects of gingerols on cells were thoroughly considered, only a few were in a medical environment. This could be due to the high quality of typical phytochemicals as well as the lack of viability in analysis. Most flavorer beneficial drugs, such as gingerols, are regulated by the Food and Drug Administration in the United States, and testing methodologies do not control the maximum quantity as research, which has lowered interest in phytochemical research. Because of a lack of funding in Japanese medical research, herbal medicine has yet to be examined in medical settings for fine affirmation, energy, and sufficiency. The majority of [6]-Gingerol research has been done on mice (in vivo) or sensitive human tissue (in vitro), and it may be used in the future to talk about various capabilities programmes for multi-goal infections.

CONCLUSION

The health-promoting perspectives of ginger are well known. It can treat a wide range of diseases via immunonutrition and anti-inflammatory responses. As a result of anti-inflammatory effect of ginger, it can reduce muscle pain after intense physical activity. Likewise, the anticancer potential of ginger is well documented and its functional ingredients like gingerols, shogaol, and paradols are the valuable ingredients which can prevent various cancers, angiogenesis and metastasis, induction of apoptosis, and inhibition of cell-cycle progression. Besides these, it improves cardiovascular disorders, diabetes mellitus, and gastrointestinal health. This review concludes to flavour ginger but some ambiguities necessitate further research before claiming its efficacy.

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Ginger	Structure of Gingerol





Amla as A Functional Food

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ABSTRACT

Phyllanthus emblica Linn. Or *Emblica officinalis* Gaertn. commonly known as Indian gooseberry or Amla. *Emblica officinalis* (Amla) are widely used in the Indian system of medicine (Ayurveda, Unani and Siddha). According to believe in ancient Indian mythology, it is the first tree to be created in the universe. It belongs to the family of Euphorbiaceae. It is the richest natural source of Vitamin C. *Emblica officinalis* (EO) primarily contains tannins, alkaloids, phenolic compounds, amino acids and carbohydrates. Its fruit juice contains the highest amount of vitamin C, Ellagic acid, Chebulinic acid, Quercetin, Chebulagic acid, Emblicanin-A, Emblicanin-B, Gallic acid and ascorbic acid etc. all parts of amla are useful in the treatment of various diseases but the most important part is fruit. Amla fruit is widely used in the Indian system of medicine as diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, anti-pyretic, hair tonic, ulcer preventive and also useful for common cold, fever; as alone or in combination with other plants. The fruit is used as a major constituent in several Ayurvedic preparations such as Chyavanprash and Rasayana which promotes health and longevity. Regular use of *Emblica officinalis* improves immunity, fight against cancers, chronic diseases like hypertension, high Cholesterol, Diabetes, influenza, Chronic cough and cold, Chronic infections, Chronic fatigue and Chronic inflammatory conditions. According to Ayurveda amla is one of the best herbs for Diabetes, bleeding disorders, strength and stamina promoter. *E. officinalis* is also suitable for used as a anti-aging, sunscreen and general purpose for skin care products. *Emblica officinalis* possesses antioxidant, immunomodulatory, adaptogenic, cardioprotective, nephroprotective, hepatoprotective, antipyretic, analgesic, cytoprotective, antitussive, gastroprotective, wound healing and antidiarrheal properties. It is also used as a hair tonic. Its





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applications for memory enhancing, ophthalmic disorders, reducing cholesterol level, prevent peptic ulcer, anaemia etc. The effects of EO in neutralizing snake venom and as an antimicrobial are also included.

Keywords: Medicinal Aromatic plant, *Phyllanthus emblica*, Chemical Components, Nutritious Assessment, Therapeutic Usages.

INTRODUCTION

Mother Nature has gifted mankind with tremendous medicinal plants to create a disease free and healthy life. Abundant medicinal plants are presented in the Indian traditional systems of medicine (like Ayurveda, Unani, siddha), mostly used one amongst them is Indian gooseberry or Amla, also known as *Phyllanthus emblica* Linn. (Syn. *Emblica officinalis* Gaertn.) belongs to the family Euphorbiaceae,¹ which is an important medicinal herb in Ayurveda and Unani systems of medicine. It is enormously used as a tonic to restore the lost body's energy and vigor. Amla is a small to medium sized deciduous tree, found in throughout India, Pakistan, Uzbekistan, Sri Lanka, South East Asia, China and Malaysia. It grows about 8-18m height with thin light grey bark, leaves are simple, light green, subsessile, closely set along the branchlets looks like pinnate leaves; flowers are greenish yellow; fruits are globose, fleshy, pale yellow with six obscure vertical furrows enclosing six trigonous seeds in two seeded three crustaceous cocci. Amla is highly nutritious and is one of the richest sources of vitamin-C, amino acids and minerals.² It contains several chemical constituents like tannins, alkaloids and phenols.³ Among all hydrolysable tannins, Emblicanin A and B; gallic acid, ellagic acid are reported to possess biological activity. Almost all parts possess medicinal properties, particularly fruit, which has been used in Ayurveda as a powerful rasayana and in customary medicine in the treatment of diarrhoea, jaundice, inflammation and several other ailments.⁴ Amla fruit is widely used in the Indian system of medicine as alone or in combination with other plants and is used to treat common cold and fever, as diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, anti-pyretic, hair tonic; to prevent ulcer and dyspepsia. Pharmacological research reports on amla reveals its analgesic,⁵ anti-tussive,⁶ anti-atherogenic,⁷ adaptogenic;⁸ cardio,⁹ gastro,¹⁰ nephro,¹¹ neuro¹² protective and anti cancer¹³ properties. Amla is also reported to possess chemopreventive,¹⁴ radio,¹⁵ chemo¹⁶ and immunomodulatory,¹⁷ free radical scavenging,¹⁸ antioxidant,¹⁹ anti-inflammatory,²⁰ anti-mutagenic activities. These properties are efficacious in the prevention and treatment of various diseases like cancer, atherosclerosis, diabetes, peptic ulcer, anemia, liver, heart diseases and various other disorders. The present work is a trial to understand the nutritional value, traditional uses, biochemical constituents and important medicinal values of Amla by emphasizing the mechanisms behind the activities and enlightens the therapeutic applications and clinical trials. It also summarizes the research done on amla from the past 5 years and also specifies the aspects that warrant future research establishing its activity and use in several diseases.

CHEMICAL CONSTITUENTS

Amla is one of the most extensively studied plants. Reports suggest that it contains tannins, alkaloids and phenols.³ Fruits have 28% of the total tannins distributed in the whole plant. The fruit contains two hydrolysable tannins Emblicanin A and B,²¹ which have antioxidant properties; one on hydrolysis gives gallic acid, ellagic acid and glucose wherein the other gives ellagic acid and glucose respectively. The fruit also contains Phyllembin.²² Activity directed fractionation revealed the presence of several phytochemicals like gallic acid, corilagin, furosin and geraniin.²³ Flavonoids like quercetin, alkaloids like phyllantine and phyllantidine are found. Along with these, it primarily contains amino acids, carbohydrates and other compounds given in Table 1. Its fruit juice contains the highest concentration of vitamin-C (478.56mg/100mL). Vitamin C levels are more than those in oranges, tangerines and lemons.^{24,25} The composition of fruit pulp of *Emblica officinalis* are given in Figure 1





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In comparison with apple, the edible fruit tissue is rich with proteins 3-fold and ascorbic acid 160-fold and contains considerably higher concentration of most minerals and amino acids. Glutamic acid, proline, aspartic acid, alanine, and lysine are 29.6%, 14.6%, 8.1%, 5.4% and 5.3% respectively of the total amino acids. Some of the phytochemicals are shown in Figure 2.28 Pulp portion of fruit, after drying found to contain: gallic acid 1.32%, tannin, gum 13.75%; albumin 13.08%; crude cellulose 17.08%; mineral matter 4.12% and moisture 3.83%. Amla fruit ash contains chromium 2.5 ppm, zinc 4 ppm and copper 3 ppm. Compounds isolated from amla fruit are gallic acid, ellagic acid, 1-O-galloyl-beta-D-glucose, 3,6-di-O-galloyl-D-glucose, chebulinic acid, quercetin, chebulagic acid, corilagin, 1,6-di-O-galloyl beta-D-glucose, 3-Ethylgallic acid (3-ethoxy 4,5-dihydroxybenzoic acid) and isostrictinin.²⁶ Amla fruit also contains flavonoids, kaempferol-3-O-alpha L-(6''-methyl) rhamnopyranoside and kaempferol-3-O-alpha L-(6''-ethyl) rhamnopyranoside.

TRADITIONAL USES

Ayurveda, Siddha, Unani systems of India, Tibetan, Sri Lankan and Chinese systems of medicine utilize Amla for a variety of ailments. It is considered as rasayana (rejuvenator)⁴ and used in delaying the degenerative and senescence related processes. In folk medicine, the fruits, which are sour, astringent, bitter, acrid, sweet and anodyne. Exert several beneficial effects include cooling, ophthalmic, carminative, digestive, stomachic, laxative, dyspepsia, aphrodisiac, rejuvenative, diuretic, antipyretic and tonic. They are useful in vitiated conditions of tridosha, diabetes, cough, asthma, bronchitis, cephalgia, ophthalmopathy, dyspepsia, colic, flatulence, hyperacidity, peptic ulcer, erysipelas, skin diseases, leprosy, haematogenesis, inflammations, anaemia, emaciation, hepatopathy, jaundice, diarrhoea, dysentery, haemorrhages, leucorrhoea, menorrhagia, cardiac disorders, intermittent fevers and premature greying of hair (Hair tonic).²⁸ Amla is also stated to have hepato, cardio, nephro and neuroprotective effects; antioxidant, anti-inflammatory, analgesic, antipyretic and restorative properties. List of amla traditional applications are given below.

As a Vermifuge: Juice of the fruit with honey is used. The recommended dose is from 1 to 3 drachms.

Appetizer: Use of pickles and preserves made from the green fruits.

Irritability of the bladder, In retention of urine, To the forehead in cephalgia: Use a paste of the fruit alone or with *Nelumbium speciosum*, Saffron and rose water. Applying it over the affected region.

As a febrifuge and in diabetes: Using an infusion of the seeds.

For hiccup and for painful respiration: Use of juice or extract of the fruit combined with honey and pipili.

For hemorrhage, diarrhea and dysentery: Using dried fruit. A decoction prepared from the fruit combined with *T. chebula* and *T. bellerica* is useful in chronic dysentery and biliousness, in doses of 1 oz. once or twice daily.

For diarrhea of children

A compound powder of the amla seed, Chitrak root, chebulic myrobalan, pipili and paleone is given in suitable doses, according to age, in warm water twice daily, morning and at bed time. Tender shoots given in butter-milk cure indigestion and diarrhea; green fresh leaves combined with curds have a similar effect. Leaves are used as infusion with fenugreek seeds in chronic dysentery and as a bitter tonic. Soak one tola of the seeds in a tinned vessel during the night. Grind it. Add cow's milk and use. This is a good remedy for biliousness.

For anemia, jaundice and dyspepsia

Use dried fruit with iron. A fermented liquor prepared from the root is used in jaundice, dyspepsia, cough, etc. –

Take 20 to 40 grains of Dhatri Leha for anemia, jaundice and dyspepsia.

Dhatri Arista is used for jaundice, dyspepsia, indigestion, and cough.





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For burning in the vagina: A mixture of the fruit juice and sugar is prescribed as a remedy for burning in the vagina. Juice of the bark combined with honey and turmeric is a remedy for gonorrhoea.

To stop nausea and vomiting: A powder of the amla seed and red sandalwood is given with honey, to stop emesis.

For bleeding of the nose: Seed fried in ghee and ground in conjee is applied as Lep to the forehead to stop bleeding from the nose.

For scabies or itch: Apply the seed burnt, powdered and mixed in oil for scabies or itch.

As a restorative invigorator:

Make a powder from an equal quantity of amla seed and root of *Withania somnifera*. Add ghee and honey. Mix well. This is a restorative invigorator, especially in winter days. Combine half a drachm each of amla seed and gokhru. Grind and make them into a powder. Mix with 15 grains of essence of Gulancha. Give this in early morning with ghee and sugar. This is a nutrient tonic.

Other uses

It helps in regulating blood sugar.

It is very powerful anti-inflammatory herb, a wonderful antioxidant and a natural source of Vitamin C. Amla helps scavenge free radicals. Amla is powerful food for the brain and helps lower cholesterol. Amla also helps maintain the functioning of the liver, increases haemoglobin, red blood cell count. It is useful for Cough, Bronchitis, and Asthma. Amla cleanses the mouth, strengthens the teeth. Its decoction is used in hyperacidity and with honey as an anthelmintic. The presence of Amla results in an enhanced cell survival, decreased free radical production and higher antioxidant levels. There are various classic Ayurvedic preparations, such as Chyawanprash in which Amla is used as a chief ingredient. It helps improve intelligence and memory power. Triphala and Brahmarasayana are other classic medicines in which Amla is being used since time immemorial.

The Ayurvedic description of amla

According to the Ayurvedic classifications, amla fruit exerts the following properties: Rasa (taste): Sour and astringent are the most dominant, but the fruit has five tastes, including sweet, bitter, and pungent. Veerya (nature): Cooling, treatment of burning sensation in inflammation and fever which are considered to be manifestations of pitta (fire) agitation. Vipaka (taste developed through digestion): Sweet. Guna (qualities): Light, dry. Doshas (effect on humors): Quiets all three doshas: vata, kapha, pitta, and is especially effective for pitta. Based on this, Amla has been considered the best of the Ayurvedic rejuvenative herbs. Inimitably, amla exerts natural balance of tastes (sweet, sour, pungent, bitter and astringent), that stimulates brain to rebalance the three main components (water, fire and air in the body) of all physiological functions.

POTENTIAL THERAPEUTIC APPLICATIONS

Amla fruit has been said to be useful against many several diseases, including cancer, diabetes, hepatic disorders and heart diseases. A list of scientifically explored therapeutic applications and pharmacological activities are presented in figure 3. Research has been done with amla evaluating its role as an antioxidant, in ulcer prevention, for people with diabetes, for mental and memory effects, and its anti-inflammatory benefits. Amla extract supplements are not only retain the lost body energy and vigor, also be helpful in those undergoing radiation therapy i.e., protect the cancer cells that the radiation is trying to destroy. Amla Tonic has a hematinic and lipolytic function useful in Scurvy and Jaundice. It prevents indigestion and controls acidity as well as it is a natural source of anti-ageing. Clinical tests on patients suffering from pulmonary tuberculosis have shown that the high concentration of vitamin C in amla fruit is more quickly assimilated in the body than the synthetic vitamin. Even though Amla is a known herb in Ayurveda and several other indigenous systems of medicine, some of its medicinal properties are yet to be explored and evidenced scientifically for human use.





CONCLUSION

Now a days, research on Indian traditional medicinal plants has gained a new recommence. Although, the other systems of medicine are effective they come with a number of undesired effects that often lead to serious complications. Being natural, herbal medicine alleviates all these problems. *Embllica officinalis* (Amla) has an important position in Ayurveda- an Indian indigenous system of medicine. Amla due to its strong antioxidant and biological properties prevent innumerable health disorders as it contains essential nutrients and highest amount of vitamin C. It can be used as a possible food additive or in nutraceuticals and biopharmaceutical industries. Several researchers revealed that various extracts and herbal formulations of amla showed potential therapeutic benefits against various diseases and the results are similar to standard drugs. In this review, we tried to make a summary the traditional and scientifically proven uses of amla and tried to establish their basic mechanisms. Even though, amla has various medicinal properties since ages, there is a colossal necessity to scientifically explore and evident its medicinal values at molecular level with help of various latest biotechnological tools and techniques.

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Table 1: Amla fruit: Chemical constituents

Type	Chemical Constituents
Hydrolysable Tannins	Emblcanin A and B, Punigluconin, Pedunculagin, Chebulinic acid (Ellagitannin), Chebulagic acid (Benzopyran tannin), Corllagin (Ellagitannin), Geraniin (Dehydroellagitannin), Ellagotannin
Alkaloids	Phyllantine, Phyllembin, Phyllantidine
Phenolic compounds	Gallic acid, Methyl gallate, Ellagic acid, Trigallayl glucose
Amino acids	Glutamic acid, Proline, Aspartic acid, Alanine, Cystine, Lysine
Carbohydrates	Pectin
Vitamins	Ascorbic acid
Flavonoids	Quercetin, Kaempferol
Organic acids	Citric acid





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Medicinal importance	Description
Healing options	<ul style="list-style-type: none"> Protects cells against free radical damage and provides antioxidant protection. To treat skin disorders, respiratory infections, and premature aging. Useful in haemorrhage, diarrhoea, dysentery and diabetes. Prevents infection and healing of ulcers. Laxative to relieve constipation in piles.
Immunity booster	Increases white blood cell counts and other measures of strengthened immunity in rodents given with Amla tonic.
Promotes vigor	One tablespoon juice with honey daily morning for few days.
Respiratory disorders	Useful in tuberculosis of the lungs, asthma and bronchitis.
Diabetes	High vitamin C content, is effective in controlling diabetes. Amla juice with bitter gourd juice, used daily for 2 months stimulates the pancreas and enables to secrete insulin.
Heart disorders	<ul style="list-style-type: none"> Effective remedy for heart disease. Tones up the functions of all the organs of the body and builds up health by destroying the heterogeneous or harmful and disease causes elements. It also renews energy.
Eye disorders	<ul style="list-style-type: none"> With honey is useful in preserving eyesight. Beneficial in the treatment of conjunctivitis and glaucoma. Reduces intra ocular tension in a remarkable manner when juice mixed with honey and taken twice daily.
Rheumatism	Teaspoonful powder with 2 teaspoonful of jiggery, twice daily for a month.
Scurvy	Rich source of vitamin C, best remedy for scurvy. Amla powder with sugar in equal quantities, 3 times a day with milk.
Diarrhoea and dysentery	<p>A drink made from <i>amla</i> mixed with lemon juice and <i>misri</i>s considered highly beneficial in controlling acute ancillary dysentery.</p> <p>One tablespoonful of the paste of leaves mixed with honey or butter-milk is an effective Medicare in the treatment of diarrhoea and dysentery.</p>
Ageing	<ul style="list-style-type: none"> Revitalizing effects. Prevent ageing and maintains strength in old age. Improves body resistance, strengthens heart, hair and glands of body. Rejuvenating effect on all organs. It is said that the great ancient sage Muni Chyawan rejuvenated himself in his late 70s and regained his virility by the use of amla.
Hair tonic	<ul style="list-style-type: none"> Enriches hair growth and pigmentation. Dried fruit boiled in coconut oil till solid matter becomes charres, prevents greying. The water in which dried amla pieces are soaked overnight is also nourishing to hair. This water should be used for the last rinse while washing the hair.





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Home remedy	Way of use
Stabilizer of blood sugar	Amla seeds or dried amla powder in the form of capsules with bitter gourd juice daily.
Natural cholesterol remedy	It strengthens the heart muscles and causes a significant decrease in total cholesterol, LDL cholesterol, VLDL cholesterol and triglycerides. A 500 mg capsule of dried Amla powder can be added to your daily routine after consulting with doctor.
Treats hypertension	High vitamin-C helps control blood pressure. Amla choorna (powder) or in the form of triphala tablets or decoction. Triphala, a combination of amla and two other herbs is an excellent medication for high blood pressure.
Natural cure for Anemia	Amla is rich in Vitamin-C or ascorbic acid, an essential ingredient that helps in the absorption of Iron.
Anti-ageing	Fresh amla fruit has revitalizing effect on the body as it contains several nutrients and helps in preserving the stamina in aged people.
Herbal cough remedy	Add a teaspoon of Amla juice or powder to a glass of warm milk and drink this thrice a day. This will clear an unpleasant throat, adding some ghee to this decoction will clear a cough. Mix amla powder with honey and suck this mixture twice a day to cure a chronic dry cough. Amla is invaluable in the treatment of tuberculosis, asthma and bronchitis.
Natural eye tonic	Fresh Amla juice or dried Amla capsules are a good supplement to improve near-sightedness, cataract and glaucoma. It reduces intra ocular tension and corrects the vision.
Promotes hair growth	Dried amla fruits are boiled in coconut oil and then ground to form amla oil. This is a very effective conditioner and prevents balding and greying of hair. For oily hair, mix half a cup of Amla juice, half a cup of lime juice and some water. Apply this to make an anti-grease hair wash.
A pitta pacifier	Amla boiled in coconut water and the ground mixture is applied to the scalp. Amla oil is an excellent way to reduce heat associated with summer season. It is a good remedy to pacify pitta conditions.
Treats white spots on the nails	As a source of Vitamin C, serves as an effective remedy in vitamin deficit condition. Addition of Amla juice/powder in diet overcomes this condition.
Remedy for menstrual disorders	White discharge can be relieved with powdered and dried Amla Seeds. Mixture of amla with honey and saunf (fennel) or mixing it with squished banana and consuming.

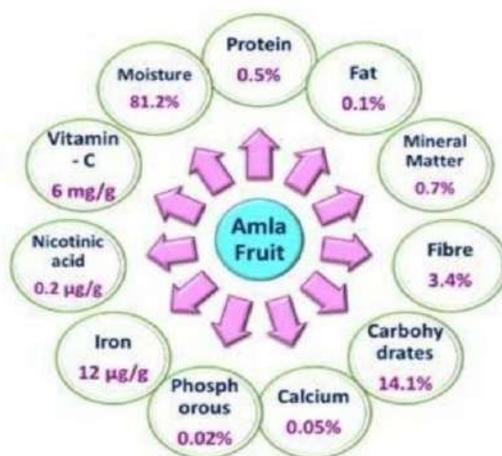


Figure 1: Amla fruit pulp: Composition





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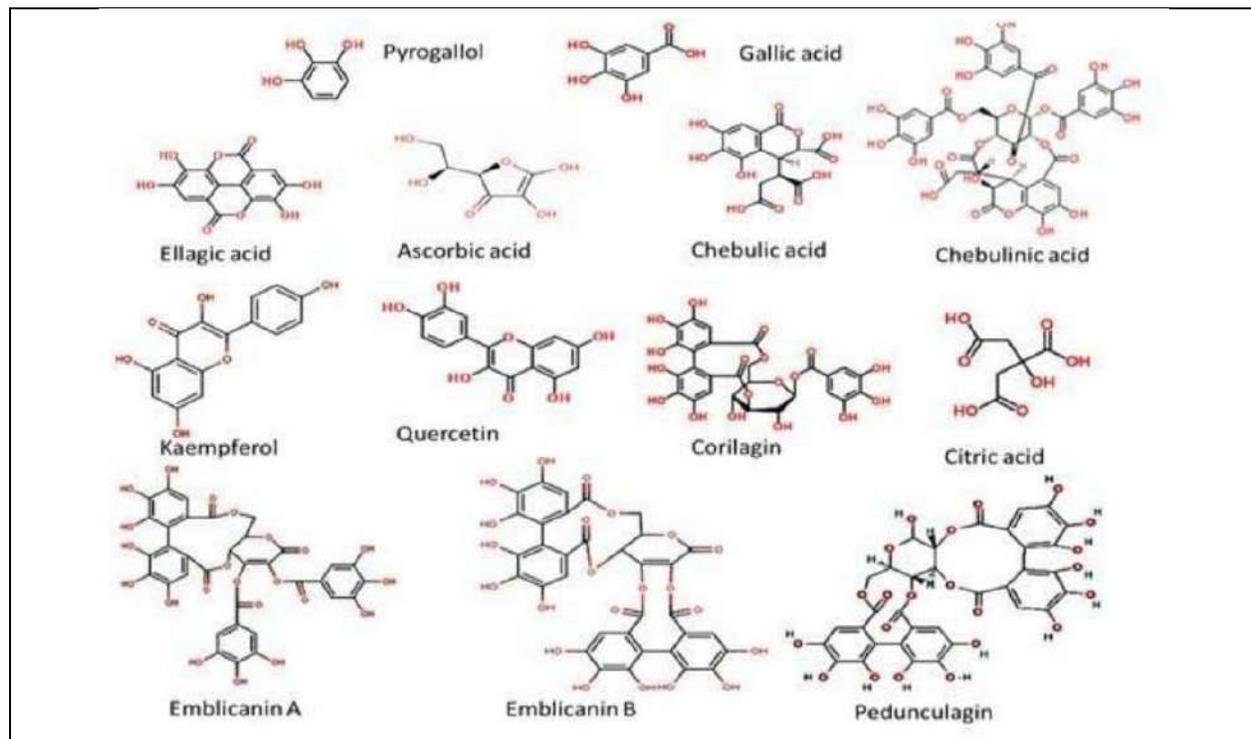


Figure 2: Amla fruit: Structures of chemical constituents

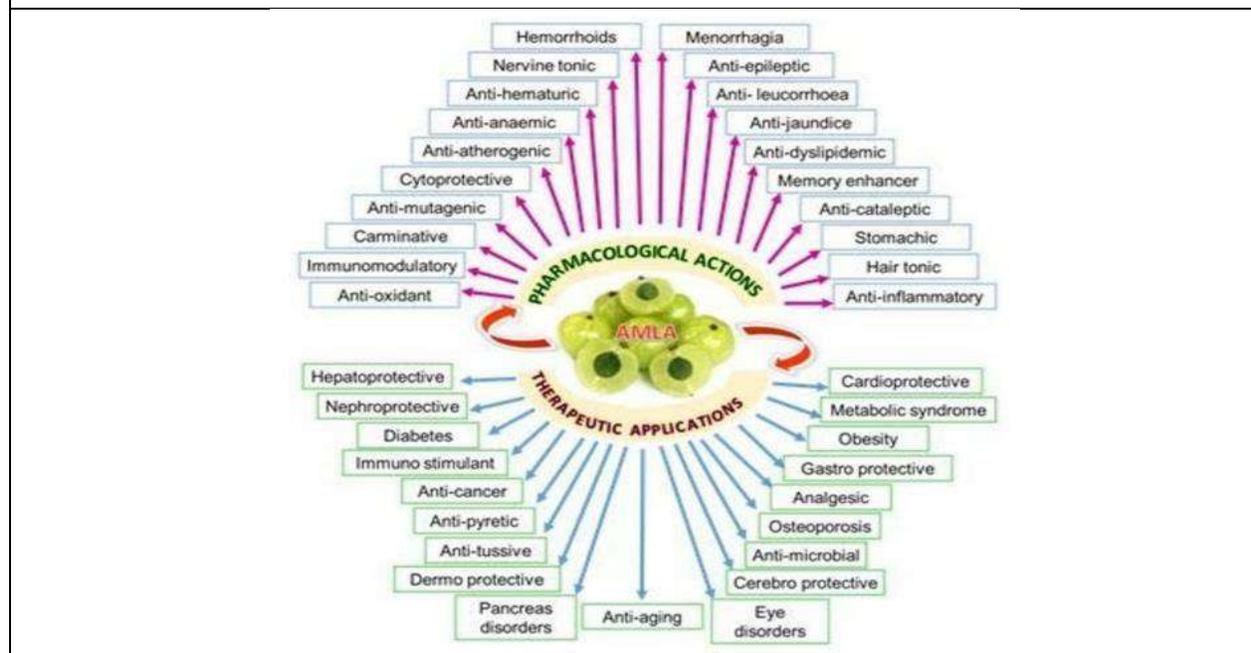


Figure 3: Amla: Pharmacological actions and therapeutic applications





Neem in Preventing Covid 19 Infection

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ABSTRACT

Neem (*Azadirachta indica* A. Juss) is one of India's most important traditional medicinal plants. Each portion of the neem tree has medical properties and can thus be exploited commercially. Apart from the chemistry of neem compounds, significant progress has been made in the biological activities and therapeutic applications of neem over the previous five decades. It is today regarded as a valuable source of unique natural components for the creation of medications and commercial products for a variety of ailments. This study focuses on the biological activities of some of the isolated neem compounds, pharmacological properties of neem extracts, clinical research, and potential medical applications of neem, as well as their safety assessment. The leaves, blossoms, seeds, fruits, roots, and bark of the neem tree have long been used to cure inflammation, infections, fever, skin ailments, and dental problems. The therapeutic properties of neem leaf have been documented in detail. Immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic, and anticarcinogenic effects have been demonstrated in neem leaf and its compounds.

Keywords: *Azadirachta indica*, medicinal plants, immune modulatory, pharmacology, clinical research, therapeutic properties, covid 19

INTRODUCTION

Neem is a fast growing tree that usually reaches a height of 15-20 m, and under very favorable conditions up to approximately 30-35 m. As a rule it is a evergreen tree, but under extreme circumstances, such as extended dry



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periods, it may shed most of nearly all of its leaves. The branches spread widely. The fairly dense crown is roundish or oval and may reach a diameter 15-20 m in old free standing specimens. The trunk is relatively short, straight and may reach a girth of 1.5-3.5 m. The bark is hard fissured or scaly and whitish-gray to reddish-brown. The sap wood is grayish-white and the heart wood reddish. The root system consists of a strong taproot and well developed lateral roots. The lateral surface root may reach over 18 m. Vesicular-arbuscular mycorrhiza (VAM) is associated with the rootlets categorized neem as a highly VAM dependant plant species. The leaves are unpaired, pinnate, 20-30 cm long and the medium to dark green leaflets, which number up to 31, are approximately 3-8 cm long. The terminal leaf is often missing. The petioles are short. The shape of mature leaflets is more or less asymmetric. Natural hybrids between *A. indica* and *A. Siamensis*, found in Thailand on places where both species grow together, have an intermediate position regarding the shape and consistency of the leaflets. The white, fragrant flowers are arranged in axillary, normally more or less drooping panicles which are up to 25 cm long. The glabrous fruits are olive-like drupes which vary in shape from elongate ovoid to nearly roundish and when ripe are 1.4-2.8 x 1.0-1.5 cm. They are green when young and yellowish-green to yellow, rarely reddish when mature. The fruit skin (exocarp) is thin and the bitter-sweet pulp (mesocarp) is yellowish-white and very fibrous. The mesocarp is 0.3-0.5 cm thick. The white hard 'shell' (endocarp) of the seed encloses one, rarely two and very rarely three elongated seed kernels having brown testa.

Geographical Distribution

The neem tree is native to the Indian subcontinent, but it has since spread throughout Asia, Africa, the Americas, Australia, and the South Pacific islands, primarily in the drier (arid) tropical and subtropical zones. It is extensively dispersed in India, with several states having it. In Myanmar, it is especially common in the country's centre regions. In the South Pacific neem occurs in the Fiji Islands. In Australia it was first introduced about 60-70 years ago. In Indonesia, neem exists mainly in the low-lying northern and eastern parts of Java and in the drier islands to the east (Bali, Lombok, Sumbawa). In the Philippines it was introduced during the seventies and eighties of the last century. In China, *A. indica* was planted on subtropical island of Hainan and southern China. In Nepal neem trees are found in the southern, low-lying areas (Tarai region). In Sri Lanka it is widespread in the drier northern parts of the island. The Fiji Islands are home to neem in the South Pacific. It was first introduced in Australia around 60-70 years ago. Neem is found primarily in the low-lying northern and eastern sections of Java, as well as the drier islands to the east, in Indonesia (Bali, Lombok, Sumbawa). It was first introduced in the Philippines in the 1970s and 1980s of the previous century. *A. indica* was first planted in China on the subtropical island of Hainan and in southern China. Neem trees can be found in Nepal's southern, low-lying areas (Tarai region). In Sri Lanka, it is common in the island's drier northern regions. Neem trees grow along the coast of Iran all the way to the Arabian Peninsula's Chat el Arab in Iraq. In Qatar and Abu Dhabi, neem trees were planted along roads and parks with desalted saltwater irrigation. To provide shade for tourists, a vast plantation was constructed on the Arafat plains near Makkah.

Chemical Constituents of Neem

The important chemical constituents were present in the leaf crude extracts of neem that can be endorsed to cultivation on a domestic plantation. The appropriate crude extracts for selective bioactive organic compounds can be chosen on the basis of GC-MS analysis. Therefore the identified good number of chemical compounds from various extracts of neem might have some ecological benefit for different ailments. Result from this study suggested that the chloroform crude extracts of neem could be used as a natural antioxidant. According to the World Health Organization (WHO), herbal medicine is used by the majority of the world's population for health care. Azadirachta Indica A. Juss, often known as neem plant, is a prominent medicinal plant in Asia and Africa that has been utilised for a variety of medical reasons since ancient times. Because of its high concentration of biologically active ingredients, it is utilised in a variety of traditional treatments. Nimbidin, Nimbin, Nimbolide, Gedunin, Azadirachtin, Mahmoodin, Cyclic trisulphide, and others are antipyretic, anti-inflammatory, antibacterial, antigastric ulcer, antiarthritic, spermicidal, antifungal, antimalarial, hypoglycemic, immunomodulatory, diuretic, and antitumour chemical components.





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Phytochemical Constituents of Neem

Aqueous extracts of *Azadirachta indica* (Neem) was subjected to in vitro antibacterial assay against human pathogenic *Escherichia coli* and *Salmonella* sp by cup diffusion method. The plant leaves were effective against all the tested organisms. Minimum Bactericidal Concentration (MBC) value of 5mg/l was obtained against *Escherichia coli* and *Salmonella* sp were found to be resistant with all the solvent extracts except water. A qualitative phytochemical analysis was performed for the detection of secondary plant metabolites [viz., alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins] and reducing sugars. Thin layer chromatography (TLC) was also performed by using different solvent system for the analysis of lipid, alkaloids, flavonoids present in plant extract. The active components separated through TLC were subjected to antimicrobial activity against the pathogens. The present study will be successful in identifying candidate plant with different antimicrobial activity which could be further exploited for isolation and characterization of the novel phytochemicals in the treatment of infectious diseases especially in light of the emergence to produce more effective antimicrobial agents. *Azadirachta indica* contains a diverse range of compounds, several of which have pharmacological potential. Triterpenes are the most commonly used therapeutic compounds among all of these compounds. Nimbin (triterpene) in particular has been shown to have antipyretic, fungicidal, antihistamine, and antiseptic properties.

Also, Nimbin has anti-inflammatory and antioxidant properties, which help to reduce damage by reducing the production of reactive oxygen species. Flavonoids, which act as inhibitors of prostaglandin biosynthesis, as well as endoperoxides and enzymes such as protein kinases and phosphodiesterases, are all found in Neem and are all involved in inflammation. Oil extracts are the most commonly used form of Neem, and extensive phytochemical analysis has confirmed the presence of high levels of triterpenes, flavonoids, and saponins, while other components such as catechins and nimbins appear to be present in lower levels. Limonoids, tannins, alkaloids, terpenoids, reducing sugar, catechins, sterols, and gallic acid are among the other metabolites found in Neem extracts. The leaf of the Neem tree appears to have developed a specific set of glycoproteins known as neem leaf glycoprotein (NLGP) that, when tested on mammalian subjects, demonstrated immune-modulatory activity, potentially limiting tumour growth by modulating local and systemic immunity. Scientists named Dash, Dixit, and Sahoo (2017) recently conducted an analysis involving leaf extracts (aqueous and methanoic) that revealed high levels of saponins, tannins, and glycosides in the aqueous extracts. While methanoic extracts contained the highest concentrations of alkaloids, tannins, and flavonoids. Proline is a current treatment for neurological disorders such as Alzheimer's and Parkinson's disease, Type 2 Diabetes Mellitus, and Polycythemia, according to biochemical study of leaf extracts.

Neem as Immune Booster

Neem aids in the strengthening of your immune system as well as the internal cooling of your body. It has antibacterial and antifungal characteristics, so it keeps your skin clean, radiant, and healthy. Blood-purifying properties are also found in neem. It aids in the removal of toxins and pollutants from the blood, resulting in a stronger immune system. Immunomodulatory effects of neem oil were studied in mice. The animals were treated intraperitoneally (i.p.) with neem oil; control animals received the emulsifying agent with or without peanut oil. Peritoneal lavage, collected on subsequent days, showed a maximum number of leukocytic cells on day 3 following treatment with neem oil; peritoneal macrophages exhibited enhanced phagocytic activity and expression of MHC class-II antigens. Neem oil treatment also induced the production of gamma interferon. Spleen cells of neem oil-treated animals showed a significantly higher lymphocyte proliferative response to in vitro challenge with Con A or tetanus toxoid (TT) than that of the controls. Pre-treatment with neem oil, however, did not augment the anti-TT antibody response. The results of this study indicate that neem oil acts as a non-specific immunostimulant and that it selectively activates the cell-mediated immune (CMI) mechanisms to elicit an enhanced response to subsequent mitogenic or antigenic challenge. For millennia, neem, or *Azadirachta indica*, has been known to have tremendous health-promoting effects. Since ancient times, Neem has been used in traditional medicinal cures in numerous countries, and it still has a particular therapeutic significance today. Neem pills can not only help you boost your immune system, but they can also help you cure a variety of health issues. Diabetes, Acne, Blood Impurities, Eczema, Dermatitis, Infections, and other conditions can all be helped with Neem Capsules. Neem improves the body's ability





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to fight illnesses by boosting the immune system. It also aids in the regulation of blood sugar levels, which is beneficial to diabetics. Neem capsules can also help to prevent high fevers, malaria, viral flu, dengue fever, and other infectious disorders.

Benefits of Neem Leaves

While neem holds immense importance for its medicinal properties, it is still a little less favoured food ingredient due to its bitter flavour. Even though we've been told by our parents and grandparents to chew a neem leave every day, but its bitter flavour always holds us back. But what if we would make a sweet and flavourful chutney out of its leaves? Neem chutney is an excellent way to add neem to our regular diet without the bitterness! It is a super easy and quick recipe with neem leaves grounded with jaggery, kokum, cumin and salt. The jaggery and cumin used in the chutney helps diminishing the bitter taste of neem and makes it a flavourful treat that you can pair with multiple dishes including rice, roti or paratha!

Neem for Immune Support

Neem has been discovered as a "immunomodulator," which means it helps the innate immune system's ability to self-regulate — either up in times of defence or down to avoid over-activity, which can lead to a "cytokine storm," or dysregulation of the immune system. "The ethanol extract of neem leaves demonstrated in vitro antibacterial activity against both Staphylococcus aureus and MRSA," according to research. Another study found that Neem had the same hepatoprotective (liver-protective) properties as milk thistle in mice, while one study concluded that Neem leaf extract "enhanced wound healing activity."

Neem in Preventing Covid 19 Infection

Neem is known to be a powerful immunostimulant, which makes it excellent to have at this time when the entire world is under threat from new coronavirus infection. Neem can help you in effective fight against the coronavirus. It can boost your immune function and can help you recover faster if you get the COVID-19 infection by any chance. Consuming Neem leaves can prevent you from being critical illness due to coronavirus infection and experience debilitating symptoms associated with it. Extract from the bark of the Neem tree may help treat and reduce the spread of SARS-CoV-2, the virus that causes COVID 19. According to a new study published in the journal Virology, components of Neem bark may target a wide spectrum of viral proteins, implying that it could be used as an antiviral drug against emerging coronaviruses like SARS-CoV. The researchers anticipate using the Neem-based medicine for COVID-

9 in the same way that people take penicillin for strep throat, allowing patients to return to their normal lives without risk of hospitalisation or death. The researchers predicted that Neem bark extract would bind to the SARS-CoV-2 spike protein at various locations, preventing virus entry into host cells, based on computer modelling. The SARS-CoV-2 virus uses the spike protein to enter and infect cells. The Neem bark extract was evaluated in SARS-CoV-2 human lung cells in Nagel's lab at the University of Colorado. The extract was found to be as effective as an anti-infection medicine, as well as reducing virus multiplication and spread following infection.

Every part of the neem tree has distinct medicinal properties, making it the most versatile medicinal tree in the world. Even though the benefits of the neem tree have been traditionally known in the Indian subcontinent, in the last decade or so, modern research has brought the humble neem tree into the spotlight. Researchers have found that neem products are virtually nontoxic and are compatible with beneficial insects, pollinators and bees. They are environmentally benign, sustainable, renewable and affordable. Neem has many incredible medicinal benefits, but one of the most important things is that it kills cancerous cells. Everyone has cancerous cells in their body, but they are normally disorganized. However, if you create certain situations in the body, they will get organized. As long as these cells are loafing around by themselves, it is not an issue. If they all gather in one place and hit it off, it becomes a problem. This is like a shift from petty crime to organized crime. It is a serious problem. If you consume neem every day, it keeps the number of cancerous cells in the body within a certain limit, so that they will not gang up against your system.





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CONCLUSION

Global health and medical practice seek to merge alternative medicine with evidence-based medicine for a better understanding of the metabolic process and its effects in the human body. An example is the use of complementary medicine like phytotherapy. *Azadirachta indica* (Neem), a tree originally from India and Myanmar, called by many “The village pharmacy” or “Divine tree” because of its many health properties. In recent times, Neem-derived extracts have been shown to work from anywhere from insect repellent, to supplements to lower inflammation, diabetic control, and even to combat cancer. Herein, we state the health benefits found in diverse compounds and extracts derived from Neem, highlighting the mechanisms and pathways in which Neem compounds produce their effects, while warning that the improper and unstandardized conditions to produce extracts can lead to health issues, particularly certain compounds might have damaging effects on the liver and kidneys.

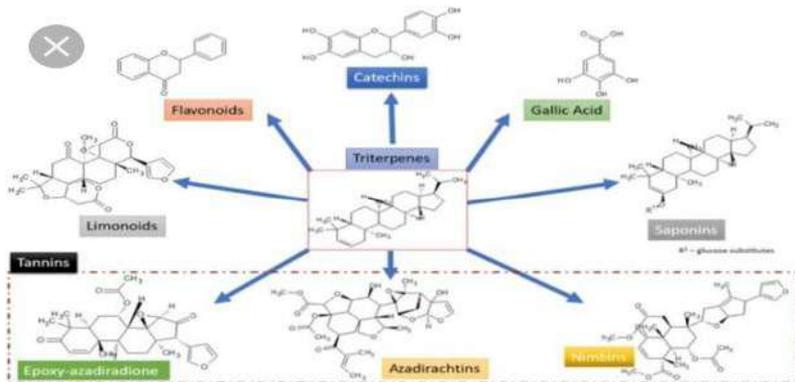
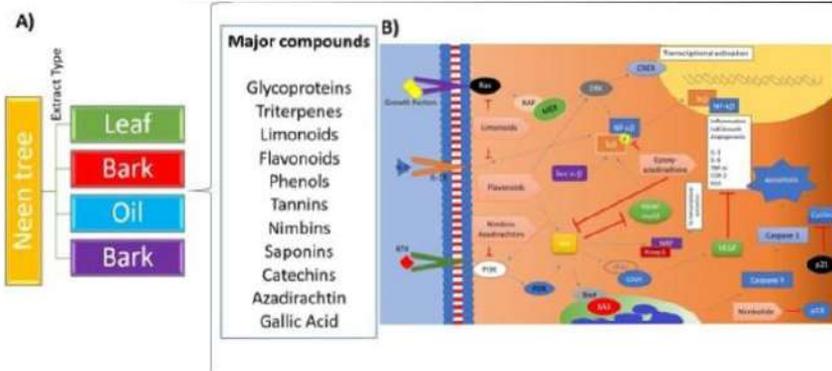
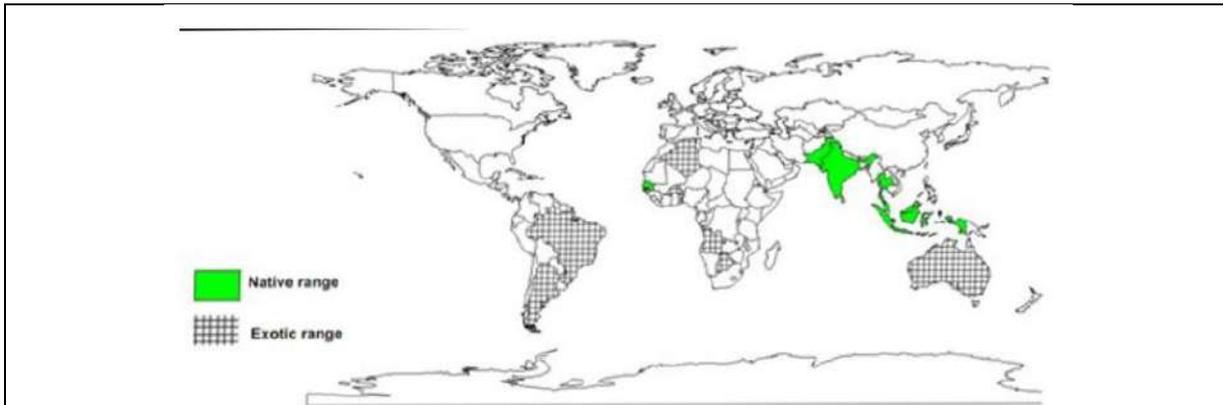
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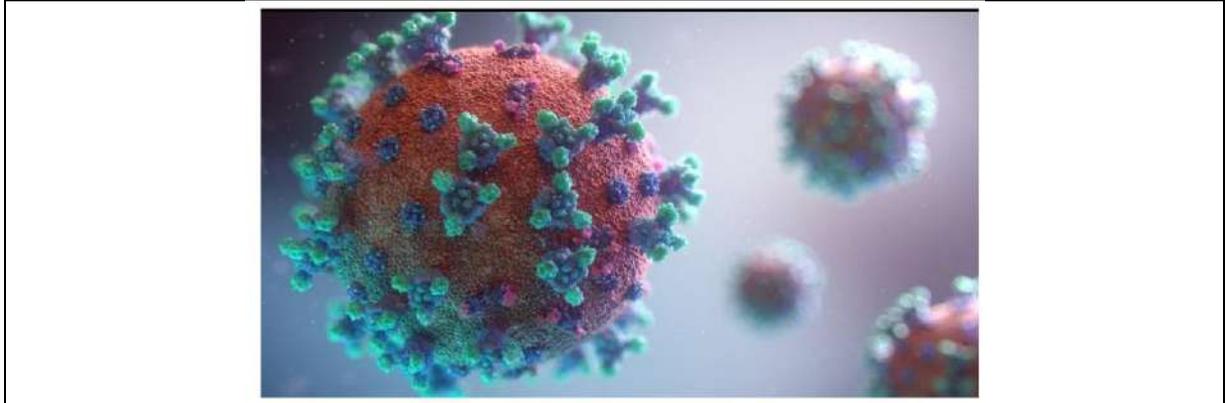


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Vibriosis in Shrimp Aquaculture

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ABSTRACT

Aquaculture is the fastest growing food sector globally and is established itself as high protein resource to fulfil the food demand since the natural resources exhibits over exploitation. But, presently, the biggest problem faced by the aquaculture industry worldwide is diseases caused due to various biological and non-biological agents. Among the groups of microorganisms that cause serious losses in shrimp culture, the best known are bacteria because of the devastating economic effects they have on affected farms. Bacterial diseases, mainly due to *Vibrio*, have been reported in penaeid shrimp culture systems implicating at least 14 species and they are *Vibrio harveyi*, *V. splendidus*, *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum*, *V. vulnificus*, *V. campbelli*, *V. fischeri*, *V. damsella*, *V. pelagicus*, *V. orientalis*, *V. ordalii*, *V. mediterrani*, *V. logei* etc.

Keywords: Aquaculture, Shrimp, Luminous bacteria, probiotics, non-antibiotics substances.

INTRODUCTION

Vibriosis is one of the major disease problems in shellfish and finfish aquaculture. Vibriosis is a bacterial disease responsible for mortality of cultured shrimp worldwide. *Vibrio* species are widely distributed in culture facilities throughout the world. *Vibrio*-related infections frequently occur in hatcheries, but epizootics also commonly occur in pond reared shrimp species. Vibriosis is caused by gram-negative bacteria in the family Vibrionaceae. Outbreaks may occur when environmental factors trigger the rapid multiplication of bacteria already tolerated at low levels within shrimp blood or by bacterial penetration of host barriers. The exoskeleton provides an effective physical



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barrier to pathogens trying to penetrate the external surface of crustaceans, as well as the foregut and hindgut. However, *Vibrio* spp. are among the chitinoclastic bacteria associated with shell disease and may enter through wounds in the exoskeleton or pores. The gills may appear susceptible to bacterial penetration because they are covered by a thin exoskeleton but their surfaces are cleaned by the setobranchs. The midgut, composed of the digestive gland (DG) and the midgut trunk is not lined by an exoskeleton and therefore seems to be a likely site for penetration of pathogens carried in the water, food and sediment.

Clinical Signs

Mortalities due to vibriosis occur when shrimps are stressed by factors such as: poor water quality, crowding, high water temperature, low DO and low water exchange. High mortalities usually occur in postlarvae and young juvenile shrimps. *P. monodon* larvae suffered mortalities within 48 hr of immersion challenge with strains of *V. harveyi* and *V. splendidus*. Mortalities involving vibriosis have been reported in market sized *P. monodon*. Adult shrimps suffering vibriosis may appear hypoxic, show reddening of the body with red to brown gills, reduce feeding and may be observed swimming lethargically at the edges and surface of ponds. *Vibrio* spp. also cause red-leg disease, characterised by red colouration of the pleopods, periopods and gills, in juvenile to adult shrimps and may cause mortality of up to 95% during the warm season. Eyeball necrosis diseases is caused by *V. cholerae*. The eyeballs of infected shrimps turn brown and fall away and mortality occurs within a few days. Six *Vibrio* species, including *V. harveyi* and *V. splendidus* cause luminescence, which is readily visible at night, in infected postlarvae, juveniles and adults. Infected postlarvae may also exhibit reduced motility, reduced phototaxis and empty guts.

Diagnosis

Diagnosis of vibrio infection is based on clinical signs and the histological demonstration of rod-shaped *Vibrio* bacteria in lesions, nodules or haemolymph. Excised organs and haemolymph may be streaked on a *Vibrio*-selective (TCBS) or general marine agar plate. When investigating postlarvae, the whole animal may be crushed and then streaked onto an agar plate. Luminescent colonies may be observed after 12 to 18 hr if incubated at room temperature or 25 to 30°C. Luminescent bacteria *Vibrio* isolates may be identified by a number of methods, including: Gram stain, motility, an oxidase test, mode of glucose utilisation, growth in the presence of NaCl, nitrate reduction and luminescence.

Transmission

Vibrio species exist in the water used in shrimp culture facilities and the biofilm, which is formed on different water contact structures of hatcheries and farms. Bacteria enter shrimps via wounds or cracks in the cuticle and are ingested with food. The primary source of *V. harveyi* in hatcheries appears to be the midgut contents of female broodstock, which are shed during spawning.

Viability

Numerous studies have been undertaken concerning the effect of freezing on vibrio which contaminate harvested shellfish. *V. vulnificus* in harvested oysters survived storage at -20 °C for 70 days. *V. parahaemolyticus*, isolated from homogenates of oyster meat was inactivated within 16 days at -15 °C when the bacterial load was very high. There is recent evidence to suggest that *V. harveyi* can survive in pond sediment even after chlorination or treatment with lime.

Present Status Of Vibriosis

Vibriosis is a common problem world-wide. The causative agent of EMS/EMD is *Vibrio parahaemolyticus*, which is causing major disease outbreak in *P. vannamei* in most of the major shrimp producing countries. *V. harveyi* continues to cause chronic mortalities of up to 30% among *P. monodon* larvae, postlarvae and adult under stressful conditions. A highly pathogenic strain of *Vibrio* sp. are also emerging and continues to cause mortalities among cultured shrimp. Problems caused by secondary vibriosis are common, but are considered minor compared to viral epidemics.





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Treatment

Vibriosis is controlled by rigorous water management and sanitation to prevent the entry of vibrios in the culture water and to reduce stress on the shrimps. Good site selection, pond design and pond preparation are also important. An increase in daily water exchanges and a reduction in pond biomass by partial harvesting are recommended to reduce mortalities caused by vibriosis. Draining, drying and administering lime/dolomite to ponds following harvest is also recommended. Luminescent vibriosis may be controlled in the hatchery by washing eggs with iodine and formaldehyde and avoiding contamination by spawner faeces. *V. harveyi* in the water column can be inactivated by Chlorine Dioxide. Probiotics are administered directly into the water or via feeds. Immunostimulants have had success in reducing shrimp mortalities associated with vibriosis. They designed to investigate an effective treatment of *Lactobacillus* sp. against vibriosis and white spot diseases in *P. monodon*. They investigated the growth of some probiotic bacteria, and their survival in the 20 ppt sea water for at least 7 days. Inhibiting activity of two *Lactobacillus* sp. against *Vibrio* sp., *E. coli*, *Staphylococcus* sp. was determined. While *V. harveyi* cultured with 40 ppm copper concentration showed decreased luminescence. Therefore, the combination of gut acidifiers, herbal extracts, prebiotics, probiotics, immunostimulants and non-antibiotic substances has superior specificity against vibriosis and Luminescent Bacteria (LB) coupled with Best Aquaculture Practices (BAP), which makes it an effective management tool for the control of Vibriosis and bacterial toxicity in aquaculture.

CONCLUSION

Vibrio is a highly diverse genus of bacteria, both in the aquatic environment and in direct association with crustaceans. In an aquaculture setting, it is nearly impossible to exclude these bacteria, and not all species/strains cause disease. Nevertheless, most *Vibrio* is opportunistic pathogens. Pathogenic strains can be devastating to aquatic organisms, not only crustaceans, but also fish, molluscs, and corals. Hence, controlling and managing the *Vibrio* population and their associated vibriosis is essential. To avoid the onset of infectious processes and thus the emergence of disease, prevention is the key mitigating factor. Prevention should include a detailed disease management plan, including immune resistance enhancement with nutrition, use of robust stock animals, and maintenance of high water quality. While emerging biotechnological innovations such as quorum sensing disruption, immune priming and phage therapy can potentially have an impact on vibriosis outbreaks, further validation of biosafety and efficacy at the farm scale is required.

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Stored Grain Pests and Advances for Their Management through Integrated Pest Management

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ABSTRACT

During the offseason, when fresh food is not available, humans have to consume stored grain food. Unfortunately, these stored grains are later infested with many pests. Foods stored in bags and bins are very much susceptible to infestation with several pests which can cause extensive post-harvest losses, spoilage, and less demand in markets, causing a huge economic crisis. Hence, successful management of stored grain pests becomes necessary to prevent these from insect pests. Current approaches for their management are one of the promising goals, as it includes preventive practices, monitoring, sanitation, and identification of main pathogens. Different management strategies of all the common stored grain pests viz. grain weevils, grain borers, grain moths, flour moths, mealworms, grain and flour beetles, booklice, mites, and parasites are enlisted here.

Keywords: Grain loss management, integrated pest management, economic loss management



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INTRODUCTION

In India, post-harvest losses caused by unscientific storage, insects, rodents, microorganisms etc., account for about 10 per cent of total food grains (Ahmad, 2021). The major economic loss caused by grain infesting insects is not always the actual material they consume, but also the amount contaminated by them and their excreta which make food unfit for human consumption. About 500 species of insects have been associated with stored grain products. Nearly 100 species of insect pests of stored products cause economic losses. Old bags, storage structures, old containers are the major source of infestation [1]. They are categorized in to Primary and secondary storage pest [2].

Stored Grain Pests

Stored grain pests cause serious post-harvest losses, almost 9% in developed countries to almost 20% or more in developing countries [3]. Pests such as various insects, pathogens, mites possess serious threats and cause severe damage to grains by producing certain enterotoxins and mycotoxins [4]. Approximately one-third of the world's production, which values almost \$100 billion has been destroyed by almost 20,000 species of field and stored grain pests [5]. The majority of stored grain pests belong to the order of Coleoptera and Lepidoptera that accounting for almost 60 and 10% respectively, Of all the stored grain pests [6]. Grains are generally attacked by several insect pests during all the stages of growth from seedlings to storage [7]. Insect pests possess a major threat to grain production and are also responsible for both direct and indirect losses of grain both in the field as well as in the storage [8]. Mihale *et al.* estimated that almost 15–100% pre-harvest losses and almost 10–60% post-harvest losses of stored grains are caused by stored grain pests in developing countries [9]. Weevils and moths are the major stored grain pests that cause huge damage to maize and sorghum [10]. Most important stored grain pests include Angoumois grain moth (*Sitotroga cerealella* (Olivier, 1789) Lepidoptera: Gelechiidae), maize weevil (*Sitophilus zeamais* Motschulsky, 1855 Coleoptera: Curculionidae), the Indian meal moth (*Plodia interpunctella* (Hubner, 1813) Lepidoptera: Pyralidae), the almond moths, *Ephestia cautella* (Walker) (Pyralidae: Lepidoptera), flour beetles (*Tribolium* spp.), the flat bark beetles *Cryptolestes* spp. (Coleoptera: Laemophloeidae) and the sap beetles *Carpophilus* spp. Stephens, 1830 (Coleoptera: Nitidulidae) [11]. The level of damage to the grains in storage gives an idea about the extent of damage [12]. Maize weevil although commonly found on maize can also attack many cereal grains such as wheat, barley, sorghum, and rice. Although, maize weevil prefers whole grains it has been reported to feed on many processed grain products including pasta and pet food [13]. Almost 10–20% losses have been reported in maize by *S. zeamais* after three months of storage [14]. Thus, millions of tons of maize are lost by stored grain pests due to inefficient storage technologies. More serious damage to maize grains is due to a larger number of adult weevils [15]. It is estimated that almost 63.85% of grain weight losses occurred due to three to six months of storage by stored grain pests. Pulses are heavily damaged by weevils and beetles in the field and also during storage time [16]. In the case of pulses, the adzuki bean weevil *Callosobruchus chinensis* (Linnaeus, 1758) (Coleoptera: Chrysomelidae) is found to be highly damaging as a stored grain pest. It is estimated that almost 50% of losses are found in important legumes such as chickpea field pea, faba bean by stored grain pests like *C. chinensis* [17]. Bruchids are found to be serious threats to faba bean and chickpea with an extent of damage sometimes reaching 90% after three months of storage.

Classification Of Pests

Stored insect pests are grouped into two types. This grouping is made according to the basis of the feeding ability of the insects in whole or previously damaged grain. They are classified as primary and secondary pests.

Primary insect pests Primary insect pests cause damage to the previously undamaged kernel or new grain. Stored grain pests are classified as major and minor pests based on the damage they cause. These insects can be classified as external feeders and internal feeders based on their feeding behavior.

(i) **External feeder:** As the name indicates these pests feed on external or surface parts of the grains such as the outside part of germ and endosperm. These pests either feed on whole seeds or damage the germinal portion of seeds and also feed on those seeds which are already damaged or attacked by other pests or are mechanically





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broken. These pests are generally visible among the seeds such as rice weevil, pulse beetle, granary weevil, Angoumois moth, etc.

(ii) **Internal feeders:** As the name indicates these pests are usually found inside the seeds. These pests mostly lay eggs inside or on the surface of grains, then spend a part or entire larval and pupal life within the grains and emerge as an adult. These pests cause significant loss of germination that is not detected externally, e.g., rice weevil, pulse beetle, granary weevil, Angoumois moth, etc.

Secondary insect pests

Secondary feeders. As the name indicates these pests are secondary because these pests attack on already infested crops these generally feed on cut and broken seeds, molds, dead insects, animal wastes, e.g., common mites, cheese mites, etc. Damage caused by these pests results in loss of germination, contamination like ball formation, and webbing besides deterioration of grains. Damage caused by these pests results in fungal activity, moisture migration across the stored grains.

- Grain in storage is subject to depredations of insects, mites, rodents, birds and moulds of which insects account for huge losses.
- In India losses during post harvest handling and storage estimated at 15 % annually.
- FAO estimate of total world losses in storage is 10% annually.
- Out of total storage in India 65 to 70 % being stored at farmer's level and 30 to 35 % by traders and Government agencies.

Various Kinds Of Stored Grain Pests

Internal feeders

- Grain weevil
- Khapra beetle
- Pulse beetle

External feeder

- Rice moth
- Red flour beetle
- Saw toothed beetle
- Cigarette beetle

Grain weevil :

Rice weevil: *Sitophilus oryzae*

Order- Coleoptera Family- Curculionidae

Nature Of Damage

- Upon hatching from eggs tiny grubs bore into grain and feed internally.
- Grubs are small white, apodous with yellow brown head. Both grubs and adults cause heavy damage in monsoon.
- Bionomics: The granary weevil is the oldest, cosmopolitan, small, brownish or blackish beetle, moderately polished having a long slender snout with a pair of stout mandibles or jaws, and having chewing-type mouthparts [18].

Symptoms Of Damage

- Hollowed out grains
- Kernels reduced to powder
- Heating takes place during heavy infestation , which is known as dry heating.

Khapra Beetle

S.N-*Trigoderma granarium*

Order –Coleoptera Family- Dermestidae





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Nature of Damage

- Being a primary pest, grub alone is destructive to grain starting with germ portion, surface scratching and devouring the grain.
- It reduces grain into frass.
- Excessive moulting creates public discrimination, loss of market appeal due to insanitation caused by the cast skins, frass, and hair.

Symptoms of Damage

- Presence of cast skins, frass and hair on bags .

Pulse Beetle

S.N- *Callosobruchus maculatus*

Order – Coleoptera Family –Bruchidae

Nature of Damage-

Young grub bores into the grain eat up the grain kernel and completes the development. Symptoms indicative of its infestation are

Symptoms of Damage

- Damaged grain unfit for consumption.
- Damaged grain converted to flour by traders give off flavour.

External Feeders

RICE MOTH -*Corcyra cephalonica*

Order- Lepidoptera , Family –Galleriidae

Nature of Damage

- Caterpillar alone is responsible for damage. It prefers partially damaged grains and feed.
- It pollutes food grains with frass, moults and dense webbing. In case of whole grains, kernels are bound into lumps up to 2 kg with the following.

Symptoms of Damage

- Grain converted to webbed mass
- Damaged grain / flour with bad odour unfit for consumption.

Red Flour Beetle

Rust red flour beetle-*Tribolium castaneum*

Order- Coleoptera , Family –Tenebrionidae

Nature of Damage

- Both adults and larvae are incapable of feeding on sound grain.
- Flour beetles are secondary pests of all grains and primary pests of flour and other milled products.

Symptoms of Damage

Flour greyish and mouldy giving disagreeable odour.

Saw Toothed Beetle

S.N – *Oryzaephilus surinamensis*

Order –Coleoptera , Family-Silvanidae

Symptoms of Damage

- Roughening of grain surface producing off odour
- Heating of grain with higher percentage of broken.

Cigarette Beetle

S.N – *Lasioderma serricorne*

Order – Coleoptera , Family –Anobiidae





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- Head and prothorax bent down to give humped nature.
- Grubs are yellowish with light brown head.
- Eggs are laid in and about the substance on which it feeds.
- Pupation is in silken cocoon covered with bits of food.
- Both grubs and adults bore holes into tobacco products like cigarettes, (cigars) and chewing tobacco.

Management of stored grain pests

- Disinfestation of stores by treating walls, dunnage, ceilings of empty godown with malathion 50 EC 1: 100 or DDVP 100EC 1: 300 @ 3 litres / 100m²(DDVP is a constant and fumigant)
- Maintenance of good storage conditions by providing dunnage, leaving gangway or alleyway of 0.75 – 1.0 mt all around for aeration, inspection and operations
- Air charging or treating alley ways with malathion 1: 100 or DDVP 1: 300 @ 1 litre of spray fluid per 270 m³
- Stack spraying over the bags with malathion 50 EC 1: 100 @ 3 litres/ 100m². Do not spray directly on food grains.

Prophylactic treatment of seeds or grains for small scale storage

- If for seed purpose, mix 1 Kg of activated Kaoline or Lindane 1.3 D or malathion 5 D for every 100 Kg of seed, store in gunny or polythene lined bags
- If for grain purpose, mix 1 Kg of activated Kaoline for every 100 Kg of grain and store
- To protect pulse grains, activated kaoline or any edible oil @ 1Kg/100 kg of grain.
- Mix neem seed kernel 1 kg for every 100 kg of cereals or pulses and store never mix synthetic insecticides with grains meant for consumption. This is legal offence.

Curative measures

Integrated Management Of Stored Produce Pests

The control methods of stored produce pests can be categorized into preventive and curative measures.

Preventive measures

- Brush the cracks, crevices and corners to remove all debris in the store houses.
- Clean and maintain the threshing floor/yard free from insect infection and away from the vicinity of villages.
- Clean the machines like harvester and thresher before their use.
- Made the trucks, trolleys or bullock carts free from infestation.
- Clean the godowns/ storage structures before storing the newly harvested crop to eliminate various bio stages of pest hiding.
- Provide a metal sheet upto a height of 25 cm at the bottom of the wood in doors to arrest the entry of rats.
- Fix up wire meshes to windows, ventilators, gutters, drains etc., to prevent entry of rats, birds and squirrels.
- Remove and destroy dirt, rubbish, sweepings and webbings etc from the stores.
- Close all the rat burrows found in godown with a mixture of broken glass pieces and mud plastered with mud/ cement.
- Plaster the cracks, crevices, holes found on walls, and floors with mud or cement and white wash the stores before storing of grains.
- Provide dunnage leaving gangway or alleyway of 0.75 to 1 m all around to maintain good storage condition.
- Store the food grains in rat and moisture proof storage structures.
- Disinfest the storage structures receptacles by spraying malathion 50 EC @ 3 lit 100 m before their use.

Curative Measures

I) Ecological Methods

- Manipulate the ecological factors like temperature, moisture content and oxygen through design and construction of storage structures/ godown and storage to create ecological conditions unfavourable for attack by insects.
- Temperature above 420 C and below 150 C retards reproduction and development of insect while prolonged temperature above 450 C and below 100 C may kill the insects.
- Dry the produce to have moisture content below 10% to prevent the buildup of pests.
- Kill the pests bio stages harbored in the storage bags, bins etc., by drying in the sun light.





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- Store the grains at around 10 % moisture content to escape from the insects attack.
- Manipulate and reduce oxygen level by 1% to increase the CO₂ level automatically, which will be lethal to all the stages of insects.

II) Physical Methods

- Provide a super heating system by infrared heaters in the floor mills and food processing plants to obtain effective control of pests since mostly the stored produce insects die at 55 –600 C in 10 – 20 minutes.
- Modify the storage atmosphere to generate low oxygen (2.4% and to develop high carbon di oxide (9.0 – 9.5) by adding CO₂ to control the insects.
- Seed purpose: Mix 1 kg of activated kaolin (or) lindane 1.3 D (or) malathion 5 D for every 100 kg of seed and store/pack in gunny or polythene lined bags.
- Grain purpose: Mix 1 kg activated kaolin for every 100 kg of grain and store. To protect the pulse grains, mix activated kaolin at the above dosage or any one of the edible oils at 1 kg for every 100 kg of grain or mix 1 kg of neem seed kernel for every 100 kg of cereal / pulse and store.
- Do not mix synthetic insecticides with grains meant for consumption.

III) Cultural Methods

- Split and store pulses to escape from the attack by pulse beetle since it prefers to attack whole pulses and not split ones.
- Store the food grains in air tight sealed structures to prevent the infestation by insects.

IV) Mechanical Methods

- Sieve and remove all broken grains to eliminate the condition which favour storage pests.
- Stitch all torn out bags before filling the grains.

V) Chemical Methods

- Treat the walls, dunnage materials and ceilings of empty godown with malathion 50 EC 10 ml/L (or) DDVP 76 WSC 7 ml/L1 at 3 L spray solution/10 sq.m.
- Treat the alleyways and gangways with malathion 50 EC 10 ml/L or DDVP 76 WSC 7 ml/ L (1 L of spray fluid/270 m³).
- Spray malathion 50 EC 10 ml/ L with @ 3 L of spray fluid / 100 m² over the bags.
- Do not spray the insecticides directly on food grains.
- Use knock down chemicals like lindane smoke generator or fumigant strips pyrethrum spray to kill the flying insects and insects on surfaces, cracks and crevices.
- Use seed protectants like pyrethrum dust, carbaryl dust to mix with grains meant for seed purposes only.
- Decide the need for shed fumigation based on the intensity of infestation.
- Check the black polythene sheets or rubberized aluminium covers for holes and get them ready for fumigation.
- Use EDB ampoules (available in different sizes 3 ml, 6 ml, 10 ml, 15 ml and 30 ml) at 3 ml/quintal for wheat and pulses and 5 ml/ quintal for rice and paddy (Do not recommend EDB for fumigation of flour oil seeds and moist grains)
- Use EDCT (available in tin containers of 500 ml, 1 liter and 5 litres) at 30 – 40 litres/ 100 cubic meter in large scale storage and 55 ml/quintal in small scale storage. FUMIGATION Use fumigants like ethylene dibromide (EDB), ethylene dichloride carbon tetra chloride (EDCT), aluminium phosphide (ALP) to control stored produce pests effectively. Apply aluminum phosphide (available in 0.6 g and 3 gram tablets) @ 3 tablets (3 gram each) per tonne of food grains lot with help of an applicator. Choose the fumigant and work out the requirement based on the following guidelines.
- 3 tablets of aluminum phosphide 3 g each per tonne of grain.
- 21 tablets of aluminium phosphide 3 g each for 28 cubic meters
- Period of fumigation is 5 days Mix clay or red earth with water and make it into a paste form and keep it ready for plastering all round the fumigation cover or keep ready sand snakes. Place the required number of aluminium





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phosphide tablets in between the bags in different layer. Cover the bags immediately with fumigation cover. Plaster the edges of cover all round with wet red earth or clay plaster or weigh down with sand snakes to make leaf proof. Keep the bags for a period of 5-7 days under fumigation based on fumigant chosen. Remove the mud plaster after specified fumigation periods and lift cover in the corner to allow the residual gas to escape. Lift the cover after few hours to allow aeration.

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MOBILE *Aeromonas septicaemia*- A Review

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ABSTRACT

Motile *Aeromonas septicaemia* is most commonly caused by *Aeromonashydrophila*. It is an opportunistic pathogen causing disease in fish under stress. The bacterium produces a number of pathogenic factors, and the most important among them are the haemolysin and the aerolysin, provoking the disease. Freshwater and saltwater fish species are susceptible. The disease is manifested clinically with haemorrhages, ulcerations, abscesses, ascitic fluid and anaemia. Mortality rates are high, and they incur substantial economic losses, thereby necessitating timely measures of control for prevention and treatment. The objective of this study was to investigate factors that predispose catfish to virulent *Aeromonashydrophila* (vAh) infection and establish a waterborne challenge model that mimics natural occurrence of MAS. Results of this study indicated that wounding on the fish body surface was one of the key factors that predisposed catfish to vAh infection via waterborne route. Therefore, the aim of this overview was to provide up-to-date information related to the control of motile *Aeromonas septicaemia* in fish through application of chemotherapeutic drugs, phytobiotics, probiotics, yeast extracts, vaccines and disinfectants.

Keywords: Motile *Aeromonas septicaemia*, Virulent *Aeromonashydrophila* Waterborne challenge

INTRODUCTION

Since the 2009 outbreak of motile *Aeromonas septicemia* (MAS) in West Alabama and East Mississippi, the disease has cost catfish aquaculture losses of about three million pounds of food-size fish annually. A new virulent clonal population of *Aeromonashydrophila* (vAh) was untypical condition that determined to be responsible for the MAS outbreaks. To date, primary or obvious field conditions leading to the disease outbreaks were largely unknown





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and none of recommended management practices that have worked in the past seemed to be effective at limiting or preventing the outbreaks. Induction of MAS in laboratory trials can assist in evaluating virulence of field isolates of *A. hydrophila*, assessing predisposing factors and developing prevention methods against MAS. Currently, intraperitoneal (IP) injection (i.e. delivering bacterial cells into fish intraperitoneal cavity by a syringe) is the main method employed in laboratories to compare relative virulence of *A. hydrophila* isolates and to examine effect of prophylactic treatments on prevention of MAS.

The challenge method via IP injection is effective and reproducible, but is unsuitable with the natural infection process (i.e. the waterborne route). Another challenge method is immersion of fish in water containing vAh cells. This practice, referred to waterborne or immersion challenge, is more natural, closely simulating infection route under aquatic conditions, but resulting mortality was low even at very high concentration of vAh cells. Crucial factors predisposing catfish to MAS have not yet been determined with the waterborne challenge method. The objective of this study was to investigate those factors that may result in an increased susceptibility of catfish to vAh infection under laboratory conditions and establish a reproducible waterborne challenge model that mimics natural occurrence of MAS. The effectiveness of the waterborne challenge model was tested using four field isolates including *A. hydrophila* and *Aeromonas veronii*.

Rearing of Channel Catfish

Channel catfish (*Ictalurus punctatus*) used in this study were purebred species (Delta Select) obtained from Warmwater Aquaculture Research Unit. Tank water was supplied by heated and de-chlorinated city water at flow rate of 0.5–0.6 L min⁻¹. The typical parameters of the water were as follows: dissolved oxygen was 7.0 ± 0.4 mg L⁻¹, ammonia content was 0.67 ± 0.05 mg L⁻¹, nitrate concentration was 0.17 ± 0.09 mg L⁻¹, hardness was 96.9 ± 8.1 mg L⁻¹ (as CaCO₃), alkalinity was 102 mg L⁻¹ (as CaCO₃), and pH was 7.3 ± 0.1. Aeration was generated from air pump and constantly supplied via an air-stone. Fish were fed with Aquamax Grower 400 (crude protein ≥45% and crude fat ≥16%) at rate of about 3% of fish weight once daily. No feed was provided in the day when experimental challenge was performed.

Disease sign

Gross pathology of MAS can range from few external or internal signs in peracute cases, to hemorrhagic septicemia in acute cases, to abscesses and large ulcers in chronic cases. Severe MAS outbreaks often display a range of lesions indicating variation in the progression of disease in individuals. External signs seen in fish with typical septicemia include reddened fins, inflammation of the anus, diffuse hemorrhages on the skin, exophthalmia and abdominal swelling. Scaled fish may display protruding scales (lepidorthisis) due to edema of the scale pockets, also fin erosion, focal scale loss and ulcers may be seen (Figure 3). One sign that is commonly seen in catfish with MAS caused by vAh is iridial hemorrhages in the eye. Internal signs include bloody ascites, diffuse hemorrhages in the intestines, connective tissue, visceral fat and musculature, swollen friable kidney and spleen.

Virulence factor

The range of virulence factors encoded by *A. hydrophila* includes adherence proteins, catalysts, nucleases, and toxins that may be expressed differently depending upon the respective environment. The role of the adhesin *minD* in virulence is its ability to mediate mucosal adherence, increase biofilm formation, and facilitate cell division as well as motility. Another element of host evasion is the nuclease encoded by the *ahn* gene of *A. hydrophila* J-1 which shows no significant change in hemolytic activity or growth in vitro; however, when Δ ahn mutants are introduced into fish and mice models, virulence is attenuated.

Confirmatory Diagnosis

Genomic DNA based assays are the most commonly used methods to confirm a motile aeromonad identification.





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DNA extraction and controls: DNA is extracted from affected fish tissues (lesion, posterior kidney) or bacterial cells using a commercially available kit. The DNA can be quantified and evaluate for purity spectrophotometrically. Alternatively, a suspension of approximately 2 µl of an individual bacterial colony can be suspended in 100 µl of sterile PBS heated to 95°C for 5 min and used as PCR templates. All PCR assays should use a representative negative control (a non *Aeromonas* or non-VAh bacterial isolate, on non-infected tissue) prepared using the same reagents. Likewise a positive control should be included in each analysis to rule out assay failure.

Molecular analysis to confirm motile *Aeromonas* species: Confirmation and more precise identification to the species or strain level is accomplished using sequence analysis of the 16S ribosomal RNA gene or housekeeping genes such as *rpoD* (which encodes sigma factor 70 of RNA polymerase) or the *gyrB* genes (which encodes the β-subunit of DNA gyrase). A more rapid confirmation that the organism is an aeromonad can be achieved using genus specific PCR targeting specific sequences of the glycerophospholipid-cholesterol acetyltransferase gene.

Conventional PCR for detection of VAh strain: The VAh strain can be differentiated from other *Aeromonas* spp. by amplifying a 167-bp region of a predicted open reading frame unique to VAh strains. Reactions consist of 13 µl of PCR super mix, 20 p mol of each September 20141.2.9 Motile *Aeromonas septicaemia* -5 primer, 5 µl of template (genomic DNA or cell suspension) and nuclease-free water to bring the total volume to 25 µl, using thermal cycling conditions.

CONCLUSION

Motile *Aeromonas* Septicemia (MAS) is a common bacterial disease, caused by *Aeromonas*, which affects warmwater fish, both in commercial production systems and in natural waters. Frequently, MAS outbreaks are stress-related and elimination of the underlying stress factor may be sufficient to resolve the disease outbreak. It is important to run antibiotic sensitivity tests prior to using antibiotics for controlling *Aeromonas* outbreaks. Many strains of *Aeromonas* are resistant to commonly-used antibiotics, and it is important to determine which drug should be used before spending time and money on an ineffective product. Anytime an *Aeromonas* infection persists as a chronic problem, it is important to make an effort to determine if an underlying stress factor is causing the fish to have insufficient immune protection from the bacteria. Evaluate the water quality, nutrition, handling, and use of drugs and chemicals. Deficiencies in any of these areas could predispose fish to *Aeromonas* infections. Cleanliness and good management practices will reduce *Aeromonas* outbreaks in a fish production unit.

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Amla as a Immune Booster

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ABSTRACT

Emblica officinalis Gaertn. or *Phyllanthus emblica* Linn, commonly known as Indian gooseberry or amla, is arguably the most important medicinal plant in the Indian traditional system of medicine, the Ayurveda. Various parts of the plant are used to treat a range of diseases, but the most important is the fruit. The fruit is used either alone or in combination with other plants to treat many ailments such as common cold and fever; as a diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, alterative, antipyretic, anti-inflammatory, hair tonic; to prevent peptic ulcer and dyspepsia, and as a digestive. Preclinical studies have shown that amla possesses antipyretic, analgesic, antitussive, antiatherogenic, adaptogenic, cardioprotective, gastroprotective, anti-anemia, antihypercholesterolemia, wound healing, antidiarrheal, antiatherosclerotic, hepatoprotective, nephroprotective, and neuroprotective properties. In addition, experimental studies have shown that amla and some of its phytochemicals such as gallic acid, ellagic acid, pyrogallol, some norsesquiterpenoids, corilagin, geraniin, elaeocarpusin, and prodelphinidins B1 and B2 also possess antineoplastic effects.

Keywords: Medicinal Aromatic plant, *Phyllanthus emblica*, Vitamin C, Chemical Components, Nutritious Assessment, Diabetesmellitus, Therapeutic Usages.

INTRODUCTION

Amla is one of the richest sources of vitamin-C, amino acids and minerals and hence it is highly nutritious. It contains several chemical constituents like tannins, alkaloids and phenols. Hydrolysable tannins, Emblicanin A and B; gallic acid, ellagic acid are reported to possess biological activity. All parts of Amla possess medicinal





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properties, particularly fruit, which has been used in Ayurveda as a powerful rasayana and in customary medicine in the treatment of diarrhoea, jaundice, inflammation and several other ailments. In the Indian system of medicine Amla fruit is widely used as alone or in combination with other plants .

Amla is used to treat common cold and fever, as diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, anti-pyretic, hair tonic; to prevent ulcer and dyspepsia. From the Pharmacological research reports on Amla we know about its analgesic, anti-tussive, anti-atherogenic, adaptogenic; cardio, gastro, nephro, neuro protective and anticancer properties. Amla possess chemo-preventive, radio, chemo and immunomodulatory, free radical scavenging, antioxidant, anti-inflammatory, anti-mutagenic activities. In the prevention and treatment of various diseases like cancer, atherosclerosis, diabetes, peptic ulcer, anemia, liver, heart diseases and various other disorders these properties are efficacious.

Taxonomical Classification

Kingdom	Plantae
Class	Magnoliopsida
Subclass	Rosidae
Division	Magnoliophyta
Family	Euphorbiaceae
Order	Euphorbiales
Genus	<i>Phyllanthus</i> L.
Species	<i>Phyllanthus</i> Linn. <i>emblic</i>

Chemical Constituents

These fruits contain high amounts of ascorbic acid (vitamin C), and have a bitter taste that may derive from a high density of ellagitannins, such as emblicanin A (37%), emblicanin B (33%), punigluconin (12%), and pedunculagin (14%). Amla also contains punicafolin and phyllanemblinin A, phyllanemblin other polyphenols, such as flavonoids, kaempferol, ellagic acid, and gallic acid. Amla natural product juice contains the most elevated grouping of nutrient C (478.56mg/100mL). Fruits contain 28% of the absolute tannins which dispersed in the entire plant of Amla. The organic product contains two hydrolysable tannins Emblicanin A and B, 21 .

Leaves: It contains gallic acid, chebulic acid, ellagic acid, chebulinic acid, chebulagic acid, amlic acid, alkaloids phyllantine and phyllantidine.

Seeds: A fixed oil, phosphatides and a small quantity of essential oil. Its Contains linolenic acid (8.78%), linoleic (44%), oleic (28.40%), steric (2.15%), palmitic (2.99%) and miristic acid (0.95%).

Barks: Contain leukodelphinidin, tannin and proanthocyanidin. Roots: Contain ellagic acid and lupeol.

Amla as immune booster

A strong immune system should always be a priority and now with the pandemic spreading its fangs across the globe, it is all the more important now. A strong immune system is the first line of defence against the virus, which can cause life-threatening complications. And the easiest way to do so is by eating a healthy diet. A healthy and balanced diet, which is full of nutrients is our best bet to gear up our immunity. One food, which is cheap, easily available and can help boost immunity is amla. Amla, also called Indian gooseberries, helps boost immunity and keep diseases at bay.





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Chromium in amla

Amla has chromium that can help reduce bad cholesterol in the body. If you know that heart is another vital organ that can get affected by the COVID 19 virus. Thus, its best to have a heart-healthy diet.

Rich in antioxidants

Amla has various antioxidants that can help neutralize the free radicals and thus save you from many diseases. Amla also has anti-bacterial properties that can help detoxify your system and even reduce acne and dandruff.

Can help burn fat

Obesity is linked with not just one but many diseases including heart disease and diabetes. Amla can help achieve a healthy BMI by aiding weight loss. The fibre in amla keeps one fuller for longer and improves the digestion.

Helps relieve symptoms of cold and cough

We all know COVID 19 is a respiratory infection and thus, it's important to strengthen the respiratory system. Amla can help you do that. Amla has been long used to relieve the symptoms of cough and cold, which often lead to chest congestion. Vitamin C in amla also improves immunity.

Excellent Anti-inflammatory Properties

Numerous studies have shown the effectiveness of Amla extract for hay fever, arthritis, osteoporosis and joint pain. This plant is capable of acting at the cellular level, reducing the expression of proinflammatory cells such as cytokines, cox and other mediators that are present in chronic diseases such as rheumatoid arthritis, cancer, among others.

Diabetes Control

The soluble fiber in amla berries dissolves quickly in the body, which helps to slow the rate your body absorbs sugar. This can help reduce blood sugar spikes. Amla berries also have a positive effect on **blood glucose** and lipid counts in people with type 2 diabetes.

DISCUSSION

Medicinal plants play a key role in our healthcare system as an incredible source of natural medicine from the ancient time. Herbal products have low or no side effect, so use of these products increasing steadily all over the world. A most essential medicinal plant of Ayurveda, an Indian indigenous system of medicine, is Amla. Amla is most important Ayurveda plant, because of its highest vitamin C content property, strong antioxidant property and essential biological property, which is familiar to prevent various numerous health sicknesses. Amla can be used in nutraceuticals and pharmaceutical industries as a food additive. Various extracts and herbal formed from of Amla are applied against various diseases, whose result is very much similar to regular drugs. The traditional use of *P. emblica* (Amla) is effective almost all of the human diseases, but not all of them have been authorized through clinical research. Now it needs to explore its medicinal values through various biotechnological tools and techniques at its molecular level.

CONCLUSION

Amla can be used as a natural source for the future drug development because of its traditional uses on numerous potential medications. Therefore, our vigilant attention must be required for increasing the usefulness of Amla for the treatment of numerous diseases and its development as a conventional, potential and safe dose





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Fig.1. Amla



Fig.2. Amla Tree





Ginger and Its Benefits

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ABSTRACT

We, as well as the new diseases we face, are growing rapidly in the new and developed world. Diabetes is one of the most common and silent killers. This is a metabolic disorder that causes a rise in blood sugar levels. Insulin is a hormone that moves sugar from the bloodstream to the cells, where it is stored and later used for energy. The body of a diabetic is either unable to produce enough insulin or is unable to adequately use the insulin it does produce. Untreated excessive glucose from this virus can begin to harm human organs because it remains in the body indefinitely and gradually reduces the cut-off of inhibiting subsequent illnesses. In the human body, there are four forms of diabetes. Each one has its own set of symptoms to deal with and control. As a result, there is a lot of interest in using nature to benefit people in the medical field. There is a lot of interest now in different sorts of herb and spice plants for medical purposes. Ginger is one of the most helpful plants among them, and it is utilized in a variety of ways in many parts of the world. *Zingiber officinale* is its botanical name, and it is primarily known and used as a spice and an old remedy. This plant's medicinal properties are used to treat a variety of ailments ranging from minor ailments to serious diseases. Currently, some research is being conducted in order to aid in the cure or prevention of the disease. If used properly, ginger can be quite beneficial. It may help you lower your blood sugar levels and regulate insulin production in your body. The highly effective piece of the ginger rhizome can be utilized to transport glucose into human muscle cells without the usage of external insulin, perhaps assisting in the management of excessive glucose levels.

Keywords: Ginger, Benefit, medical, symptoms, diseases, diabetes





INTRODUCTION

In today's environment, sickness attaches itself to our bodies like a parasite inside the host. It signifies that the sickness is progressing at the same time as we are. In the current scenario, rapid virus mutation has been proved to be lethal to the entire world. In the current scenario, but throughout the ages, we have been dealing with a very widespread ailment known as diabetes. It appears normal and has no major symptoms, but it begins to weaken our immune system, making it more vulnerable to other diseases. According to the report, about 463 million people, or nearly 8.9% of the global population, will be affected by this disease by 2020, making it the seventh greatest cause of death on the planet. Among the other types of diabetes, type 2 diabetes accounts for roughly 89 percent of cases. It has equal rates in both men and women, and it is expected to rise in the near future. It virtually doubles a person's chances of dying sooner than expected. Diabetes is responsible for 4.3 million deaths worldwide. The blood sugar levels of those with diabetes stay high. This could be because insulin isn't created at all in the body, or isn't produced in appropriate amounts. Perhaps it isn't as beneficial to the body as it should be. Type 1 diabetes is the most basic and prevalent kind of diabetes, affecting around 5% of the population. Obesity is related to this kind. Gestational diabetes is a kind of diabetes that develops during pregnancy. Type 1 diabetes is an autoimmune condition in which the body's immune system assaults the pancreas, preventing the body from producing insulin. In type 2, there may be protected from the effects of insulin on the body or a flaw in insulin release. Adults who are overweight are more likely to develop type 2 diabetes. There are a number of factors that contribute to elevated blood glucose levels in diabetics. Furthermore, the key factor is the body's protection against insulin in the body, which largely ignores insulin discharge. The beta cells of the pancreas are unable to produce insulin as a result of this factor. As a result, a person with type 2 diabetes may experience a combination of insulin release and mobility problems. Despite the fact that type 2 diabetes is more common in adults, type 1 diabetes is caused by the immune system attacking and destroying the beta cells in the body. What causes this insusceptible framework attack is currently unknown, but the end outcome is the body's insulin production being completed without the help of anybody else. Regardless, modern science is seriously considering many types of plants in order to develop a treatment for this condition, and one of them is a very common sprouting plant. An investigation discovered that 83 percent of 453 cancer patients had used at least one discretionary drug, with many of these improvements being natural (Elvin-Lewis, 2001). zingiberene species are members of a family of plants that have been used in cooking, cosmetics, and medicine for centuries, notably in Asian regions. In animal model studies, several spices and flavours, particularly those from the Zingiberene family, were found to provide excellent protection against degenerative diseases like tumours (Milner *et al.*, 2001; Suhr, 2002). Several members of the family have been identified as having cancer prevention, mitigation, and anticancer exercises (Rao *et al.* 1995, Lee *et al.*, 1998, Suhr *et al.*, 1999). Ginger has been discovered to offer a variety of organic properties, including cancer prevention, relaxing, antibacterial, anticancer, neuroprotective, cardiovascular, respiratory, ant obesity, antidiabetic, antinausea, and antiemetic properties. It may aid in the reduction of blood sugar levels and the regulation of insulin synthesis in the body. The medicinal component of ginger can increase glucose uptake into human muscle cells without the use of external insulin, which could aid in the management of high blood sugar levels. This chapter of the literature review summarises previous research on the history and study of diabetes, as well as the study of phytochemicals found in ginger. It covers the quantity of phytochemicals in ginger, as well as their effects on numerous disorders, particularly diabetes. This chapter contains the following information:

History Of Ginger

Zingiber officinale Roscoe was classified as a member of the Zingiberoside family by Wagner in 1980 and is associated with the Zingiber variety. The name Zingiber comes from the Sanskrit word "zingiber," which refers to the projections on the rhizome. It was given by English botanist William Roscoe (1753-1831). (Kutzer, 1999). Ginger grows to be a straight, evergreen shrub that grows to be 1 to 4 feet tall. The shoot rises 13 jerks above the ground and is encircled by the two masterminded edge's sheathing bases, which look like leaves. It produces white parties as well as pink bloom buds that develop into yellow fledglings. The design known as rhizome, which fundamentally means horn-shaped, grows ginger in an impartial way, at the edge fixed with extending portion The entire rhizome



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has a striated and hard surface. It's around 5 to 16cm long, 1.6 to 6cm wide, and 2.2cm thick, and the masking tone can be yellow, white, or red depending on the approach. Wanger mentions it. South-eastern Asia has ginger as a neighbour. The Arabs knew about the Mediterranean area, and journalists like Discords about 41–91 AD and Pliny the Elder around 25–80 AD portrayed it. Ptolemy discovered that ginger was produced in Ceylon, a city in Sri Lanka, circa 150 AD. During the Middle Ages, raw and protected ginger was imported into Europe. Ginger is one of the key flavours that have been presented from Asia in the fourteenth century England, showing up in Europe for the punch exchanges. Due to their similar taste, the dicots in the Asarum family are commonly referred to as wild ginger. In 2019, the total global production of ginger was 2.8 million tonnes, with India accounting for 34% of total global production. One pound of ginger was worth the same as a sheep. Ginger was used by Eclectic doctors in the nineteenth century to promote perspiration, improve craving, and treat disease, as well as a good counterirritant. According to Shrimad Bhagwat Geeta's Hirayama, ginger is a key component of Ayurveda, India's traditional medicine, and is known as Sunitha in Ayurveda. It was used to prevent excessive blood hardening in conductors and veins, lower cholesterol, and prevent joint aggravation. In traditional Chinese medicine, ginger is thought to be a sharp, dry, warming punch that can be utilised to relieve discomforts brought on by a chilly, saturated environment. It was also employed to treat depletion issues, disease, going revealed, toothache, snakebite, and respiratory conditions, as well as as a stomach related guide and antinausea fix. Ginger is a long-lasting flavour that is frequently prepared in tropical areas and occasionally naturalises. It mostly multiplies vegetatively since different cultivars only seldom sprout is fruitless (Suvarna *et al.* 1999; Flowers of India, 2016). It is listed as invading in Taiwan's Invasive Species Database (Taiwan Invasive Species Database, 2016) and is a weed in Puerto Rico and Queensland, Australia.

Ginger

Ginger is used as a zest, flavouring specialist, embellishment, medicinal, and food additive all over the world, and it's done fresh, in a fresh glue, or dried, in a dry powder. Although the shapes of new and dried ginger are significantly different, new ginger can be substituted for dried ground ginger. Ginger's strong, sweet-smelling scent is pervading the room. Within the Indian and Pakistani subcontinents, ginger is known as "Adak" (neighbourhood name) and is used in a variety of recipes.

Nutritional Composition of Ginger**Chemical Composition**

Ginger includes greater or much less half of carbs, 9% protein and loose amino acids, 6-8 % unsaturated fats and fatty oils, 3-6 bris, and 3-6% tough fibre (on dry remember quantity premise) contingent upon assortment, topography, and climatic conditions (Leung, 1984, Tang, 1992). Some African ginger assortments encompass 5. ninety-8 and 3.72g/100 proteins and fats (Shrine Adel, 2010). Dissolvable and insoluble strands are likewise decided in ginger. Ginger is a first-rate wellspring of crucial micronutrients like potassium, magnesium, copper, manganese and silicon. Potassium and manganese help to bring together protection from infection and ensure the coating of heart, veins and urinary entries. Silicon advances solid skin, hair, teeth, and nails and assists with absorbing calcium. Limited quantity of nutrients A, E and a few measures of B-nutrients and Nutrient Care further more decided in ginger rhizome (Adel and Prakash, 2010).

Diabetes

Diabetes is a condition in which your blood glucose, often known as blood sugar, is abnormally high. Your main source of energy is blood glucose, which comes from the food you eat. Insulin, a hormone produced by the pancreas, aids glucose absorption into cells for use as energy.

Isolation Of Components From Ginger**Gingerol**

Gingerol, accurately as [6]-gingerol, may be a phenol phytochemical compound located in new ginger that enacts zest receptors at the tongue. Atomically, the chemical irritant is a relative of chemical irritant associated pipeline, the



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combinations that are alkaloids, but the bioactive pathways are detached. it's normally located as an impactful yellow oil with inside the ginger rhizome, but will likewise body a low-liquefying semi-transparent strong. This substance compound is located taking all matters collectively people from the Zingiberene family.

Biological Activity of Gingerol

In a pre-medical meta-exam of gingerol intensifies anticancer, calming, opposed to contagious, most cancers prevention agent, neuroprotective and gastroprotective residences had been accounted for, which don't forget reads for Vitro and A couple of in-vivo examines have recommended that gingerols inspire sound glucose guiding principle for diabetics. Numerous examinations were across the influences of gingerols on a huge scope of malignant growths which include leukaemia, prostate, bosom, skin, ovarian, lung, pancreatic and There has now no longer been a number of medical attempts bent on note gingerols physiological leads to human. whereas a huge wide range of the substance additives related to the influences of gingerols on cells was completely contemplated, few were in an exceedingly medical setting. this can be because of the very smart quality in normal phytochemicals and also the absence of viability in analysis. Most flavourer healthful drugs, that embody gingerols, are below the constraints of the Food and Drug Administration with inside u. s. and take a glance at techniques that have currently not controlled the maximum amount as research which has pale the motivation in phytochemical research. Herbal medicine is untested for fine affirmation, energy, and adequacy in medical settings because of an insufficiency of finance in Jap medical research. Most exploration on [6]-Gingerol has been on each mouse subject (in-vivo) or on delicate human tissue (in-vitro) and is maybe applied in an exceedingly whereas to talk some capability programs for multi-goal infectious.

CONCLUSION

This study looked at the therapeutic medication potentials of ginger, which belongs to the Zingiberoside family of plants. Additionally, since the mechanism of action was done that antidiabetic medication holdings were intermediary, the volumes and outcomes varied. Though this research suggests that ginger has anti-diabetic or warning sign properties, dosages and goods vary; most notably, the instruments of achievement complete which anti-diabetic properties intercede were highlighted. According to these studies, ginger's anti-diabetic properties include comprehensive restorative effects on duct secretor -cells, cumulative insulin comprehension, hormone-like activity, and marginal aldohexose eating. Various devices hold coupled amalgamation of internal organ polyose via the sweetener of glycogen regulatory protein countenance inside the liver, the discomfiture of supermoleculometabolizing enzymes, stimulation of duct gland insulin release, and blockage of liverwort glucose building.

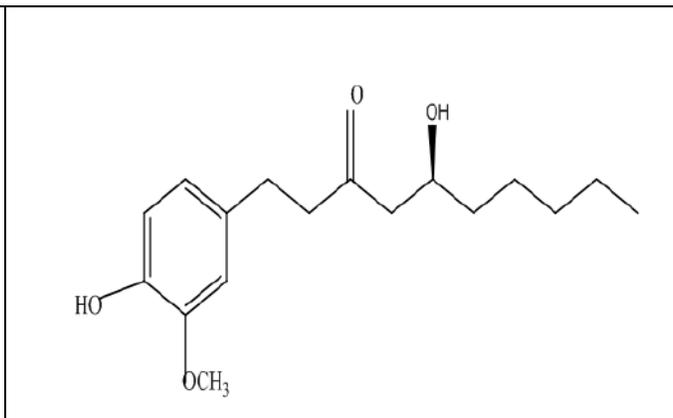
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	 <chem>CCCC(O)C(=O)CCc1ccc(O)c(OC)c1</chem>
<p>Ginger (<i>Zingiber officinale</i>)</p>	<p>Structure of Gingerol</p>





Ginger and Colon Cancer

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ABSTRACT

Colon cancer is a type of cancer that begins in the large intestine (colon). The colon is the final part of the digestive tract. Colon cancer typically affects older adults, though it can happen at any age. It usually begins as small, noncancerous (benign) clumps of cells called polyps that form on the inside of the colon. Over time some of these polyps can become colon cancers. Polyps may be small and produce few, if any, symptoms. For this reason, doctors recommend regular screening tests to help prevent colon cancer by identifying and removing polyps before they turn into cancer. If colon cancer develops, many treatments are available to help control it, including surgery, radiation therapy and drug treatments, such as chemotherapy, targeted therapy and immunotherapy. Colon cancer is sometimes called colorectal cancer, which is a term that combines colon cancer and rectal cancer, which begins in the rectum.

Keywords: Ginger, Colon Cancer, drug, treatments, Oleoresins

INTRODUCTION

Ginger originated from Maritime Southeast Asia. It is a true cultigen and does not exist in its wild state. The most ancient evidence of its domestication is among the Austronesian peoples where it was among several species of ginger cultivated and exploited since ancient times. They cultivated other gingers including turmeric (*Curcuma longa*), white turmeric (*Curcuma zedoaria*), and bitter ginger (*Zingiber zerumbet*). The rhizomes and the leaves were used to flavour food or eaten directly. The leaves were also used to weave mats. Aside from these uses, ginger had religious significance among Austronesians, being used in rituals for healing and for asking protection from spirits.



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It Contains gingerol, which has powerful medicinal properties . It Can treat many forms of nausea, especially morning sickness . It May help with weight loss . It Can help with osteoarthritis .ItMay drastically lower blood sugars and improve heart disease risk factor Anticancer activities of ginger against colorectal cancer have been well documented. Numerous *in vitro* studies showed that ginger and its active components inhibit growth and proliferation of colorectal cancer cells . In a study, 6-gingerol inhibited growth of colon cancer HCT116 cells . The suppression of tumor growth was found to be linked with the inhibition of leukotriene A4 hydrolase activity, which was further confirmed by *in silico* approach . Besides these, various other mechanisms were reported to be involved in 6-gingerol-induced cell growth inhibition and apoptosis in human colorectal cancer cells. These include protein degradation as well as down regulation of cyclin D1, NAG-1 beta-catenin, PKCepsilon, an GSK-3 β pathways . Radhakrishnan *et al.* reported that the anticancer activity of 6-gingerol could be associated with the inhibition of ERK1/2/JNK/AP-1 pathway. Whole ginger extract also prevent the primary stage of colon carcinogenesis. Administration of ginger extract to the mice pretreated with carcinogen 1,2-dimethylhydrazine (DMH) inhibited the levels of fecal bile acids, neutral sterols, tissue cholesterol, HMG CoA reductase, free fatty acids, triglycerides, phospholipase A, and phospholipase C . Thus, ginger supplementation reduced the risk of colon cancer markedly by virtue of its hypolipidemic and antioxidative effects. Ginger extract not only inhibits carcinogenesis of colorectal cancer cells but also enhances the anticancer effects of chemotherapeutic drug 5-fluorouracil. It has also shown that ginger extract synergistically increases the apoptotic efficacy of Gelam honey . As *in vitro*, 6-gingerol effectively suppresses tumor growth in nude mice . For improving the colon cancer therapeutic efficiency of ginger extract, a multiparticulate system (ginger extract loaded with coated alginate beads) has been designed . Preclinical evaluation against DMH-induced colon cancer in male Wistar rats showed that this bead has significantly better recession of the cancers compared to free ginger extract . Cysteine-conjugated shogaols have also been reported to cause death of colon cancer cells through the activation of the mitochondrial apoptotic pathway . Hexahydrocurcumin extracted from ginger was also found to be cytotoxic to colorectal cancer cells. It has been observed that treatment of SW480 colon cancer cells with hexahydrocurcumin (100 μ M) resulted in apoptosis , indicating its potential as anticancer agent. Besides ginger rhizome, exposure of ginger leaf extract exhibited reduced cell viability and induced apoptosis to human colorectal cancer HCT116, SW480, and LoVo cells. This anticancer activity of ginger leaf extract was attributed to the increased expression of ATF3 through ERK1/2 activation in human colorectal cancer cells . Another compound zerumbone, a sesquiterpene from the edible ginger (*Zingiber zerumbet* Smith), has been shown to enhance the radiosensitivity of colon cancer cells. It enhanced radiation-induced DNA damage and inhibited nuclear expression of DNA repair proteins ataxia-telangiectasia mutated (ATM) and DNA-PKcs

History of Ginger

Europe saw ginger for the first time in the 1st century when the ancient Romans traded with the India. When the Rome fell, Europe forgot about ginger until Marco Polo brought it again from his travel to the East .The fast development of human society and therefore the improvement of standard life, cancer as a growing threat, is that the second leading non contagious sickness of death globally next solely to upset. Malignant increase is stated to be a essential purpose for dying, and there had been kind of 9.6 million times of dying in 2018. A few exam works have exhibited that regular items. As of late, ginger has been commonly explored for its anticancer houses in opposition to numerous malignant increase types, like bosom, cervical, colorectal, and prostate disorder. The viable structures of interest consist of the restraint of multiplication and the popularity of apoptosis in malignant increase. The cytotoxic influences and hidden structures of ginger in prostate malignancy had been assessed each in vivo and in vitro. The anticancer additives on the whole consist of the enlistment of apoptosis and the restraint of the enlargement of malignancy cells. It is belongs to the Zingiberaceae family, The rhizome is the part which is normally used as a spice. It's often called ginger root or simply ginger. Ginger can be used in different form or state like raw, dried, powdered or as an oil. It's a very common ingredient in cooking recipes as a spice and flavouring agent. It's sometimes added to processed foods and decorative. Here are 11 health benefits of ginger that are discovered and supported by scientific researchers. Ginger is a flowering plant that which is fast found in Southeast Asia. It's known as the healthiest spices Effect of Ginger in Colon Cancer . on the planet in between all other spices. In a meta investigation taking a goose at numerous phytochemical impacts on colon malady, 2 specific examinations utilizing mice noticed



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[6]-gingerol intensifies instigated programmed cell death in malignant growth cells by meddling with the mitochondrial film. there have been likewise noticed instruments connected with the disturbance of G1 stage super molecules to prevent the propagation of malignant growth cells that is in addition a related advantage of alternative vital malignant neoplasm studies. the elemental system by which chemical irritant phytochemicals follow abreast of disease cells is by all accounts protein interruption . Many Years before British surgeon Dr. James Lind discovered that lime could prevent scurvy; fifth-century Chinese sailors were using ginger's vitamin C nutritive value for the same purpose on long voyages. The cultural outlook on aphrodisiacs in the seventeenth century was another factor in the reduction of its usage as a therapeutic agent.

Ginger

Ginger is burned-thru worldwide as zest, seasoning specialist, embellish, medication, and food additive and is carried out each new, in a brand-new glue, or dry, in a dry powder. New ginger can be fill in for dried ground ginger, albeit the forms of new and dried ginger are quite unique. The heady perfume of ginger is entering into and sweet-smelling. Ginger is called as "Adrak" (neighbourhood name) withinside the subcontinent like India and Pakistan and is a crucial element of numerous dishes.

Nutritional Composition of Ginger**Chemical Composition**

The chemical composition and the antimicrobial characteristics of ginger and ginger oil, respectively. The estimated chemical composition of ginger was: moisture ($15 \pm 0.033\%$), fiber ($17 \pm 0.03\%$), ash ($6.5 \pm 0.001\%$), protein ($5.2 \pm 0.1\%$), fat ($8.0 \pm 0.003\%$) and carbohydrates ($48.3 \pm 1.16\%$). Ginger essential oil was collected by steam distillation process. The physicochemical analysis of the oil was carried out, and the acid value was found to be 2.9, saponification value 25, ester value 30.22, free fatty acids 1.45 g oleic acid/100 g oil and the refractive index 1.5060% at 27 deg C. The inhibitory effect of ginger oil was detected for growth of six microorganisms: the bacteria *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurim*, the mould *Aspergillus niger*, *Aspergillus flavus* and the yeast *Sacharomyces cerevisiae*. The results indicated that ginger oil has a potent antimicrobial activity against all tested organisms. The highest antibacterial activity was detected against *E.coli*, where 23 and 30mm were the inhibition zone diameters at the lower and his higher oil concentrations, respectively. The higher antimicrobial activity, among all tested organisms was found against moulds where complete inhibition (100%) was recorded

Bioactive Components

Ginger is abundant in active constituents, such as phenolic and terpene compounds . The phenolic compounds in ginger are mainly gingerols, shogaols, and paradols. In fresh ginger, gingerols are the major polyphenols, such as 6-gingerol, 8-gingerol, and 10-gingerol. With heat treatment or long-time storage, gingerols can be transformed into corresponding shogaols. After hydrogenation, shogaols can be transformed into paradols [2]. There are also many other phenolic compounds in ginger, such as quercetin, zingerone, gingerenone-A, and 6-dehydrogingerdione . Moreover, there are several terpene components in ginger, such as β -bisabolene, α -curcumene, zingiberene, α -farnesene, and β -sesquiphellandrene, which are considered to be the main constituents of ginger essential oils [16]. Besides these, polysaccharides, lipids, organic acids, and raw fibers are also present in ginger

Antioxidant Activity

It has been known that overproduction of free radicals, such as reactive oxygen species (ROS), plays an important part in the development of many chronic diseases. It has been reported that a variety of natural products possess antioxidant potential, such as vegetables, fruits, edible flowers, cereal grains, medicinal plants, and herbal infusions . Several studies have found that ginger also has high antioxidant activity . The antioxidant activity of ginger has been evaluated in vitro via ferric-reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) methods. The results revealed that dried ginger exhibited the strongest antioxidant activity, because the number of phenolic compounds was 5.2-, 1.1-, and 2.4-fold higher than that of fresh, stir-fried, and carbonized ginger, respectively. The antioxidant activity of different gingers had a



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tendency to be the following: dried ginger > stir-fried ginger > carbonized ginger > fresh ginger. This was mainly associated with their polyphenolic contents. When fresh ginger was heated, dried ginger with higher antioxidant activity was obtained, because fresh ginger contains a higher moisture content. However, when dried ginger was further heated to obtain stir-fried ginger and carbonized ginger, the antioxidant activity decreased, because the processing could change gingerols into shogaols. Additionally, a fraction of the dried ginger powder abundant in polyphenols showed high antioxidant activity based on data from FRAP, oxygen radical absorbance capacity, and cellular antioxidant activity assays. Besides, the type of extraction solvent could have an effect on the antioxidant activity of ginger. An ethanolic extract of ginger showed high Trolox-equivalent antioxidant capacity and ferric-reducing ability, and an aqueous extract of ginger exhibited strong free radical scavenging activity and chelating ability [16]. Moreover, ethanolic, methanolic, ethyl acetate, hexane, and water extracts of ginger respectively inhibited 71%, 76%, 67%, 67%, and 43% of human low-density lipoprotein (LDL) oxidation induced by Cu^{2+} . Results from a xanthine/xanthine oxidase system showed that an ethyl acetate extract and an aqueous extract had higher antioxidant properties than ethanol, diethyl ether, and *n*-butanol extracts did.

Antimicrobial Activity

The spread of bacterial, fungal, and viral infectious diseases has been a major public threat due to antimicrobial resistance. Several herbs and spices have been developed into natural effective antimicrobial agents against many pathogenic microorganisms. In recent years, ginger has been reported to show antibacterial, antifungal, and antiviral activities. Biofilm formation is an important part of infection and antimicrobial resistance. One result found that ginger inhibited the growth of a multidrug-resistant strain of *Pseudomonas aeruginosa* by affecting membrane integrity and inhibiting biofilm formation. In addition, treatment with ginger extract blocked biofilm formation via a reduction in the level of bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP) in *Pseudomonas aeruginosa* PA14. Moreover, a crude extract and methanolic fraction of ginger inhibited biofilm formation, glucan synthesis, and the adherence of *Streptococcus mutans* by down regulating virulence genes. Consistent with the in vitro study, a reduction in caries development caused by *Streptococcus mutans* was found in a treated group of rats

Cytotoxicity

Cancer is documented to be a dominant cause of death, and there were approximately 9.6 million cases of death in 2018. Several research works have demonstrated that natural products such as fruits and medicinal plants possess anticancer activity. Recently, ginger has been widely investigated for its anticancer properties against different cancer types, such as breast, cervical, colorectal, and prostate cancer. The potential mechanisms of action involve the inhibition of proliferation and the induction of apoptosis in cancer

Colon Cancer

Colon cancer is a type of cancer that begins in the large intestine (colon). The colon is the final part of the digestive tract. Colon cancer typically affects older adults, though it can happen at any age. It usually begins as small, noncancerous (benign) clumps of cells called polyps that form on the inside of the colon. Over time some of these polyps can become colon cancers. Polyps may be small and produce few, if any, symptoms. For this reason, doctors recommend regular screening tests to help prevent colon cancer by identifying and removing polyps before they turn into cancer. If colon cancer develops, many treatments are available to help control it, including surgery, radiation therapy and drug treatments, such as chemotherapy, targeted therapy and immunotherapy. Colon cancer is sometimes called colorectal cancer, which is a term that combines colon cancer and rectal cancer, which begins in the rectum.

Isolation Of Components From Ginger**Gingerol**

Gingerol, accurately as [6]-gingerol, may be a phenol phytochemical compound located in new ginger that enacts zest receptors at the tongue. Atomically, chemical irritant is a relative of chemical irritant associated piperine, the combinations that are alkaloids, but the bioactive pathways are detached. it's normally located as an impactful



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yellow oil with inside the ginger rhizome, but will likewise body a low-liquefying semitransparent strong. This substance compound is located taking all matters collectively people from the Zingiberene family.

Biological Activity of Gingerol

In a pre-medical meta-exam of gingerol intensifies anticancer, calming, opposed to contagious, most cancers prevention agent, neuroprotective and gastroprotective residences had been accounted for, which don't forget reads for vitro and A couple in-vivo examines have recommended that gingerols inspire sound glucose guiding principle for diabetics. Numerous examinations were across the influences of gingerols on a huge scope of malignant growths which include leukaemia, prostate, bosom, skin, ovarian, lung, pancreatic and There has now no longer been a number of medical attempting bent on note gingerols physiological leads to human. whereas a huge wide range of the substance additives related to the influences of gingerols on cells were completely contemplated, few were in an exceedingly medical setting. this can be because of the very smart quality in normal phytochemicals and also the absence of viability in analysis. Most flavorer healthful drug, that embody gingerols, are below the constraints of the Food and Drug Administration with inside the u. s. and take a glance at techniques have currently not control the maximum amount as research which has pale the motivation in phytochemical research. Herbal medicine is untested for fine affirmation, energy and adequacy in medical settings because of a insufficiency of finance in Jap medical research. Most exploration on [6]-Gingerol has been on each mouse subjects (in-vivo) or on delicate human tissue (in-vitro) and is maybe applied in an exceedingly whereas to talk some capability programs for multi-goal infectious.

Oleoresins

Ginger oleoresin is gotten by permeating the powdered rhizomes of ginger. Zingiber officinale with unstable solvents. Ginger contains 1 to 2% of unstable oil and 5-8% of sharp oleoresin. Zingiberene is the main constituent of oil of ginger. Oil is utilized for seasoning of a wide range of food items and confectionary and discovers restricted use in scent. Oleoresin, industrially is called Gingering contains impactful standards viz, gingerol and shogaol separated from the unpredictable oil of ginger and are used as a fragrant, carminative, stomachic and energizer. In ginger oleoresins extraction was examined utilizing a watery two-stage framework, with beta - cyclodextrin, sodium carbonate, and sodium chloride as extractants. Results showed clear shared partition of the water. Generally, appropriation coefficient of gingerols, the principle accumulates present in ginger oleoresins, was 0.21-0.32, with an extraction pace of as much as 71.6%.

CONCLUSION

- Regardless of advances in the therapy of bosom disease, there stays a necessity to conquer helpful opposition and create novel therapies for metastatic bosom malignancy.
- Ongoing investigations have exhibited that BCSCs might be answerable for opposition.
- Crosstalk between the essential tumor and the stroma or microenvironment is allegedly conceivably answerable for the relocation and obtrusive nature of metastatic bosom disease.
- To address these worries, consideration should be centered around intensifies that explicitly influence different subatomic targets related with undifferentiated cells and the metastatic tumor micro-environment.
- Phytochemicals have demonstrated viable at focusing on various flagging pathways and BCSCs in bosom malignancy.

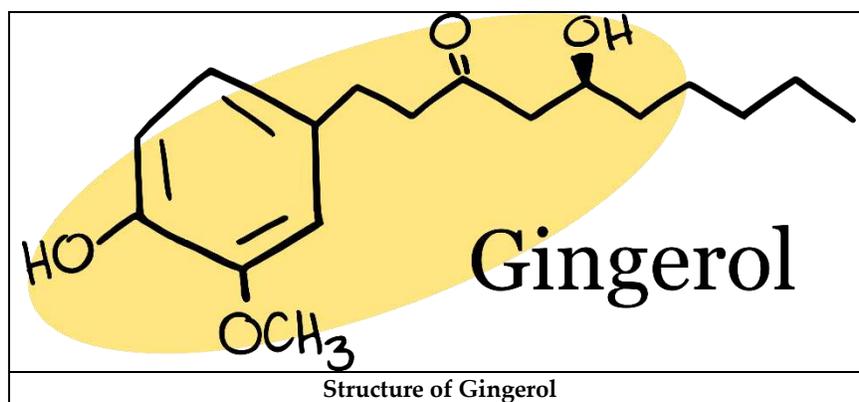
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Amla as A Personalized Medicine

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ABSTRACT

Phyllanthus emblica Linn. Or *Emblica officinalis* Gaertn. commonly known as Indian gooseberry or Amla is one of the most important medicinal plants in Indian traditional systems of medicine (Ayurveda, Unani and Siddha). It is a well-known fact that all parts of amla are useful in the treatment of various diseases. Among all, the most important part is fruit. Amla fruit is widely used in the Indian system of medicine as diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, anti-pyretic, hair tonic, ulcer preventive and for common cold, fever; as alone or in combination with other plants. Phytochemical studies on amla disclosed major chemical constituents including tannins, alkaloids, polyphenols, vitamins and minerals. Gallic acid, ellagic acid, emblicanin A & B, phyllembein, quercetin and ascorbic acid are found to be biologically effective. Research reports on amla reveals its analgesic, anti-tussive, antiatherogenic, adaptogenic; cardio, gastro, nephro and neuroprotective, chemopreventive, radio and chemo modulatory and anticancer properties. Amla is also reported to possess potent free radical scavenging, antioxidant, anti-inflammatory, anti-mutagenic, immunomodulatory activities, which are efficacious in the prevention and treatment of various diseases like cancer, atherosclerosis, diabetes, liver and heart diseases. In this article, we discuss the nutritional value, biochemical constituents, traditional uses, medicinal value of amla and its use as a household remedy. We also emphasized the mechanisms behind the pharmacological activities based on the recent research reports and tried to summarize the results of research done from the past 5 years with proper specifications on the future prospects in a pharmacological perspective.



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Keywords: Amla, traditional uses, chemical constituents, pharmacological activities, mechanisms, therapeutic applications

INTRODUCTION

Mother Nature has gifted mankind with tremendous medicinal plants to create a disease free and healthy life. Abundant medicinal plants are presented in the Indian traditional systems of medicine (like Ayurveda, Unani, siddha), mostly used one amongst them is Indian gooseberry or Amla, also known as *Phyllanthus emblica* Linn. (Syn. *Emblia officinalis* Gaertn.) belongs to the family Euphorbiaceae, which is an important medicinal herb in Ayurveda and Unani systems of medicine. It is enormously used as a tonic to restore the lost body's energy and vigor.

Amla is a small to medium sized deciduous tree, found in throughout India, Pakistan, Uzbekistan, Sri Lanka, South East Asia, China and Malaysia. It grows about 8-18m height with thin light grey bark, leaves are simple, light green, sub-sessile, closely set along the branchlets looks like pinnate leaves; flowers are greenish yellow; fruits are globose, fleshy, pale yellow with six obscure vertical furrows enclosing six trigonous seeds in two seeded three crustaceous cocci.

Amla is highly nutritious and is one of the richest sources of vitamin-C, amino acids and minerals. It contains several chemical constituents like tannins, alkaloids and phenols. Among all hydrolysable tannins, Emblicanin A and B; gallic acid, ellagic acid are reported to possess biological activity. Almost all parts possess medicinal properties, particularly fruit, which has been used in Ayurveda as a powerful rasayana and in customary medicine in the treatment of diarrhoea, jaundice, inflammation and several other ailments. Amla fruit is widely used in the Indian system of medicine as alone or in combination with other plants and is used to treat common cold and fever, as diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, anti pyretic, hair tonic; to prevent ulcer and dyspepsia.

MORPHOLOGY OF AMLA

- Amla tree is a small to medium sized deciduous tree with an average height of 18 m, with thin light grey bark exfoliating in small thin irregular flakes, exposing the fresh surface of a different color underneath the older bark. The average girth of the main stem is 70 cm. In most cases, the main trunk is divided into 2 to 7 scaffolds very near to the base. Leaves are 10 -13 mm long, 3 mm wide, closely set in pinnate fashion which makes the branches feathery in general appearance. After setting of the fruits leaves develop. Flowers are unisexual, 4 to 5 mm in length, pale green in color, borne in leaf axils in clusters of 6 to
- Fruits are fleshy, almost depressed to globose shape, 2.1-2.4 cm in diameter, 5.3-5.7 g in weight, 4.5-5.0 mL in volume. The stone of the fruit is
- 6 ribbed, splitting into three segments each containing usually two seeds; seeds are 4-5 mm long and 2-3 mm wide, each weighing 572 to 590 mg.

THEORY

An ethanol extract of 'Amla' *Emblia officinalis* Gaertn. was examined for its antisecretory and antiulcer activities employing different experimental models in rats, including pylorus ligation Shay rats, indomethacin, hypothermic restraint stress-induced gastric ulcer and necrotizing agents (80% ethanol, 0.2 M NaOH and 25% NaCl). Oral administration of Amla extract at doses 250 mg/kg and 500 mg/kg significantly inhibited the development of gastric lesions in all test models used. It also caused significant decrease of the pyloric-ligation induced basal gastric secretion, titratable acidity and gastric mucosal injury. Besides, Amla extract offered protection against ethanol-induced depletion of stomach wall mucus and reduction in nonprotein sulfhydryl concentration.





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Histopathological analyses are in good agreement with pharmacological and biochemical findings. The results indicate that Amla extract possesses antisecretory, antiulcer, and cytoprotective properties.

CHEMICAL COMPOSITION OF AMLA

Hydrolysable Tannins Emblicanin A and B, Punigluconin, Pedunculagin, Chebulinic acid (Ellagitannin), Chebulagic acid (Benzopyran tannin), Corilagin (Ellagitannin), Geraniin (Dehydroellagitannin), Ellagotannin Alkaloids Phyllantine, Phyllembin, Phyllantidine Phenolic compounds Gallic acid, Methyl gallate, Ellagic acid, Trigallayl glucose Amino acids Glutamic acid, Proline, Aspartic acid, Alanine, Cystine, Lysine Carbohydrates Pectin

Leaves: It contains gallic acid, chebulic acid, ellagic acid, chebulinic acid, chebulagic acid, amlic acid, alkaloids phyllantine and phyllantidine.

Seeds: A fixed oil, phosphatides and a small quantity of essential oil. Its Contains linolenic acid (8.78%), linoleic (44%). Oleic (28.40%), steric (2.15%), palmitic (2.99%) and miristic acid (0.95%).

Barks: Contain leukodelphinidin, tannin and proanthocyanidin. Roots: Contain ellagic acid and lupeol.

PHYTOCHEMICAL

In recent years, many scientific kinds of literature have recorded different Phyto-constituents of *E. officinalis*. The amla fruit includes numerous bioactive components including isostrictinin, ellagic acid, apigenin, chebulinic acid, quercetin, gallic acid, chebulagic acid. The tannins also found in the fruit extract of amla are pedunculagin, emblicanin A, phyllaemblicin B, emblicanin B and punigluconin. 100 g of edible fruit have been reported to be 470–680 mg of Vit. C. In ethanolic extracts of amla, recently identified Quercetin and β-sitosterol. Another study confirmed the existence of 5 major plants, including 5-hydroxymethylfurfural, 1, 2, 3-benzenetriol (synonym: Pyragallol), 2-acetyl-5-methylfuran. Recently two new chalconoid analogues, emblirol B and emblirol A, have been isolated from the roots of emblica with a molecular formula C₁₉H₂₄O₆. The discovery of various phytochemicals in different sections of amla is very significant and understand its therapeutic role, along with mechanistic action in the attack against different disorders. Also, known phytocompounds can be docked for the understanding of their goals and related therapeutic activities. Vitamins Ascorbic acid Flavonoids Quercetin, Kaempferol Organic acids Citric acid

ANTIOXIDANT ACTIVITIES

Because of behavioral and biochemical abnormality changes due to cold stress, a study on EO has been carried out. The oral dispensation of Triphala by approximately one g/kg of the animal body until the 48-day period prevented cold stress-induced behavioral and biochemical abnormalities of albino rats.

Hence the deemed shielding drug against stress is Triphala supplement. In the aging rats, accelerated urea-nitrogen status is also suppressed by feeding Amla. Sun Amla ethyl acetate extract (EtoAc). Conspicuous lessening of thiobarbituric acid-reactive elements volumes of serum, renal homogenate and mitochondria in old age mice are due to the oral intake of this extract. Thus urging that oxidative stress undergoing would be ameliorated by Amla.

+

Moreover, EtoAc extracts from Amla or sun Amla extracts have substantially prevented up-grades in the COX-2 aorta of aged mice of nitric oxide synthase (iNOS) and cyclooxygenase. Restricting the NF-κB activation in the aged rats, the expression levels of COX-2 and iNOS are leveled down by the EtoAc extracts of Amla or Sun Amla. Ultimately for the prevention of age-associated renal ailments, Amla will come to be a very fruitful antioxidant. Chashan the increase in renal gamma-glutamyl transpeptidase (GGT) activity triggered via hexachlorocyclohexane (HCH) was declined by pre-feeding Amla. Amla feeding was recognized to be an excellent part of a hepatic antioxidant mechanism and to deplete cytotoxic products which could be caressed by feeding HCH.





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WHAT IS PERSONALIZED MEDICINE

A form of medicine that uses information about a person's own genes or proteins to prevent, diagnose, or treat disease. In cancer, personalized medicine uses specific information about a person's tumor to help make a diagnosis, plan treatment, find out how well treatment is working, or make a prognosis. Examples of personalized medicine include using targeted therapies to treat specific types of cancer cells, such as HER2-positive breast cancer cells, or using tumor marker testing to help diagnose cancer. Also called precision medicine.

HEALTH BENEFITS OF AMLA

- **It gives a boost to your immunity:** Vitamin C rich amla can give a boost to your immunity. Vitamin C is important for children, adults, and the elderly.
- **It is good for your heart health:** Content of Vitamin C in amla is great for your heart health. It strengthens and thickens arteries in your heart. This is especially important for people who have high levels of bad cholesterol.
- **Amla is good for skin and hair:** Health skin and hair is vastly dependent on your intake of Vitamin C. Vitamin C helps collagen perform in the right way, thus improving your skin quality and making it tighter. Amla gives you the glowing skin you have always aspired for. You can mix amla powder with yoghurt and apply it as a face mask. For hair, you can mix amla powder with water of coconut oil or sesame oil and massage it deep into your scalp. It can help in getting rid of dandruff, dry skin on your scalp, and improve your hair quality.
- **It reduces inflammation:** Free radicals in the body can damage heart, skin and even hampers our immunity. This is because free radicals cause inflammation, which is basically the root causes of a majority of diseases. Antioxidants in amla help in neutralising free radicals and reduce inflammation in the body.
- **Amla is a rich source of fibre:** You must include fibre in your diet for a healthy digestive system. However, make sure that you don't go overboard with consuming fibre as it can irritate your bowel and increase risks of irritable bowel syndrome. Include just the right amount of fibre in your diet to stay away from constipation, acidity and stomach ulcers. Amla stimulates the production of acid in the stomach, thereby reducing hyperacidity and ulcers.
- **It is great for diabetes:** Amla contains chromium which is great for stabilising your blood sugar levels. It improves the insulin sensitivity of your cells, which can be beneficial for people with type 2 diabetes.

DISCUSSION

Medicinal plants play a key role in our healthcare system as an incredible source of nature medicine from the ancient time. Herbal products have low or no side effect, so use of these products increasing steadily all over the world. A most essential medicinal plant of Ayurveda, an Indian indigenous system of medicine, is Amla. Amla is most important Ayurveda plant, because of its highest vitamin C content property, strong antioxidant property and essential biological property, which is familiar to prevent various numerous health sicknesses. Amla can be used in nutraceuticals and pharmaceutical industries as a food additive. Various extracts and herbal formed from of Amla are applied against various diseases, whose result is very much similar to regular drugs. The traditional use of P.emblica(Amla) is effective almost all of the human diseases, but not all of them have been authorized through clinical research. Now it needs to explore its medicinal values through various biotechnological tools and techniques at its molecular level. It can be act as the miscellaneous subject for the research and development of alternative and complementary medicine in future.

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AMLA USED AS PERSONALIZED MEDICINE

Sl no.	Diseases / Treatment for	Parts used	Preparation/Administration/Dose
1	Boils and spots	Fruit pericarp	Decoction with cow ghee
2	Constipation	Fruit	Pickled or preserved in sugar is the fruit or fresh fruit. Used when dry, every day one or two fruit.
3	Dental problems	Root, leaves, node	The roots of 10 grammes are collected and ground. Take twice a day after dinner.
4	Diabetes	Fruit	Juice tablespoon combined with a cup of bitter gourd juice, Two months every day.
5	Diarrhoea	Fruit, Bark, root, leaves	Fruit decoction is combined with acid or bark of the fruit's astringency. Decoction and development of the root solution create a catechu like astringent extract.
6	Diuretic	Fresh fruit	Fruit paste + saffron +Crocus sativus + Nelumbium speciosum
7	Fever	Leaves, fresh fruit, seed	The leaves and seed decoctions are applied, dried grapes and sugar are applied, or fresh fruits and compounds comprising equal amounts of emblica.
8	Gonorrhoea	Bark	Bark juice is administered in combination
9	Hair growth and prevent greying	Fruit	Fresh fruits or crushed fruits. Dried fruit boiled in coconut oil prevents greying. Dried Amla pieces are soaked overnight in water , nourishing to hair.
10	Eye disorder	Seed	Seed infusion applied externally.





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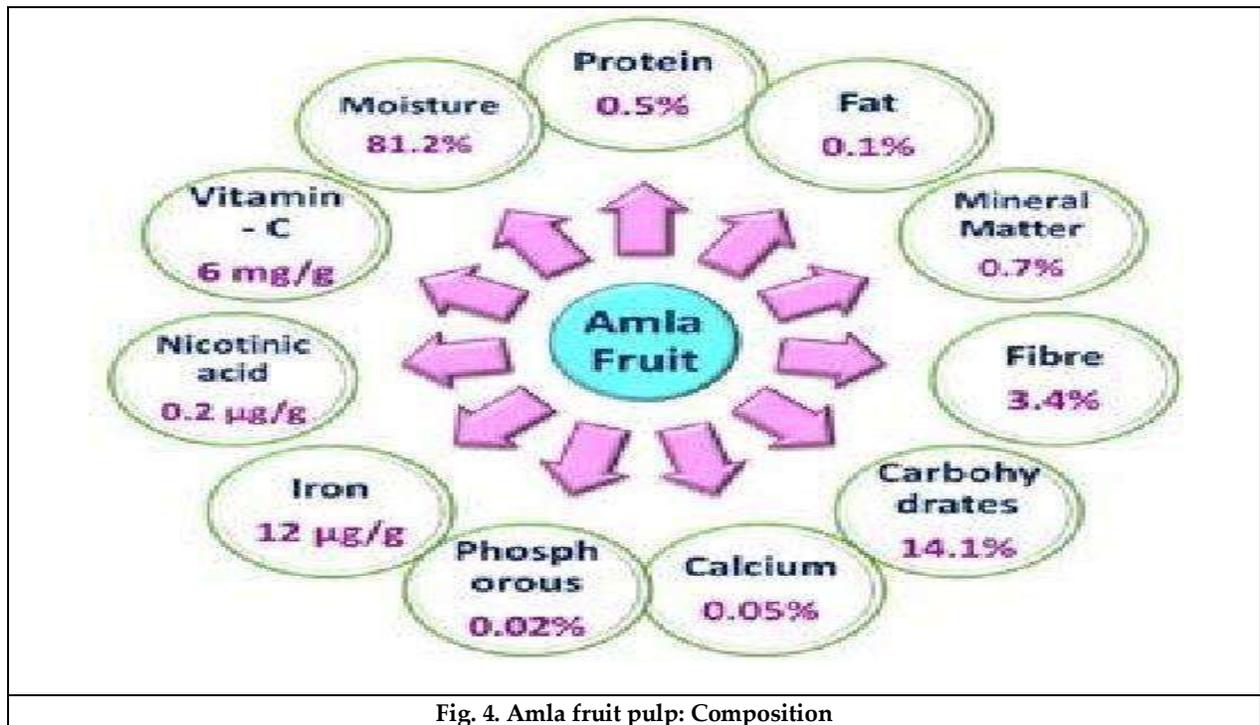


Fig. 4. Amla fruit pulp: Composition





Cocoa as Functional Food

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ABSTRACT

Chocolate has been consumed as confection, aphrodisiac, and folk medicine for many years before science proved its potential health benefiting effects. Main compounds of cocoa and chocolate which contribute to human health are polyphenols that act as antioxidants and have potential anti-inflammatory, cardioprotective, antihepatotoxic, antibacterial, antiviral, antiallergenic, and anticarcinogenic properties. This paper gives a short overview of scientific literature regarding cocoa polyphenols and influence of cocoa and chocolate on human health. Although research on health benefits of dark chocolate and cocoa is quite extensive nowadays and shows potentially beneficial effects of dark chocolate and cocoa, there are still lots of unknowns and some controversies. This is obviously an area that needs more research in order to determine factual influence of chocolate on health. For many years, chocolate was used as a confection, aphrodisiac, and folk medicine before science demonstrated its potential health benefits Polyphenols, which serve as antioxidants and have possible anti-inflammatory, cardioprotective, antihepatotoxic, antibacterial, antiviral, antiallergenic, and anticarcinogenic characteristics, are the main chemicals in cocoa and chocolate that contribute to human health. This study provides a brief overview of the scientific literature on cocoa polyphenols and their impact on human health. Despite the fact that research on the health advantages of dark chocolate and cocoa is rather comprehensive presently and reveals that dark chocolate and cocoa may have potentially beneficial effects, there are still many unknowns and some disagreements. This is clearly an area in which additional research is needed in order to identify the true impact of chocolate on health.



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Keywords: anti-inflammatory, cardioprotective, antihepatotoxic, antibacterial, antiviral, antiallergenic, anticarcinogenic, Cocoa, functional Food.

INTRODUCTION

Humans have been eating cocoa and chocolate for thousands of years. Cocoa pods were sacred to the Mayans as emblems of fertility, life, and godly nourishment. Cocoa was utilised as payment by the Aztecs, who felt it brought them wisdom and strength. Xocolatl was a dark, unsweetened cocoa-based drink prepared by the Aztecs and Mayas. They seasoned it with jalapeño peppers and corn meal, but they had never heard of sugar. Columbus introduced cocoa beans from America to Europe in 1492, although they were uninteresting to Europeans at the time. They had never heard of sugar. Columbus introduced cocoa beans from America to Europe in 1492, although they were uninteresting to Europeans at the time. In 1528, Hernan Cortez brought cocoa to Spain, along with the recipe for Chocolate, Sugar, vanilla, nutmeg, cloves, allspice, and cinnamon were added to the original recipe in Spain, and aphrodisiac properties were discovered. For many years, chocolate was used solely for pleasure, but studies in the last 20 years have revealed that dark chocolate and cocoa may provide health benefits due to their high polyphenol content. Polyphenols are a large mix of biologically active secondary metabolites found in plants that serve as cell wall support materials, colourful attractants for birds and insects, and defence mechanisms in response to various environmental stresses (wounding, infection, excessive light, or UV irradiation) .. They are divided into four groups based on the number of phenolic rings and the structural elements that connect these rings: phenolic acids, lignans (known as phytoestrogens; flaxseed and flaxseed oil are the primary sources), flavonoids (the most abundant polyphenols in human diets), and stilbenes (resveratrol is under investigation for its anticarcinogenic properties). Anthocyanins, flavonols, flavanols (catechins in tea, red wine, and chocolate), flavanones (primary source is citrus fruit), flavones, and isoflavones (major source is citrus fruit).

Cocoa Polyphenols

Flavan-3-ols or catechins, anthocyanins, and proanthocyanidins are the three primary categories of polyphenols found in unfermented cocoa beans, with typical amount of 120–180 g/kg. (-)-epicatechin is the most abundant polyphenol component in fresh cocoa beans, with an average amount of 21–43 mg/g of defatted sample, followed by (+)-catechin and their dimers and trimers. Cocoa bean colour is brown and purple due to complex alteration products of catechin and tannin, and leucoanthocyanins are present as glycosides. In 1528, Hernan Cortez brought cocoa to Spain, along with the recipe for Chocolatl. Sugar, vanilla, nutmeg, cloves, allspice, and cinnamon were added to the original recipe in Spain, and aphrodisiac properties were discovered. For many years, chocolate was used solely for pleasure, but studies in the last 20 years have revealed that dark chocolate and cocoa may provide health benefits due to their high polyphenol content. Polyphenols are a large mix of biologically active secondary metabolites found in plants that serve as cell wall support materials, colourful attractants for birds and insects, and defence mechanisms in response to various environmental stresses (wounding, infection, excessive light, or UV irradiation). They are divided into four groups based on the number of phenolic rings and the structural elements that connect these rings: phenolic acids, lignans (known as phytoestrogens; flaxseed and flaxseed oil are the primary sources), flavonoids (the most abundant polyphenols in human diets), and stilbenes (resveratrol is under investigation for its anticarcinogenic properties). Anthocyanins, flavonols, flavanols (catechins in tea, red wine, and chocolate), flavanones (primary source is citrus fruit), flavones, and isoflavones (major source is citrus fruit).

Counet *et al* research 's demonstrated that genetic characterization influences polyphenol content in cocoa. They discovered that Criollo cultivars contained more procyanidins than Forastero and Trinitario beans. Furthermore, crop season and country of origin have an effect on polyphenols in cocoa beans. The polyphenol content of cocoa beans is greatly influenced by processing. Polyphenols diffuse with cell liquid from storage cells during fermentation and are subjected to oxidation (both nonenzymatic and polyphenol-oxidase-catalyzed), polymerisation, and protein reactions .Anthocyanins are hydrolyzed to anthocyanidins and a sugar component, leucocyanidins are dimerised and (-)-epicatechin and soluble polyphenol content are reduced to 10–20% Hurst *et al.* investigated the



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levels of flavan-3-ol monomers in cacao beans during fermentation, drying, and roasting. They claimed that unripe and only (-)-epicatechin and (+)-catechin are found in ripe cacao pods. Both of these compounds were depleted during fermentation, but (-)-catechin was formed due to heat-induced epimerization. Additional polyphenol loss occurs during drying, primarily due to nonenzymatic browning reactions, Roasting results in significant polyphenol loss due to thermolabileflavanols and quinones, which complex with amino acids and proteins and polymerize with other polyphenols. According to Hurst *et al.*, the loss of (-)-epicatechin and (+)-catechin during this processing step is partially attributed to heat-induced epimerization to (-)-catechin. All of these processes are required to produce the distinctive cocoa aroma. Polyphenols impart an astringent and bitter aroma to cocoa and contribute to sensory panel perception of “cocoa flavour”. However, nowadays, processes are carried out in such a way that as much polyphenol as possible is preserved while maintaining a satisfactory aroma. The composition and content of polyphenols are further altered during the chocolate-making process, owing primarily to relatively high temperatures and the presence of oxygen [8]. As a result, despite having a higher polyphenol content, dark chocolate had comparable antioxidant activity to pomegranate juice.

Bioavailability of Cocoa Polyphenols

Polyphenol bioavailability is generally affected by polyphenol chemical structure, food matrix, food processing factors, and interactions with other constituents in diet, as well as some host-related factors (genetic aspects of individuals, gender and age, disorders and physiological condition, and microbiota metabolism and enzyme activity in the colon). Vegetables and fruits, green and black tea, red wine, coffee, chocolate, olives, and some herbs and spices, as well as nuts and algae, are the richest sources of polyphenol. Furthermore, some polyphenols are specific to specific foods, while others are found in all plant products, so food is generally thought to contain complex mixtures of polyphenols. The most absorbable substances are isoflavones and phenolic acids, followed by catechins, flavanones, and quercetin glucosides, whereas proanthocyanidins, anthocyanidins, and galloylated tea catechins are poorly absorbed. Sucrose increased bioavailability of polyphenols, but formulation also influenced the extent of sucrose impact. Schramm *et al.* observed enhanced uptake of aglycone flavanols when they were consumed immediately after carbohydrate-rich meal. Peters *et al.* concluded that sucrose addition to green tea resulted in delay of catechin absorption, partly due to viscosity increase, but it also improved catechin uptake by intestine.

Influence of Cocoa Polyphenols on Health

Unlike vitamins, polyphenols are not essential components of human diet. Nevertheless, they are consumed on daily basis due to their ubiquitous presence in fruits and vegetables. Many researches have shown that polyphenols and/or polyphenol-rich foods have an important role in health preservation due to antioxidant properties. The antioxidant activity of cocoa and chocolate was shown to be correlated with their catechin and procyanidin contents. Antioxidant properties of polyphenols highly depend on the arrangement of functional groups around the nuclear structure. Free radical scavenging capacity is primarily attributed to hydroxyl groups, and aglycones are more potent antioxidant than their corresponding glycosides. Polyphenols can act as proton donor-scavenging radicals, inhibitors of enzymes that increase oxidative stress, chelate metals, bind carbohydrates, and proteins. These properties enable them to act as anticarcinogenic, anti-inflammatory, antihepatotoxic, antibacterial, antiviral, and antiallergenic Polyphenols. This is supported by research of Hollenberg *et al.* who established relationship between high consumption of cocoa beverages and very low blood pressure levels, reduced frequency of myocardial infarction, stroke, diabetes mellitus, and cancer in Kuna Indians residing in archipelago on the Caribbean Coast of Panama, unlike Kuna Indians residing on Mainland. Another study, conducted on elderly men free of chronic diseases in Zutphen, Netherlands, showed that consumption of cocoa reduced blood pressure and decreased risk of cardiovascular and all-cause death by 45–50%. Grassi *et al.* observed decrease of blood pressure by short-term administration of dark chocolate in healthy and glucose-intolerant, hypertensive subjects. However, they investigated only 15 subjects per research and these findings should be taken with reserve. Djoussé *et al.* associated frequent consumption of dark chocolate with lower prevalence of cardiovascular diseases in men and women independently of traditional risk factors estimated based on health questionnaire. This association was perceived both in smokers and nonsmokers, as well as in subjects under and above 60 years of age. The research included large number of examinees, but data about consumption of chocolate were self-reported and there was no differentiation between dark and milk chocolate.



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Hot cocoa beverage was proven to successfully reduce LDL cholesterol, increase HDL cholesterol, and suppress LDL oxidation in research of Baba *et al.* [43]. Atherosclerotic cholesterol profile (cholesterol:HDL ratio) in patients with diabetes was improved after 8-week chocolate consumption without affecting weight, inflammatory markers, insulin control, or glycaemic control. In addition to lowering blood pressure levels, cocoa polyphenols might be involved in cholesterol control. Waterhouse *et al.* (1996) reported polyphenols from chocolate inhibited LDL oxidation by 75%, compared to 37–65% of red wine (adopted from) In addition, Vinson *et al.* reported that dark chocolate had higher quality of phenol antioxidants expressed as IC50 for LDL + VLDL oxidation compared to red wine and black tea, with high lipoprotein bound antioxidant activity, which is very important in prevention of heart diseases. A survey implemented by a group of experts showed that in the case of similar absorption, about 50 g of dark chocolate should be eaten to provide equivalent flavonoids to about 200 mL of red wine, which has been shown to reduce heart attack risk for an average adult. Flavanol-rich cocoa increases blood flow to key areas of brain increasing blood oxygenation level-dependent response to cognitive task switching paradigm in healthy young people and could be useful in treatment of cerebrovascular flow (CBF) demenstroke, Alzheimer's disease , and stroke . Chandranayagam *et al.* reported that tannin-rich chocolate can be considered as functional food which effectively antagonizes adverse effects of arsenic intoxication. However, this research was conducted on Sprague Dawley rats and should yet be confirmed by research on humans.

CONCLUSION

Recent researches have shown that cocoa and dark chocolate could have beneficial impact on our health, mainly on cardiovascular system. However, part of the researches could be arguable, since either a small number of examinees were included or information about type of chocolate and consumption was scarce and/or ambiguous. In addition, since chocolate is a rich source of sugar and saturated fat, it is questionable whether chocolate consumption can be recommended in vascular health promotion because of its contribution to total calorie intake and impact on weight. More systematic approaches should be applied in human studies to reduce possible misinterpretation of data – more examinees, longer test periods, and larger age differences should be involved in addition to controlled chocolate administration with specified polyphenol content and composition. Individual nutritive preferences which could have great impact on results should also be taken into consideration. Chocolate and cocoa contain not only polyphenols but also methylxanthines which could additionally contribute to the health impact of these foods. There is need for additional researches that would elucidate the extent of polyphenols and methylxanthines health impact and possible synergy of these compounds in chocolate, with respect to energy contribution. Obviously, elucidation of cacao and chocolate impact on human health is rather a complex problem and should be addressed as such data should not be lightly interpreted but closely examined and reassessed before withdrawing conclusions.

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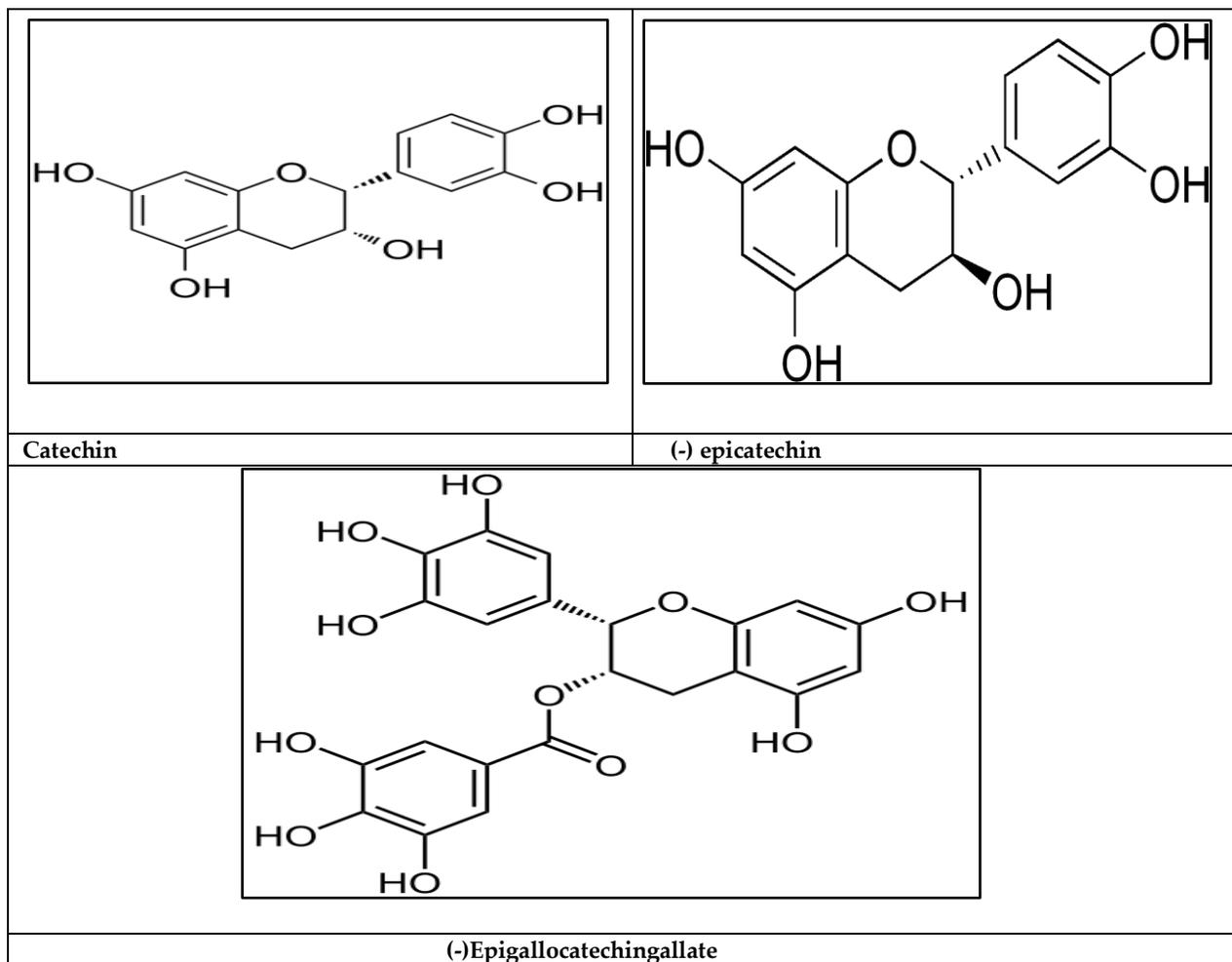
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Role of Ginger in Biofertilizer

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ABSTRACT

Ginger, a famous functional food, has been utilised for ages all throughout the world. Its metabolic activities, on the other hand, remain unknown, posing a barrier to a better understanding of its functional components. The metabolic profiles of ginger in rats were examined using UPLC–Q/TOF-MS in this work. Based on the summarised fragmentation patterns and self-building chemical database, the researchers were able to characterise 92 components of ginger. In addition, four representative molecules were chosen to investigate ginger's usual metabolic routes. As a result, 141 ginger-related xenobiotics were identified, followed by a summary of the pungent phytochemicals' metabolic sites. These findings suggested that *rol* and *-shogaol* were the most potent components of ginger *in vivo*. Meanwhile, their main metabolic reactions were hydrogenation, demethylation, glucuronidation, sulfation, and thiolation. These findings add to our understanding of ginger metabolism, which will aid in the discovery of functional components and additional mechanism study.

Keywords: Ginger, gigerol, biofertilizers

INTRODUCTION

In recent years, functional foods have gained popularity within health and wellness circles. Also known as nutraceuticals, functional foods are highly nutritious and associated with a number of powerful health benefits. For example, they may protect against disease, prevent nutrient deficiencies, and promote proper growth and development. This article looks at the definition, benefits, and potential uses of functional foods. Functional foods are ingredients that offer health benefits that extend beyond their nutritional value. Some types contain supplements



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or other additional ingredients designed to improve health. The concept originated in Japan in the 1980s when government agencies started approving foods with proven benefits in an effort to better the health of the general population. Some examples include foods with vitamins, minerals, probiotics, or fibres. Nutrient-rich ingredients like fruits, vegetables, nuts, seeds, and grains are often considered functional foods as well. Oats, for instance, contain a type of fibre called which has been shown to reduce inflammation, enhance immune function, and improve heart health. Similarly, fruits and vegetables are packed with, which are beneficial compounds that help protect against disease. Nutraceutical and functional food are gaining much popularity and capturing the global market.

Nowadays more than 470 nutraceutical and functional food items are marketed with proven health claims. The statistical analysis of the market shows that worth of nutraceuticals and functional foods was estimated above \$197 billion and 30-60 billion US\$ in Japan and USA respectively. The term functional food was defined as "Food which not only provides basic nutrients but also possesses many therapeutic benefits". The division between functional and nutraceuticals foods is that the nutraceuticals foods are enriched with active components to get targeted health benefits. The utilization of these products not only aimed to get health benefits, but they can also have preventive and curative effects for many ailments ranging from cardiovascular diseases to cancer. Researchers have confirmed that various phytochemicals and bioactive components are present in these indigenous herbs and medicinal plants that ensure their medicinal attribute and thus are an important part of modern functional and nutraceutical foods. Researches have also depicted that a handsome number of phytochemicals and bioactive moieties are present in herbal plants among them ginger has rich phytochemical profile as it possesses nutraceutical potential against various physiological threats especially due to the presence of 6-gingerol.

The inclusions of processed foods, changing living patterns and irregular dietary habits are the leading causes of physiological disorders and diet related complexions like diabetes, cancer, CVD, and high cholesterol levels are increasing day by day. Prevention from these disorders is a major public health concern worldwide especially developed and underdeveloped nations. Then involvement of phytochemicals in diet for diseases prevention was widely spread and documented from ancient times due to their safer and high pharmacological values. Recently, analysis proves therapeutic properties of diet have created a revitalization to improve human health and nutrition research. Nutraceuticals have been claimed to have a physiological benefit or provide protection against many diseases Cardiovascular agents, Antiobese agents, Anti-diabetics, Anticancer agents, Immune boosters, Chronic inflammatory, disorders Degenerative diseases. Among these ailments, very common ones are obesity, CVD, High serum triglyceride level, declined levels of high-density lipoproteins (HDL), hypertension, and impaired glucose tolerance

History of Ginger

Zingiber officinal Roscoe was classified as a member of the Zingiberoside family by Wagner in 1980 and is associated with the Zingiber variety. The name Zingiber comes from the Sanskrit word "zingiber," which refers to the projections on the rhizome. It was given by English botanist William Roscoe. Ginger grows to be a straight, evergreen shrub that grows to be 1 to 4 feet tall. The shoot rises 13 jerks above the ground and is encircled by the two masterminded edge's sheathing bases, which look like leaves. It produces white parties as well as pink bloom buds that develop into yellow fledglings. The design known as rhizome, which fundamentally means horn-shaped, grows ginger in an impartial way, at the edge fixed with extending portion. The entire rhizome has a striated and hard surface. It's around 5 to 16cm long, 1.6 to 6cm wide, and 2.2cm thick, and the masking tone can be yellow, white, or red depending on the approach. Wanger mentions it. South-eastern Asia has ginger as a neighbour. The Arabs knew about the Mediterranean area, and journalists like Discords about 41–91 AD and Pliny the Elder around 25–80 AD portrayed it. Ptolemy discovered that ginger was produced in Ceylon, a city in Sri Lanka, circa 150 AD. During the Middle Ages, raw and protected ginger was imported into Europe. Ginger is one of the key flavours that have been presented from Asia in the fourteenth century England, showing up in Europe for the punch exchanges. Due to their similar taste, the dicots in the Asarum family are commonly referred to as wild ginger. In 2019, the total global production of ginger was 2.8 million tonnes, with India accounting for 34% of total global production. One pound of





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ginger was worth the same as a sheep. Ginger was used by Eclectic doctors in the nineteenth century to promote perspiration, improve craving, and treat disease, as well as a good counterirritant. According to ShrimadBhagwatGeeta's Hirayama, ginger is a key component of Ayurveda, India's traditional medicine, and is known as Sunitha in Ayurveda. It was used to prevent excessive blood hardening in conductors and veins, lower cholesterol, and prevent joint aggravation. In traditional Chinese medicine, ginger is thought to be a sharp, dry, warming punch that can be utilized to relieve discomforts brought on by a chilly, saturated environment. It was also employed to treat depletion issues, disease, going revealed, toothache, snakebite, and respiratory conditions, as well as a stomach-related guide and antinausea fix. Ginger is a long-lasting flavor that is frequently prepared in tropical areas and occasionally naturalizes. It mostly multiplies vegetatively since different cultivars only seldom sprout is fruitless (Suvarna et al. 1999; Flowers of India, 2016). It is listed as invading in Taiwan's Invasive Species Database (Taiwan Invasive Species Database, 2016) and is a weed in Puerto Rico and Queensland, Australia.

Ginger

Ginger (*Zingiberofficinale*) commonly known as “Adrak” widely used in Pakistani and Indian cuisines over 2500 years .It belongs to family “Zingiberaceae” which is very famous due its medicinal herbal plants like, cardamom and turmeric . It has been cultivated in South-East Asia from thousands of years. After that, it gains much popularity in European and African countries due to its therapeutic effects. Currently, the ginger and its products are used in many traditional medicinal systems, due to its rich phytochemistry and diseases preventive properties Ginger is known by different names in different languages like in Arabic “Zanjbeel” Frech “Gingembre” etc.Ginger is advanced and modified name of different words. Historically on the basis of its appearance linked with Sanskrit word “Srngaveram” means “Horn root”, in Greek with “Ziggiberis” and in Latin, “Zinziberi”. In Indo-Pak in Urdu it is known as “Adrak

METHODS AND MATERIALS

Three promising lines of *Zingiber officinale* var. rubrum L, i.e. Balitro 1, Balitro 2, and Balitro 3, were grown in Cibinong and Sukamulia experimental stations from October 2005 to August 2006. These experimental stations represent two different agroecological conditions. The first site (in Cibinong) is located in 220 m above sea level (asl.), soil type is classified as Latosol and the soil fertility was low. The second site (in Sukamulia) is located in 450 m asl. Type of soil is Latosol, and the soil fertility was medium. The crops were planted with standard operational procedures of good agricultural practices for ginger, except for fertilizers application. Fertilizers applied were 10 ton compost + 90 kg bio fertilizer + 300 kg Zeolite + 300 kg rock phosphate per hectare. The treatments were arranged in Randomized Block Design with 3 treatments and 9 replications. The planting space was 60 x 40 cm. Observed parameters were crop yield (fresh and dry weights) and quality of dried rhizome.

RESULTS AND DISCUSSIONS

Crop yield Yields of three promising lines of ginger were significantly affected by ecological conditions. Yield of ginger grown at Cibinong ranged from 3.82 to 4.48 ton fresh ginger/ha, while those at Sukamulia ranged from 5.91 to 7.31 tons fresh ginger/ha. These differences may be due to soil conditions of each experimental site. Some characteristics of Sukamulia soil are relatively higher in C-organic content (2.15%) and its sand fraction (50.62%) compared to those of Cibinong soil. These are likely to be main factors and resulting in the difference in yield of rhizomes. Sudiarto and Gusmaini (2004) reported that organic material content of soil play an important role on the rhizome growth. In addition, high sand fraction of soil texture in Sukamulia induces more loose soil aggregation. The rhizome usually grows and expands optimally in loose soil. The low yield of ginger rhizome at Cibinong site was also caused by pest infestation. During the growing period, about 25% of ginger population was infested by *Phylosticta* sp. As a result, ginger yield at Cibinong decreased about 30% compared with ginger yield at Sukamulia. shows that actual yields of three promising lines were relatively different. The difference was noted when ginger was cultivated in optimal condition. The yields of three promising lines in Cibinong were not different. On the other



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hand, yields of three promising lines cultivated in Sukamulia were significantly different, where Balitro 3 showed higher yield (7.31 ton fresh rhizome/ha or 1.07 ton dried rhizome/ha) than the other two lines. The result also showed that ginger yield treated with biofertilizer and rock phosphate were below of their potential yield. Bermawie et al. (2004) reported that potential yields of the three tested clones were about 10 tons/ha that is mean the ginger yield only reached about 70% of its potential yield if treated with biofertilizers and rock phosphate. This result demonstrates that the use of organic fertilizers and natural minerals may substitute the use of chemical fertilizers. It seemed that application of 10 tons compost + 90 kg bio fertilizer + 300 kg Zeolite + 300 kg rock phosphate per hectare provide ginger nutrient requirement. Application of rock phosphate combined with phosphate solubilizing bacteria in the form of biofertilizer increased P availability. Some active microorganisms of the biofertilizers are *Azospirillum lipoferum* Beijerincki, *Azotobacter vinelandii* Beijerincki, *Aeromonas punctata* Zimmermann, and *Aspergillus niger* van Tiegham (Yusrondan Januwati, 2005). Supanjani et al. (2006) reported that integration of P rocks with inoculation of P-solubilising bacteria increased P availability from 12 to 21% in the soil as compared with control, and subsequently improved nutrient (N, P, and K) uptake by the plant. The availability of applied nutrients was the dominant factor affecting to rhizome growth. Compost and rock phosphate are categorized as slow release fertilizers, so all the nutrients given can not be taken optimally by the crop. Some of nutrients might be in form of organic compounds, which are not readily available for crop growth. On the other hand, chemical fertilizers are rapid release fertilizers, so nutrients can be used by plant efficiently.

Ginger quality

Quality of dried rhizomes of three promising lines of *Z. officinale* var. *rubrum*. The essential oil content in ginger cultivated in Cibinong ranged from 4.17 to 4.43%, while in Sukamulia from 2.78 to 3.35%. The similar pattern was also found in gingerols content. Gingerols content of ginger grown in Cibinong was higher (0.188-0.225%) compared to those in Sukamulia (0.016-0.109%). This indicated that syntheses of essential oil and gingerols were strongly affected by crop growth conditions. It seems that under environmental stress conditions plant synthesizes more constituents. Medicinal plant species mostly react to their environment by secreting secondary metabolites (Pedneault et al., 2005). Environmental stresses arise from conditions that are unfavorable for the optimal growth and development of organisms (Levitt, 1972; Guy, 1999). Environmental stresses can be classified either as abiotic or biotic. Abiotic stresses are produced by inappropriate levels of physical components of the environment, including water stress. Pedneault et al. (2005) reported that phenolic compound concentration from *T. officinale* was 6.2 times higher in fields (31.2 mg/g dry weight) compared to hydroponics (5.0 mg/g dry weight)

CONCLUSION

The increasing demand of nutritional therapies motivates the researchers and processors of food to introduce some food products with therapeutic potential. Although, many investigations are done still areas need to be explored to find out the role of therapeutic components, their mechanism of action, the effect of processing, application, and safety. Moreover, garlic demonstrated superior effects as compared to ginger alone. Several studies reported the lipid-lowering effect of ginger (Shoail et al., 2016); a meta-analysis-based study reported the favorable effect of ginger on Triglycerol level and LDL-C while having no effect on TC and HDL-C. Moreover, the low dose of ginger was found to have a greater lowering influence on total cholesterol and Triglycerols. Ginger is also thought to be capable of combating common influenza viruses and influenza-like symptoms. Fresh ginger in the airway epithelium proved effective against plaque formation induced by a human respiratory syncytial virus (HRSV). Due to its properties, ginger is also developed to improve its functionality in the form of nanoparticles as a drug delivery with various advantages to increase prevention.

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Neem as Personalized Medicines

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ABSTRACT

Azadirachta indica, also known as neem, has gained worldwide reputation in recent years. Because of its extensive spectrum of medical benefits, Neem is widely utilized in Ayurveda, Unani, and Homoeopathic treatment, and has become a modern medical cynosure. Neem produces a wide range of physiologically active chemicals that are both chemically and structurally varied. From various portions of the neem plant, more than 140 chemicals have been identified. The leaves, blossoms, seeds, fruits, roots, and bark of the neem tree have long been used to cure inflammation, infections, fever, skin ailments, and dental problems. The therapeutic properties of neem leaf have been documented in detail. Immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, and antiviral properties have been demonstrated in neem leaf and its compounds.

Keywords: *Azadirachta indica*, Phytochemical constituents, chemical constituents, anti-oxidant activity, Medicinal properties

INTRODUCTION

Azadirachta indica is a fast-growing evergreen tree that can be found in, Africa, and North America. Because of its therapeutic characteristics, it has been utilized in Ayurvedic medicine for over 4000 years. In Sanskrit, neem is known as 'arista,' which means 'perfect, complete, and imperishable.' Arishtha has been sanskrit name for the neem tree, which means 'reliever of sickness,' and is thus known as 'Sarbarogaribarini. The neem tree is primarily grown on the Indian subcontinent. Meliaceae, the mahogany family, includes neem. Humans utilized neem widely to treat a variety of diseases. Humans have been using neem since prehistoric times. It's a deciduous tree that loses a lot of its





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leaves in the dry winter months. The branches are long and splayed out. The crown is dense and roundish, with a diameter of 20–25 m (66–82 ft). The upright, pinnate leaflets are 20–40 cm (8–16 in) long, with 20–30 medium to dark green leaflets ranging in length from 3–8 cm. Neem trees can grow to be 15–30 metres tall (49–98 feet), with rounded crowns and thick grooved bark. The serrated leaflets of the compound leaves are usually evergreen, but they do fall during periods of intense drought. The omnipotent tree neem is a holy gift from nature. The fruit has a sweet-tasting flesh and is a silky yellow-green drupe. The neem tree is a remarkable plant that has been designated by the United Nations as the "Tree of the Twenty-First Century". In 1992, the National Academy of Science of the United States produced a paper titled "Neem: A Tree for Solving Global Problems." It is one of the medicinal plants with a wide variety of therapeutic benefits, and every part of the tree is utilized as medicine both locally and in pharmaceutical businesses after preparation. It kills bacteria, fungi, viruses, ticks, and mites, among other microorganisms and ectoparasites.

Taxonomical classification

Kingdom :	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order :	Sapindales
Family :	Meliaceae
Genus :	<i>Azadirachta</i>
Species :	<i>A. indica</i>

Chemical Constituents of Neem

Azadirachtaindica L., *Azadirachtaindica* L., *Azadiracht* Due to its abundant source of numerous types of components, (neem) plays a therapeutic role in health management. *Azadirachtin* is the most active ingredient, followed by *nimbolinin*, *nimbin*, *nimbidin*, *nimbidol*, *sodium nimbin*, *gedunin*, *salannin*, and *flavonoid*.

Leaves: It contain *nimbin*, *nimbanene*, *6-desacetylnimbinene*, *nimbandiol*, *nimbolide*, *ascorbic acid*, *n-hexacosanol* and *amino acid*, *17-hydroxyazadiradione*, and *nimbiol*. It's also contain *quercetin* and β -*sitosterol* and *polyphenolic flavonoids*.

Seeds: It include important components such as *gedunin* and *azadirachtin*.

Bark: It consists of *Nimbn* (0.04%), *nimbinin* (0.001%), *nimbidin* (0.4%), *nimbosterol* (0.03%), *essential oil* (0.02%), *tannins* (6.0%), a bitter principle *margosine*, and *6-desacetyl nimbinene*

Neem oil: *Resins*, *glucosides*, and *fatty acids* are all contained in *neem oil*, as well as *sulphur* (0.427 percent), a bitter yellowish molecule generated from the alcoholic extract of the oil, which is assumed to be an *alkaloid*; and *resins*, *glucosides*, and *fatty acids*.

Flowers: It have been found to contain a *flavonoid*. The compounds *nimbicetin* and *kaempferol* are interchangeable. The bitter components identified in dried bark are the same as those found in seed oil, and the bitter component *bakayanin* has been discovered in the pericarp of the fruit.

Phytochemicals in neem

Neem can be thought of as a "storehouse" for a variety of phytochemicals. A total of 300 phytochemicals have been isolated from the neem tree. *Isoprenoids* and *non-isoprenoids* are the two most important groups of phytochemicals extracted from diverse regions of neem. The most well-known *isoprenoids* are *diterpenoids*, *vilasinins*, *triterpenoids*, and *limonoids*, while *non-isoprenoids* include *proteins*, *carbohydrates* (*polysaccharides*), *sulphur compounds*, *tannins*, *polyphenolics* such as *flavonoids* and their *glycosides*, *dihydrochalcone*, *coumarin*, *aliphatic compounds* and





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phenolic acids. The leaves, fruit, seeds, oil, roots, bark, and twigs of the neem tree contain a great number of phytochemicals that have been proven to have antibacterial, antiviral, antifungal, anti-inflammatory, antiplasmodial, antiseptic, antipyretic, anti-diabetic, and anti-ulcer characteristics. Oil extracts are the most often utilised form of Neem, and comprehensive phytochemical investigation has verified the presence of high levels of triterpenes, flavonoids, and saponins, as well as lower levels of catechins and nimbins. Dash, Dixit, and Sahoo (2017) conducted a study involving leaf extracts (aqueous and methanoic) and discovered high quantities of saponins, tannins, and glycosides in the aqueous extracts. Alkaloids, tannins, and flavonoids were found in the highest amounts in methanoic extracts. When tested on human subjects, the leaf of the Neem tree appeared to have generated a particular collection of glycoproteins known as neem leaf glycoprotein (NLGP), which showed immune-modulatory activity, perhaps inhibiting tumour progression by influencing local and systemic immunity.

Antioxidant Activity of Neem

Because of their relevance, antioxidant molecules have recently garnered a lot of attention. Antioxidant substances limit auto oxidation through a radical scavenging method in which they provide one unpaired electron to a free radical, lowering the amount of free radicals. Many Indonesian medicinal plants, such as neem, have active chemical components that have antioxidant properties (*Azadirachta indica* A. Juss). This plant has a long history of usage in both natural insecticides (biopesticides) and natural medicine. The goal of this research was to find active chemical components in neem leaf extracts, as well as to determine neem leaf antioxidant activity and the best solvents for extracting active compounds from neem leaves. Free radicals, also known as reactive oxygen species, are one of the leading causes of disease. On the other hand, neutralising free radical activity is an important step in disease prevention. Antioxidants aid in the stabilisation and deactivation of free radicals before they attack targets in biological cells, as well as the activation of antioxidative enzymes that aid in the regulation of free radical and reactive oxygen species damage. Medicinal herbs have been found to have antioxidant properties. Fruits, seeds, oil, leaves, bark, and roots of plants play an important role in disease prevention due to their high antioxidant content. The main cause of disease is free radicals, also known as reactive oxygen species. The neutralisation of free radical activity, on the other hand, is a key step in disease prevention. Antioxidants help to stabilise and deactivate free radicals before they assault targets in biological cells, and they also help to activate antioxidative enzymes that help to regulate the damage produced by free radicals and reactive oxygen species. Antioxidant activity has been reported in medicinal plants. Because of their high antioxidant content, plants' fruits, seeds, oil, leaves, bark, and roots play a significant role in disease prevention.

What is Personalized Medicine

A type of medication that prevents, diagnoses, or treats disease by using knowledge about a person's own genes or proteins. Personalized medicine in cancer uses particular information about a person's tumour to aid in diagnosis, therapy planning, determining how well medication is appropriate, and determining a prognosis. Targeted medicines to cure particular forms of cancer cells, such as HER2-positive breast cancer cells, are examples of personalised medicine, as is tumour marker testing to aid in cancer diagnosis. Precision medicine is another term for it.

Health Benefits of Neem

The neem tree, also known as '*Azadirachta indica*,' is an Indian tree. In Sanskrit, the word neem is arista, which means "perfect, imperishable, and complete." The tree's seeds, roots, and bark, in addition to its leaves, contain essential chemicals with medicinal and cosmetic benefits. In Ayurveda, the tree is thought to represent 'excellent health.' The health advantages of neem leaves, a traditional powerful medicine, have been discussed for years. It would be unusual to find an Indian home or community without a neem tree. So, over 130 distinct types of biological components, such as nimbin and nimbandial, are found in neem leaves, which assist the body heal and promote a healthy lifestyle. The anti-aging benefits of neem are well-known. Neem defends the skin from harmful UV radiation, pollution, and other environmental causes because to its antioxidant capabilities. Neem's nutrients and fatty acids help to retain skin suppleness and diminish wrinkles and fine lines. You and your skin will appear



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revitalised and youthful as a result of this. Neem is also useful in the treatment of fungal infections. Its antimicrobial and antifungal qualities keep hazardous bacteria and fungi at bay. As a result, it protects the skin and keeps skin disorders at bay. For better health and well-being, Ayurveda, which uses natural methods for therapy and healing, has been using neem tree extracts as a crucial element. Some of neem's herbal cures, health advantages, and magical characteristics are listed below;

Neem used as Personalized Medicines**Pharmacy under a single tree**

Neem tree extracts have been used in numerous home cures used by Indians since time immemorial, not just in Ayurvedic medicine. Neem is used to cure hair and skin problems.

Antifungal and antibacterial properties

The leaves of the neem tree are used to cure bacterial and fungal illnesses. They're used to treat warts and chicken pox, among other things. The paste is either applied to the affected area or the individual is forced to bathe in neem water. It can also be used to treat fungal infections in the feet.

Beneficial to your bones

Milk isn't the only thing that helps you create strong bones. Also the neem leaves are high in calcium and minerals, which aid in the formation of strong bones and the reduction of inflammation. Neem leaves and neem oil are commonly administered to elderly patients to treat arthritic pain and any stiffness or pain that comes with age in traditional medicine. Massaging the skin with neem oil on a regular basis might also assist to promote good bone health.

Insecticide

Toward off insects, keep neem-soaked cotton near your windows or burn neem leaves. It is highly effective and is used to combat the mosquito problem.

Assists with oral hygiene

Our oral hygiene prevents the accumulation of hazardous pathogens and infection-causing bacteria. With its active qualities, neem acts to reduce the growth of microorganisms and germs in your body. For the same reason, many people like chewing neem leaves on a daily basis. It also keeps your breath fresh, keeps your saliva pH balanced, and protects your teeth from decay. Neem is an active ingredient in several types of toothpaste for the same reason.

Enhances immunity

Many Ayurvedic specialists advise taking neem pills on a daily basis. Neem tea is also commonly used to treat fevers, particularly those caused by malaria. Because neem is bitter, the tea takes on a similar flavour, but it works like magic.

The toothbrush of nature

It is an age-old Indian habit to chew neem twigs for oral hygiene and care. People in Indian households used to wash their teeth with neem twigs. These days, you may get neem-based toothpaste to keep your teeth healthy. It protects against all types of dental infections and disorders thanks to its antibacterial, anti-inflammatory, and antifungal qualities.

Hair that is thick and lengthy

Neem also improves the condition of hair and encourages hair development. Neem paste can also be used to condition hair. Neem is a great approach to get rid of dandruff because of its antibacterial, antifungal, and anti-inflammatory characteristics. This strengthens hair follicles, which promotes hair growth. It gives the roots the necessary food and training, making them stronger and more lustrous.





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Treat a skin disorder

Ayurveda has a number of formulations for treating skin problems. It's because it's a natural detoxifier. Sczema and other skin infections are treated with it.

Neem's healing properties

Neem has the ability to heal wounds without leaving unsightly scars. It also helps to avoid septic infections. Because of its antibacterial characteristics, neem is widely used to treat wounds. Apply a small amount of neem oil to wounds and scars on a daily basis. Neem Oil is high in essential fatty acids, which aid wound healing and keep your skin healthy.

Acne treatment

Neem is also anti-inflammatory, which helps to treat acne. Neem oil is thought to help with skin dryness, itching, and redness. It also helps to keep acne and skin imperfections at bay.

Other advantages of neem

Neem is a good moisturiser for the skin as well. The fatty acids and vitamins in neem oil hydrate and condition your skin, making it look clearer and younger. Neem oil contains vitamin E, which helps to repair damaged skin while also limiting the effects of environmental changes that might cause skin damage. Neem is often used in bath powders, shampoos, skin lotions, and toothpaste, and several companies have begun promoting neem leaf capsules for improved immunity. With the threat of dengue fever looming, it's also a great way to keep insects at bay.

CONCLUSION

Natural products or their derivatives are becoming increasingly popular in the treatment and prevention of diseases due to their lack of negative effects. Neem and its constituents have therapeutic properties and have been utilised in traditional medicine around the world, particularly in the Indian Subcontinent, since ancient times. Clinical investigations have shown that neem is effective in preventing a variety of disorders. Active components have been shown to have a chemopreventive effect in a variety of tumours by modulating many cell signalling pathways. To understand the actual mechanism of action in illness management, a detailed study based on animals should be conducted.

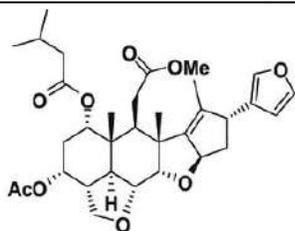
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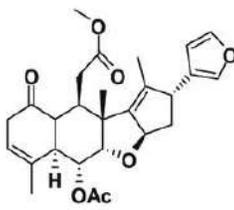


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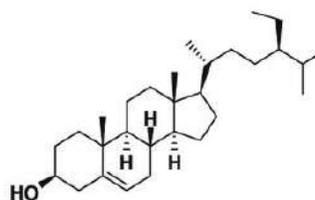
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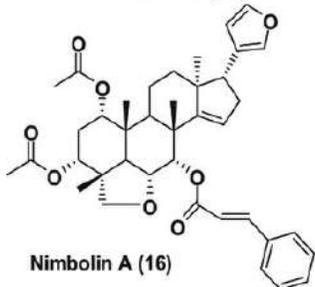
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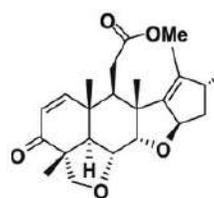
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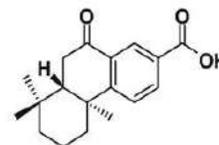
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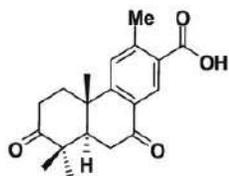
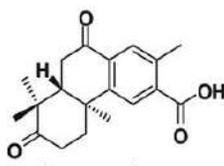
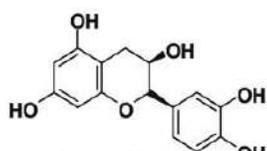
Nimbolin A (16)



Azadiramide A (17)

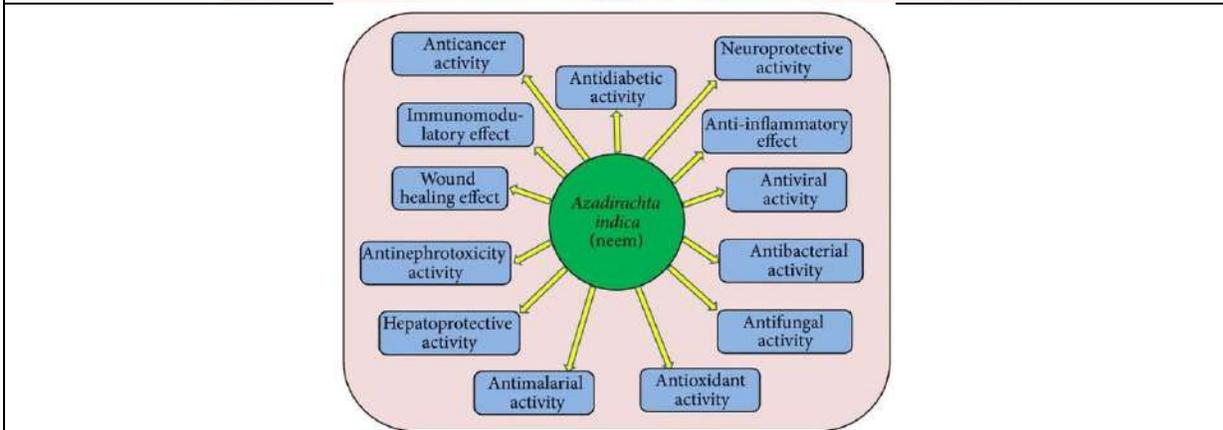


Margolone(18)

Margolonone
(19)Isomargolonone
(20)Catechin
(21)epicatechin
(22)quercetin
(23)



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The Benefits of Ginger in Pharmaceuticals

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ABSTRACT

Ginger, a famous personalised medicine, has been utilised for ages, all throughout the world. Its metabolic activities, on the other hand, remain unknown, posing a barrier to a better understanding of its functional components. The widely used spice ginger contains important biochemical compounds such as phenolics, terpenes, polysaccharides, lipids, organic acids, and raw fibers. Based on the summarised fragmentation patterns and self-building chemical database, the researchers were able to characterise 92 components of ginger. In addition, four representative molecules were chosen to investigate ginger's usual metabolic routes. As a result, 141 ginger-related xenobiotics were identified, followed by a summary of the pungent phytochemicals' metabolic sites. These findings suggested that [6]-gingerol and [6]-shogaol were the most potent components of ginger in vivo. Meanwhile, their main metabolic reactions were hydrogenation, demethylation, glucuronidation, sulfation, and thiolation. These findings strengthen the understanding of ginger metabolism, which will aid in the discovery of functional components and additional mechanism studies. This review article is mainly focused on the importance of phytochemicals present in ginger for combating chronic diseases.

Keywords: phytochemicals, xenobiotics, phenolics, terpenes, antinausea, anticancerous





INTRODUCTION

Over the last few years, the usage of "natural" or alternative treatments has skyrocketed. In the belief that complementary and alternative medicine, nutritional supplements and herbal medicines will have a favourable effect, an increasing number of older persons are utilising them without consulting a physician. Ginger (*Zingiber officinale*) is one of the most extensively used dietary condiments in the world today. It is a member of the Zingiberaceae family, which includes medicinal plants such as turmeric and cardamom (Barrett et al, 1990). Due to its numerous health advantages, ginger is getting popularity day by day as a component of making personalized medicine. Ginger is made up of rhizomes, which are underground stems that grow horizontally and develop roots underneath while sprouting leaves and new stems on top. Buds appear at regular intervals along each stem. Ginger is not only well-known as a spice and an essence but also one of the oldest herbal and aromatic cures, particularly in Asian countries such as China and India, as well as the Middle East. It has been used to treat inflammation and diarrhoea in China for more than 2,000 years. Ginger is directly employed in traditional Asian food to add flavour and soft drinks such as coffee and tea by slicing it up or simply making use of its powder. The essential oil present in the enticing slice of ginger has been treasured and exploited by perfume manufacturers since antiquity. Ginger is a tropical plant native to the Indo-Malaysian rain forests. The optimum growing condition for ginger is in lush, damp, tropical environments. The ginger cultivation is said to have begun in southern Asia, but it has now expanded to East Africa and the Caribbean. Ginger is a perennial plant and it produces beautiful crimson blooms in a variety of shapes, including torch and honeycomb that are often used to decorate booths, dwellings, and other areas in South Pacific seasonal festivals.

History of ginger

Cardamom and turmeric are members of the same plant family as ginger. The presence of ketones, particularly gingerols, which appear to be the principal component of ginger examined in much health-related scientific research, contributes to its pungent scent. The rhizome, or horizontal stem from which the roots grow, is the most commonly consumed part of ginger. Ginger's current name stems from the Middle English word *gingivere*, while the Sanskrit word *srngaveram*, which means "horn root," refers to its look and dates back over 3000 years. It was known as *ziggiberis* in Greek and *zinziberi* in Latin. Ginger is unusual in that it does not grow wild and its origins are unknown. Ginger has been used as a tonic root by Indians and Chinese for over 5000 years to treat a variety of diseases, and the plant is currently grown across the humid tropics, with India being the major producer. Long before history was written, ginger was utilised as a flavouring ingredient. It was a highly valuable commodity that was brought from India to the Roman Empire over 2000 years ago, when it was prized for its medical virtues. Even after the fall of the Roman Empire, ginger remained a highly sought-after item in Europe, with Arab merchants leading the trade in ginger and other spices for centuries. A pound of ginger was equivalent to the cost of a sheep in the thirteenth and fourteenth centuries. It was being imported in preserved form to be used in sweets. The gingerbread man, which became a favourite Christmas treat, is linked to Queen Elizabeth I of England. Ginger is burned-through worldwide as zest, seasoning specialist, embellishment, medication, and food additive and is carried out each day, in a brand-new glue, or dry, in a dry powder. New ginger can be substituted for dried ground ginger, albeit the forms of new and dried ginger are quite unique. The heady perfume of ginger is entering into and sweet-smelling. Inside the subcontinent, like India and Pakistan, ginger is known as "Adrak" (neighbourhood name) and is an important ingredient in a variety of recipes.

Uses of Ginger

Fresh, dried, pickled, preserved, crystallised, candied, and powdered or ground gingers are just some of the ways it is utilised. The scent is powerful and spicy, with a peppery and slightly sweet flavour. Since the concentration of essential oils in ginger increases over time, the collection period is determined by the rhizome's intended usage. If obtaining oil is the main objective behind ginger production then it can be picked at 9 months or longer. Ginger is frequently pickled in sweet vinegar that gives it a pink colour and makes it popular in sushi. The root of ginger picked at 8-9 months has a tough skin that must be removed before eating, and it is used dry or pulverised into





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powdered ginger. This is the most prevalent kind in our spice cabinets, and it's used in biscuits, cakes, and curry powder. Ginger that has been candied or crystallised is boiled in sugar syrup and then dusted with granulated sugar. The rhizomes are soft with a mild flavour and are best used fresh or preserved. The five months old unripe ginger has a very thin peel. Ginger has recently been discovered to have biological properties such as antioxidant (Nile and Park, 2015), anti-inflammatory (Zhang et al, 2016), antibacterial (Kumar et al, 2014) and anticancer (Citronberg et al, 2013). Ginger can pave the path to suppress and cure chronic diseases such as neurodegenerative diseases (Ho et al., 2013), cardiovascular diseases (Akinyemi et al, 2015) obesity (Suk et al, 2017) diabetes (Wei et al, 2017), nausea and respiratory infections.

Bioactive Components

Ginger has a lot of active ingredients such as phenolic and terpenoids. The phenolic compounds of ginger are mainly comprised of gingerols, shogaols, and paradols (Mao et al, 2019). Gingerols, such as 6-gingerol, 8-gingerol, and 10-gingerol, are the most abundant polyphenols in fresh ginger (Mao et al, 2019). The conversion of gingerols to shogaols is induced through heat treatment or long-term preservation (Jung et al, 2018). Shogaols can be converted to paradols after hydrogenation (Stoner, 2013). Some other phenolic compounds such as quercetin, zingerone, gingerenone-A, and 6-dehydrogingerdione are also found in ginger (Schadich et al, 2016; Ji et al, 2017). Additionally, ginger includes numerous terpene components, such as -bisabolene, -curcumene, zingiberene, -farnesene, and -sesquiphellandrene, that are the major ingredients of ginger essential oils. In addition to these, ginger contains polysaccharides, lipids, organic acids, and raw fibres (Mao et al, 2019).

Ginger as medicine for pregnancy

The usage of ginger, which has been the most extensively, used herbal treatment in the therapy of pregnancy-related nausea and vomiting for decades, is a common source of exposure during pregnancy. In addition, ginger is supposed to promote human health and strengthen the immune system, which has led to an increase in popularity in recent years. Ginger is now commonly found in everyday items sold in Danish supermarkets, such as teas and shots. The usage of ginger as a health supplement may lead to increasing and ongoing intake during pregnancy.

CONCLUSION

Ginger is not only a popular dietary condiment for flavouring food, but it is also a medicinal herb that has been used to treat a range of maladies for thousands of years. According to chemical and metabolic investigations, ginger contains hundreds of chemicals and metabolites. Gingerols and shogaols, particularly [6]-gingerol and [6]-shogaol, have been the most widely researched bioactive components. The content of each component is clearly influenced by the ginger rhizome's origins and treatment. Over the last few years, there has been a significant surge in research interest in establishing the role of natural chemicals in disease prevention. Despite the abundance of research studies, many of the findings are based on phenomena and provide descriptive and observational data rather than mechanistic data. More research on the kinetics of ginger and its constituents in animals and people, as well as the consequences of long-term ingestion, is needed. It is necessary to identify specific molecular targets and methods of action.

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- Helps with digestion
- Immunity booster
- Reduce muscle pain and soreness
- Prevents cancer
- Reduces nausea
- Heals ulcers
- Anti-fungal property
- Improve diabetes

Fig: List of health benefits of ginger





Gastroprotective Effects of “NEEM”

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ABSTRACT

Neem (*Azadirachta indica* A. Juss) is one of the most versatile medicinal plants, widely distributed in the Indian subcontinent. Neem is a rich source of limonoids that are endowed with potent medicinal properties predominantly antioxidant, anti-inflammatory and anticancer activities. The Vedas called Neem, "Sarvaroganivarini", which means one that cures all ailments and ills. This tree is considered to be of divine origin. According to Indian Mythology, amrita (ambrosia or the elixir of immortality) was being carried to heaven and a few drops of it fell on the Neem tree. *A. indica* shows therapeutic role in health management due to rich source of various types of ingredients. The most important active constituent is azadirachtin and the others are nimbolin, nimbin, nimbidin, nimbidol, sodium nimbin, gedunin, salannin and quercetin. The chemicals present in neem have medicinal promise. Among the chemicals, the most commonly employed medicinal agent is triterpenes. Antipyretic, fungicidal, antihistamine and antiseptic effects have been demonstrated for nimbin (triterpene). Its easy to get anti-oxidants from neem and cost-effective method to include natural extracts to your diet such as those derived from Neem, which can be found in teas and other beverages. It boosts up dental and oral health and other potential benefits. Drink up a glass of neem juice every morning to get rid of toxins from your body. It purifies blood and improves blood circulation. According to Ayurveda, neem is one of the best herbs for diabetes, bleeding disorders, strength and stamina promoter. Neem is also used as anti ageing, sunscreen and general purpose for skin care products. It is used as a hair tonic. Applications of neem are leprosy, eye disorders, bloody nose, intestinal worms, stomach upset, loss of appetite, skin





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ulcers, cardiovascular diseases, fever, diabetes, gingivitis and liver problems. Its leaf is used for birth control and to cause abortions.

Keywords: *Azadirachta indica*, therapeutic role, anti-inflammatory, anticancer.

INTRODUCTION

Mother nature has gifted mankind with tremendous medicinal plants to create a disease free and healthy life. Abundant medicinal plants are present in the Indian traditional systems of medicine (like Ayurveda, Unani and Sidha), mostly used one amongst them in Neem/Indian liliac (*Azadirachta indica*), belongs to the family Meliaceae, which is an important medicinal herb in Ayurveda and Unani systems of medicine. The incidence of peptic ulcer disease (PUD) has declined in the recent years although the economic burden, morbidity and mortality due to the disease are huge. Peptic ulcer is a break in the gastric or duodenal mucosa that arises when the normal mucosal defensive factors are impaired or are overwhelmed by aggressive luminal factors such as acid and pepsin. There are three major causes of peptic ulcer disease: non-steroidal inflammatory drugs (NSAIDs), chronic *Helicobacter pylori* infection and an acid hyper secretory state such as Zollinger-Ellison syndrome. Over 99% of peptic ulcers are caused by infection with *H. pylori* or by uses of NSAIDs.² Aspirin, which inhibits cyclooxygenase, is rapidly deacetylated to salicylate. Salicylate is toxic to cells and affects mucosal barrier function, reduces cytosolic adenosine triphosphate, stimulates sodium transport, and increases proton dissipation from surface epithelial cells. Cyclooxygenase inhibition makes the gastric mucosa more susceptible to injury, inhibits mucus and bicarbonate secretion, alters the physicochemical nature of mucus, stimulates fundic but not antral [3H] thymidine incorporation, and reduces epithelial surface hydrophobicity.³ It decreases mucin, surface active phospholipids, bicarbonate secretion and mucosal proliferation and also produces damage by formation of free radicals.⁴ Several agents that enhance the healing of peptic ulcers may be divided into three categories: 1) Acid anti secretory agents a) H₂ receptor blocker (ranitidine, famotidine, cimetidine) b) Anti muscarinic agent (pirenzepine, telenzepine) c) Proton pump inhibitor (omeprazole, lansoprazole, pantoprazole), 2) Mucosal protective agents (sucralfate, carbenexolone, prostaglandin analogue), and 3) Agents that promote eradication of *H. pylori*.⁵ Neem or *Azadirachta indica* is native to tropical South East Asia. It is a tree which has been used for thousands of years in agricultural and medicine fields for its beneficial properties. Neem has been extensively used in Ayurvedic, unani and homeopathic medicine.⁶ Neem oil, bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, constipation, rheumatism, chronic syphilitic sores, skin diseases and malaria.^{7,8} Neem oil and leaf contain mainly glycerides and 2% bitter principles. Among them Nimbidin, Nimbin, Nimbinin and Nimbidiol are important. Neem leaves contain some ascorbic acid and some amino acid also.⁹ The values of the leaves have been proven by their immune modulatory, anti-inflammatory, anti hyperglycemic, anti ulcer, anti malarial, anti fungal, anti bacterial, anti viral, antioxidant, antimutagenic and *anti* carcinogenic property.¹⁰ Although to prevent gastric damage, many of the drugs are available like PPI, H₂ blocker, sucralfate, they also have adverse effect and are also expensive. Newer drugs are continuously searched worldwide which have minimum adverse effect and maximum benefit. We therefore planned to evaluate gastroprotective potential of neem leaves extract following consumption of aspirin.

Chemical Constituents

Nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol, amino acid, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol are chemical components identified in the leaves of neem. The cellular matrix of the seeds contains 40–45 percent of the oil in neem seeds. The oil is brownish yellow in colour, non-drying, and has an acerbic flavour and odour (Johnson and Morgan 1997). Neem oil contains several useful bioactive compounds from the limonoids class of triterpenoids.



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Nimbn (0.04%), nimbinin (0.001%), nimbidin (0.4%), nimbosterol (0.03%), essential oil (0.02%), tannins (6.0%), a bitter principle margosine, and 6-desacetyl nimbinene are all found in the trunk bark. Tannins (12-16%) and non-tannins (12-16%) are found in the stem bark (8-11 percent). Sulphur (0.427%), a bitter yellowish compound derived from the alcoholic extract of the oil, which is thought to be an alkaloid; resins; glucosides; and fatty acids are all found in neem oil. Flower- A flavonoid has been discovered in flowers. Nimbicetin and kaempferol are the same thing. The bitter components present in the dried bark are the same as those found in the seed oil, and in the bitter component bakayanin was discovered in the fruit's pericarp.

Traditional Uses Of Neem

The herbal remedies from medicinal plant like neem are used traditionally since the dawn of civilization. WHO has estimated that as many as 80% of world's population rely on herbal traditional medicines as their primary health care. Over 3000 years, the neem tree is well known in India and its neighbouring countries. Neem products are believed by Siddha and Ayurvedic practitioners to be anthelmintic, antifungal, antidiabetic, antibacterial, contraceptive and sedative. It is considered as a major component in Siddha medicine and Ayurvedic and Unani medicine and is particularly prescribed for skin diseases. All the parts of neem plant are used against various human ailments as traditional medicine for household remedies. This tree was usually used as natural pesticide, planting and afforestation as shade trees and to protect against erosion and definitely as medicinal plants. Neem plants are used to cure different ailments such as stomach ulcers, jaundice and to overcome a variety of infections and parasitic diseases, ranging from leprosy, chicken pox and malaria. Infusions and teas made from leaves are used to alleviate malaria attacks, intestinal complaints, treat dental, headache, stimulating the appetite, heartburn and as insects repellent.

List of Neem's traditional applications are given below-

Wound healer: Make a paste out of the neem leaves and dab it on your wounds or insect bites a few times a day till it heals.

Goodbye dandruff: Boil a bunch of neem leaves till the water turns green, allow it to cool. After washing your hair with shampoo, cleanse it with this water.

Eye Trouble: Boil some neem leaves, let the water cool completely and then use it to wash your eyes. This will help any kind of irritation, tiredness or redness.

Treat that zit: Grind a few neem leaves, make a paste and apply it daily till the acne dries out. The paste also helps any kind of eruptions, dark spots and chronic ulcers.

Ear ailments: Blend some neem leaves and add some honey to it. Use a few drops of this mix to treat any ear boils. Other skin disorders: Turmeric combined with a paste of neem leaves can also be used for itching, eczema, ring worms and some mild skin diseases.

Boost immunity: Crush some neem leaves and take them with a glass of water to increase your immunity.

Other Uses Of Neem

1.Treats Acne- Neem has an anti-inflammatory property which helps reduce acne. Azadirachta indica also helps reduce skin blemishes.

2. Nourishes Skin- Neem is a rich source of Vitamin E which help repair damaged skin cells.

3. Treats Fungal Infections- Neem has scientifically proven antifungal property which helps treat fungal infections.

4. Useful in Detoxification- Neem can prove useful in detoxification both internally and externally. Consumption of neem leaves or powder stimulates kidneys and liver increasing the metabolism and eliminating the toxins out of the body. Externally, neem scrubs or paste can be used to remove germs, bacteria, dirt, etc from your skin preventing rashes and skin diseases.

5. Increases Immunity- The herbal remedies from medicinal plant like neem are used traditionally since the dawn of civilization. WHO has estimated that as many as 80% of world's population rely on herbal traditional medicines as their primary health care. Over 3000 years, the neem tree is well known in India and its neighbouring countries. Neem products are believed by Siddha and Ayurvedic practitioners to be anthelmintic, antifungal, antidiabetic,



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antibacterial, contraceptive and sedative. It is considered as a major component in Siddha medicine and Ayurvedic and Unani medicine and is particularly prescribed for skin diseases. All the parts of neem plant are used against various human ailments as traditional medicine for household remedies. This tree was usually used as natural pesticide, planting and afforestation as shade trees and to protect against erosion and definitely as medicinal plants. Neem plants are used to cure different ailments such as stomach ulcers, jaundice and to overcome a variety of infections and parasitic diseases, ranging from leprosy, chicken pox and malaria. Infusions and teas made from leaves are used to alleviate malaria attacks, intestinal complaints, treat dental, headache, stimulating the appetite, heartburn and as insects repellent.

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Boost immunity: Crush some neem leaves and take them with a glass of water to increase your immunity.

Ayurvedic Description Of Neem

In the world of Ayurveda, neem is a popular medicinal herb that has been part of traditional remedies that date back almost 5000 years. Neem is considered a boon for mankind by nature. Use of neem has been recommended by Ayurveda for a wide range of diseases. Such usage are attributed to its purification effect on blood. Scientific research on neem demonstrates it to be a panacea. It is suggested to be an antibacterial, anthelmintic, antiviral, anticancer and more importantly immuno modulatory agent. Quietens all three doshas: Vata, kapha and Pitta and is especially effective for Pitta. Based on this, neem has been considered the best of the Ayurvedic rejuvenative herbs.

Potential Therapeutic Applications Of Neem

Neem has been said to be useful against many several diseases, including cancer, diabetes, hepatic disorders, as well as heart diseases. A list of scientifically explored therapeutic and pharmacological applications of neem are presented in the figure given below. Neem has been widely used in treatment of diseases because it is a rich source of antioxidants. It contains 60 different types of biochemically active components including steroids and terpenoids. Various parts of neem tree are known to possess antifungal, anticancer, anti fertility and other biological activities. Neem is said to be effective in treatment of malaria,, tuberculosis, rheumatism, arthritis, jaundice, parasitic diseases as well as skin treatments. The extracts of various plant parts have also been found to be beneficial in cardiovascular diseases, hepatitis, fungal infections, psoriasis, eczema, lice and ulcers. Neem is also used in cosmetic products for treatment of acne and pimples and improving skin elasticity. It prevents indigestion and controls acidity. It is a natural source of anti-ageing. Even though neem is a known herb in Ayurveda and several other indigenous systems of medicine, some of its medicinal properties are yet to be explored and evidenced scientifically for human use.

CONCLUSION

Now a days, research on Indian traditional medicinal plants has gained a new commerce. Although the other systems of medicine are effective, they come with a number of undesired effects that often lead to serious complications. Being natural, herbal medicine alleviates all these problems. *Azadirachta indica* (Neem) has an





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important position in Ayurveda - an Indian indigenous system of medicine. Neem due to the presence of countless phytochemicals and biological properties, prevent innumerable health disorders. It can be used as a possible food additive or in nutraceuticals and bio pharmaceutical industries. Several researchers revealed that various extracts and herbal formulations of neem showed potential therapeutic benefits against various diseases and the results are similar to standard drugs. In this review, we have tried to make a summary - The traditional and scientifically proven uses of neem and also tried to establish their basic mechanisms. Even though, neem has various medicinal properties since ages, there is a colossal necessity to scientifically explore and evident its medicinal values .

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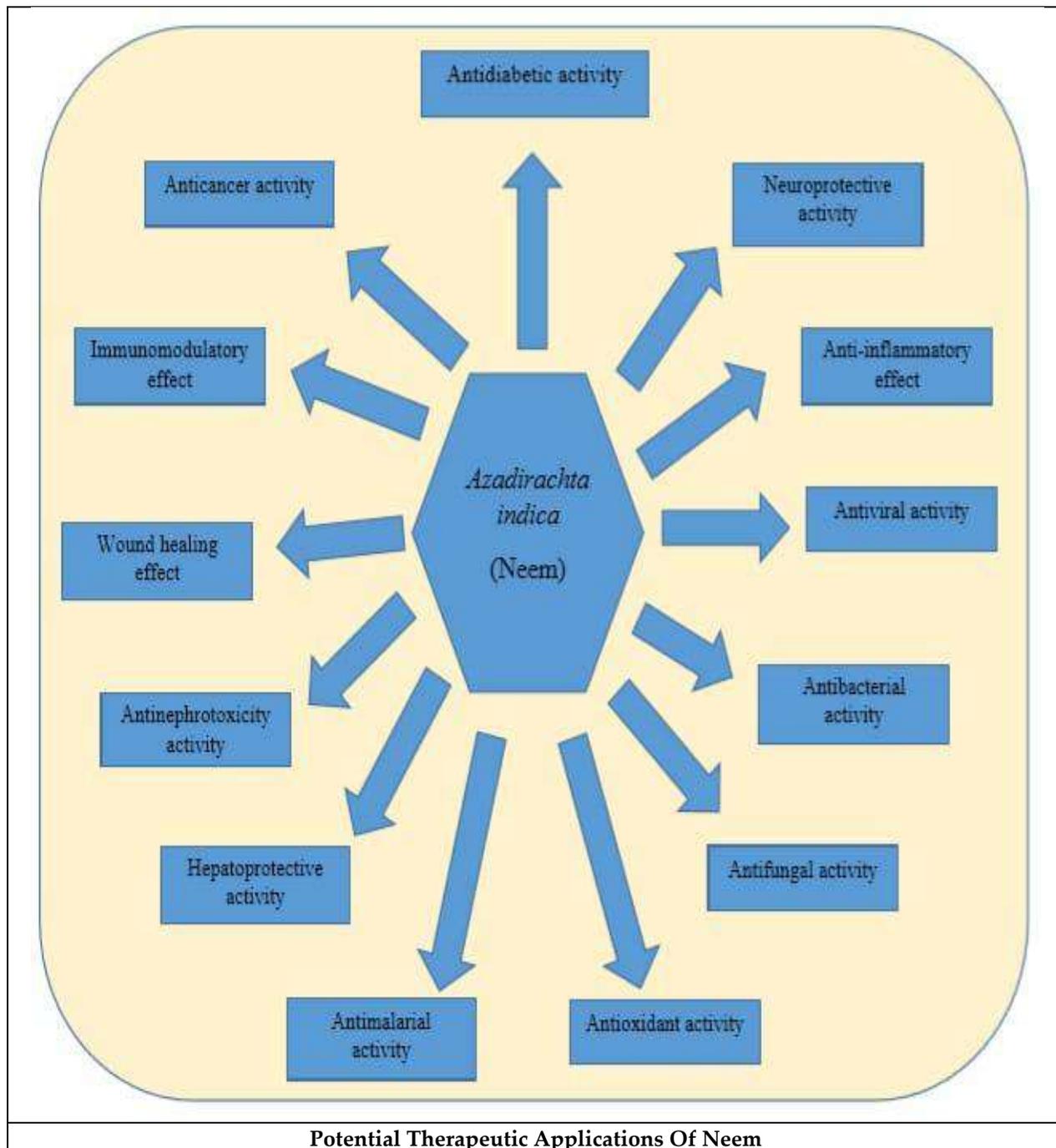
Part	Medicinal uses
Leaf	Leprosy, eye problem, epistaxis, intestinal worms, anorexia, biliousness, skin ulcers
Bark	Analgesic, alternative and curative of fever
Flower	Bile suppression elimination of intestinal worms and phlegm
Fruit	Relieves piles, intestinal worms, urinary disorders, epistaxis, phlegm, eye problem, diabetes, wounds and leprosy
Twig	Relieves cough, asthma, piles, phantom tumor, intestinal worms, spermatorrhea, obstinate urinary disorders, diabetes
Gum	Effective against skin diseases like ring worms, scabies, wounds and ulcers
Seed pulp	Leprosy and intestinal worms
Oil	Leprosy and intestinal worms
Root, bark, leaf, flower and fruit together	Blood morbidity, ulcers, burning, biliary afflictions, leprosy, skin sensation and itching

Ayurvedic Description Of Neem





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Tulsi as a Biofertilizer and Biopesticide

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ABSTRACT

Tulsi has an incredible regular clinical properties/behaving property towards the bringing down the glucose level in body, cardiovascular sickness (due to having the property of lessening the cholesterol), it is likewise brings down the circulatory strain, it is additionally has been utilized for vexed stomach, cough, cold, protozoal disease and headache. It has an extraordinary immunomodulatory property (Due to presence of nutrient C), it acts an enemy of disease, alleviates fever, battle's acne, eye health, oral wellbeing, diminishes respiratory issues, Kidney-stones, advances wellbeing heart. The most fascinating property of tulsi is it is used as partner in nursing insect repellent (Due to presence of ursolic corrosive in tulsi which goes about as partner in nursing against richness (preventative) agent. For the development of these medicative plants we utilize some normal biofertilizers. The microorganisms present in natural manure reestablish the normal supplement pattern of the dirt and construct natural matter. The most utilization of the natural microorganisms are to advances the development of medicative plants. "plant development advancing rhizobacteria" (PGPR). it is extremely useful for advancing the dirt ripeness and reestablishing the regular supplement to establish necessities by giving natural supplements intensive microorganisms and their items and don't contain synthetic substances that are harmful to the living soil.

Keywords: *Ocimum sanctum*, Tulsi, Holy Basil, Medicinal plant, Antimicrobial, Hypoglycaemic, Immunomodulatory, Anti-stress, Antioxidant, Anti-inflammatory, biofertiliser, biopesticide

INTRODUCTION

Plants are one of the most significant wellspring of medicines. Among them *Ocimum* species having a place with the family Lamiaceae are vital for their remedial possibilities. *Ocimum sanctum* Linn. (Tulsi), *O. gratissimum* Linn. (Slam Tulsi), *O. canum* Sims (DulalTulsi), *O. basilicum* Linn. (Boycott Tulsi), *O. kilimandscharicum* Guerke (Camphor



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Basil), *O. americanum* Linn. (Ancient Basil) and *O. micranthum* Willd. are examples of known significant species. Among them Holy Basil, *Ocimum sanctum* has been well recorded for its therapeutic potential. Tulsi is a fragrant shaggy enduring growing up to 1.5 m in tallness with profusions of white blooms and marginally purple colored foliage. This herb has been referred to from as soon as the Vedic period and is held holy by the Hindus. Furthermore, is frequently planted around sanctuaries and utilized in rosaries. It is native to India, arrived at Western Europe in the sixteenth century. In several antiquated frameworks of medication including Ayurveda, Greek, Roman, Siddha and Unani, *O. sanctum* has tremendous number of therapeutic applications for example, in cardiopathy, haemopathy, leucoderma, asthma, bronchitis, catarrhal fever, otalgia, hepatopathy, lumbago, hiccups, ophthalmia, gastropathy, genitourinary problems, ringworm, verminosis and skin illnesses, and so on. It is, normally utilized in cough, cold, mild indigestion, heartburn, lessened hunger and disquietude. Other names of tulsi are: it is known as Tulasi in Sanskrit language, Holy Basil in English. Different names employed for Tulsi area product Krishna tulsi, Manjari, Thulasi, Tulsi and Trittavu.

Botanical Identification of *Ocimum Tenuiflorum*

Tulsi plants area product unremarkably found throughout Asian nation. The plant shall grow crazy in tropical heat areas. Plant height is about 2-4 feet. Flowering begins in the winter time (to February) december. Tulsi leaves have a marked Associate in Nursing decisive aroma and an style that is astringent. There area product some substances which are biologically active Ursolic acid, luteolin and apigenin which will be removed from Tulsi leaves. Though it's the leaves that area product typically utilized, the flowers, seeds and origins conjointly realize smart use. There area unit 2 main forms of Tulsi: the dark selection or Krishna Tulsi and therefore the selection that is light-weight avatar Tulsi. Tulsi conjointly includes a connection that is shut Thai basil. This is glabrescent and swish as opposed to the Tulsi that is slightly furry. Also, Thai basil includes a anise that is robust licorice scent whereas Tulsi or Holy Basil includes a lemon like and spicy taste that is resembling the taste of clove.

Morphology of Tulsi

Holy basil is an erect, many-expanded subshrub, 30-60 cm tall with bushy stems. Leaves are green or purple; they are straightforward, petioled, with an applaud cutting edge up to 5 cm long, which generally has a marginally toothed edge; they are unequivocally scented and have a decussate phyllotaxy. The purplish blossoms are put in close whorls on stretched racemes. The three primary morphotypes developed in India and Nepal are Ram tulsi (the most widely recognized type, with expansive radiant green leaves that are somewhat sweet), the more uncommon purplish green-leaved (Krishna or Shyamtulsi) and the normal wild vanatulsi (e.g., *Ocimum gratissimum*).

Chemical Composition of Tulsi

The chemical composition of Tulsi is very complicated. It is eugenol, or 1-hydroxy-2-methoxy-4-allylbenzene. This chemical formula contains many phyto-chemicals referred as compounds. These numerous compounds present in entire plant consist of antioxidant, adaptogenic, anti-inflammatory, antibacterial and immune-enhancing properties. With these properties when anyone consumes Tulsi in any form their body gets prepared to fight against the diseases and other health problems. Some of the phytochemical constituents of tulsi are oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, and β -caryophyllene (about 8%). Tulsi essential oil consists mostly of eugenol (~70%) β -elemene (~11.0%), β -caryophyllene (~8%), and germacrene (~2%), with the balance being made up of various trace compounds, mostly terpenes.

Benefits of Tulsi

Normal Immunity Booster: Tulsi is rich in Vitamin C and zinc. It thus acts as a natural immunity booster and keeps infections at bay. It has immense anti-bacterial, anti-viral and anti-fungal properties which protect us from a variety of infections. Tulsi leaves extract increases the T helper cells and natural killer cells activity, boosting the immune system.

Reduces Stress & Blood Pressure: Tulsi contains compounds *Ocimumosides A* and *B*. These compounds reduce stress and balance the neurotransmitters serotonin and dopamine in the brain. Anti-inflammatory properties of Tulsi reduce inflammation and blood pressure.



**Niharika Patnaik and Preetha Bhadra****Anti-cancer properties**

Phytochemicals present in Tulsi have strong antioxidant property. Thus, they help in protecting us from skin, liver, oral and lung cancers.

Useful in Kidney stones & Gouty Arthritis

Tulsi detoxifies the body and has diuretic properties. It decreases the level of uric acid in the body, which is the main reason why kidney stones are formed. Reduction in uric acid levels also provides relief to patients suffering from Gout.

Good for Heart Health

Tulsi has a profound effect on treatment and prevention of cardiovascular diseases by means of lowering blood lipid content, suppressing ischemia and stroke, reducing hypertension, and also due to its higher antioxidant properties.

Medicinal Properties of Tulsi

Antioxidant activity: It has significant ability to scavenge highly reactive free radicals. Antioxidant bioassay-directed extraction of fresh leaves and stems of tulsi extract yielded: cirsilin, cirsimaritin, isothymusin, isothymonin, apigenin, rosmarinic acid and appreciable quantities of eugenol. Eugenol is a major component of the volatile oil, and other compounds also demonstrated good antioxidant activity. **Anti-inflammatory activity:** Gas liquid chromatographic analysis of fixed oil of *O. sanctum* revealed the presence of five fatty acids (stearic, palmitic, oleic, linoleic and linolenic acids). The triglyceride fraction of the oil showed higher protection compared to fixed oil against carrageenan-induced paw edema and acetic acid-induced writhings in rats and mice, respectively. Linolenic acid present in *O. sanctum* fixed oil has the capacity to block both the cyclo-oxygenase and lipoxygenase pathways of arachidonate metabolism and could be responsible for the anti-inflammatory activity of the oil.

Antimicrobial activity:

The narrowest spectrum of antibacterial activity was observed in *sanctum*. The crude aqueous extract of leaf possesses some antibacterial and immunomodulatory active principles. *Neisseria gonorrhoeae* clinical isolates and WHO strains were found to be sensitive to extracts. Aqueous extract of the plant showed growth inhibition for *Klebsiella*, *Escherichia coli*, *Proteus* and *Staphylococcus aureus*. Alcoholic extract showed growth inhibition for *Vibrio cholerae*. The ethanolic extracts from the leaves showed better activity against the β -lactamase producing methicillin-resistant *Staphylococcus aureus* strains. The essential oil also showed potent anthelmintic activity in the *Caenorhabditis elegans* model. **Anti-ulcer activity:** Holy basil is reported to possess potent anti-ulcerogenic as well as ulcer-healing properties and it is due to its ability to reduce acid secretion and increase mucous secretion. The fixed oil of tulsi was found to possess significant anti-ulcer activity against Aspirin-, Indomethacin-, alcohol-, histamine-, reserpine-, serotonin- and stress-induced ulceration in experimental animal models. Significant inhibition was also observed in gastric secretion and Aspirin-induced gastric ulceration in pylorus ligated rats. The lipoxygenase inhibitory, histamine antagonistic and antisecretory effects of the oil could probably have contributed towards anti-ulcer activity.

Biofertilizer

A biofertilizer is a substance which contains living miniature organic entities which, when applied to seeds, plant surfaces, or soil, colonize the rhizosphere or the inside of the plant and advances development by expanding the inventory or accessibility of essential supplements to the host plant. Biofertilizers add supplements through the regular cycles of nitrogen fixation, solubilizing phosphorus, and animating plant development through the amalgamation of development advancing substances. The miniature life forms in biofertilizers reestablish the dirt's normal supplement cycle and construct soil natural matter. Using biofertilizers, solid plants can be developed, while upgrading the supportability and the wellbeing of the dirt. Biofertilizers can be anticipated to decrease the utilization of engineered manures and pesticides, however they are not yet ready to supplant their utilization. Since they



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assume a few parts, a favored logical term for such advantageous microbes is "plant-development advancing rhizobacteria" (PGPR).

Role of Tulsi as a Biofertilizer

Natural composts add wholesome components through typical natural cycles, solubilizing phosphorus and invigorating plant development through the shaping of development advertising substances. Microorganisms in regular composts reestablish the normal supplement time frame related with the dirt and fabricate soil normal matter. The work of normal manures ensures better plant development, rising the house and imperativeness of this dirt. A most smoking clinical term for accommodating microorganism that fill fluctuated roles is "plant development advancing rhizobacteria" (PGPR). they region unit exceptionally supportive in improving soil ripeness and meeting plant supplement necessities by giving normal supplements through microorganisms and their item and don't have synthetics that are terrible for living soil (Vessey, 2003). Kalmegh, Ashwagandha, Tulsi, sort Azadirachta, Curcuma, Ocimum, unpracticed leaflike veggies and so on they're fundamental medicative blossoms brought up in old Ayurvedic abstract works. These medicative blossoms region unit created in fluctuated medicine that treat temperature to extreme jaundice and cardiopathy to disease growths.

Blossoms region unit likely the most inventory of micronutrients and supplements. They're lower in fat, worked in dietary fiber, made in supplement B, supplement C, K and metal, in like manner as containing bioactive mixtures like phenolic pitch compounds, flavonoids, alkaloids, tannins, lignans, phenols, terpenes and so forth. Numerous phytochemicals like xanthophyl, β -cryptoxanthin, carotenoid and β -carotene are isolated and perceived. They're great for rising invulnerable perform and diminishing the chance of disease and cardiopathy. Rejuvenating ointments eliminated from the leaves of *Ocimum sanctum* L. are situated to forestall the extension of *E. coli*, *B. anthracis* and *P. aeruginosa*. Tulsi has against tuberculous action and represses the development of *M. TB* and conjointly has antifungal and antiviral assignment (Unander et al., 1990). The double blend and ethanolic concentrate of spinach an unpracticed green has shown strong inhibitor and hostile to diarrheal exercises. Extra metabolites become cell reinforcements that region unit wide used in wellbeing supplements as they are analyzed for the obstruction of illnesses like malignant growth cancers, coronary cardiopathy as well as hypoxia and even have really a couple of modern applications as additives in food and corrective cosmetics items. Sporadic and low germination is the most drawback inside the spread of numerous medicativeplants. The applying of manures like synthetic composts builds efficiency and development regardless declines soil conditions and conjointly impacts individuals. The enormous and ill-advised use of substance manures winds up in an expedient crumbling of this physical, synthetic and natural properties of the dirt. To stay away from the remainder of the harmfulness of synthetic composts, utilizing natural cultivating techniques is consistently better.

Bio-manures region unit environmental and lessen these abnormalities and further develop germination. They conjointly downsize substance data sources and upgrade yield each subjectively and quantitatively. Crafted by regular composts was accounted for to be incredible for developing veggies and grains by a few staff (Hadas and Okon, 1987). The dynamic elements of bio-composts rely on the overall yield of biomass that more is reliant upon harmless to the ecosystem condition qualities, with respect to the strategy of regular agrotechniques, on water organization and conjointly in regards to the utilizations of manures. That the improvement in regards to the yield, the development of the dirt design, the actual properties and particularly its liquid maintenance capacity will generally be accomplished by normalizing precise cultivating and rehearses uniquely with significance those boundaries could be the great asset inside the jungles as well concerning its property. The work of natural excrement alongside natural manures conveys a decent chance to extend crop creation with lower costs. Most regular composts contain microorganisms like *Rhizobium*, *Azotobacter*, *Azospirillum* and eubacteria (BGA). Bio-composts region unit regular natural manures that embrace fertilizer, slurry, worm castings, peat, green growth, fertilizer, humic substance and discharge and so forth moreover as bio-separates like *Rhizobacteria*, *Azotobacter*, *Azospirillum*, phosphate solubilizing microorganism, castor and *Trichoderma*.





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Biopesticides: Biopesticides, a constrictor of 'natural pesticides', incorporate a few kinds of bug the executives mediation: through savage, parasitic, or synthetic connections. The term has been related generally with natural vermin control - and by suggestion, the control of living life forms. Administrative positions can be impacted by open insights, Hence, in the EU, biopesticides have been characterized as "a type of pesticide in light of miniature organic entities or normal items". The US EPA expresses that they "incorporate normally happening substances that control bothers (biochemical pesticides), microorganisms that control bugs (microbial pesticides), and pesticidal substances delivered by plants containing added hereditary material (plant-joined protectants) or PIPs". They are gotten from organic entities including plants, microorganisms and different microorganisms, parasites, nematodes, and so forth. They are in many cases significant parts of coordinated bug the board (IPM) programs, and stand out enough to be noticed as substitutes to engineered synthetic plant insurance items (PPPs).

Role of Tulsi as a Biopesticide

Farming has an influence that is vital a very agricultural nation like Asian country. Additionally to satisfying the suppers needs of India's general population that continues to develop it conjointly plays a task in up the nation's economy. The utilization of upset innovation somewhere in the range of 1960 and 2000 aggregated wide kinds of harvest yields per region unit, expanding food offer in non-industrial nations by 12-13%. Geographic region and Asian country were the essential agricultural nations to bring up the effect of GR on rice yield assortments. Inputs like composts and pesticides have really helped tons with this regard. Anyway as anyone might expect truth, suppers weakness and condition that is financial win inside our country. Crafted by compound biopesticides and composts has set off an effect that is negative the climate by contacting soil richness, water hardness, development of bug resistance, genetic variety in plants, aggregated unhealthful deposits through the normal sensation and creature sustenance, thusly expanding medical issue and bunches of extra. This made it critical to present measures fit for taking advantage of the said difficulties.

The work of biopesticides and biofertilizers will assume a very part that is significant a very addressing these difficulties in home approach. Pesticides and Environmental Safety Biopesticides square measure natural science pesticides that square measure present substances that administration bothers with non-harmful instruments. Biopesticides live creatures (normal adversaries) or their thing (phytochemicals, microbic items) or results (semiochemicals) which is utilized for the treatment of unsafe plant parasites. They result less dangers to your current circumstance and wellbeing that is person. Extra usually used biopesticides dwell life forms, that action that is square to the parasite of interest. These typify biofungicides (Trichoderma), bioherbicides (Phytophthora) and bioinsecticides (Bacillus thuringiensis). There are additionally various plant thing which could as of now be appropriately utilized being an inventory that is extreme of. The protectives remembered for the plant typify substances that square measure grew normally in the adjustment that is hereditary of. The joining of the Bt series, PI, lectins, chitinase etc to the plant appointment all together that the transgenic plant combines substance obliterates the designated pesterer as models square measure. The potential edges to agribusiness and wellbeing that is public through crafted by biopesticides square measure wide. The consideration in biopesticides uses the gigantic advantages with respect to item that is such are:

- Intrinsically less destructive and less weight that is natural.
- Intended to zero in on exclusively a chose parasite or, now and again, certain objective creatures.
- typically compelling in appallingly sums which are minuscule rarely breaks down apace, subsequently bringing about less perceivability and for the most part starts by staying away from contamination proborganism.
- At the point when utilized as a component of Integrated Pesterer Management (IPM) programs, biopesticides will contribute considerably. Biopesticides 193.

Biopesticides in Asian country Biopesticides address totally two or three 0.89% (like in 2005) of the compound market in Asian country and is anticipated to increment altogether in the years which can return. In India, to the genuine point exclusively twelve sorts of biopesticides are enrolled underneath the bug powder Act, 1968. Nim tree





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pesticides upheld *Eubacteriumthuringensis*, NPV and *Trichoderma* square measure the numerous biopesticides utilized and made in Asian country. Though north of a hundred ninety artificial materials measure that is square to be used as substance pesticides. Most biopesticides measure that is square in open wellbeing, aside from some that square measure found in agribusiness. Besides, i) transgenic plants and ii) supportive organic entities known as bioagents: they're valuable for pesterer the board in Asian country.

DISCUSSION

Tulsi is used to treat insect bites. Tulsi is also used to treat heart disease and fever. Tulsi is also used to treat respiratory problems. Tulsi is used to cure fever, common cold and sore throat, headaches and kidney stones. It is an erect plant with branched sub-shrubs. The leaves are green coloured with a sharp aroma and flavour. Tulsi leaves are oval-shaped with a slightly sharp tip, and the edges are slightly toothed.

CONCLUSION

Tulsi has been broadly utilized for relieving different illnesses because of its extraordinary remedial. Various pharmacological impacts like hypoglycaemic, immunomodulatory, antistress, hostile to fiery, hostile to ulcerogenic, against hypertensive, CNS depressant, radioprotective, antitumour and antimicrobial of *O. sanctum* have been concentrated by different specialists. These investigations help in laying out a logical reason for helpful purposes of the plant. Notwithstanding, considerably more studies are as yet expected to investigate other likely exercises of this plant.

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Role of Ginger in Personalized Medicine

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ABSTRACT

Ginger, a famous personalised medicine, has been utilised for ages all throughout the world. Its metabolic activities, on the other hand, remain unknown, posing a barrier to a better understanding of its functional components. The metabolic profiles of ginger in rats were examined using UPLC-Q/TOF-MS in this work. Based on the summarised fragmentation patterns and self-building chemical database, the researchers were able to characterise 92 components of ginger. In addition, four representative molecules were chosen to investigate ginger's usual metabolic routes. As a result, 141 ginger-related xenobiotics were identified, followed by a summary of the pungent phytochemicals' metabolic sites. These findings suggested that [6]-gingerol and [6]-shogaol were the most potent components of ginger in vivo. Meanwhile, their main metabolic reactions were hydrogenation, demethylation, glucuronidation, sulfation, and thiolation. These findings add to our understanding of ginger metabolism, which will aid in the discovery of functional components and additional mechanism study.

Keywords: Ginger, Colon Cancer, personalized medicine

INTRODUCTION

Over the last few years, the usage of "natural" or alternative treatments has skyrocketed. In the belief that complementary and alternative medicine nutritional supplements and herbal medicines will have a favourable effect, an increasing number of older persons are utilising them without consulting a physician. Ginger (*Zingiber officinale*) is the most extensively used dietary condiment in the world today, believe it or not. It's actually a member of the same plant family as turmei-ic and cardamom, which explains why the health benefits of ginger are so remarkable. Ginger



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is made up of rhizomes, which are underground stems that grow horizontally and develop roots underneath while sprouting leaves and new stems on top. Buds appear at regular intervals along each stem. Not only is ginger well-known as a spice and an essence, but it is also one of the oldest herbal and aromatic cures, particularly in Asian countries such as China and India, as well as the Middle East. It has been used to treat inflammation and diarrhoea in China for more than 2,000 years. It is directly employed in traditional Asian food and soft drinks such as coffee and tea by slicing it up or simply making use of its powder. The enticing slench of ginger is the result of an essential oil in its composition that has been prized and exploited by perfume makers since antiquity. Ginger is a tropical plant native to the Indo-Malaysian rain forests. It grows best in lush, damp, tropical environments. TIS cultivation is said to have begun in southern Asia, but it has now expanded to East Africa and the Caribbean. Ginger's perennial plant produces bright crimson blooms in a variety of shapes, including torch and honeycomb, that are commonly utilised in South Pacific seasonal events to decorate stalls, residences, and even clothes.

History of ginger

Cardamom and turmeric are members of the same plant family as ginger. The presence of ketones, particularly gingerols, which appear to be the principal component of ginger examined in many health-related scientific research, contributes to its pungent scent. The rhizome, or horizontal stem from which the roots grow, is the most commonly consumed part of ginger. Ginger's current name stems from the Middle English word *gingivere*, while the Sanskrit word *srngaveram*, which means "horn root," refers to its look and dates back over 3000 years. It was known as *ziggiberis* in Greek and *zinziberi* in Latin. Ginger is unusual in that it does not grow wild and its origins are unknown. Ginger has been used as a tonic root by Indians and Chinese for over 5000 years to treat a variety of diseases, and the plant is currently grown across the humid tropics, with India being the major producer. Long before history was written, ginger was utilised as a flavouring ingredient. It was a highly valuable commodity that was brought from India to the Roman Empire over 2000 years ago, when it was prized for its medical virtues. Even after the fall of the Roman Empire, ginger remained a highly sought-after item in Europe, with Arab merchants leading the trade in ginger and other spices for centuries. A pound of ginger was equivalent to the cost of a sheep in the thirteenth and fourteenth centuries. It was being imported in preserved form to be used in sweets by the Middle Ages. The gingerbread man, which became a favourite Christmas treat, is linked to Queen Elizabeth I of England.

Ginger

Ginger is burned-thru worldwide as zest, seasoning specialist, embellish, medication, and food additive and is carried out each new, in a brand-new glue, or dry, in a dry powder. New ginger can be fill in for dried ground ginger, albeit the forms of new and dried ginger are quite unique. The heady perfume of ginger is entering into and sweet-smelling. Ginger is called as "Adrak" (neighbourhood name) withinside the subcontinent like India and Pakistan and is a crucial element of numerous dishes.

Uses of Ginger

Fresh, dried, pickled, preserved, crystallised, candied, and powdered or ground ginger are just some of the ways it's utilised. The scent is powerful and spicy, with a peppery and slightly sweet flavour. As the concentration of essential oils in ginger grows with age, the period when it is collected is determined by the intended use of the rhizome. Ginger can be picked at 9 months or longer if the main objective is to obtain the oil. Ginger is frequently pickled in sweet vinegar, which colours it pink and is popular in sushi. The root of ginger picked at 8-9 months has a tough skin that must be removed before eating, and it is used dry or pulverised into powdered ginger. This is the most prevalent kind in our spice cabinets, and it's used in biscuits, cakes, and curry powders. Ginger that has been candied or crystallised is boiled in sugar syrup and then dusted with granulated sugar. The rhizomes are soft with a mild flavour and are best used fresh or preserved. Ginger collected at 5 months is not yet ripe and has a very thin peel.

Bioactive Components

Ginger is abundant in active constituents, such as phenolic and terpenecompounds. The phenolic compounds in ginger are mainly gingerols, shogaols, and paradols. In fresh ginger, gingerols are the major polyphenols, such as 6-



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gingerol, 8-gingerol, and 10-gingerol. With heat treatment or long-time storage, gingerols can be transformed into corresponding shogaols. After hydrogenation, shogaols can be transformed into paradols. There are also many other phenolic compounds in ginger, such as quercetin, zingerone, gingerenone-A, and 6-dehydrogingerdione. Moreover, there are several terpene components in ginger, such as β -bisabolene, α -curcumene, zingiberene, α -farnesene, and β -sesquiphellandrene, which are considered to be the main constituents of ginger essential oils. Besides these, polysaccharides, lipids, organic acids, and raw fibers are also present in ginger

Ginger as medicine for pregnancy

The usage of ginger, which has been the most extensively used herbal treatment in the therapy of pregnancy-related nausea and vomiting for decades, is a common source of exposure during pregnancy. In addition, ginger is supposed to promote human health and strengthen the immune system, which has led to an increase in popularity in recent years. Ginger is now commonly found in everyday items sold in Danish supermarkets, such as teas and shots. The usage of ginger as a health supplement may lead to increasing and ongoing intake during pregnancy.

CONCLUSION

Ginger is not only a popular dietary condiment for flavouring food, but it is also a medicinal herb that has been used to treat a range of maladies for thousands of years. Ginger contains hundreds of chemicals and metabolites, according to chemical and metabolic investigations. Gingerols and shogaols, particularly [6]-gingerol and [6]-shogaol, have been the most widely researched bioactive components. The content of each component is clearly influenced by the ginger rhizome's origins and treatment. Over the last few years, there has been a significant surge in research interest in establishing the role of natural chemicals in disease prevention. Despite the abundance of research studies, many of the findings are based on phenomena and provide descriptive and observational data rather than mechanistic data. More research on the kinetics of ginger and its constituents in animals and people, as well as the consequences of long-term ingestion, is needed. It is necessary to identify specific molecular targets and methods of action.

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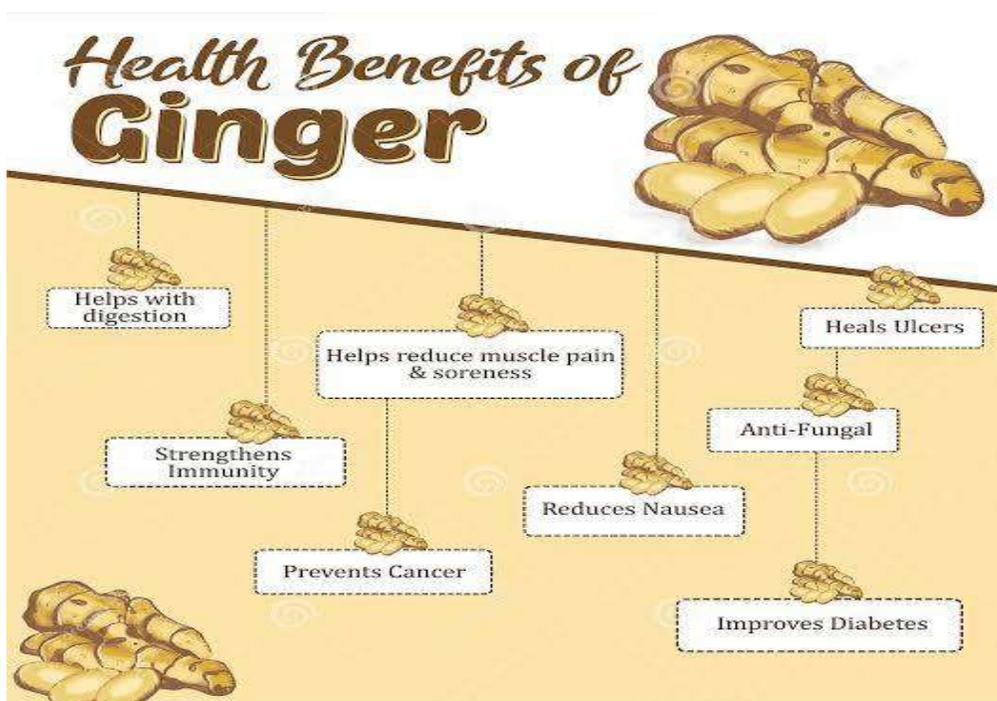
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Clove as a Functional Food

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ABSTRACT

Clove (*Syzygium aromaticum*) is a highly valued spice that has been used for centuries as a food preservative and for a wide range of medicinal purposes. Clove is native to Indonesia, but it is now grown in many parts of the world, including Brazil's state of Bahia. This plant is a rich source of polyphenols like eugenol, eugenol acetate, and gallic acid, and it has a great deal of potential for pharmaceutical, skincare products, food, and agricultural applications. This review covers the most important studies on the bioactivity of clove and eugenol. Clove has more antioxidants and antibacterial properties than many fruit and vegetables, as well as spices, and it deserves special attention. A new application of clove as a larvicidal agent is an intriguing strategy for combating dengue, which is a serious health problem in Brazil and other tropical countries. The various studies reviewed in this work confirm the traditional use of clove as a food preservative and medicinal plant, highlighting the plant for various applications.

Keywords: Spice, Clove, Aromatic plant, Volatile, Antioxidant, Larvicidal

INTRODUCTION

Flavours such as clove, oregano, mint, thyme, and cinnamon were used as food preservatives and herbal medicines for hundreds of years due to its antioxidant and antimicrobial activities. Many research findings now confirm seasoning plants' antibacterial, fungicidal, antiviral, and anti - carcinogenic characteristics. Clove, in specific, has caught the interest of research groups due to its powerful antimicrobial and antioxidant characteristics that





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distinguish it from other spices[1]. *Syzygium aromaticum* (*S. aromaticum*) (synonym: *Eugenia caryophyllata*), as well known as clove, is a medium-sized plant (8-12 m) native to the Maluku islands in east Indonesia. For middle ages, the trade of cloves and the search for this valuable spice helped fuel the economic and social development of all this Asian region[2]. The clove plant is normally cultivated in coastal areas at maximum altitude of 200 metres above sea level. After 4 years of plantation, the production of flower buds, which is the marketed part of this tree, begins. Before flowering, flower buds are collected during the maturation phase. The collection could be performed manually or chemically to use a natural phytohormone which liberates ethylene in the vegetal tissue, resulting in premature maturation[13].

Clove

The Latin word *Caryophyllon* and the Greek word *Karuophullon* were combined to form the term clove. [32] [33] These words were shortened to *clou de girofle* in Old French and *clow of gilofer* in Middle English. [32] [33] The term was first introduced in English in the 15th century. Cloves are considered to be indigenous to Maluku Island (Moluccas), an island in Indonesia known historically as the "Spice island." The name "cloves" comes from the French word *clou*, which indicates "nail," because the buds have the shape of a small irregular nail. According to FAO, Indonesia produces approximately 80% of the world's clove output in 2005, followed by Madagascar and Tanzania. Cloves are also grown in Pakistan, India, Sri Lanka, Mauritania, and the West Indies. Cloves, nutmeg, and pepper were valued highly in Roman times, and Pliny the Elder famously complained that "there is no year in which India does not drain the Roman Empire of 50 million sesterces"[4]. Arabs traded cloves in the financially rewarding Indian Ocean trade during the Middle Ages, thanks to the Terms of The treaty with Spain and a separate treaty with the Sultan of Ternate. The Portuguese brought large amounts of cloves to Europe, mostly from the Maluku Islands. Clove was a valuable spice at the time[16].

The Dutch finally took over the trade in the seventeenth century. During in the Portugese and Dutch momocracies, both countries worked hard to sustain tight control over production and sales. The French succeeded in introducing the clove tree into Mauritius with great difficulty in 1770, and their cultivation was later introduced in Guyana, Brazil, most of the West Indies, and Zanzibar, which exports more cloves than any other country[13]. Two examples of a clove found at a trading port in Sri Lanka, dated around 900-1100AD, represent another archaeological find. According to Chinese records from the Sui Dynasty, ships from the Austonsian polities of Java, Sri Lanka, and Butuan primarily exported cloves from the Moluccas. Before the establishment of Southeast Asian maritime trade, there were several putative historical mentions of "cloves" reported from China, South Asia, and the Middle East[20]. Clove flourishes in the humid tropics' rich loamy soils and it can be grown successfully in the red soils of Kerala's midlands and the hilly terrain of the Western Ghats at higher elevations in Tamil Nadu and Karnataka. Flowering thrives in cooler climates with evenly distributed rainfall. The chosen site for clove cultivation must have proper drainage because the crop cannot tolerate waterlogged conditions. It grows well in areas with annual rainfall of 150-300 cm. Clove grows in India from sea level to 1500 m above sea level[15].

Functional Food

Functional foods resemble traditional foods in appearance, with the former being consumed as part of the regular diet. Functional foods, in contrast to the conventional foods, have demonstrated physiological benefits and can reduce the risk of chronic disease beyond basic nutritional functions, such as gut health maintenance [3]. Food is referred to as "functional food" when it is cooked or prepared using "scientific intelligence," with or without knowledge of how or why it is being used. Thus, functional food provides the body with the vitamins, fats, proteins, carbohydrates, and other nutrients it requires for survival and wellbeing [4]. Eating habits and food consumption and production trends have health, environmental, and social consequences. Food choices form in early childhood. Even so, it is very well understood that the early stages of life (the intrauterine period and the first year of life) are sensitive to nutritional factors [12]. Food habit development is a complicated process which can be influenced by various factors such as region, religion, family structure and lifestyles, income, prices, mental stress, and technical advances. Several observational studies research published over the last 50 years have clearly proven that diets high





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in fruits, vegetables, and dietary fibres (plant-based foods) prevent and reduce the risk of chronic diseases (e.g., cardiovascular disease, obesity, diabetes) and promote good people's health. Scientific research linking plant-based foods and health has resulted in the recognition that plant bioactive compounds have anti-oxidant and other beneficial properties [10,11]. The human gut is inhabited by a diverse variety of bacterial species, the latter of which perform important metabolic and immune functions, all of which have a significant impact on the host's nutritional and health status [5]. According to a consensus definition, probiotics are "living microorganisms that, when consumed in sufficient numbers, exert health benefits beyond inherent basic nutrition" [6,7]. Alternatively, probiotics are loosely defined as "live microorganisms belonging to natural biota with low or no pathogenicity, but with functions important to the host's health and well-being" [8,9].

Clove-Derived Chemical Constituents

Clove is a major source of phenolic compounds including such flavonoids, hydroxybenzoic acids, hydroxycinnamic acids, as well as Clove hydroxyphenyl propens. Eugenol is the primary biologically active compound found in clove, with concentrations ranging from 9 381.70 to 14 650.00 mg per 100 g of fresh plant material [6]. Gallic acid is the compound found in the highest concentration (783.50 mg/100 g fresh weight) among the phenolic acids. Other gallic acid derivatives, such as hydrolyzable tannins, have higher concentrations (2 375.8 mg/100 g)[1]. Caffeic, ferulic, elagic, and salicylic acids are some of the other phenolic acids found in clove. In lower concentrations, clove contains flavonoids such as kaempferol, quercetin, and its derivatives (glycosylated). Clove flower buds contain up to 18 percent volatile oils concentration levels. Approximately 89 percent of clove essential oil is eugenol, with the remaining 5 to 15% being eugenol acetate and -cariofileno[7]. -humulen is another important compound found in clove essential oil at concentrations up to 2.1 percent. Other volatile compounds found in clove essential oil in lower concentrations include -pinene, limonene, farnesol, benzaldehyde, 2-heptanone, and ethyl hexanoate.

Nutritional Content and Culinary/Dietary Uses of Clove

Clove originally belonged to the generally regarded as safe (GRAS) food products list. It is used to flavour foods and beverages especially rice, masala and soups. 100 g of clove contains 274 calories, 66 g carbohydrates, 6 g protein, 13 g fat, and is high in vitamins A and B6 as well as iron, calcium, magnesium, and phosphorus[25]. Clove spice is pungent and astringent, and it improves blood circulation, digestive health, and metabolic activity while also alleviating stomach issues. 100 g of ground clove contains the following nutrients: Water 5.40–6.86 g; Food energy 323 (Kcal); Protein 5.98 g; Fat 20.06 g; Carbohydrate 61.22 g; Ash 5.88 g; Ca 0.646 g; P 105 mg; Na 243 mg; K 1102 mg; Fe 8.68 mg; Thiamin 0.115 mg; Riboflavin 0.267 mg; Niacin 1.458 mg; Ascor. Clove has been used in some food formulations to improve their nutritional, preservative, and biological properties[27]. A study on the sensory, antioxidant, and maillard reaction profiles of rye-buckwheat cakes enhanced with selected spices, including clove, discovered that total flavonoid content, antioxidant capacity, browning properties, and overall acceptability of cakes enriched with clove, allspice, and spice were elevated and best. The inclusion of a combination of essential oils of cloves, rosemary, and *Origanum vulgare* L. (oregano), and also vitamin E, to substitute conventional chemical antioxidants in dog feed significantly enhanced beagle food quality and health. Another study compared the flavours of clove, ginger, and *Cymbopogon citratus* (DC.) Stapf (lemon grass) in cakes. Other culinary applications of clove include the use of clove as an actual study for *Allium cepa* L. (onions), *Solanum lycopersicum* L. (tomatoes), salads, herbal teas, and soups, as well as the flavouring of meat products, cookies, pastries, sandwiches, pickles, puddings, chewing gums, spiced fruits, chocolates, fizzy drinks, and sweets[28].

Health Benefits in Clove

Antioxidants abound in cloves. These compounds assist your body in fighting free radicals, which cause cell damage and can lead to disease. The antioxidants found in cloves can help reduce your risk of developing heart disease, diabetes, and certain cancers by removing free radicals from your system. Cloves are full of antioxidants. These compounds allow your body fight free radicals, which damage the cells and can lead to disease. By removing free radicals from your system, the antioxidants discovered in cloves can help to reduce your risk of developing heart disease, diabetes, and certain cancers. The dried, unopened flower buds of the tropical evergreen tree *Eugenia caryophyllata* L. Merr and Perry (Myrtaceae), also known as *Syzygium aromaticum* and *Eugenia aromatica*, are used





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to make whole cloves. Clove trees are indigenous to Indonesia's Maluku Islands, but they are now grown in Madagascar, Tanzania, the West Indies, China, and Malaysia. This hard, brown nail-shaped spice gets its name from the Latin *clavus*, which means "nail." Clove, also known as *ding xiang* or "nail spice" in traditional Chinese medicine, was used to treat indigestion, nausea, vomiting, and infections, among other things.¹ Even today, clove is said to be a cure-all for a variety of ailments, including coughs and colds, diarrhoea, digestive disorders, diabetes, toothaches, memory loss, erectile dysfunction, and arthritis.^{2,3} Clove is being used in a variety of dishes, including ham, stewed fruits, pickles, curries, pies, salads, and spiced alcoholic beverages.¹ Clove is known to mask spoiled food bad smells by interfering with odour maps in the forebrain's olfactory bulb.⁴ It is also used in perfumes, oral care products, and soaps and detergents. Cloves are added to tobacco in Indonesian kreteks, aromatic high-tar cigarettes. Cloves are high in beta-carotene, which contributes to their rich brown colour. The pigments in the carotene family are important antioxidants and provitamins. Carotene pigments can be converted into vitamin A, which is essential for eye health.

Reduced Inflammation

Cloves contain a number of compounds that have been linked to anti-inflammatory properties. The most important of these compounds is eugenol. Eugenol has been shown to reduce the inflammatory response in the body, lowering the risk of diseases like arthritis and aiding in symptom management.

Fewer Free Radicals

Eugenol is a powerful antioxidant as well. Antioxidants abound in cloves. These compounds assist your body in fighting free radicals, which cause cell damage and can lead to disease. The antioxidants found in cloves can help reduce your risk of developing heart disease, diabetes, and certain cancers by removing free radicals from your system.

Reduced Ulcers

Cloves can aid in the prevention of stomach ulcers. The majority of ulcers are caused by thinning of the mucus layers that protect your stomach lining. Preliminary research indicates that cloves can thicken this mucus, lowering your risk of developing ulcers and aiding in the healing of existing ulcers.

Improved Liver Function

Cloves may also improve liver function. According to some studies, the eugenol found in cloves can help reduce the symptoms of liver cirrhosis and fatty liver disease. It may also help with overall liver function.

Antioxidant Activity

Recently, the United States Department of Agriculture created a database with the polyphenol content and antioxidant activity of various foods in collaboration with universities and private companies. Pérez-Jiménez *et al.* classified the 100 richest dietary sources of polyphenols based on this database[8]. According to the findings, spice plants have the highest polyphenol content, followed by fruits, seeds, and vegetables. Among spices, clove contained the most polyphenols and antioxidant compounds. The main phenolic compounds in 26 spices were identified and quantified using high performance liquid chromatography, followed by an ABTS in vitro antioxidant activity analysis. The findings revealed a strong relationship between polyphenol content and antioxidant activity. Clove (buds) had the highest antioxidant activity and polyphenol content, with (168.6600.024) mmol of Trolox/100g dried weight and (14.3800.006) g of gallic acid (equivalents/100g dried weight, respectively. Phenolic acids (gallic acid), flavonol glucosides, phenolic volatile oils (eugenol, acetyl eugenol), and tannins were the most common types of phenolic compounds discovered. It was emphasised that clove has enormous potential as a radical scavenger and as a commercial source of polyphenols. High performance liquid chromatography was used to identify and quantify the main phenolic compounds in 26 spices, which was followed by an in vitro antioxidant activity analysis. The antioxidant activity of clove and caraway was tested using various in vitro models, including b-carotene-linoleate, ferric thiocyanate, 1,1-diphenyl-2-picryl hydroxyl (DPPH) radical, hydroxyl radical, and reducing power model



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systems, and the antioxidant activity of clove and caraway was found to be comparable to butylated hydroxytoluene (BHT), a synthetic compound commonly used as food preservative.

Antimicrobial Activity

Clove has been shown to have antimicrobial properties against a variety of bacteria and fungi. Sofia *et al.* investigated the antimicrobial activity of various Indian spice plants, including mint, cinnamon, mustard, ginger, garlic, and clove [18]. The only sample that demonstrated complete bactericidal activity against all of the food-borne pathogens tested, *Escherichia coli* (*E. coli*), *Staphylococcus aureus*, and *Bacillus cereus*, was a 3 percent aqueous extract of clove. Clove extract, at a concentration of 1%, also demonstrated good inhibitory activity. Dorman and Deans [19] tested the antibacterial activity of black pepper, geranium, nutmeg, oregano, thyme, and clove against 25 strains of Gram positive and Gramnegative bacteria in another study. Thyme, oregano, and clove were the oils with the broadest spectrum of activity. The antibacterial activity of clove, oregano (*Origanum vulgare*), bay (*Pimenta racemosa*), and thyme (*Thymus vulgaris*) essential oils against *E. coli* O157:H7 was tested, and the different grades of inhibition of these essential oils were demonstrated [20]. Similarly, formulations containing eugenol and carvacrol encapsulated in a nonionic surfactant were tested against four strains of two important foodborne pathogens, *E. coli* O157:H7 and *Listeria monocitogenes*, and the results support the use of eugenol to inhibit the growth of these microorganisms on food-contact surfaces [21].

Antiviral

Clove, oregano (*Origanum vulgare*), and bay (*Pimenta racemocarpa*) have antibacterial properties. The antiviral activity of eugenin, a compound isolated from *S. aromaticum* and *Geum japonicum*, was tested against herpes virus strains and found to be effective at 5 g/mL, implying that one of eugenin's major targets is viral DNA synthesis via inhibition of the viral DNA polymerase [34]. Aqueous extracts of *S. aromaticum* (L.) Merr. et Perry, as well as other plants such as *Geum japonicum* Thunb., *Rhus javanica* L., and *Terminalia chebula* Retz., demonstrated strong antiherpes simplex virus type 1 (HSV-1) activity when combined with acyclovir in another study. This synergistic activity was stronger in the brain than in the skin, and it was also demonstrated that these combinations were not toxic to mice[35].

Citotoxicity of Eugenol

Several molecular targets for cancer prevention and treatment have been identified after several years of intensive research. Eugenol was chosen as a possible molecule that could disrupt several cell-signaling pathways, specifically the nuclear factor kappa B. (NF-KB). This factor is activated by free radicals, resulting in the expression of genes that suppress apoptosis and induce, among other things, cellular transformation, proliferation, invasion, and metastasis[36]. The ability of eugenol and borneol to modulate resistance against the damaging effects of H₂O₂ on DNA of different strains of human cells: malignant HepG2 hepatome cells, malignant Caco-2 colon cells, and nonmalignant human VH10 fibroblast was tested. The results showed that eugenol had a significant anti-oxidative potential at all concentrations tested. It was also demonstrated that eugenol had stronger citotoxic effects than borneol. In terms of toxicity, eugenol had strong genotoxic (DNA-damaging) effects on human VH10 fibroblasts, medium genotoxic effects on Caco-2 colon cells, and non-DNA-damaging effects on HepG2 hepatome cells[37]. Nonetheless, the National Toxicology Program concluded that eugenol was not carcinogenic to rats based on several long-term carcinogenicity studies. In another study, eugenol inhibited the growth of the malignant melanoma WM1205Lu of both anchorage-dependent and anchorage-independent growth, decreased tumour size, and inhibited melanoma invasion and metastasis by inhibiting the two E2F family transition factors[38]. Although there have been numerous reports of eugenol's antioxidant activity, at high concentrations eugenol may be prooxidant. Atsumi et al[39] investigated the cytotoxicity, reactive oxygen species (ROS) production, and intracellular glutathione levels of eugenol and isoeugenol in a human submandibular cell line (HSG cells). In the absence of oxidative stress, it was discovered that eugenol acts as an antioxidant at low concentrations but as a prooxidant at high concentrations. Eugenol increased ROS levels in the presence of oxidative stress at low concentrations (5-10 mol/L), but decreased them at high concentrations (500 mol/L). As a result, it can be concluded that eugenol cytotoxicity occurs in the presence of oxidative stress in a ROS-independent manner. Another study found that eugenol inhibits the enzyme



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MMP-9, which is linked to metastasis, in human fibrosarcoma cells, implying that it could be used to prevent oxidative stress-related metastasis[32].

Agricultural and Insecticidal Applications

Clove essential oil could also be used as a pesticide. Park and Shin recommended that clove essential oil could be used to control the Japanese insects *Reticulitermes speratus* Kolbe[35]. Similarly, Eamsobhana *et al.* discovered that clove essential oil at 5% has 100% repellent activity against the chigger *Leptotrombidium imphalu*, that could be a safer and less expensive alternative to synthetic repellents that are commonly associated with harmful side effects[37]. A formulation containing 10% clove essential oil was effective against the bite of *Aedes aegypti* (L.) and *Anopheles dirus* Peyton and Harrion, with protection times of (80.3310.56) and (60.0010.00), respectively, with soy bean oil serving as a control[32]. The structure-activity relationship of the main clove oil constituents and synthetic derivatives of eugenol against *Aedes aegypti* (Diptera: Culicidae) larvae was investigated in a recent study. Due to the lack of a treatment drug or vaccine, larvicidal methods are one of the most effective strategies for combating dengue. Eugenol showed promising results and could be a viable alternative to common insecticides[33]. Clove oil can also be used to anaesthetize a variety of fish. Long term exposure, on the other side, can result in death and subacute morbidity[35]. Hekimoglu and Ergun[31] determined the best dose to anaesthetize the angelfish. This research will aid in the transportation and handling of this fish, which is one of the most difficult to handle in an aquarium. Clove oil has the potential to suppress potato tuber germination by influencing lipid peroxidation and the enzyme activities of catalase, glutathione-S-transferase, peroxidase, polyphenol oxidase, and superoxide dismutase[36].

CONCLUSION

Clove may be viewed as the undefeated leader of all anti-oxidants known to date. A drop of clove oil has 400 times the anti-oxidant power of wolfberries or blueberries. Clove is a medicinally powerful herb with a long history and tradition. Clove is helpful to one's physical, mental, and emotional health. Clove has anti-fungal, anti-viral, anti-microbial, anti-diabetic, anti-inflammatory, antithrombotic, anaesthetic, pain relieving, and insect repellent properties. Cloves are one of Mother Nature's most powerful antiseptics. Eugenol is the primary constituent responsible for the clove bud's medicinal properties. According to global trade, clove is the most important spice in the world.

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Luminous Vibriosis in Shrimp Aquaculture

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ABSTRACT

Healthy shrimp culture system is always in harmony with the ecology of the pond environment. This can be manipulated by developing a dense heterotrophic bacterial community that takes care of waste generated in the system through in situ bioremediation. Considering the importance to reduce an occurrence of luminous vibriosis in shrimp aquaculture, countless studies have been carried out with an objective to screen anti-vibrio biological agents, which can be used as an alternative to antibiotics. In such studies, microalgae, bacteriophage, and probiotic bacteria have been found to have potential benefits in reducing vibriosis. Eco-based shrimp farming, green water technology, bio-floc technology, phage therapy, and integrated multi-trophic aquaculture (IMTA), since their inception, hold a promising alternative to antibiotics in the near future. This article seeks to secure all the available information on different biological agents, their involvement in lowering *Vibrio* load, and strategies to control *Vibrio* infection in shrimp aquaculture.

Luminescent *Vibrio harveyi* is a natural microflora of marine and coastal water bodies and is associated with mortality of larval shrimp in penaeid shrimp hatcheries. It is also known that the bacteriophages occur virtually in all places where their hosts exist. In this study, distribution of luminescent *V. harveyi* and the bacteriophages affecting these hosts was examined in a commercial *Penaeus monodon* hatchery during three shrimp larval production cycles, including a cycle affected by luminescent bacterial (LB) disease outbreak. During the larval production cycle affected by LB disease (LBD), luminescent *V. harveyi* could be recovered from 52% of the hatchery samples, whereas during luminescent bacterial disease-free larval production cycle (LBDF), these bacteria could be recovered from only about 9% of the samples. The predominant source of luminescent bacteria was the brood shrimp and their rearing tanks in maturation





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and spawning facilities. 73% of the maturation and 80% of the spawning tank water samples harbored LB during LBD, whereas, only 20% and 32% of the maturation and spawning tanks respectively harbored LB during LBDF. LB could be isolated from 17% of the water samples in tanks from nauplius stage onwards with increasing counts that subsequently lead to LB disease.

Keywords: Luminous bacteria, Shrimp mortality, *Vibrio harveyi*

INTRODUCTION

In order to reduce the introduction of high organic matter into the coastal environment and thus avoid severe damage to the production system (Nygaard et al. 1992), either closed pond systems or recycle systems are necessary. These closed and recycle systems increase the problems associated with bacterial pathogens. It is recognized that damage to shrimp stocks is frequently associated with bacterial diseases that are mostly caused by luminous bacteria. The problem seems to be common among the Asian countries where shrimp farming is the main aquaculture activity. This presentation describes the species composition (incidence and intensity) of luminous bacteria in shrimp farms and the coastal environment in Thailand, their pathogenicity and recent studies on the chemotherapy and biological control. It is hoped that the information will contribute towards further investigations leading to successful resolution of the luminous disease problem. Bacteriophages affecting the luminescent *V. harveyi* could be isolated from as many as 36% (21% and 43% of the samples analysed during LBDF and LBD respectively) of a total of 181 water samples drawn from various sources in the hatchery, using 27 luminescent *V. harveyi* hosts by agar overlay technique. The maturation tank water samples were found to be the predominant source of bacteriophages, followed by spawning tank water samples as observed with the LB. Sixty five bacteriophages, 13 during LBDF and 52 during LBD were isolated, which were grouped in to seven types based on their plaque morphology

Pathogenesis and Host Susceptibility

Among the luminous bacterial species reported from shrimp ponds, hatcheries and moribund shrimp, only *V. harveyi* has been confirmed to cause mortality of shrimp. The disease is widely known as "luminous disease". Epizootics of this disease occur several times a year and are expanding throughout shrimp farming areas along the coast. A number of shrimp farmers have suffered severely from damage of long duration caused by this disease. The bacterial pathogenesis resulted in mortalities up to 100% for naupliar to zoeal stages of *P. merguensis*. Living and dead shrimp larvae and even the sea water in disease outbreak areas were luminescent in dim light. Other gross features of the diseased shrimp were milky white bodies, weakness, swimming disorders and loss of appetite, eventually leading to death. Using luminous media (LM) (Baumann & Baumann 1981), luminescent bacterial colonies could be isolated from the diseased specimens as well as from hatchery water. Based on 47 phenotypic characters, the causative agents were identified to *V. harveyi*.

Prevention and Control

Prevention and control of luminous disease in shrimp are generally achieved by chemical treatment. Chemotherapeutants may be applied in the feed or in the water. Usually antibiotics are applied in the feed while disinfectants (and sometimes antibiotics as well) are applied in the water. To prevent the luminous disease in hatchery operations, water should be filtered through a biofilter system prior to chemical treatment with 60% active chlorine at 10-30 g/ton or commercial grade formalin at 50-100 ml/ton. During treatment aeration is necessary. Antibiotics treatment via the water is a common practice during rearing the early larval stages (nauplius to post larvae 3-4). Application of antibiotics at 3-5 g/ton into the rearing water, especially during periods of metamorphosis has been found to greatly reduce luminous bacteria and stock mortality. Oxytetracycline (OTC) was the main drug used for several years in all areas of the country. The effectiveness of OTC gradually declined after repeated application for long periods due to the development of bacterial resistance. Recently, various drugs have been tested against luminous and nonluminous vibrios, including resistant strains. Therefore, alternative drugs can





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be recommended based on sensitivity tests. After harvesting, treatment of the larval rearing facilities by flushing or vigorous spraying with freshwater has been suggested as a routine practice. Antibiotics are usually applied in grow-out pond by addition to pelleted feed at approximately 3-5 g/kg administered to the shrimp for 7-10 days. Oxytetracycline and oxolinic acid were commonly used in the past. Recently they appear to be of limited value due to the development of bacterial resistance. Several drugs claimed to be effective against luminous bacteria are supplied in commercial grade although many show very low bacterial growth inhibition.

Phytochemicals

To preserve and protect the environment as well as human health as a best alternative, different parts of *Azadirachta indica* (Neem) tree have been studied by Chitmanat et al. (2005). Neem leaves containing nimbin, azadirachtin and meliantr oil have been reported to possess a variety of properties, including insecticidal and antiviral from ancient times. Indian almond (*Terminalia catappa*) and garlic (*Allium sativum*) have been said as an alternative to chemicals to treat fish ectoparasites, *Trichodina* sp. infections in tilapia (*O. niloticus*) fingerlings. Both Indian almond and garlic had low acute toxicity to tilapia fingerlings, treating the trichodina as is caused by *Trichodina*. The Indian almond, commonly used as herb in Taiwan, prevents the fish diseases. It is claimed to be a wound healing substance for Siamese fighting fish hurt after matches in Thailand as well. The immunostimulant effects of the dietary intake of 3 plants (viz., *Viscum album*, *Urtica dioica* and *Zingiber officinale*)-extracts on rainbow trout (*Oncorhynchus mykiss*) have also been narrated by the authors. Christy bapita et al. (2007) observed the immunostimulatory effect of aqueous extract of *Eclipta alba* (Bhangra) leaf (oral administration as feed supplement) in tilapia fish, *Oreochromis mossambicus*.

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CONCLUSIONS

Disease problems still exist and are associated with serious crop losses. The survey data on luminous bacteria has shown that they are members of the natural estuarine and brackish water environment. The studies also suggest that they induce disease more frequently in dry season than the rainy season and that their is related to salinity levels and effluent from farming operations. Only *V. harveyi* has been confirmed to be a pathogenic agent associated with shrimp mortality. In hatcheries, luminous disease can be controlled by chemical treatment with disinfectants and antibiotics, while in grow-out ponds, effective treatment and control methods are still uncertain. Some experiments indicate effective growth inhibition of *V. harveyi* by probiotics and biological treatments. However, effectiveness has not yet been proven in field trials. Although luminous pathogens exist naturally in the water, they certainly need carbon and nitrogen (C+N) for growth and multiplication. Thus, the first action to take in reducing their numbers is to be careful not to overfeed the shrimp and thus supply plentiful nutrients in the pond and the adjacent environment.



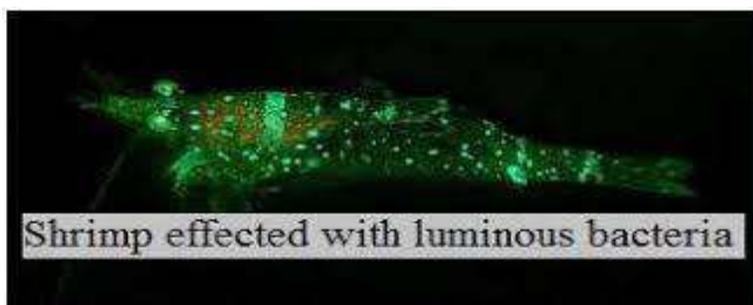


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Drugs	MIC ranges (ug/ml)	IC s (ug/ml)	
		50% *1	90% *2
Oxytetracycline	6.25 - 100	15.00	69.20
Oxolinic acid	12.5 - 100	28.20	56.70
Norfloxacin	0.8 - 100	65.50	100
Chloramphenicol	3.13 - 50	8.75	45.20
Trimethoprim	3.13 - 25	9.37	15.00
Kanamycin	100 -> 100	>100	>100





Nutraceutical Effect of Ginger

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ABSTRACT

Ginger, a famous functional food, has been utilised for ages all throughout the world. The metabolic profiles of ginger were examined using UPLC–Q/TOF-MS. These findings add to our understanding of ginger metabolism, which will aid in the discovery of functional components and additional mechanism studies. It contains phenolic chemicals, terpenes, polysaccharides, lipids, organic acids, and raw fibers, among other chemical elements. The phenolic chemicals in ginger, such as gingerols and shogaols, are primarily responsible for providing health benefits. Multiple biological effects of ginger have been discovered, including antioxidant, anti-inflammatory, antibacterial, anticancer, neuroprotective, cardiovascular protective, respiratory protective, antiobesity, antidiabetic, antinausea, and antiemetic properties. Due to the presence of bioactive components in ginger, it can be used to develop functional foods or nutraceuticals for human health. This review article concentrates on the therapeutic effects of phytochemicals present in ginger.

Keywords: Functional foods, Nutraceuticals, Phytochemicals, Metabolism

INTRODUCTION

Functional foods are becoming incredibly popular in health and wellness circles in recent years. This is also known as nutraceuticals and can be defined as the compounds that are highly nutritious and associated with a number of health beneficiaries. They provide protection against diseases, prevention for nutrient deficiencies, and promotion of proper growth and development. The definition, benefits, and potential implications of functional foods are discussed in this article. Functional foods are ingredients that have health benefits in addition to their nutritional





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value. Sometimes supplements or other additional ingredients are designed to improve health. In the 1980s, the concept of functional foods has been originated in Japan with government approval. The revolutionary decision was taken by visualizing the health benefits of functional foods on the general population. Some examples include foods with vitamins, minerals, probiotics, or fibers. Fruits, vegetables, nuts, seeds, and grains, that are highly nutritious, are commonly considered as functional foods. Oats, for instance, contain a type of fibre that has been shown to reduce inflammation, enhance immune function, and improve heart health. Similarly, fruits and vegetables are packed with phytochemicals that help protect against disease. Nutraceutical and functional foods are gaining popularity and capturing the global market. Nowadays, more than 470 nutraceutical and functional food items are marketed with proven health claims. The statistical analysis of the market shows that the worth of nutraceuticals and functional foods was estimated at \$197 billion and \$30-60 billion in Japan and the USA, respectively. The term “functional food” was defined as Food that not only provides basic nutrients but also possesses many therapeutic benefits. The differentiation between functional and nutraceutical foods is that the nutraceutical foods are enriched with active components to get targeted health benefits.

The utilisation of these products are not only aimed at getting health benefits, but they can also have preventive and curative effects for many chronic diseases, ranging from cardiovascular diseases to cancer. Researchers have confirmed that various phytochemicals and bioactive components are present in these indigenous herbs and medicinal plants that ensure their medicinal attributes and thus are an important part of modern functional and nutraceutical foods. Researchers have also depicted that a handsome number of phytochemicals and bioactive moieties are present in herbal plants. Among them ginger has a rich phytochemical profile as it possesses nutraceutical potential against various physiological threats, especially due to the presence of 6-gingerol. The inclusions of processed foods, changing living patterns, and irregular dietary habits are the leading causes of physiological disorders, and diet-related completions like diabetes, cancer, CVD, and high cholesterol levels are increasing day by day. Prevention of these disorders is a major public health concern worldwide, especially in developed and underdeveloped nations. Then involvement of phytochemicals in diet for diseases prevention was widely spread and documented from ancient times due to their safer and high pharmacological values. Recently, analysis proves therapeutic properties of diet have created a revitalization to improve human health and nutrition research. Nutraceuticals have been claimed to have a physiological benefit or provide protection against many diseases such as cardiovascular diseases, obesity, diabetics, cancer, chronic inflammatory disorders, CVD, high serum triglyceride level, declined levels of high-density lipoproteins (HDL), hypertension, and impaired glucose tolerance (Shoib et al, 2016). They can be used as immune boosters as well.

History of Ginger

Zingiber officinal Roscoe was classified as a member of the Zingiberoside family by Wagner in 1980 and is associated with the Zingiber variety. The name Zingiber comes from the Sanskrit word "zingiber," which refers to the projections on the rhizome. It was given by the English botanist William Roscoe. Ginger grows to be a straight, evergreen shrub that grows to be 1 to 4 feet tall. The shoot raises 13 jerks above the ground and is encircled by the two masterminded edges' sheathing bases, which look like leaves. It produces white parties as well as pink bloom buds that develop into yellow fledglings. The design known as rhizome, which fundamentally means horn-shaped, grows ginger in an impartial way, at the edge fixed with an extending portion. The entire rhizome has a striated and hard surface. It's around 5 to 16 cm long, 1.6 to 6 cm wide, and 2.2 cm thick, and the masking tone can be yellow, white, or red depending on the approach. Southeast Asia has ginger as a neighbour. The Arabs knew about the Mediterranean area, and journalists like Discords about 41–91 AD and Pliny the Elder around 25–80 AD portrayed it. Ptolemy discovered that ginger was produced in Ceylon, a city in Sri Lanka, circa 150 AD. During the middle ages, raw and protected ginger was imported into Europe. Ginger is one of the key flavours introduced from Asia in fourteenth-century England, first appearing in Europe for punch exchanges. Due to their similar taste, the dicots in the Asarum family are commonly referred to as wild ginger. In 2019, the total global production of ginger was 2.8 million tonnes, with India accounting for 34% of the total global production. One pound of ginger was worth the same as a sheep. Ginger was used by eclectic doctors in the nineteenth century to promote perspiration, improve cravings, and treat disease, as well as a good counterirritant. According to ShrimadBhagwatGeeta's Hirayama,





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ginger is a key component of Ayurveda, India's traditional medicine, and is known as Sunitha in Ayurveda. It was used to prevent excessive blood hardening in arteries and veins, lower cholesterol, and prevent joint aggravation. In traditional Chinese medicine, ginger is thought to be a sharp, dry, warming punch that can be used to relieve discomforts brought on by a chilly, saturated environment.

It was also employed to treat depletion issues, disease, going blind, toothaches, snakebite, and respiratory conditions, as well as a stomach-related guide and antinausea fix. Ginger is a long-lasting flavour that is frequently prepared in tropical areas and occasionally naturalizes. It mostly multiplies vegetatively since different cultivars only rarely sprout, being fruitless (Andallu et al., 2003). It is listed as invading in Taiwan's Invasive Species Database (Taiwan Invasive Species Database, 2016) and is a weed in Puerto Rico and Queensland, Australia. Ginger (*Zingiberofficinale*), commonly known as "Adrak", has been widely used in Pakistani and Indian cuisines for over 2500 years. It belongs to the family "Zingiberaceae", which is very famous due to its medicinal herbal plants like cardamom and turmeric. It has been cultivated in South-East Asia for thousands of years. After that, it gained popularity in European and African countries due to its therapeutic effects. Currently, the ginger and its products are used in many traditional medicinal systems due to their rich phytochemistry and disease-preventive properties. Ginger is known by different names in different languages like in Arabic "Zanjbeel" and French "Gingembre" etc. "Ginger" is an advanced and modified name for different words. Historically the basis of its appearance is linked with the Sanskrit word "Srngaveram", which means "Horn root", in Greek with "Ziggiberis", and in Latin, "Zinziberi". In Indo-Pak, in Urdu, it is known as "Adrak."

Functional Food

Pickled ginger, biscuits, candies, gingerbread, beer (gingerale), powder, and syrup are all examples of ginger's widespread use in food processing (Vasala, 2012). Processed ginger in the form of ginger candy was able to reduce the rate of vomiting in pregnant women in the first trimester (Anita et al., 2020). Adding ginger extract to turmeric tea increases antioxidant activity. It is due to phenolic compounds in ginger, which play a role in eliminating free radicals and radicals (Lugito et al., 2019). Ginger oleoresin has more 6-gingerol, shogaol, and zingerone than vitamin E. (Sueishi et al., 2019). Gingerol and shogaol compounds in ginger function as a spicy flavour and zingiberene that gives a warm feeling (Panjaitan et al., 2012). Using ginger powder in processed meatballs adds a pungent fragrance, flavor, and taste due to the presence of zingiberol and zingiberene compounds. Proteolytic enzymes also influence colour in ginger meatballs, livestock, myoglobin, and haemoglobin concentrations, as well as nonenzymatic browning reactions between meat proteins and sugar reduction. Meatball thickness is determined by the filler used, type, or meat part used (Kusnadi, 2012). Peanut oil rancidity can be prevented by ginger phenolic components like gingerol and shogaol.

Health Effects

Plants are a good source of phytochemicals that significantly possess antioxidants and reduce many ailments like oxidative stress, cancer, mutagenic, diabetes, CVD, infections, inflammation, and other carcinogenic conditions. The linkages between diabetes with other complications and the confirmation of side effects of synthetic medicines mean that researchers are focusing on the development of botanical novel anti-diabetic drugs. For thousands of years, due to its rich phytochemistry, ginger has been considered a remedy for many health complications, from colds to cancer. It is a good source of many functional and nutraceutical components like gingerols, sterols, paradols, etc. (Ali et al., 2008). All these components have been shown to have strong antioxidant potential and are helpful in scavenging free radicals. The administration of ginger in the in vivo studies on rats claimed many therapeutic effects like improvement in antioxidant status, prevention of lipid peroxidation, carcinogens, glucose, LDL lowering effects, and many others (Sasidharan and Nirmala et al., 2010). It is believed that ginger is useful as an antioxidant, hypocholesterolemic, hypoglycemic, anti-inflammatory, anti-nausea, and anticancer agent, as well as having a protective impact against various diseases. It can reduce biosynthesis or prostaglandin formation, reducing pain intensity (Khan et al., 2012). The concentration of 10% and 20% ginger extract cream has been shown to reduce elderly pain (Setyawan and Tasminatun, 2013). Fresh ginger extract from water has optimum efficacy as an analgesic for 25 min, while extracts from ethanol extraction have analgesic effects for up to 30 min.





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Antioxidant properties of ginger

Free radicals are the highly reactive moieties produced during food processing and in biological systems as a result of many generative and degradative reactions. Many health problems are linked to advancements in food processing, dietary habits, and free radical production. Extra antioxidant supplementation through dietary components is critical for organism survival in such circumstances of imbalance. Antioxidants are those compounds that eliminate these active moieties by binding them with their own active sites and reduce the risk of different health complications.

Gingers Hyperglycaemic/ Anti-Diabetic Effects:

The change in dietary style and low physical activity generate many lifestyle-related disorders like high blood pressure, obesity, CVD, and many others. Among these, diabetes mellitus is a rapidly growing health complication and one of the foremost reasons for casualties in the world. It has been estimated that if these diseases continue to increase at the current rate, then by 2030 they will harm about 367 million people worldwide. Shogaol or -gingerol significantly inhibited TNF- α -mediated down-regulation of adiponectin expression in 3T3-L1 adipocytes (Isa et al., 2008). Islam and Choi have explored that in HFD and STZ induced diabetic rats, ginger plays an important role in lowering the glucose level and increasing the insulin levels to control the blood glucose levels.

Potential of ginger to increase body immunity

In addition to these health benefits, ginger is currently being targeted by the community as it is believed that it can increase the body's immune system to prevent the COVID-19 outbreak. COVID-19 is an infectious disease caused by SARS-CoV-2, a type of coronavirus that spreads through droplets from the respiratory tract, such as coughing or sneezing. The lungs are the organs most affected by this virus, as the virus enters its host cells through the angiotensin 2 converting enzyme (ACE2), most commonly found in alveolar lung type II cells. One way to prevent this virus is to increase the immune system of the body to fight the infection when it enters the body. Ginger can also boost the body's immune system as it contains non-nutritional compounds with antioxidant properties. Ginger can also boost the body's immune system as it contains non-nutritional compounds with antioxidant properties. Antioxidants present in ginger, play a role in counteracting free radicals entering the body, and due to this reason, free radicals cannot do any damage to the cells of the body's immune system.

CONCLUSION

The increasing demand for nutritional therapies motivates the researchers and processors of food to introduce some food products with therapeutic potential. Although many investigations have been conducted to date to reveal the role of therapeutic components, their mechanism of action, the effect of processing, application, and safety, there are still areas that need to be explored to find out. Moreover, garlic demonstrated superior effects as compared to ginger alone. Several reports have shown the lipid-lowering effect of ginger (Shoaib et al., 2016). A meta-analysis-based study reported the favourable effects of ginger on triglycerol levels and LDL-C while having no effect on TC and HDL-C. Moreover, the low dose of ginger was found to have a greater lowering influence on total cholesterol and triglycerols. Ginger is also thought to be capable of combating common influenza viruses and influenza-like symptoms. Fresh ginger in the airway epithelium proved effective against plaque formation induced by the human respiratory syncytial virus (HRSV). Due to its properties, ginger is also being developed to improve its functionality in the form of nanoparticles as a drug delivery system, with various advantages for increased prevention.

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Ginger in Lung Cancer

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ABSTRACT

Lung cancer is a type of cancer that begins in the lungs. Your lungs are two spongy organs in your chest that take in oxygen when you inhale and release carbon dioxide when you exhale. Lung cancer is the leading cause of cancer deaths worldwide. People who smoke have the greatest risk of lung cancer, though lung cancer can also occur in people who have never smoked. The risk of lung cancer increases with the length of time and number of cigarettes you've smoked. If you quit smoking, even after smoking for many years, you can significantly reduce your chances of developing lung cancer. Smoking causes the majority of lung cancers – both in smokers and in people exposed to secondhand smoke. But lung cancer also occurs in people who never smoked and in those who never had prolonged exposure to secondhand smoke. In these cases, there may be no clear cause of lung cancer. Exactly when an individual has cell breakdown in the lungs, they have uncommon cells that gather to outline a tumour. As opposed to common cells, danger cells create without solicitation or control, pulverizing the sound lung tissue around them. Such tumour is called undermining tumours. Right when the illness cells spread, they hold organs of the body back from working suitably.

Key words: Ginger, Lung Cancer, tumor



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INTRODUCTION

Ginger (*Zingiber officinale*) is a flowering plant whose rhizome, ginger root or ginger, is widely used as a spice and a folk medicine.[2] which belongs to the Zingiberaceae family and the *Zingiber* genus, has been commonly consumed as a spice and an herbal medicine for a long time . Ginger root is used to attenuate and treat several common diseases, such as headaches, colds, nausea, and emesis. Many bioactive compounds in ginger have been identified, such as phenolic and terpene compounds. The phenolic compounds are mainly gingerols, shogaols, and paradols, which account for the various bioactivities of ginger. In recent years, ginger has been found to possess biological activities, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. In addition, accumulating studies have demonstrated that ginger possesses the potential to prevent and manage several diseases, such as neurodegenerative diseases, cardiovascular diseases, obesity, diabetes mellitus, chemotherapy-induced nausea and emesis and respiratory disorders. In this review, we focus on the bioactive compounds and bioactivities of ginger, and we pay special attention to its mechanisms of action.

HISTORY OF GINGER

Ginger first appeared in the southern parts of the ancient China. From there, it spread to India, Maluku Islands (so-called Spice Islands), rest of the Asia and West Africa. Europe saw ginger for the first time in the 1st century when the ancient Romans traded with the India. When the Rome fell, Europe forgot about ginger until Marco Polo brought it again from his travel to the East. In the Middle Ages, a price of a half a kilogram of ginger was the same as of one sheep. In the 15th century, with the rediscovery of the New World, Ginger was brought to the Caribbean where it started to grow with ease. Today, India is the greatest producer of ginger in the world Name “ginger” came a long way, but its root is in Sanskrit word “srngaveram” which means “horn body” and describes its root. While it grows, it has white and pink buds which bloom into yellow flowers. When the stalk withers, the rhizome is harvested and immediately scalded (which kills it) to prevent sprouting.

GINGER

Ginger is in the family Zingiberaceae, which also includes turmeric (*Curcuma longa*),[4] cardamom (*Elettaria cardamomum*), and galangal. Ginger originated in Maritime Southeast Asia and was likely domesticated first by the Austronesian peoples. It was transported with them throughout the Indo-Pacific during the Austronesian expansion (c. 5,000 BP), reaching as far as Hawaii. Ginger is one of the first spices to have been exported from Asia, arriving in Europe with the spice trade, and was used by ancient Greeks and Romans.[5] The distantly related dicots in the genus *Asarum* are commonly called wild ginger because of their similar taste. Although used in traditional medicine and as a dietary supplement, there is no good evidence that consuming ginger or its extracts has any effect on human health or as a treatment for diseases.[2][6] In 2019, world production of ginger was 4.1 million tonnes, led by India with 44% of the world total. Ginger (*Zingiber officinale*) is a flowering plant whose rhizome, ginger root or ginger, is widely used as a spice and a folk medicine.[2] It is a herbaceous perennial which grows annual pseudostems (false stems made of the rolled bases of leaves) about one meter tall bearing narrow leaf blades. The inflorescences bear flowers having pale yellow petals with purple edges, and arise directly from the rhizome on separate shoots.[3]

Nutritional Composition of Ginger

Chemical Composition

Garlic contains around 65% water, 28% carbo-hydrate (fructans), 2.3% organosulfur compounds, 2% solid of teins (allinase), 1.2% free amino ruinous (arginine) and 1.5% fiber. The amazing substance allicin (diallyl thiosulfate) is capable for the conventional compelling smell and for its helpful genuine ties (Macpherson *et al.*, 2005; Li, 2000; Tune and Milner, 2001). Basic sulfur containing reinforces present in garlic are gamma-glutamyl-s-allyl-cysteine and S-allyl-L- cysteins sulf-oxides (alliin). These moreover go about as harbingers two or three different mixtures The amazing substance allicin (diallyl thiosulfate) is capable for the conventional compelling smell and for its helpful



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Thiosulfinates

Impedance of garlic bulb accomplishes the strategy of thiosulfi-nates, for example, allicin, by the development of blends (Lawson and Hughes, 1992). Other thiosulfinates present in garlic are allyl methyl-methyl allyl-and trans-1 propenyl- thiosulfinate which are delicate in nature.

Organosulfur volatiles

Diallyl disulfide (Fathers), diallyl sulfide (DAS), diallyl trisulfide (DTS) and sulfur dioxide are shaped by the rot of allicin. The enormous volatiles that are perceived from upset garlic and garlic chief oil are, DAS, Fathers, DATS, methyl allyl disulfide, methyl allyl trisulfide vinyl dithiins and ajoenes. The enormous sulfide blends of garlic oil are DAS (5%), allyl methyl (37%), dimethyl (6%), mono-to-hexasulfides beside confined measure of allyl 1-propenyl and methyl 1-propenyl di-tri-and tetra sulfides.

Diels-birch Vinyl dithiins:

These are the thermal degradation products of allicin. It is formed by type of mechanism involving diels-alder dimerization of thioacrolein, which is obtained by the beta elimination of allicin. The oil macerate of raw garlic is a very rich source of 2-vinyl-4H-1,3 dithiin.

Ajoene

The design includes E and Z isomers of 4, 5, 9-trithiadodeca-1-oxide and 6-11-triene-9-oxide. Another ajoene is E-4, 5, 9-tritiradeca-1,7-diene-9 oxide which is an organosulfur com-pound present in oil macerated concentrate of garlic

Water soluble organosulfur compounds

The significant compound, S-allyl-L-cysteine (SAC) which is derived from gamma glutamyl-S- allyl-L-cysteine, is predomi-nantly found in the watery and alcoholic concentrates of garlic

Physiological Effect

For any segment to have physiological impacts it should be consumed by the body and the degree of ingestion can be concentrated by following its bio availability utilizing in vitro or in vivo models. The bio availability of alliin was concentrated in a mouse murine model by oral organization with a measurement level of 10 mg/mouse. The outcomes showed that alliin was available in stomach, digestive tract and liver to the degree of 7.2%, 22.4% and 2.5%.

Phytochemical Composition

Ginger is abundant in active constituents, such as phenolic and terpene compounds. The phenolic compounds in ginger are mainly gingerols, shogaols, and paradols. In fresh ginger, gingerols are the major polyphenols, such as 6-gingerol, 8-gingerol, and 10-gingerol

Bioactive Compound of Ginger

Bioactive Components. Ginger is abundant in active constituents, such as phenolic and terpene compounds. The phenolic compounds in ginger are mainly gingerols, shogaols, and paradols. In fresh ginger, gingerols are the major polyphenols, such as 6-gingerol, 8-gingerol, and 10-gingerol. With heat treatment or long-time storage, gingerols can be transformed into corresponding shogaols. After hydrogenation, shogaols can be transformed into paradols. There are also many other phenolic compounds in ginger, such as quercetin, zingerone, gingerenone-A, and 6-dehydrogingerdione. Moreover, there are several terpene components in ginger, such as β -bisabolene, α -curcumene, zingiberene, α -farnesene, and β -sesquiphellandrene, which are considered to be the main constituents of ginger essential oils. Besides these, polysaccharides, lipids, organic acids, and raw fibers are also present in ginger.



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It has been known that overproduction of free radicals, such as reactive oxygen species (ROS), plays an important part in the development of many chronic diseases. It has been reported that a variety of natural products possess antioxidant potential, such as vegetables, fruits, edible flowers, cereal grains, medicinal plants, and herbal infusions. Several studies have found that ginger also has high antioxidant activity. The antioxidant activity of ginger has been evaluated in vitro via ferric-reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) methods. The results revealed that dried ginger exhibited the strongest antioxidant activity, because the number of phenolic compounds was 5.2-, 1.1-, and 2.4-fold higher than that of fresh, stir-fried, and carbonized ginger, respectively. The antioxidant activity of different gingers had a tendency to be the following: dried ginger > stir-fried ginger > carbonized ginger > fresh ginger. This was mainly associated with their polyphenolic contents. When fresh ginger was heated, dried ginger with higher antioxidant activity was obtained, because fresh ginger contains a higher moisture content. However, when dried ginger was further heated to obtain stir-fried ginger and carbonized ginger, the antioxidant activity decreased, because the processing could change gingerols into shogaols. Additionally, a fraction of the dried ginger powder abundant in polyphenols showed high antioxidant activity based on data from FRAP, oxygen radical absorbance capacity, and cellular antioxidant activity assays. Besides, the type of extraction solvent could have an effect on the antioxidant activity of ginger. An ethanolic extract of ginger showed high Trolox-equivalent antioxidant capacity and ferric-reducing ability, and an aqueous extract of ginger exhibited strong free radical scavenging activity and chelating ability. Moreover, ethanolic, methanolic, ethyl acetate, hexane, and water extracts of ginger respectively inhibited 71%, 76%, 67%, 67%, and 43% of human low-density lipoprotein (LDL) oxidation induced by Cu²⁺. Results from a xanthine/xanthine oxidase system showed that an ethyl acetate extract and an aqueous extract had higher antioxidant properties than ethanol, diethyl ether, and *n*-butanol extracts did. Ginger extract showed antioxidant effects in human chondrocyte cells, with oxidative stress mediated by interleukin-1 β (IL-1 β). It stimulated the expression of several antioxidant enzymes and reduced the generation of ROS and lipid peroxidation.

Neuroprotection

Some individuals, especially elderly people, have a high risk for neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD). Recently, many investigations have revealed that ginger positively affects memory function and exhibits anti-neuroinflammatory activity, which might contribute to the management and prevention of neurodegenerative diseases.

Cytotoxicity

Cancer is the leading cause of morbidity and mortality worldwide, characterized by irregular cell growth. Cytotoxicity or killing tumor cells that divide rapidly is the basic function of chemotherapeutic drugs. However, these agents can damage normal dividing cells, leading to adverse effects in the body. Cytotoxicity is defined as the toxicity caused due to the action of chemotherapeutic agents on living cells. Cytotoxicity is the quality of being toxic to cells. Examples of toxic agents are an immune cell or some types of venom, e.g. from the puff adder (*Bitis arietans*) or brown recluse spider (*Loxosceles reclusa*). The cytotoxic influences and hidden structures of ginger in prostate malignancy had been assessed each in vivo and in vitro. The anticancer additives on the whole consist of the enlistment of apoptosis and the restraint of the enlargement of malignancy cells.

LUNG CANCER

Cellular failure in the lungs is malevolence that begins in the lungs. Exactly when an individual has cell breakdown in the lungs, they have uncommon cells that gather to outline a tumour. As opposed to common cells, danger cells create without solicitation or control, pulverizing the sound lung tissue around them. Such tumour is called undermining tumours. Right when the illness cells spread, they hold organs of the body back from working suitably.





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Types of Lungs Cancer

Non-small cell Lungs cancer (N small cell cancer unit)

About 85% to 90% of lung cancers are N small cell cancer unit. The essential subtypes of N small cell cancer unit are adenocarcinoma, squamous molecular carcinoma, and massive molecular carcinoma. These sub-types, which begin from special kinds of lung cells are assembled collectively as Nsmall cell cancer unit due to the fact their medication and prognose (outlooks) are regularly comparable.

Adenocarcinoma

Adenocarcinoma begin withinside the compartments that could generally secretes materials consisting of mucous. These kinds of lungs most cancers happen especially in present day or former smokers, however it's also the maximum not unusual place kind of lung most cancers visible in non-smokers. It is extra not unusual place in girls than in men, and its miles much more likely to arise in more youthful human beings than different sorts of lung most cancers. Adenocarcinoma is normally observed withinside the external elements of the lung and is most expected to be experimental earlier than it has meal.

Squamous cell carcinoma

Squamous molecular carcinoma instigates in squamous cells, which strength be plane cells that line the interior of the airlines within the lungs. They were consistently connected to a record of smoking and have a propensity to be situated withinside the prime a part of lungs, close to a chief airway.

Large cell carcinoma

Big molecular carcinomas can be seamed in any portion of the lung. It has a propensity to mature and unfold swiftly, that could be made it more problematic to treat. A subtype of big molecular carcinoma, recognized as big molecular neuroendocrine carcinomas, is a fast-developing utmost cancer this is selfsame much like small molecular lung most cancers.

Cancers that spread to Lungs

Cancers those begin in different types of organs (which includes breast, pancreas, kidney or skin) can be spread (metastasized) into the lungs, however those were not lung cancer. For example, most of the cancers those begins off evolve withinside breast and spreaded to the lungs continues to be the breast most cancers, now is not lungs cancer. Action for metastatic utmost cancers to the lungs is chiefly based absolutely on in which it on track.

ISOLATION OF COMPONENTS FROM GINGER

Gingerol

Gingerol, accurately as [6]-gingerol, may be a phenol phytochemical compound located in new ginger that enacts zest receptors at the tongue. Atomically, chemical irritant is a relative of chemical irritant associated piperine, the combinations that are alkaloids, but the bioactive pathways are detached. it's normally located as an impactful yellow oil withinside the ginger rhizome, but will likewise body a low-liquefying semitransparent strong. This substance compound is located taking all matters collectively people from the Zingiberene family.

Biological Activity of Gingerol

In a pre-medical meta-exam of gingerol intensifies anticancer, calming, opposed to contagious, most cancers prevention agent, neuroprotective and gastroprotective residences had been accounted for, which don't forget reads for vitro and A couple in-vivo examines have recommended that gingerols inspire sound glucose guiding principle for diabetics. Numerous examinations were across the influences of gingerols on a huge scope of malignant growths which include leukaemia, prostate, bosom, skin, ovarian, lung, pancreatic and There has now no longer been a number of medical attempting bent on note gingerols physiological leads to human. whereas a huge wide range of the substance additives related to the influences of gingerols on cells were completely contemplated, few were in an exceedingly medical setting. this can be because of the very smart quality in normal phytochemicals and also the absence of viability in analysis. Most flavorer healthful drug, that embody gingerols, are below the constraints of the



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Food and Drug Administration within the U.S. and take a glance at techniques have currently not control the maximum amount as research which has pale the motivation in phytochemical research. Herbal medicine is untested for fine affirmation, energy and adequacy in medical settings because of a insufficiency of finance in Jap medical research. Most exploration on [6]-Gingerol has been on each mouse subjects (in-vivo) or on delicate human tissue (in-vitro) and is maybe applied in an exceedingly whereas to talk some capability programs for multi-goal infectious.

Oleoresins

Ginger oleoresin is gotten by permeating the powdered rhizomes of ginger. Oleoresins are semi-solid extracts composed of resin and essential or fatty oil, obtained by evaporation of the solvents used for their production.^[1] The oleoresin of conifers is known as crude turpentine or gum turpentine, which consists of oil of turpentine and rosin. Most oleoresins are used as flavors and perfumes, some are used medicinally (e. g., oleoresin of dry Cannabis infructescence). Oleoresin capsicum is commonly used as a basis for tear gases. There are also uses known in the manufacture of soaps of cosmetics, as well as coloring agents for foods.

ANTICANCER ACTIVITY

The anticancer activity of ginger is attributed to its ability to modulate several signaling molecules like NF- κ B, STAT3, MAPK, PI3K, ERK1/2, Akt, TNF- α , COX-2, cyclin D1, cdk, MMP-9, survivin, cIAP-1, XIAP, Bcl-2, caspases, and other cell growth regulatory proteins.

CONCLUSION

- Ginger root is remarkable for its potassium and manganese content.
- Experimental studies showed that ginger and its active components including 6-gingerol and 6-shogaol exert anticancer activities against GI cancer.
- Potassium is essential for maintaining up regular blood flow, muscle and nerve work, whilst manganese assimilates beneficial vitamins and minerals.
- Ginger incorporates vitamins A, C and E, beta-carotene and zinc. Nutrient C and zinc invigorate the insusceptible framework, and guard the frame from infection and irritation.
- Ginger is fruitful in hindering 5-lipoxygenase, a protein that propels damage improvement. It diminishes the peril of dangerous improvement or even assistants withdraw tumours within the lungs.
- In specific examinations, ginger has been got down to effect lungs threat cells via way of means of : Crippling the development of dangerous improvement cells, Disturbing the development instance of damage cells, Controlling by and large conveyed combos to cause fall apart of cells, executing off risk cells via way of means of reducing off electricity advent and stimulating the arrival of artificial compounds that specific contamination cells.

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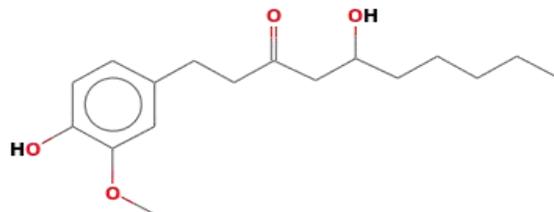


Fig. 1. Structure of Gingerol





Garlic as Immuno Booster- Review

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ABSTRACT

The benefits of garlic to health have been proclaimed for centuries; however, only recently have *Allium sativum* and its derivatives been proposed as promising candidates for maintaining the homeostasis of the immune system. The complex biochemistry of garlic makes it possible for variations in processing to yield different preparations with differences in final composition and compound proportion. In this review, we assess the most recent experimental results, which indicate that garlic appears to enhance the functioning of the immune system by stimulating certain cell types, such as macrophages, lymphocytes, natural killer (NK) cells, dendritic cells, and eosinophils, by mechanisms including modulation of cytokine secretion, immunoglobulin production, phagocytosis, and macrophage activation. Finally, because immune dysfunction plays an important role in the development and progress of several diseases, we critically examined immunoregulation by garlic extracts and compounds isolated, which can contribute to the treatment and prevention of pathologies such as obesity, metabolic syndrome, cardiovascular disorders, gastric ulcer, and even cancer. We concluded that *A. sativum* modulates cytokine secretion and that such modulation may provide a mechanism of action for many of their therapeutic effects.

Keywords: Garlic, Immune System, Diseases, Disorders, Therapeutic Effects

INTRODUCTION





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Plants of the genus *Allium* are known for their production of organosulfur compounds, which possess interesting biological and pharmacological properties. Among these, garlic (*Allium sativum*) is one of the most widely used ones. When extracted and isolated, these compounds exhibit a broad spectrum of beneficial effects against microbial infections as well as cardioprotective, anticancerigenic, and anti-inflammatory activity. Preparations of garlic are mainly liquid (aqueous, oil, or solvent extracts) or solid (dried garlic powder and fresh cataplasm). These extractions can be based on water formulations, oils, or by using solvents as alcohols. Composition of the extracts depends on the source of the garlic strain, age, storage conditions, and type of processing, and the effects of the extracts are influenced by the method of consumption. Biological effects of different garlic preparations and extracts are summarized in The wide variety of effects that has been reported of garlic preparations and extracts with beneficial and useful properties may be due to their numerous compounds (organosulfur and others) contained in different concentrations, which is being a challenge to separate and identify compounds with potential beneficial properties on the human immune and cardiovascular systems. A comprehensive classification of the different compound derived from garlic, as well as their biological effects reported, is actually in preparation and will be published elsewhere (Rodrigo-Arreola et al., in preparation). The presence and potency of garlic compounds vary with respect to mode of garlic preparation and extraction. Additionally, the proportion of these compounds is poorly controlled with the methods used to generate different garlic preparations, the main problem being reproducibility and validation of the real effects observed.

Kingdom	Plantae
Order	Asparagales
Family	Amaryllidaceae
Subfamily	Allioideae
Genus	<i>Allium</i>
Species	<i>A. sativum</i>

Main Organosulfur Compounds Purified from Garlic Preparations

The presence of garlic compounds varies with respect to mode of garlic preparation and extraction as follows: (1) fresh bulbs main compounds are S-allyl-L-cysteine sulfoxide (alliin) and γ -glutamyl cysteine derivatives; (2) in steam distilled oils, sulfide family compounds are the main compounds; (3) powder from crushed and dried garlic contains alliin and diallyl disulfide (DADS); (4) macerates (ground garlic) are enriched extractions with sulfide family compounds, dithiines, and (E-Z)-ajoene compounds, and (5) AGE (soaked, sliced, aged garlic extract in ethanol solution) contains S-allyl-L-cysteine (SAC) and S-allyl mercaptocysteine (SAMC). Garlic compounds can be divided in several groups or families of compounds. Among these families, we find γ -glutamyl cysteine derivatives, the primary precursor components of the alliin and allyl methyl cysteine (methiin) compound families, that produce, by enzymatic action of alliinase (alliin lyase, EC: 4.4.1.4), the diallylthiosulfinate (Allicin) and allyl methyl thiosulfinate (AM) compound families, which are precursors of several organosulfur compound families (i.e., the ajoene and dithiin families). Additionally, garlic preparations contain nonorganosulfured compounds, such as tetrahydro-beta-carbolines, fructans, and glucose-linked β -D-fructofuranosyl, identified in AGE preparations.

Immunomodulatory Properties of *Allium sativum*

Immunomodulation is one of the main targets for synthetic drugs and chemicals. However, its high cost, anticipated toxicity, and adverse event effects render it undesirable for the patients. In contrast, the use of herbal plants as health promoters is gaining increasing attention in both consumers and scientific circles. In the literature, several plants have been listed that exhibit immunomodulatory actions, like modulation of cytokine secretion; phagocytosis promotion and macrophage activation; immunoglobulin production; allergic reactions and lymphocyte proliferation. Recently, garlic has been suggested as a promising candidate for maintaining the homeostasis of the immune system. Several studies have been carried out in animal models to examine the effect of different garlic components and formulations on immunomodulatory activities



**Ishmapriyadarshini Sahoo et al.****Modulation of Cytokine Secretion by Garlic Derivatives**

Herbal medicines with immunomodulatory activity alter the immune function through the dynamic regulation of molecules such as cytokines and chemokines. Altering cytokine expression and targeting their receptors may offer therapeutic potential. Current pharmacological strategies include cytokine antagonist, agonist, inhibition, and stimulation models. However, in light of the adverse events experienced with cytokine-targeted therapy, it could be useful to consider the use of phytotherapy in the modulation of cytokine expression. Recently, Quintero-Fabián et al. examined the effects of alliin in lipopolysaccharide- (LPS-) stimulated 3T3-L1 adipocytes. Incubation of cells for 24 h with 100 $\mu\text{mol/L}$ alliin prior to LPS (100 ng/mL) stimulation for 1 h prevented an increase in the expression of proinflammatory genes *IL-6*, *MCP-1*, and *Egr-1* and in the protein levels of IL-6 and MCP-1. Interestingly, the phosphorylation of ERK1/2, which is involved in LPS-induced inflammation in adipocytes, decreased following alliin treatment. Furthermore, gene expression profile by microarray evidences an upregulation of genes involved in immune response and downregulation of genes related with cancer. Indeed SAC, caffeic acid (CA), uracil, diallyl trisulfide (DATS, as known as Allitridin), diallylsulfide (DAS), and other garlic-derived compounds can inhibit transcription factor NF- κ B, a master regulator, inhibiting the transcription of several cytokine genes involved in proinflammatory responses, such as *TNF- α* , interleukin-1beta (*IL-1 β*), *IL-6*, *MCP-1*, and *IL-12(p70)*.

Phagocytosis Promotion and Macrophage Activation

The Th1 cytokine pattern is essential for controlling parasite load during the early phase of malaria infection. Feng et al. found that allicin administered to Balb/c mice postinfected with *Plasmodium yoelii* reduced parasitemia and prolonged survival due to the enhancement of proinflammatory mediators such as interferon-gamma (IFN- γ); additionally, allicin treatment stimulated the expansion of CD4⁺ T cells and macrophages. The antimicrobial activity of allicin was demonstrated by modulation of the cytokines activating macrophages that controlled the parasitic infection.

Immunoglobulin Production

Modulation by means of a Th2 profile aids in the generation of an efficient humoral immune response. Washiya et al. investigated, in a mouse model, the effects of an oil-macerated garlic extract that contained Z-ajoene. The authors found that fecal IgA levels increased after 3 weeks of treatment and concluded that ajoene may have exerted an influence on B-cell stimulation or interleukin secretion. Hanieh et al. proved that dietary *Allium sativum* and *Allium cepa* at low doses in white Leghorn chickens, following immunization with Newcastle Disease Virus (NDV), Sheep red blood cells (SRBC), and *Brucella abortus* (BA), enhanced anti-NDV, anti-SRBC, and anti-BA antibody production. The authors concluded that enhanced T cell proliferation with dietary garlic might have directly/indirectly enhanced B-cell proliferation and differentiation. However, opposite results have been reported with garlic in the induction of antibody secretion. Jafari et al. reported that supplementing broilers with garlic do not have any beneficial effects on antibody production. Therefore, more studies with garlic and its derivatives are necessary in order to clarify the mechanism implicated in immunoglobulin production.

Antiallergic and Allergic Properties of Garlic

An allergic reaction involves the secretion of immunoglobulin E (IgE) and inflammatory mediators by immune cells. Kyo et al. found that AGE possesses antiallergic properties. In a rat basophil cell line, RBL-2H3, these authors induced histamine release with monoclonal antibodies, and after AGE administration, this significantly inhibited the antigen-specific histamine release. In addition, in a mouse model, orally administered (o.a.) AGE significantly decreased the index of immunoglobulin IgE-mediated skin reaction. Zare et al. investigated the effect of intraperitoneal (i.p.) injections of AGE on an established allergic-airway inflammation murine model and observed that AGE treatment caused a significant decrease in the hallmark criteria of allergic-airway inflammation. On the other hand, dietary garlic lectins have been shown to release histamine from mast cells and basophils as a result of their interaction with cell-surface IgE molecules. Recently, Clement et al. isolated three immunomodulatory proteins (QR-1, QR-2, and QR-3) from raw garlic. In humans, skin prick test (SPT) using QR-1 and QR-2 on atopic and nonatopic subjects revealed that ~26% (in the case of QR-2) of atopic subjects demonstrated a positive reaction, compared with negative reactions in the case of nonatopic (normal) subjects. QR-2 induced histamine release from



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leukocytes to a much greater degree in the case of atopics compared with nonatopics. Results noted the propensity of garlic lectins to nonspecifically activate mast cells and basophils in atopics as a result of the higher density of IgE in these patients.

Immunostimulatory Activities of Garlic

Fructooligosaccharides (FOS) are fructans that are naturally present in garlic. Chandrashekar et al. isolated fructans present in AGE: high molecular weight (>3.5 kDa; HF) and low molecular weight (<3 kDa; LF), which were assessed in an immunostimulatory mouse model. Both HF and LF displayed mitogenic activity and activation of macrophages including phagocytosis. These activities were comparable with those of known polysaccharide immunomodulators, such as zymosan and mannan. Additionally, similar results have been obtained with immunoproteins QR-1, QR-2, and QR-3, present in garlic and identified as lectins or agglutinins were previously described as ASA II and ASA I, and their mitogenic and comitogenic properties were confirmed as comparable with potent mitogenic lectins ConA and PHA. On the other hand, it is well known that fructans selectively stimulate some beneficial bacteria in colon, modulating different immune responses. Despite increasing evidence, the different components in garlic responsible for effective immune stimulation or inhibition are not known conclusively, and it is likely that several components are responsible for its immunopharmacological mechanisms. Therefore, further research on garlic fructans may cast light on the underlying mechanisms of immunomodulation and should aid in identifying potential uses of garlic fructans in various therapeutic applications.

Effects of Garlic Compounds/ Extracts on Cells of the Immune System

Different studies have shown that garlic compounds are able to perform antiapoptotic, antiparasitic, proapoptotic, anticancerigenic, and immunomodulatory effects on different cells. It was observed in a murine macrophages cell line infected with *Leishmania* that AGE induced IL-12 production and, in addition, INF- γ and inducible nitric oxide synthase (iNOS) were over expressed. However, in peripheral blood monocytes, AGE upregulated IL-10 and decreased IL-12 production, which might cause down regulation of proinflammatory cytokines TNF- α , IL-6, INF- γ , and IL-2 by T cells and it acts as negative feedback in the signaling proinflammatory response. Additionally, DADS decrease NO production, proinflammatory cytokines, and protein expression in a mouse leukaemic monocyte/macrophage cell line. Therefore, garlic compounds could act as immunomodulatory agents on the macrophages response.

Other studies conducted in mice have been shown that DATS can enhance the antiviral immune response to murine cytomegalovirus (MCMV), by blocking Treg *in vivo* in chronic MCMV infection. Additionally, the protein fraction of fresh garlic stimulates the peripheral blood T-lymphocyte proliferation and increases CD8⁺ subpopulation in treated animals, causing an increase in delayed-type hypersensitivity responses, promoting an efficient cellular response. However, these studies did not assess the cytokine profile, which could provide more information about the immunomodulatory role of different garlic protein subfractions. It has been documented that garlic or its compounds induce a variety of immunomodulatory activities in leukocyte cytokine production. In Th1 cells, inflammatory cytokine production is reduced significantly in the presence of garlic extract and/or its compounds, revealing a potential therapeutic use in inflammatory conditions such as inflammatory bowel disease (IBD) and malaria. However, it is also known that garlic oil shifts the Th1-Th2 balance toward the Th2 type.

Furthermore, garlic derivatives exert both stimulatory and inhibitory effects on whole blood cultures of monocytes and lymphocyte proliferation and LPS-induced TNF- α generation through IL-10 production, which controls proinflammatory cytokines. Moreover, other compounds, such as allicin, exert negative effects on human T-cell migration through fibronectin by down regulating actin reorganization. Even more so, protein fraction 4, isolated from AGE, enhances the cytotoxic activity of human peripheral blood lymphocytes (HPBL) in synergy with IL-2 and independently from INF- γ or TNF- α . Finally, the $\gamma\delta$ -T population, as a unique type of T cell that recognizes and responds to pathogen-associated molecular patterns (PAMP), increases its proliferation by AGE supplementation in healthy humans. Taken together, these data strongly suggest that garlic compounds and its derivatives are involved in the cellular immune response, acting as immunoregulators; however, more studies are needed to clarify its use in



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immunotherapy. Proteins isolated from garlic modulate NK cell line activity in the mesenteric lymph node of mouse, while AGE modulates the number and the activity of NK cells in patients with various advanced cancers and also increases NK activities against different cancerous cell lines. Moreover, in healthy subjects, AGE increases the NK cell population. Therefore, garlic acts as a proliferation inductor for this cell type. Mature dendritic cells (DC) can activate naïve lymphocytes and play a critical role in the induction of primary immune response. Allicin treatment could promote the maturation of DC by increasing the expression of costimulatory molecules such as CD40, yielding an enhancement of the proinflammatory immune response in a rodent malaria model. However, it was not possible to establish whether the 14 kDa protein isolated from AGE induced mouse DC *in vitro* maturation by an increase in the expression of the CD40 molecule in DC. Consequently, future studies are needed to determine the effect of garlic on DC.

Garlic allergens have been reported as causing hypersensitivity reactions in both patients and animal models, such as dermatitis, rhinoconjunctivitis, asthma, urticaria, and anaphylaxis after ingestion of garlic. This can be due to cross-reactivity in patients with oral allergy. Recently, it was demonstrated that a 56-kDa protein of alliin lyase is the major IgE-binding protein in patients allergic to garlic. Alliin lyase contains a carbohydrate with free terminal α -D-glucopyranoside or α -mannopyranoside residues, thought to bind human IgE in subjects with allergy and to lead to cross-reactivity. Additionally, three protein components from raw garlic displayed hemagglutination and mannose-binding activities; one of these induces histamine release from human leukocytes; likewise, garlic lectins are able to evoke immunogenicity. However, the molecular basis of the interaction between food allergens and the immune system is not clear.

Role of Garlic Compound in Inflammatory disorders

Numerous research works have shown the immunomodulatory and immunotherapeutic potentials of AGE as a whole, including free radical-mediated anti-inflammatory, anticancer, and antiangiogenic effects, as well as improving hyperglycemia and dyslipidemia, cardiovascular diseases, infectious diseases, autoimmune diseases, and allergy, which have been shown in both animal models and cell lines. It is known that the aqueous garlic extract exerts antioxidant action by scavenging reactive oxygen species (ROS) and enhancing cellular antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. In addition, garlic represents an important source of antioxidants due to phytochemicals such as DAS and SAMC.

Metabolic Syndrome

The metabolic syndrome is a cluster of abnormalities including hypertension, insulin resistance, hyperlipidemia, glucose intolerance, and abdominal obesity. This syndrome frequently precedes type 2 diabetes and atherosclerosis. The role of garlic has been studied in some of these pathologies, and their effects on the immune system components associated with the proinflammatory state of metabolic syndrome include modulation of oxidative stress (OS), proapoptotic signal pathways, inflammatory mediators, and cellular activities.

Cardiovascular Disorders

Cardiovascular diseases (CVD) continue to accelerate globally and remain the largest cause of deaths worldwide. CVD include diseases of the heart, vascular diseases of the brain, and diseases of blood vessels. Plasma markers of inflammation have also been evaluated as potential tools for prediction of the risk of coronary events. Among these are markers of systemic inflammation, such as high-sensitivity C-reactive protein (CRP), and acute-phase protein, serum amyloid A, cytokines such as IL-6, and adhesion molecules such as soluble intercellular adhesion molecule type 1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). The participation of ROS and the activity of endothelial nitric oxide synthase (eNOS) in vascular alterations have been reported.

Several studies *in vitro*, have confirmed the cardio protective effects of garlic on primary cultured cardiac myocytes, fibroblasts, and endothelial cells, by reducing the production of ROS and blocking ROS-dependent extracellular signal-regulated kinase (ERK)1/2, JNK1/2, AKT, NF- κ B, and SMADS signaling. However, garlic powder exerts no detectable effects on CRP, TNF- α , ICAM-1, lipid concentrations, and risk markers for inflammatory processes



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associated with subjects with atherosclerosis and CVD; additionally, AGE does not change plasma cholesterol level or ICAM-1 expression in a rabbit model of atherosclerosis. However, studies do not reflect the entire population-at-risk for atherosclerosis and cardiovascular diseases because these studies underwent adverse events in disease course (e.g., significant numbers of subjects withdrew from the study). In contrast, recent data showed that long-term administration of aqueous garlic was capable of attenuating VCAM-1 expression in fructose-fed rats. Therefore, garlic compounds reduce vascular inflammation.

Atherosclerosis is recognized as a complex disease characterized by an excessive inflammatory, fibrofatty, and proliferative response to damage in the vascular endothelium and involving several cell types, particularly smooth muscle cells, monocyte-derived macrophages, T-lymphocytes, and platelets. Clinical reports have revealed the potential benefits of garlic as a modulator of multiple cardiovascular features through lowering low-density lipoproteins (LDL) and blood pressure, reducing platelet aggregation and adhesion, preventing LDL oxidation, and reducing the progression of atherosclerosis. However, it is known that some garlic compounds, such as DADS and allyl mercaptan, did not inhibit the transcriptional activity of factor NF- κ B employing human umbilical endothelial cells, suggesting that they play a pivotal role in atherogenesis by regulating the expression of proinflammatory genes and of NF- κ B-regulated genes, suggesting that NF- κ B is not the major target of DADS and allyl mercaptan. Accordingly, there are differential effects among different organosulfur compounds of garlic; thus, more research is needed to discriminate the beneficial effects accurately and to ascribe these to specific garlic compounds.

Obesity

Obesity is associated with low-grade chronic inflammation characterized by abnormal cytokine production, increased acute-phase reactants, and other mediators in response to excess nutrients in metabolic cells. Activation of a network of inflammatory signaling pathways in the cell eventually causes the activation of specialized immune cells and leads to an unresolved inflammatory response within the tissue. Thus, macrophage, mast-cell, and NK-cell infiltration is present in obese adipose tissue, which participates in the inflammatory changes in obesity and contributes to insulin resistance. Garlic 1,2-vinyldithiin reduces the secretion of IL-6 and MCP-1, -2 in human preadipocytes treated with macrophage factors. Both molecules are associated with inflammation and the metabolic complications of obesity. Recently, our group demonstrated that allicin prevents the increase of genes and proteins related with the proinflammatory state induced by LPS in 3T3-L1 adipocytes, through the toll-like receptor-4 (TLR-4) pathway and possibly, by regulating ERK1/2 activity.

Ulcerogastric Pathologies

In gastric pathophysiology, T and B cells are clearly involved. OS causes damage to lipids, proteins, and DNA. In this respect, garlic has been studied as a gastroprotective agent. AGE capsules have been capable of resolving indomethacin-induced OS in gastric tissue through a reduction of TNF- α and malondialdehyde levels and reduction of myeloperoxidase activity, as well as increasing total glutathione, superoxide dismutase, and catalase activities in animal model. Additionally, garlic oil administered to rats prior to ethanol administration induced a decrease in ulcer index and lipid peroxidation and ameliorated the decrease in antioxidant enzyme levels caused by ethanol. Therefore, garlic can be considered an excellent preventive and protective agent to reduce gastric pathologies. The anti-inflammatory effect of the garlic extract by IL-10 deregulation and the reduction of IL-12 production in Inflammatory bowel disease (IBD) prevents IL-12 from binding to its receptor on T and NK cells, causing inhibition of the production of IFN- γ .

Cancer

Numerous health benefits have been ascribed to organosulfur compounds, including its immunomodulatory properties in cancer. A report in the literature noted an association between garlic consumption and decreased incidence of distal colon cancer in women in a cohort study. It has been proposed that allicin presents antitumor activity *in situ*. More specifically, cultured Ehrlich ascites carcinoma (EAC) cells treated with tamoxifen and supplemented with allicin resulted in cytotoxic damage markers and a decrease in TNF- α levels. Hence, a beneficial role of allicin is suggested as an adjuvant to tamoxifen treatment in cancer. Recent work also showed that SAC and



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DATS cause inhibition of PI3K/Akt, JNK apoptotic pathways in human ovarian, and T24 human bladder cancer cells. Even more allicin induces apoptosis through JNK pathway activation and mitochondrial Bax translocation in cells human ovarian cell line SKOV3. Other studies have demonstrated the role of protein fractions from garlic bulbs in tumor growth and intratumor-infiltrated T lymphocytes in mice transplanted with mammary tumor cells, as well as a significant decrease in the size of mouse mammary tumor and complete suppression of growth of Human erythroleukemia cell line HEL in a dose-dependent manner. Recently, our group evidenced that alliin treatment of 3T3-cell-derived adipocytes is capable of downregulating several cancer-related genes. Thus, garlic compounds could significantly affect the tumor development, thorough, at least, their antiproliferative action. Other groups have shown that fraction 4 of AGE, combined with IL-2 administration, could be employed in tumor immunotherapy, because these increase the cytotoxicity of T-cell lineage, and it has been proposed that the sulfhydryl-group hydrophobic portion of proteins, as well as estrogen receptors with cysteine residues in hormone-binding, could be target of inhibition from organosulfur compounds of garlic, (e.g., allyl sulfides). This may be of greater benefit in the prevention of hormone-responsive carcinogenesis. Thus, while total sulfur may be comparable, marked differences in specific organosulfur components likely exist among the preparations studied, which strongly suggest that the antitumor effect of allyl sulfur compounds may be related with both their anti-inflammatory and their immunostimulatory properties.

CONCLUSION

Garlic is one of the most employed seasonings for cooking. In addition to its use as a food additive, garlic has been long used in traditional medicine with protective and curative purposes. At present, the trend toward the use of natural remedies with fewer side effects has given rise to garlic consumption as an alternative therapy for diseases such as cardiovascular diseases, cancer, and microbial infections. Different dietary garlic formulations, such as powder (tablets), garlic oil (capsules), and aged garlic extracts (tablets, capsules, and liquids), have been incorporated into the globally increased market of garlic bioactive compounds. However, the variety of manufacturing processes of garlic comprises important issues when choosing a garlic supplement, due to that these processes can markedly influence the composition of the garlic product and thus its biological effects. Garlic as an herbal medicine or its different bioactive molecules and formulations have been extensively probed in *in vitro/in vivo* animal models to examine its anti-inflammatory and immunomodulatory properties. One of the main mechanisms observed is through modulation of cytokine profiles and, on the other hand, direct stimulation of immune cells. Although there is sufficient scientific evidence on the beneficial effects of garlic as therapy under different pathological conditions in animal models, human clinical studies are scarce and methodologically weak, with short duration and a reduced number of patients. Therefore, it is mandatory to establish general criteria to finally probe the variety of nutritional and health-promoting properties of garlic.

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Table 1 Biological effects of different types of garlic preparations and extracts.

Preparations/extract	Effect
Dehydrated garlic powder/ slices/ crushed	Diminish serum cholesterol
Aqueous extracts	Antibacterial
	Antiparasitic
	Modify immune response
	Lipid metabolism
	Cardiovascular-protective effects
Oil extracts	Antibacterial
	Acaricidal
	Modify Immune response
Chloroform extract	Inhibiting ROS formation and attenuating the activities of adhesion molecules
Hexane extract	Cytotoxic
	Modify immune response
AGE	Antioxidant
	ROS scavenger and anti-inflammatory
	Inhibits development of preneoplastic lesions





Vitamin C as Immune Booster

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ABSTRACT

Vitamin C is an essential micronutrient for humans, with pleiotropic functions related to its ability to donate electrons. It is a potent antioxidant and a cofactor for a family of biosynthetic and gene regulatory enzymes. Vitamin C contributes to immune defense by supporting various cellular functions of both the innate and adaptive immune system. Vitamin C supports epithelial barrier function against pathogens and promotes the oxidant scavenging activity of the skin, thereby potentially protecting against environmental oxidative stress. Vitamin C accumulates in phagocytic cells, such as neutrophils, and can enhance chemotaxis, phagocytosis, generation of reactive oxygen species, and ultimately microbial killing. It is also needed for apoptosis and clearance of the spent neutrophils from sites of infection by macrophages, thereby decreasing necrosis/NETosis and potential tissue damage. The role of vitamin C in lymphocytes is less clear, but it has been shown to enhance differentiation and proliferation of B- and T-cells, likely due to its gene regulating effects. Vitamin C deficiency results in impaired immunity and higher susceptibility to infections. In turn, infections significantly impact on vitamin C levels due to enhanced inflammation and metabolic requirements. Furthermore, supplementation with vitamin C appears to be able to both prevent and treat respiratory and systemic infections. Prophylactic prevention of infection requires dietary vitamin C intakes that provide at least adequate, if not saturating plasma levels (i.e., 100–200 mg/day), which optimize cell and tissue levels. In contrast, treatment of established infections requires significantly higher (gram) doses of the vitamin to compensate for the increased inflammatory response and metabolic demand.

Keywords: Vitamin C deficiency, lymphocytes, B- and T-cells, Immune Booster





INTRODUCTION

The immune system is a multifaceted and sophisticated network of specialized organs, tissues, cells, proteins, and chemicals, which has evolved in order to protect the host from a range of pathogens, such as bacteria, viruses, fungi, and parasites, as well as cancer cells. It can be divided into epithelial barriers, and cellular and humoral constituents of either innate (non-specific) and acquired (specific) immunity. These constituents interact in multiple and highly complex ways. More than half a century of research has shown vitamin C to be a crucial player in various aspects of the immune system, particularly immune cell function. Vitamin C is an essential nutrient which cannot be synthesized by humans due to loss of a key enzyme in the biosynthetic pathway. Severe vitamin C deficiency results in the potentially fatal disease scurvy. Scurvy is characterized by weakening of collagenous structures, resulting in poor wound healing, and impaired immunity. Individuals with scurvy are highly susceptible to potentially fatal infections such as pneumonia. In turn, infections can significantly impact on vitamin C levels due to enhanced inflammation and metabolic requirements. Early on, it was noted that scurvy often followed infectious epidemics in populations, and cases of scurvy have been reported following respiratory infection. This is particularly apparent for individuals who are already malnourished.

Although the amount of vitamin C required to prevent scurvy is relatively low (i.e., ~10 mg/day), the recommended dietary intakes for vitamin C are up to one hundred-fold higher than that for many other vitamins. A diet that supplies 100–200 mg/day of vitamin C provides adequate to saturating plasma concentrations in healthy individuals and should cover general requirements for the reduction of chronic disease risk. Due to the low storage capacity of the body for the water-soluble vitamin, a regular and adequate intake is required to prevent hypovitaminosis C. Epidemiological studies have indicated that hypovitaminosis C (plasma vitamin C < 23 $\mu\text{mol/L}$) is relatively common in Western populations, and vitamin C deficiency (<11 $\mu\text{mol/L}$) is the fourth leading nutrient deficiency in the United States. There are several reasons why vitamin C dietary recommendations are not met, even in countries where food availability and supply would be expected to be sufficient. These include poor dietary habits, life-stages and/or lifestyles either limiting intakes or increasing micronutrient requirements (e.g., smoking and alcohol or drug abuse), various diseases, exposure to pollutants and smoke (both active and passive), and economic reasons (poor socioeconomic status and limited access to nutritious food). Even otherwise 'healthy' individuals in industrialized countries can be at risk due to lifestyle-related factors, such as those on a diet or eating an unbalanced diet, and people facing periods of excessive physical or psychological stress.

Vitamin C has a number of activities that could conceivably contribute to its immune-modulating effects. It is a highly effective antioxidant, due to its ability to readily donate electrons, thus protecting important biomolecules (proteins, lipids, carbohydrates, and nucleic acids) from damage by oxidants generated during normal cell metabolism and through exposure to toxins and pollutants (e.g., cigarette smoke). Vitamin C is also a cofactor for a family of biosynthetic and gene regulatory monooxygenase and dioxygenase enzymes. The vitamin has long been known as a cofactor for the lysyl and prolyl hydroxylases required for stabilization of the tertiary structure of collagen, and is a cofactor for the two hydroxylases involved in carnitine biosynthesis, a molecule required for transport of fatty acids into mitochondria for generation of metabolic energy.

Vitamin C is also a cofactor for the hydroxylase enzymes involved in the synthesis of catecholamine hormones, e.g., norepinephrine, and amidated peptide hormones e.g., vasopressin, which are central to the cardiovascular response to severe infection. Furthermore, research over the past 15 years or so has uncovered new roles for vitamin C in the regulation of gene transcription and cell signaling pathways through regulation of transcription factor activity and epigenetic marks. For example, the asparagyl and prolyl hydroxylases required for the downregulation of the pleiotropic transcription factor hypoxia-inducible factor-1 α (HIF-1 α) utilize vitamin C as a cofactor. Recent research has also indicated an important role for vitamin C in regulation of DNA and histone methylation by acting as a cofactor for enzymes which hydroxylate these epigenetic marks.





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Our review explores the various roles of vitamin C in the immune system, including barrier integrity and leukocyte function, and discusses potential mechanisms of action. We discuss the relevance of the immune-modulating effects of vitamin C in the context of infections and conditions leading to vitamin C insufficiency.

Vitamin C

Vitamin C (also known as ascorbic acid and ascorbate) is a water-soluble vitamin found in citrus and other fruits and vegetables, and also sold as a dietary supplement. It is used to prevent and treat scurvy. Vitamin C is an essential nutrient involved in the repair of tissue, the formation of collagen, and the enzymatic production of certain neurotransmitters. It is required for the functioning of several enzymes and is important for immune system function. It also functions as an antioxidant. Most animals are able to synthesize their own vitamin C. However, apes (including humans) and monkeys (but not all primates), most bats, some rodents, and certain other animals must acquire it from dietary sources. There is some evidence that regular use of supplements may reduce the duration of the common cold, but it does not appear to prevent infection. It is unclear whether supplementation affects the risk of cancer, cardiovascular disease, or dementia. It may be taken by mouth or by injection. Vitamin C is generally well tolerated. Large doses may cause gastrointestinal discomfort, headache, trouble sleeping, and flushing of the skin. Normal doses are safe during pregnancy. The United States Institute of Medicine recommends against taking large doses. Vitamin C was discovered in 1912, isolated in 1928, and, in 1933, was the first vitamin to be chemically produced. It is on the World Health Organization's List of Essential Medicines. Vitamin C is available as an inexpensive generic and over-the-counter medication. Partly for its discovery, Albert Szent-Györgyi and Walter Norman Haworth were awarded the 1937 Nobel Prizes in Physiology and Medicine and Chemistry, respectively. Foods containing vitamin C include citrus fruits, kiwifruit, guava, broccoli, Brussels sprouts, bell peppers, potatoes and strawberries. Prolonged storage or cooking may reduce vitamin C content in foods.

7 Impressive Ways Vitamin C Benefits Your Body

- Vitamin C is an essential vitamin, meaning your body can't produce it. Yet, it has many roles and has been linked to impressive health benefits.
- It's water-soluble and found in many fruits and vegetables, including oranges, strawberries, kiwi fruit, bell peppers, broccoli, kale, and spinach.
- The recommended daily intake for vitamin C is 75 mg for women and 90 mg for men.
- While it's commonly advised to get your vitamin C intake from foods, many people turn to supplements to meet their needs.
- Here are 7 scientifically proven benefits of taking a vitamin C supplement.

May reduce your risk of chronic disease

- Vitamin C is a powerful antioxidant that can strengthen your body's natural defenses.
- Antioxidants are molecules that boost the immune system. They do so by protecting cells from harmful molecules called free radicals.
- When free radicals accumulate, they can promote a state known as oxidative stress, which has been linked to many chronic diseases .
- Studies show that consuming more vitamin C can increase your blood antioxidant levels by up to 30%. This helps the body's natural defenses fight inflammation

May help manage high blood pressure

- Approximately one-third of American adults have high blood pressure .
- High blood pressure puts you at risk of heart disease, the leading cause of death globally .
- Studies have shown that vitamin C may help lower blood pressure in both those with and without high blood pressure.
- An animal study found that taking a vitamin C supplement helped relax the blood vessels that carry blood from the heart, which helped reduce blood pressure levels .





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- Moreover, an analysis of 29 human studies found that taking a vitamin C supplement reduced systolic blood pressure (the upper value) by 3.8 mmHg and diastolic blood pressure (the lower value) by 1.5 mmHg, on average, in healthy adults.
- In adults with high blood pressure, vitamin C supplements reduced systolic blood pressure by 4.9 mmHg and diastolic blood pressure by 1.7 mmHg, on average .
- While these results are promising, it's not clear whether the effects on blood pressure are long term. Moreover, people with high blood pressure should not rely on vitamin C alone for treatment.

May lower your risk of heart disease

- Heart disease is the leading cause of death worldwide .
- Many factors increase the risk of heart disease, including high blood pressure, high triglyceride or LDL (bad) cholesterol levels, and low levels of HDL (good) cholesterol.
- Vitamin C may help reduce these risk factors, which may reduce heart disease risk.
- For example, an analysis of 9 studies with a combined 293,172 participants found that after 10 years, people who took at least 700 mg of vitamin C daily had a 25% lower risk of heart disease than those who did not take a vitamin C supplement .
- Interestingly, another analysis of 15 studies found that consuming vitamin C from foods — not supplements — was linked to a lower risk of heart disease.
- However, scientists were unsure whether people who consumed vitamin-C-rich foods also followed a healthier lifestyle than people who took a supplement. Thus, it remains unclear whether the differences were due to vitamin C or other aspects of their diet .
- Another analysis of 13 studies looked at the effects of taking at least 500 mg of vitamin C daily on risk factors for heart disease, such as blood cholesterol and triglyceride levels.
- The analysis found that taking a vitamin C supplement significantly reduced LDL (bad) cholesterol by approximately 7.9 mg/dL and blood triglycerides by 20.1 mg/dL .
- In short, it seems that taking or consuming at least 500 mg of vitamin C daily may reduce the risk of heart disease. However, if you already consume a vitamin-C-rich diet, then supplements may not provide additional heart health benefits.

May reduce blood uric acid levels and help prevent gout attacks

- Gout is a type of arthritis that affects approximately 4% of American adults .
- It's incredibly painful and involves inflammation of the joints, especially those of the big toes. People with gout experience swelling and sudden, severe attacks of pain .
- Gout symptoms appear when there is too much uric acid in the blood. Uric acid is a waste product produced by the body. At high levels, it may crystallize and deposit in the joints.
- Interestingly, several studies have shown that vitamin C may help reduce uric acid in the blood and, as a result, protect against gout attacks.
- For example, a study including 1,387 men found that those who consumed the most vitamin C had significantly lower blood levels of uric acid than those who consumed the least .
- Another study followed 46,994 healthy men over 20 years to determine whether vitamin C intake was linked to developing gout. It found that people who took a vitamin C supplement had a 44% lower gout risk .

Helps prevent iron deficiency

- Iron is an important nutrient that has a variety of functions in the body. It's essential for making red blood cells and transporting oxygen throughout the body.
- Vitamin C supplements can help improve the absorption of iron from the diet. Vitamin C assists in converting iron that is poorly absorbed, such as plant-based sources of iron, into a form that is easier to absorb .
- This is especially useful for people on a meat-free diet, as meat is a major source of iron.
- In fact, simply consuming 100 mg of vitamin C may improve iron absorption by 67%
- As a result, vitamin C may help reduce the risk of anemia among people prone to iron deficiency.





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- In one study, 65 children with mild iron deficiency anemia were given a vitamin C supplement. Researchers found that the supplement alone helped control their anemia
- If you have low iron levels, consuming more vitamin-C-rich foods or taking a vitamin C supplement may help improve your blood iron levels.

Boosts immunity

- One of the main reasons people take vitamin C supplements is to boost their immunity, as vitamin C is involved in many parts of the immune system.
- First, vitamin C helps encourage the production of white blood cells known as lymphocytes and phagocytes, which help protect the body against infection .
- Second, vitamin C helps these white blood cells function more effectively while protecting them from damage by potentially harmful molecules, such as free radicals.
- Third, vitamin C is an essential part of the skin's defense system. It's actively transported to the skin, where it can act as an antioxidant and help strengthen the skin's barriers.
- Studies have also shown that taking vitamin C may shorten wound healing time
- What's more, low vitamin C levels have been linked to poor health outcomes.
- For example, people who have pneumonia tend to have lower vitamin C levels, and vitamin C supplements have been shown to shorten the recovery time.

Protects your memory and thinking as you age

- Dementia is a broad term used to describe symptoms of poor thinking and memory.
- It affects over 35 million people worldwide and typically occurs among older adults .
- Studies suggest that oxidative stress and inflammation near the brain, spine, and nerves (altogether known as the central nervous system) can increase the risk of dementia .
- Vitamin C is a strong antioxidant. Low levels of this vitamin have been linked to an impaired ability to think and remember .
- Moreover, several studies have shown that people with dementia may have lower blood levels of vitamin C .
- Furthermore, high vitamin C intake from food or supplements has been shown to have a protective effect on thinking and memory as you age .
- Vitamin C supplements may aid against conditions like dementia if you don't get enough vitamin C from your diet. However, additional human studies are needed to understand the effects of vitamin C supplements on nervous system health.

How much vitamin C is enough

- Adults aged 19 to 64 need 40mg of vitamin C a day.
- One should be able to get all the vitamin C you need from your daily diet.
- Vitamin C cannot be stored in the body, so you need it in your diet every day.

Good sources of vitamin C

Vitamin C is found in a wide variety of fruit and vegetables.

Good sources include:

- citrus fruit, such as oranges and orange juice
- peppers
- strawberries
- blackcurrants
- broccoli
- brussels sprouts
- potatoes
- kiwi fruits



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- lemons
- Guava
- Avocado

Lemon As A Source Of Vitamin C

Lemons are an excellent source of Vitamin C, a natural antioxidant that improves the immune system and has antibacterial and antiviral qualities. Lemon water mixed with honey can be a great way to boost the body's capability to fight diseases. *Citrus limon* (L.) Burm. f. is a tree with evergreen leaves and yellow edible fruits from the family *Rutaceae*. In some languages, *C. limon* is known as lemon. The main raw material of *C. limon* is the fruit, particularly the essential oil and juice obtained from it. The *C. limon* fruit stands out as having well-known nutritional properties, but it is worth remarking that its valuable biological activities are underestimated in modern phytotherapy and cosmetology. *C. limon* fruit juice (lemon juice) has traditionally been used as a remedy for scurvy before the discovery of vitamin C. This common use of *C. limon*, known since ancient times, has nowadays been supported by numerous scientific studies. Other uses for lemon juice, known from traditional medicine, include treatment of high blood pressure, the common cold, and irregular menstruation. Moreover, the essential oil of *C. limon* is a known remedy for coughs.

Biological Activities**Anticancer Activity**

C. limon nanovesicles have been isolated from the fruit juice using the ultracentrifugation method and purification on a 30% sucrose gradient, using an in vitro approach. The study showed that isolated nanovesicles (20 µg/mL) inhibited cancer cell proliferation in different tumour cell lines, by activating a TRAIL-mediated apoptotic cell death. Furthermore, *C. limon* nanovesicles suppress chronic myeloid leukemia (CML) tumour growth in vivo by specifically reaching the tumour site and by activating TRAIL-mediated apoptotic cell processes.

Antioxidant Activity

It has been shown that the antioxidant activity of the flavonoids from *C. limon*—hesperidin and hesperetin—was not only limited to their radical scavenging activity but also augmented the antioxidant cellular defences via the ERK/Nrf2 signalling pathway. In addition, vitamin C prevents the formation of free radicals and protects DNA from mutations. Studies have also shown a reduction in lipid peroxidation in seizures and status epilepticus was induced by pilocarpine in adult rats.

Anti-Inflammatory Activity

Various in vitro and in vivo studies have been conducted to evaluate hesperidin metabolites, or their synthetic derivatives, at their effectiveness in reducing inflammatory targets including NF-κB, iNOS, and COX-2, and the markers of chronic inflammation. The essential oil from *C. limon* (30 or 10 mg/kg p.o.) exhibited anti-inflammatory effects in mice under formalin test by reducing cell migration, cytokine production and protein extravasation induced by carrageenan. These effects were also obtained with similar amounts of pure D-limonene. The anti-inflammatory effect of *C. limon* essential oil is probably due to the high concentration of D-limonene.

Antimicrobial Activity

Acetone extracts from *C. limon* fruits have shown inhibitory activity against the Gram-positive bacteria *Enterococcus faecalis* (MIC 0.01 mg/mL) and *Bacillus subtilis* (MIC 0.01 mg/mL), and the Gram-negative *Salmonella typhimurium* (MIC 0.01 mg/mL) and *Shigella sonnei* (MIC 0.01 mg/mL). Moreover, under another study, *C. limon* essential oil showed antibacterial activity against Gram-positive bacteria (*Bacillus subtilis* (MIC 2 mg/mL), *Staphylococcus capitis* (MIC 4 mg/mL), *Micrococcus luteus* (MIC 4 mg/mL)), and Gram-negative (*Pseudomonas fluorescens* (MIC 4 mg/mL), *Escherichia coli* (100% inhibition)).

The *C. limon* essential oil exhibits inhibitory activity against *Staphylococcus mutans* (MIC 4.5 mg/mL) and effectively reduced the adherence of *S. mutans* on a glass surface, with adherence inhibition rates (AIR) from 98.3% to 100%, and



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on a saliva-coated enamel surface, for which the AIRs were from 54.8% to 79.2%. It effectively reduced the activity of glucosyltransferase (Gtf) and the transcription of Gtf in a dose-dependent manner. Ethanol and acetone extracts from fruits of *C. limon* were active against *Candida glabrata* (MIC 0.02 mg/mL) (Table 7) [Z]. On the other hand, *C. limon* essential oil ingredients, such as D-limonene, β -pinene and citral, have shown inhibitory activity against *Aspergillus niger* (MIC 90 μ L/mL at 70 °C), *Saccharomyces cerevisiae* (MIC 4 mg/mL) and *Candida parapsilosis* (MIC 8 mg/mL). Another study confirmed that *C. limon* essential oil promoted a 100% reduction in the growth of *C. albican*. Moreover, other studies have shown that *C. limon* essential oil at a concentration of 0.05% inhibits *Herpes simplex* replication to the extent of 33.3%.

Antiparasitic Effect

The effect of *C. limon* essential oil on *Sarcoptes scabiei* var. *cuniculi* has been evaluated in vitro and in vivo. The infected parts of rabbits were treated topically once a week for four successive weeks. In vitro application results showed that *C. limon* essential oil (10% and 20%, diluted in water) caused mortality in 100% of mites after 24 h post-application. In vivo application of 20% lemon oil on naturally infected rabbits showed complete recovery from clinical signs and absence of mites in microscopic examination from the second week of treatment.

Anti-Allergic Effect

Aqueous extracts from the peel of *C. limon* fruits have been used to investigate their effects on the release of histamine from rat peritoneal exudate cells (PECs). The extracts inhibited the release of histamine from rat PECs induced by the calcium ionophore A23187. Heating the extracts at 100 °C for 10 min. enhanced the inhibition of histamine release. Histamine release was inhibited to the extent of 80%. The extracts potentially suppressed inflammation in mice cavity, like indometacin, a well-known anti-inflammatory drug.

Lemon Nutritional Value Chart

Lemons are an absolute powerhouse of nutrients and consuming lemon juice every day is beneficial for the body. Here are some nutritional facts about these citrusy fruits: Carbohydrates In Lemons: Lemons are a low-calorie and a low-carb fruits. According to USDA, a 100-gm serving of lemon pulp contains just 9 gm of carbohydrates. A very small part of these carbs come from the sugars, while a major portion of the carbs is dietary fibre. Proteins In Lemons: Despite their numerous and varied health benefits, lemons are not what you would call 'protein-dense fruits'. A 100-gm serving of lemon pulp contains a mere 1.1 gm of protein, as per USDA data. However, you can squeeze lemons on a variety of protein-rich foods like chicken, smoked fish, etc. Vitamins And Minerals In Lemons: Apart from vitamin C, lemons also contain vitamins B5, B6, B1 and B2, as well as calcium, copper, iron and potassium. Lemons contain high levels of dietary fibre and this property of the fruit, combined with its low-calorie nature, make it ideal for anyone wanting to lose weight. As mentioned earlier, a majority of lemon's benefits are present due to high levels of vitamin C in it. Due to this vitamin, lemons may help reduce symptoms of rheumatism and arthritis. Due to the presence of vitamin B5, lemons may also help in quicker metabolic processing of food as well as proper formation of hormones. Vitamin B5 also helps in raising levels of good cholesterol in blood.

CONCLUSION

Overall, vitamin C appears to exert a multitude of beneficial effects on cellular functions of both the innate and adaptive immune system. Although vitamin C is a potent antioxidant protecting the body against endogenous and exogenous oxidative challenges, it is likely that its action as a cofactor for numerous biosynthetic and gene regulatory enzymes plays a key role in its immune-modulating effects. Vitamin C stimulates neutrophil migration to the site of infection, enhances phagocytosis and oxidant generation, and microbial killing. At the same time, it protects host tissue from excessive damage by enhancing neutrophil apoptosis and clearance by macrophages, and decreasing neutrophil necrosis and NETosis. Thus, it is apparent that vitamin C is necessary for the immune system to mount and sustain an adequate response against pathogens, whilst avoiding excessive damage to the host. Vitamin C appears to be able to both prevent and treat respiratory and systemic infections by enhancing various immune cell





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functions. Prophylactic prevention of infection requires dietary vitamin C intakes that provide at least adequate, if not saturating plasma levels (i.e., 100–200 mg/day), which optimize cell and tissue levels. In contrast, treatment of established infections requires significantly higher (gram) doses of the vitamin to compensate for the increased metabolic demand. Epidemiological studies indicate that hypo vitaminosis C is still relatively common in Western populations, and vitamin C deficiency is the fourth leading nutrient deficiency in the United States. Reasons include reduced intake combined with limited body stores. Increased needs occur due to pollution and smoking, fighting infections, and diseases with oxidative and inflammatory components, e.g., type 2 diabetes, etc. Ensuring adequate intake of vitamin C through the diet or via supplementation, especially in groups such as the elderly or in individuals exposed to risk factors for vitamin C insufficiency, is required for proper immune function and resistance to infections. The presented review proves that *C. limon* is a very attractive object of different scientific studies. The *C. limon* fruit is a raw material that can be used in different forms, e.g., extracts, juice and essential oil. The rich chemical composition of this species determines a wide range of its biological activity and its being recommended for use in phytopharmacology. The studies have focused on the essential oil and its main active compound—D-limonene. Extracts from *C. limon* fruits are rich in flavonoids such as naringenin and hesperetin. Current pharmacological studies have confirmed the health-promoting activities of *C. limon*, especially its anti-cancer and antioxidant properties. *C. limon* also finds increasing application in cosmetology and food production.

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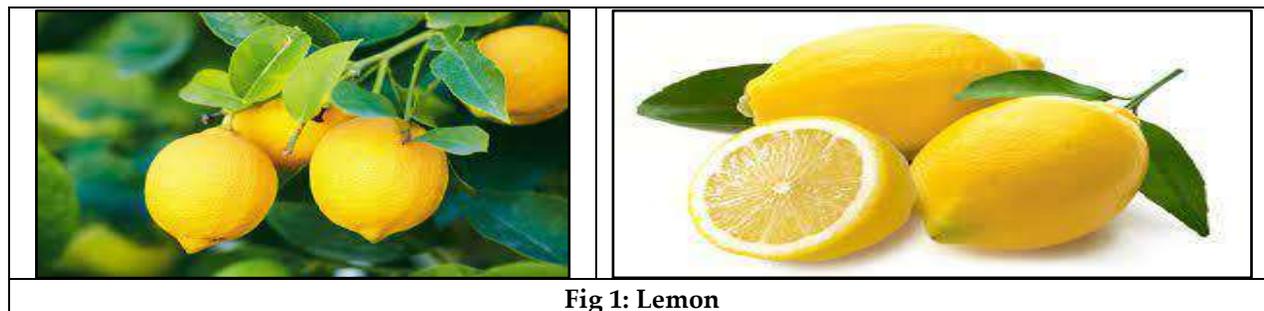


Fig 1: Lemon





Turmeric As A Personalized Medicine

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ABSTRACT

Treatment based on traditional medicine is very popular now a day, several researches are going on different types of medicinal plants which have been recognised for the treatment and prevention of different diseases. The effect of *Curcuma longa* and its chief constituent Curcumin has broad range of disease cure modulations over physiological and biochemical processes. Curcumin has a yellow polyphenolic pigment from the *Curcuma longa*. Turmeric is one of the nature's most powerful healers. The bioactive ingredient of turmeric is Curcumin. Over 2500 years in India, turmeric has been used. It has many medicinal properties which have been slowly revealing themselves over centuries. It is mainly known for its inflammatory properties which has been revealed as natural wonder and proving as beneficial treatment for health conditions and helps to cure diseases from cancer to Alzheimer. It is used as an antiseptic in India and act as active agent in curing *Staphylococcus aureus* which is a pus producing infections. It helps in decreasing Kapha and used to remove mucus in throat, watery discharges like leucorrhoea, and pus from eye, ears, and from wounds. Turmeric is also regarded as 'rasayana' herb which is a branch of Ayurvedic medicine. For desentry the roasted turmeric is used as a main ingredient. It also has a great effect in curing the aging. This review article mainly focuses on the pharmacological activities such as anti-diabetic, anti-microbial, hepato-protective activity, anti-inflammatory, anti-tumor activity and neurodegenerative diseases.

Keywords: Curcumin, Ayurvedic medicine, traditional, Alzheimer, *Staphylococcus aureus*



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INTRODUCTION

Curcuma longa L. (Zingiberaceae), also known as turmeric, is a bright yellow spice native to Southwest India. Its rhizomes are used to make a brilliant yellow spice with a variety of medical uses. Turmeric is derived from the root of *Curcuma longa*, a ginger family flowering plant. It's frequently seen in spice jars. When purchased fresh, it resembles ginger root but has a more intense yellow to golden colour. Turmeric is being used to cure skin problems, digestive problems, other aches and pains in India. Turmeric is a plant native to southern India and Indonesia that is widely grown on the mainland and in the Indian Ocean islands. This was used as a perfume and a spice in ancient times. The rhizome has a peppery scent and a slightly bitter heated flavour, as well as a vivid orange-yellow staining colour. Turmeric, a spice and colour agent used in many cuisines, is used to flavour and colour yellow mustard. At a pH of 7.4, it changes colour from yellow (acidic form) to red (basic form). Tartaric acid is found in turmeric. *Curcuma* has been identified in 133 different species all over the world (Table 13.2). The majority of them have popular names in their home countries and are utilised in a variety of medical compositions depicts some unique turmeric species. Temperatures between 20°C to 30°C are required for the turmeric plant to thrive, as well as a significant amount of annual rainfall. Turmeric, particularly its most active ingredient, curcumin, has numerous scientifically proved health advantages, including the ability to boost heart health and to prevent Alzheimer's disease and cancer. It has anti-inflammatory and antioxidant properties. It may also assist with depression and arthritic problems. India is the world's leading producer, consumer, and exporter of turmeric. Turmeric production is estimated to be over 11 lakh tonnes per year worldwide.

What Personalized Medicine?

A type of medication that prevents, diagnoses, or treats disease by using knowledge about a patient's own genes or proteins. In CF (Cystic Fibrosis) and many other diseases, personalised medicine is used to treat patients based on their symptoms. The use of digestive enzyme supplements in CF is a classic example. By enhancing the matching process between patients and medicines, as well as a patient's comprehension of the risk of major side effects, personalised medicine improves the health impact of existing treatments.

History

Turmeric's use stretches back approximately 4000 years to India's Vedic period, when it was employed as a culinary spice as well as having religious importance. By 700 AD, it had made its way to China, East Africa, West Africa, and Jamaica in the seventeenth century. Curcumin was discovered by Vogel and Pelletier roughly two centuries ago, when they isolated "yellow colouring-matter" from the rhizomes of *Curcuma longa* (turmeric) and termed it curcumin. This chemical was later discovered to be a combination of resin and turmeric oil. Vogel Jr. was born in 1842. The southern states of Telangana, Andhra Pradesh, Tamil Nadu, and Karnataka, as well as the eastern states of Orissa and West Bengal, and the western state of Maharashtra, are major turmeric producing states in India. Telangana is currently India's most important turmeric-producing state, accounting for 19.5% of total land, 33.3 percent production, and 6500 kg/ha productivity.

Origin And Distribution

Because the yellow dye is used to paint the robes of monks and priests, it migrated to Southeast Asia alongside Hinduism and Buddhism. Turmeric was also discovered before European contact in Tahiti, Hawaii, and Easter Island.

Morphology Of Turmeric

Turmeric, or *Curcuma longa*, is a tropical rhizomatous herbaceous perennial belonging to the ginger family. It grows to 3-4' tall with a leaf clump of ornamentally appealing, pleated, elliptic to lanceolate green leaves (each to 3 1/2' long). Turmeric plants grow to a height of around 1 metre (3.3 feet) and have long, simple leaves with long petioles (leaf stems). The leaves originate from rhizomes that branch just beneath the soil surface. Juvenile rhizomes typically



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pale yellow to brown-orange in colour, whereas older rhizomes are scaly and brown. The little yellow-orange blooms bloom inside the axils of waxy bracts that are typically pale green or purple-tinged.

Chemical Composition

Turmeric - Curcuminoids, a mix of curcumin, demethoxycurcumin, and bisdemethoxycurcumin, make up 3-6 percent of the polyphenolic chemicals in turmeric. Curcuminoids are important components that have a variety of biological effects. Curcumin [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6 heptadiene-3, 5-dione] is an orange-yellow component of turmeric (*Curcuma longa*), which is commonly used in curry powder. Leaves - A combination of high resolution GC and GC/MS was used to evaluate the chemical composition of *Curcuma domestica* L. leaf oil. More than 20 constituents have been discovered, with the monoterpenes -phellandrene (24.5%), 1,8-cineole (15.9%), p-cymene (13.2%), and -pinene (8.9%) being the most prominent.

Composition Of Turmeric Powder

Turmeric powder is about 60–70% carbohydrates, 6–13% water, 6–8% protein, 5–10% fat, 3–7% dietary minerals, 3–7% essential oils, 2–7% dietary fiber, and 1–6% curcuminoids.

Phytochemicals

Curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerones, and turmeronols are only a few of the phytochemical found in turmeric. Turmeric's antimicrobial effect is indicated by the presence of phytochemical such as tannins, alkaloids, phenols, steroids, flavonoids, phlobatannin, cardiac glycosides, terpenoids, triterpenes, saponin, and others. Curcumin [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6 heptadiene-3, 5-dione] is an orange-yellow component of turmeric (*Curcuma longa*), which is commonly used in curry powder.

Turmeric Used As A Personalized Medicine

From many years awareness of turmeric and its use as medicine is continuously increasing. A flowering plant, Turmeric, in the ginger family, is commonly used as a food coloring and is one of the basic ingredients in curry powder. To heal many health disorders like liver problems, digestive disorders, treatment for skin diseases and wound healing turmeric has long been used in medicinal as an anti-inflammatory. Curcumin is the active ingredient in turmeric which has been shown to have a wide range of therapeutic effect. Turmeric is used to treat rheumatoid arthritis, chronic anterior uveitis, conjunctivitis, skin cancer, small pox, chicken pox, wound healing, urinary tract infections, and liver problems. The skin, heart, liver, and lungs are the key organs that turmeric helps. Turmeric is used to treat epilepsy and bleeding disorders, as well as skin illnesses, to purify the body-mind, and to aid in the expulsion of Kapha from the lungs. Alternative, analgesic, antibacterial, anti-inflammatory, anti-tumor, anti-allergic, antioxidant, antiseptic, antispasmodic, appetiser, astringent, cardiovascular, carminative, cholagogue, digestive, diuretic, stimulant, and vulnerary are just a few of Turmeric's properties.

Health Benefits Of Turmeric

High Cholesterol levels - In overweight persons with high cholesterol, consuming turmeric extract twice daily for three months reduces total cholesterol, low-density lipoprotein (LDL or "bad") cholesterol, and triglycerides, according to research. Osteoarthritis- According to some studies, using turmeric extracts alone or in conjunction with other herbal ingredients can help people with osteoarthritis reduce pain and improve function. Turmeric was found to be about as effective as ibuprofen for reducing osteoarthritis pain in several studies. However, it does not appear to be as effective as diclofenac in relieving pain and function in osteoarthritis patients.

Itching (pruritus) - According to research, consuming turmeric three times a day for eight weeks lowers itching in persons with chronic renal disease. Early research also reveals that using a special combination product (C3



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Complex, Sami Labs LTD) combining curcumin plus black pepper or long pepper on a daily basis for four weeks reduces itching severity and improves quality of life in persons who have persistent itching caused by mustard gas.

Ulcers in stomach - According to some studies, consuming turmeric three times a day for eight weeks does not help stomach ulcers. Furthermore, using powdered turmeric four times per day for six weeks appears to be less effective than taking a traditional antacid.

Another health benefit –

- It's an antiseptic and antibacterial substance that can be used to treat cuts and burns.
- It has been demonstrated to prevent prostate cancer and slow the progression of existing prostate cancer when mixed with cauliflower.
- In mice, it stopped breast cancer from spreading to the lungs.
- It has the potential to prevent melanoma and cause existing melanoma cells to self-destruct.
- Lowers the risk of leukaemia in children.
- It's a natural liver cleanser.
- By eliminating amyloid plaque build-up in the brain, it may help to prevent and reduce the progression of Alzheimer's disease.
- May help to prevent cancer metastases in a variety of cancers.
- It's a powerful natural anti-inflammatory that works just like anti-inflammatory medicines but without the negative side effects. Has shown to be promising.

CONCLUSION

Turmeric has long been used in India as a tasty, vibrant condiment as well as an Ayurvedic medication to enhance appetite, function as a carminative, and cure gallstones and other biliary issues, as well as dyspepsia. It's used as an ointment, paste, or poultice for scabies, boils, bruises, insect bites, and other skin lesions in India, China, and other Southeast Asian countries, and as an ointment, paste, or poultice for scabies, boils, bruises, insect bites, and other skin lesions. Turmeric is also used to treat menstruation issues, pain, epilepsy, respiratory tract infections, bleeding, diarrhoea, jaundice, and rheumatic illnesses when taken orally. It has recently earned a reputation as an anti-inflammatory agent, a hypercholesterolemia therapy, an antioxidant, and a cancer preventative, and it is believed to protect cardiovascular and other degenerative changes. Curcumin is used to prevent oxidation and improve the colour of foods like butter and margarine. Turmeric is a highly prized spicy condiment that has long been used to aid digestion and cure dyspepsia and inflammatory conditions. Turmeric and its main component, curcumin, are also marketed as antioxidants, cancer, HIV, and hypercholesterolemia therapies, and heart disease prevention. However, controlled clinical trials for these indications are either missing or have not produced clearly good outcomes. For peptic ulcer disease, no therapeutic benefit has been established, and one research for dyspepsia was inconclusive. Controlled trials for arthritis and inflammation have likewise failed to show that the treatments are effective. Other applications haven't been tested in a controlled clinical investigation.

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Diseases

SL NO	DISEASES	TREATMENT
1	Liver disease	Turmeric is the best thing for liver because of its anti-inflammatory properties. Liver can be strengthened by increasing the amount of herbs and foods consumed in the spring. Turmeric contains liver protecting chemicals as found in milk thistle and artichoke plants. It reduces engorged hepatic ducts making it effective for curing the diseases like hepatitis, cirrhosis, and jaundice.
2	Cancer	Turmeric can treat a variety of disorders that can even slow the progression of cancer. This is a spice that is used to cure skin cancer and pre-cancerous disorders.
3	Atherosclerosis	Turmeric may aid avoid artery blockage which leads to heart attack or stroke. It lowers cholesterol levels and prevents LDL oxidation. Production of atherosclerotic plaque which accumulates in the walls of blood vessels. It also helps to inhibit platelet aggregation.
4	Osteoarthritis	Turmeric help to relieve the symptoms of osteoarthritis as it has the ability to reduce pain and disability.
5	Menstrual problems of women	Monthly cramps during menstruation can reduce if turmeric is taken twice a day for two weeks. Turmeric lowers digestion and menstrual cramps by acting as an antispasmodic to smooth muscles. Turmeric is a fantastic supplement for diet and menstrual cycle.
6	Bacterial infection and wounds	Turmeric is a useful an external antibiotic in preventing bacterial infections in wounds.
7	Eye disorder	Curcumin is effective as corticosteroids in the verities which is a type of eye disorder.

Anioxidant Activities

SI NO.	ACTIVITIES	OUTCOME AND FINDINGS
1	Antioxidant activity	Exerts powerful inhibitory effects against peroxide induced damage in human keratinocytes and fibroblasts, it shows herbal function in health management. Curcumin helps in detoxifying enzymes such as glutathione-S-transferase.
2	Anti-diabetic	Curcumin helps increase in gene expression such as insulin like growth factor-I, B-cell lymphoma, superoxide dismutase and GST. Activities like heme oxygenase-I gene expression and HO were significantly increased after the isolation in Islets of Langerhans.





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3	Anti-inflammatory	Curcumin shows vital effect in prevention of inflammatory process by inhibiting and modulating various molecular pathways. Its supplements linked lower plasma level tumor necrosis factor alpha, interleukin-6 and monocyte chemo attractant protein-I. Reduces infiltration in inflammatory condition.
4	Anti-microbial	Inhibits the growth of <i>Helicobacter pylori</i> strains in vitro which is isolated from patients suffering from gastrointestinal disorders. Possesses antibacterial property against Gram-positive and Gram-negative bacteria. Its exhibited inhibitory activity on methicillin-resistant <i>Staphylococcus aureus</i> strains having concentration value of 125-250 µg/ML.
5	Anti-ulcer	Antiulcer activity in indomethacin induced gastric ulceration associated with down regulation of MMP-9 and up regulation of MMP-2.
6	Anti-obesity	Curcumin therapy can reduce the inflammatory consequences of obesity. This also helps in animals to decrease NF-kB activity in liver tissue. These helps in potential health benefits for preventing obesity and associated metabolic disorders and up regulate the adipocyte energy metabolism.
7	Anti-cancer activity	Curcumin increased the activity of phase II enzymes, such as GSTs and down regulated VEGF through inhibition of PPAR in colon cancer cells.
8	Cardio preventive	Curcumin inhibits p300-HAT and finally prevent the development of heart failure.
9	Hypertension reducing	Curcumin helps in prevention of hypertension. Hypertension is increased by N-nitro-L-arginine-methyl ester which can partially be decreased by Curcumin.
10	Role in respiratory activity	Curcumin increases the expression of cathepsins K and L in lung which effect lung fibroblast cell behaviour. Its oral administration inhibits bleomycin-induced pulmonary fibrosis in rats. Its anti-inflammatory agent prevents release of TNF-α and protects against pulmonary and cardiovascular effects.
11	Anti-malarial activity	Turmeric shows cytotoxic effect in <i>Giardia lamblia</i> which inhibit the parasitic growth, induce morphological alterations and provoked apoptosis. Its oral administration showed reduced blood parasitemia by 80-90%.
12	Reduction in sperm mortality	Curcumin resulted in dose and time dependent loss of sperm motility by incubating normal human sperm.
13	Immunomodulatory activity	Curcumin imparted immunosuppression by mainly down regulating the expression CD28 and CD80 and up regulating CTLA-4. Also activate T cells, B cells, macrophages, dendritic cells, cell cycle protein, cell mediated and humoral mediated immunity.
14	Nephrotoxicity effect	Curcumin protects against diabetic nephropathy and oxidative stress against streptozotocin induced and showed protective effects against nephrotoxicity.
15	Neuro preventive	In vitro study confirmed that Curcumin improves the survival of cortical neurons induced OGD induced cell injury. In vitro levels of active oxygen decreased in chronic ischemic PC12 cells when treated with Curcumin.
16	Hepato protective activity	Carbon tetrachloride induced liver toxicity which proved that pre-treatment with picroliv, Curcumin, and ellagic acid which decrease the level of malondialdehyde to improve the antioxidant status and normalize the hepatic histo architecture.
17	Scavenger of reactive oxygen activity	Curcumin findings based on in vitro condition which is effective for scavengers of ROS and act as reactive nitrogen species.
18	Prevention of gastric lesions activity	Curcumin prevents from gastric lesions and helps in development in the gastric wall during the acute phase of gastric ulcer diseases.
	Radio sensitizer effect	PC3 is based on prostate cancer cell line and is confirmed as a major chemical





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19		component of turmeric which has radio sensitizing effects on cancer.
20	Anti-tumor activity	Curcumin seeds, leaves, flowers and stem plays major role in tumor prevention. Its chief constituent inhibit the activity of drug metabolizing enzymes i.e., cytochrome p450 and p450 reductive. It helps in the induction of apoptosis ,inhibit the proliferation of melanoma cells and is associated with down regulation of Notch-1 and NF-Kb.

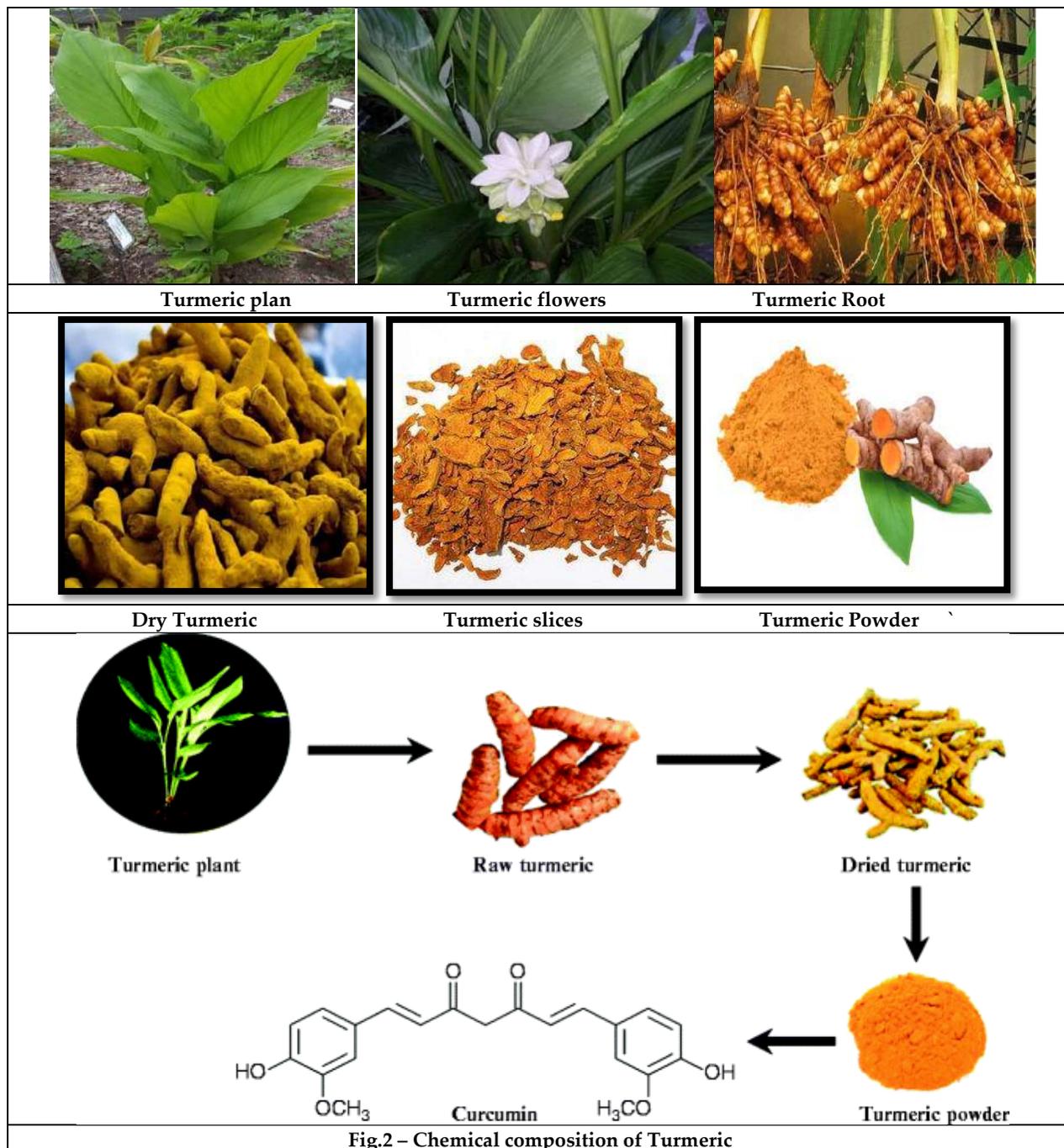


Fig.2 – Chemical composition of Turmeric





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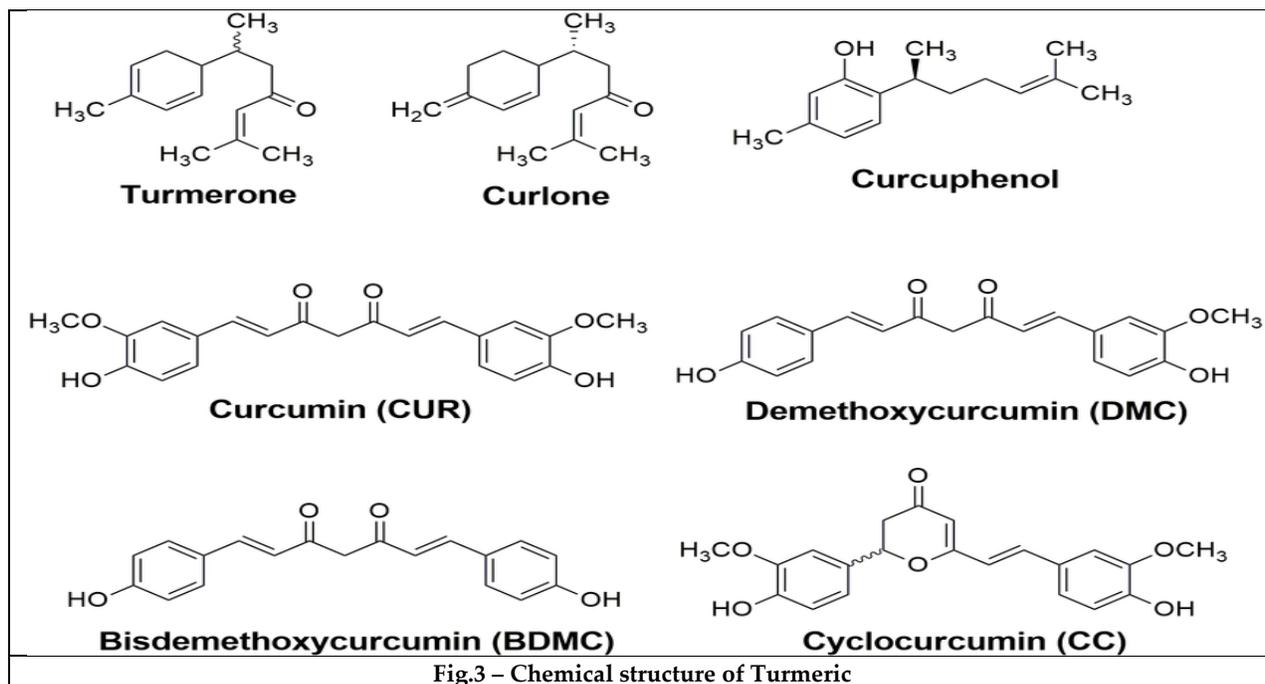


Fig.3 – Chemical structure of Turmeric





LUTEIN: The Influence on Skin and Human Health

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ABSTRACT

Lutein is a carotenoid with proven anti-inflammatory properties. Sufficient evidence shows that lutein has multiple beneficial effects, especially on eye health. Lutein, in particular, is known to improve or prevent age-related macular disease, a major cause of blindness and poor vision. In addition, many studies have reported that lutein may have beneficial effects on a variety of clinical conditions, improve cognitive function, reduce the risk of cancer, and improve cardiovascular health measurements increase. Lutein is a carotenoid present in a wide variety of species, including bacteria, algae, yeasts, and plants. Various studies confirming this pigment's antioxidant ability, playing a vital part in the prevention of age-related macular degeneration and other illnesses such as cancer, revealed its biological significance. Lutein is also necessary for the brain development of babies and must be ingested in adequate quantities to get its health advantages. Due to limited lutein bio accessibility and bioavailability in dietary sources, obtaining a physiologically useful amount of lutein is challenging. These parameters are influenced differently by the food matrix's characteristics, processing, and the presence of additional dietary components. The goal of this literature study was to investigate lutein stability and bioavailability as a result of changes and alterations connected with food technical methods.

Keywords: AREDS, AMD, Lutein

INTRODUCTION

In humans, carotenoids are important in scavenging singlet oxygen and peroxy radicals. Lutein and zeaxanthin have been demonstrated in multiple studies to help safeguard the skin and eyes from photodamage, as well as provide a variety of other health advantages. This research examines the possible merits of employing lutein as a dietary or





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aesthetic supplement. Lutein's recent advancements in health and aesthetic care are also mentioned. This paper also discusses diverse drug carrier technologies that have been investigated for lutein administration. Oxidative stress is caused by free radicals and an imbalance in antioxidant. Oxidative stress causes the breakdown of DNA, lipids, and proteins, as well as the aetiology of cancer, cardiovascular disease, diabetes, neurological illnesses, and skin aging. Antioxidants in the diet may protect against oxidative and free radical damage caused by high-energy foods. Lutein (L) and zeaxanthin (Z) are two of the most common macular carotenoids in the diet, and they are responsible for the vibrant hues of many fruits and vegetables. The placement of a single double bond distinguishes lutein (L) and zeaxanthin isomers (Zi) (Juturu, V., Bowman, J., et.al, 2016).

The macula of the retina contains these macular carotenoids. Because humans are unable to produce macular carotenoids, and hence supplementation of macular carotenoids will meet their requirements. Many lutein/zeaxanthin formulations derived from the marigold flower (*L. Tagetes erecta*) are commercially available. High-energy blue light can be filtered by lutein and zeaxanthin, which are powerful antioxidants. As a result, Xanthophylls may protect against oxidative damage caused by light. It compared the effects of lutein- and zeaxanthin-enriched meals containing 0.4 percent or 0.04 percent lutein and zeaxanthin to a typical control diet, finding a significant decrease in UVB-induced skin inflammation. Furthermore, when lutein- and zeaxanthin-fed mice were compared to control mice, apoptotic cells and cell proliferation were significantly reduced.

Lutein

Lutein is an antioxidant belonging to the carotenoid group. It is abundant in leafy vegetables, orange-yellow vegetables, and dietary supplements. Lutein is important for maintaining eye health and reducing the risk of macular degeneration and cataracts. It also protects the skin and cardiovascular system. There is no officially recommended daily intake of lutein, but studies suggest that 620 mg / day has health benefits. Most of us don't get enough lutein in our diet (Webster, A. 2018). Lutein is a carotenoid, which is a form of organic pigment. It is related with beta-carotene and vitamin A. and is commonly referred to as "the eye vitamin." The human eye contains two main carotenoids: lutein and zeaxanthin (macula and retina). It is supposed to act as a light filter, shielding the eye tissues from the harmful effects of sunlight. Egg yolks, spinach, kale, maize, orange pepper, kiwi fruit, grapes, zucchini, and squash are all high in lutein. Lutein is a carotenoid that is widely taken by mouth to prevent eye illnesses such as cataracts and a condition that causes vision loss in the elderly (age-related macular degeneration or AMD). Lutein is also utilised for a variety of different ailments, although there is no solid scientific proof to back up these claims (MD, W. 2016).

Lutein is a carotenoid that contains oxygen and is classified as a xanthophyll. Carotenoids are the pigments that give foods their natural colours of yellow, orange, and red. They're classified as an essential nutrient since our bodies can't produce them and we have to receive them from food. Carotenoids are divided into two categories. Yellow pigments are made up of xanthophylls, which include oxygen, while orange pigments are made up of carotenes, which do not contain oxygen. Lutein, together with another xanthophyll called zeaxanthin, is detected in the retina of the eye. These carotenoids are termed as macular pigments since they are abundant at the back of the eye and could be invaluable to general eye health. Lutein contains antioxidant qualities that may help with cognitive function, heart health, and anticarcinogenic, however more research is required (Landes, E., 2021).

Uses of lutein in various field

Eye health

The Age-Related Eye Disease Study (AREDS) on lutein and eye health is well-known research. Scientists investigated the effect of several performance enhancer compositions on age-related macular degeneration (AMD). In those who already had AMD, a booster incorporating lutein and zeaxanthin minimized the onset of progressive AMD by 25% over 5 years. The supplement did not prevent or cure AMD in persons who did not have it. Another carotenoid associated to eye health, beta carotene, was previously used in the supplement, but it was discovered to raise the risk of lung cancer in smokers. Using lutein and zeaxanthin instead of beta carotene had the same effect on eye health and did not raise the risk of lung cancer. Lutein is also an antioxidant, which is good for your eyes. Glaucoma,



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diabetic retinopathy, and macular degeneration are all associated with inflammation and oxidative stress. Lutein is an antioxidant that has been shown to be useful in the prevention of various eye disorders in studies. Furthermore, research shows that lutein is vital for a baby's eye development during pregnancy as well as their eyesight throughout their lives, however more research is needed to establish the best amount for pregnant and nursing women. Finally, lutein may be a useful therapy for dry eyes, while more research is needed in this area. (Landes, E., 2021).

Skin health

While we adore the long days and balmy weather of summer, the sun emits damaging UV radiation that can cause skin damage. UV radiation not only ages us, but it also has the potential to kill us. Skin cancer is caused by harmful UV radiation, which is generally curable but occasionally deadly. Recently, scientists have studied in to the lutein's UV-protective properties, particularly in human skin. According to one study, it was able to prevent some of the UVA and UVB's adverse effects. It may also aid in the protection of the skin from dangerous high-energy radiation. People's skin tone, suppleness, and colouring all improved following a 12-week lutein intake, according to a randomised, double-blind clinical experiment. Relatively similar study suggested that lutein might be a safe and effective treatment for melasma, acne scars, freckles, and age spots, among other skin disorders. (Staff, B., 2020). Although previous research has suggested that taking a daily lutein supplement can help the skin's natural antioxidant system and protect it from sun damage, this is the first time it's been tested for more specific, age-related skin benefits such as hydration, elasticity, and superficial skin lipids Halliday, J. (2019).

Antioxidants, such as lutein, may protect both the skin and the eyes.

Antioxidants such as lutein may protect both the skin and the eyes

Lutein in the Macula of the Eye

The yellow-orange carotenoids lutein and its structural look-alike, zeaxanthin, are present in spinach, kale, and collards. These antioxidants are found in high concentration in the lens and macula of the eye, where they adequately defend against UV radiation exposure. Researchers discovered that levels of these carotenoids in the macula fall with age, reaching an all-time low at the age of 60, especially when the risk of age-related macular degeneration (AMD) keeps rising. In fact, AMD patients who took lutein supplements for at least three months after being diagnosed with the disease had lutein levels that were substantially identical to healthy controls. Based health, S. (Ed.). (2019).

UV Affects Both the Eyes and the Skin

Scientists believe that lutein safeguards the skin in the same way that it protects the eye's macula from potentially harmful UV rays. Dietary carotenoids have been shown to generate in the skin and provide a significant photoprotective advantage that is proportional to their skin content. Henceforth, early research suggests that vitamin C and E, or vitamin E and carotenoids, in combination, provide higher protection against UV-induced sunburn and inflammation than separate nutrients. A recent study backs up carotenoids and antioxidants as beneficial to skin and eye health. Based health, S. (Ed.). (2019).

Anti-oxidants that protect the skin

Lutein is a carotenoid with better antioxidant capabilities than other carotenoids. The antioxidant activities of lutein are around ten and fifteen times greater than those of carotene and lycopene, respectively. UV (ultraviolet) light is protected from the body and skin by antioxidants. Lutein is a carotenoid found in high concentrations in skin cells. Furthermore, lutein and zeaxanthin isomers may reduce membrane lipid peroxidation and protect the skin against high-energy sources. Juturu, V., Bowman, J. et.al, (2016)

The Factors That Contribute to Aging Skin

As people grow older, they develop expression lines, thin wrinkles, and sagging skin, which is mostly dictated by heredity. Sun exposure, not ageing, is the primary cause of skin that appears older, wrinkled, rough, or speckled with dark patches. Sunlight promotes reddening and inflammation, as well as a reduction in the number of dermal blood vessels that give nutrients to the skin. Sunlight also wreaks havoc on proteins that are essential for skin tone



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maintenance. In addition to UV radiation, exposure to air pollution creates free radicals and damages skin cells. These effects are referred described as "photo-aging."

Maintain Skin Health to the Best of Your Ability

While you can't change your genetic make-up, there are certain things you can do to help decrease photoaging and boost skin health:

- Sunscreen should be applied to all exposed skin (via sunscreens and protective clothing)
- Drink plenty of water to keep hydrated.
- Reduce your stress levels and get enough rest.
- Avoid consuming too much alcohol and refrain from smoking.
- Consume a nutritious diet to aid in the creation of new skin cells.

More studies need to be conducted to establish the potential skin and ocular advantages of lutein and antioxidants. Furthermore, we should all consume more fruits and vegetables, specifically lutein-rich greens. Because if we compare the average American utilises just around 1-2 mg of lutein per day, it's also a good idea to take a daily "multi" to get a larger intake of lutein and other crucial antioxidants.

Heart health

Lutein ingestion from the food, along with redistributing lutein levels, have been linked to improved cardiovascular health. In one research, lutein and zeaxanthin were linked to improvements in clinical indicators in cardiac diseases. The anti-inflammatory characteristics were useful, according to the researchers, who recommend that further study be done in this area. Another study discovered that taking 20 mg of lutein every day for three months reduced cholesterol and triglyceride levels, both of which are established risk factors for cardiac diseases. However, the evidence on lutein and heart health is varied, with some studies finding no link at all. To identify lutein's involvement in heart health, additional research is needed, notably in humans. (Landes, E., 2021).

Cancer

Lutein, like other carotenoids, has been shown to enhance cancer prognosis. A high intake of lutein, along with other nutrients present in fruits and vegetables, was linked to a lower risk of pancreatic cancer in one investigation. Furthermore, lutein, along with other carotenoids, may protect against breast cancer and head and neck cancer. Overall, the study on lutein and its cancer-related effects is encouraging but not conclusive, and further human trials are required. (Landes, E., 2021).The decreased incidence of breast cancer associated with the use of lutein-rich nutritional supplements has piqued researchers' curiosity in learning more about the molecular mechanism behind lutein's growth inhibitory properties.

Kavalappa, Y. P., et.al (2020).

Brain health

According to studies, a high dietary intake of lutein and high circulating levels of lutein are linked to improved cognitive function and memory. A daily dose containing 10 mg of lutein, zeaxanthin, and meso-zeaxanthin was found to be useful in enhancing memory over the course of a year in one research. Overall, carotenoids may serve a functional role in the preventative measures of neurodegenerative illnesses, implying that they may assist improve brain health in later life, however the evidence is still conflicting (Landes, E., 2021).Lutein is the most abundant carotenoid in human brain tissue, comprising areas that affect many elements of cognition. Given that the preferential absorption of lutein and zeaxanthin in the retina and brain is presumably controlled by comparable processes, it's not unexpected that macular pigment density was shown to be highly associated with their levels in matching brain tissue.Lutein has been linked to a variety of cognitive functions, notably executive function, linguistics, knowledge, and recollection. The richness of retinal pigment in brain tissue found to be a suitable indicator for lutein contents. This might clarify why there is a strong link among retinal pigment concentration and cognitive performance in normal individuals. Erdman, J. (2015).



**Harshita Singh et al.,****Skin health**

While we adore the long days and balmy weather of summer, the sun emits damaging UV radiation that can cause skin damage. UV radiation not only ages us, but it also has the potential to kill us. Skin cancer is caused by harmful UV radiation, which is generally curable but occasionally deadly. Recently, scientists have studied in to the lutein's UV-protective properties, particularly in human skin. According to one study, it was able to prevent some of the UVA and UVB's adverse effects. It may also aid in the protection of the skin from dangerous high-energy radiation. People's skin tone, suppleness, and colouring all improved following a 12-week lutein intake, according to a randomised, double-blind clinical experiment. Relatively similar study suggested that lutein might be a safe and effective treatment for melasma, acne scars, freckles, and age spots, among other skin disorders. (Staff, B.,2020). Although previous research has suggested that taking a daily lutein supplement can help the skin's natural antioxidant system and protect it from sun damage, this is the first time it's been tested for more specific, age-related skin benefits such as hydration, elasticity, and superficial skin lipids Halliday, J. (2019).

Diabetes

Marigold (*Tagetes erecta* L.) is an ornamental plant of the Asteraceae family. The flowers are used to treat a variety of ailments, including diabetes and antioxidants. The major pigment component of marigold blooms was discovered to be lutein. Higher levels of lutein in the blood have been associated with better blood sugar balance and a reduced risk of diabetes. Lutein is more efficient than carotene at preventing oxidant-induced cell damage by suppressing cellular lipid auto-oxidation. (Landes, E., 2021).

Lutein stability and biodiversity as a useful functional food ingredient

The pharmaceutical, nutritional supplement, food, and animal and fish feed sectors make up the lutein industry. The main use of Lutein is to brighten the colours of chicken feathers and to darken the yellow of egg yolk. However, because of its unpredictability and the chemical changes that occur during food processing, lutein's usage in the food industry is restricted. High temperatures, oxygen, light, and pH extremes are among factors that can compromise the integrity of lutein. Microalgae might be a potential source of lutein in the future. Lutein is an antioxidant that has a variety of health advantages. The main challenge for incorporating lutein into food items is its low stability. The bioavailability of lutein can be increased by making nanoemulsions (Nanoemulsions and nanopigments demonstrate cosmetic roles by reversing skin damage and evening skin tone). Becerra, M. O., et.al ,2019. Lutein is a naturally occurring carotenoid that is exclusively generated by plants. Spinach, kale, and yellow carrots have significant levels. Most significantly, lutein for skin is well-known for its powerful antioxidant qualities, which are critical for protecting the skin from damaging free radicals found in the environment. Furthermore, studies have shown that by efficiently lowering the amount of oxidative stress that occurs in the skin, topical administration of lutein advantages may accidentally aid to increase moisture retention and give anti-aging effects. As a result, this topical novel antioxidant is frequently found in cosmetic and personal care products. Cosmetic products may frequently combine the advantages of numerous carotenoids, such as lutein and zeaxanthin, to provide more strong protection Paris, L. (2022).

Lutein Uses, Effectiveness, and Inefficacy**Possibly effective in the treatment of**

An eye illness that causes vision loss in older people (age-related macular degeneration or AMD). Some symptoms of AMD can be improved by taking lutein supplements by mouth for up to 36 months. When taken for at least a year at dosages more than 10 mg, and when coupled with other carotenoid vitamins, it may provide additional advantages. However, lutein does not appear to prevent AMD from worsening over time. Cataracts - Consuming more lutein in one's diet has been related to a decreased chance of acquiring cataracts. However, it's unclear if taking lutein supplements by mouth benefits patients with cataracts.





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Possibly ineffective in terms of

An illness that damages babies' lungs (bronchopulmonary dysplasia). Giving lutein and zeaxanthin to premature babies does not lessen their risk of developing bronchopulmonary dysplasia. A dangerous intestinal illness that affects preterm babies (necrotizing enterocolitis or NEC). It is not possible to prevent NEC in preterm new-borns by giving them lutein and zeaxanthin by oral. (MD, W. 2016).

Lutein in Marigold

The marigold flower (*Tagetes erecta* L.) has a lot of lutein. Mexico, Peru, Ecuador, Spain, India, and China are among the countries where it is produced for commercial reasons, because it neutralises free radicals created by the action of UV radiation on the eye retina, lutein is an excellent antioxidant in the protection of eyes. Because humans are unable to produce lutein, they must obtain it from the eating of fruits, vegetables, and/or dietary supplements. Carotenoids make about 0.1–0.2 percent of the dry matter (DM) of dried Marigold flowers, with lutein diesters accounting for 80 percent of the total. A nonpolar oleoresin extract is obtained by extracting dried and crushed flowers. Lutein, a carotenoid antioxidant, has been shown to provide additional advantages such as decreasing cancer risk, promoting immunological function, and preventing UV blue light damage to the eyes. While lutein may be found in several fruits and vegetables, marigold flowers (*Tagetes erecta* L.) are one of the most plentiful and cost-effective plant sources of lutein. Lutein is generally found esterified with fatty acids in its natural state. To be appropriate for human consumption, it must be saponified to free lutein and refined to more than 90% purity. (MD, W. 2016).

Five advantages of marigold extract for the skin

- Marigold extract is good for all skin types, but it is particularly useful to people suffering from eczema and psoriasis due to its soothing and non-irritating nature. This plant has a high amount of linoleic acid, which is a natural anti-inflammatory that works wonderfully for persons with sensitive skin.
- Linoleic acid is an omega-6 fatty acid as well. This component is critical for keeping the skin smooth, moisturised, and nourished from inside.
- Marigold has a lot of flavonoids. Flavonoids are potent antioxidants that can help to slow down the ageing process while also providing a relaxing and calming impact.
- Marigold contains healing and regenerating properties that instantly brighten dull skin. The brightening effect of marigold extract is attributed to glycoproteins contained in the plant, and it is especially beneficial during the winter months or when you are stressed.
- Marigold is also abundant in saponins (natural soap-like components), making it an excellent natural cleanser that leaves your skin feeling clean and rejuvenated. Goess, M. (2021).

Marigold Ayurvedic Uses & Skin Benefits

Flavonoids found in marigold flowers have been connected to cancer therapy owing to their anti-inflammatory properties. Marigold has been used to treat skin irritation, sensitivity, redness, and even dryness. Its essential oil and distilled flower water are both thought to be particularly effective at reducing UV radiation damage and preventing symptoms of ageing. Marigold is considered cooling in nature and so balanced for Pitta and Kapha Doshas in Ayurveda. Traditionally, a Marigold leaf paste was applied to the body to cure muscular soreness, as well as acne and sunburn on the skin Forest Essentials, F. (2020).

Health benefits of lutein from marigold

Effects

When consumed by orally, lutein is most likely harmless. It indicates that consuming up to 20 mg of lutein per day as part of one's diet or as a supplement is safe.

Warnings and Special Precautions

Lutein is probably safe during pregnancy and breast-feeding when consumed in the levels found in food. Lutein is probably safe for children when given in adequate doses by mouth. A medication containing lutein 0.14 mg daily (LUTEINofta, SOOFT Italia SpA) has been safely used in new borns for 36 weeks. (MD, W. 2016).





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CONCLUSION

Lutein(L) is a potent antioxidant, and numerous studies support its beneficial effects on eye health, skin health, heart health, brain health, and can also cure diabetes and cancer. L also has positive effects on other tissues, particularly the brain, where it has been linked to improved cognitive performance. Thus, not only should high L intake be encouraged through a diet rich in fruits and vegetables, but also supplementation, especially in the elderly and individuals at high risk of various clinical conditions. However, there are still conflicting data that need to be resolved through randomised clinical trials with large general population cohorts. As a result, this time span is likely insufficient to demonstrate significant favourable effects; thus, longer-term studies are required to better elucidate the possible beneficial role of L on human health. Current evidence suggests that higher lutein intakes may play an important role in preventing age-related macular degeneration (AMD). Improved understanding and quantification of lutein are required to determine a recommended target for these macular carotenoids, as well as a better understanding of their distinct roles in eye health. A varied diet is essential for maintaining adequate lutein in the diet (as well as other nutrients). In addition, in accordance with dietary guidelines, such a diet should include plenty of leafy green vegetables. As part of a healthy dietary pattern, there is also value in including a variety of other foods to increase variety and improve L/Z bioavailability, such as eggs and selected nuts. Intervention trials examining the efficacy of specific dietary patterns aimed at increasing macular pigment and preventing or delaying the progression of AMD are needed. Meanwhile, those at high risk of AMD or who already have AMD should be advised to increase their consumption of lutein-containing foods in their diet.

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Role of Berries in Cancer

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ABSTRACT

Dietary patterns, including regular consumption of particular foods such as berries as well as bioactive compounds, may confer specific molecular and cellular protection in addition to the overall epidemiologically observed benefits of plant food consumption (lower rates of obesity and chronic disease risk), further enhancing health. Mounting evidence reports a variety of health benefits of berry fruits that are usually attributed to their non-nutritive bioactive compounds, mainly phenolic substances such as flavonoids or anthocyanins. Although it is still unclear which particular constituents are responsible for the extended health benefits, it appears that whole berry consumption generally confers some anti-oxidant and anti-inflammatory protection to humans and animals. With regards to cancer, studies have reported beneficial effects of berries or their constituents including attenuation of inflammation, inhibition of angiogenesis, protection from DNA damage, as well as effects on apoptosis or proliferation rates of malignant cells. Berries extend effects on the proliferation rates of both premalignant and malignant cells. Their effect on premalignant cells is important for their ability to cause premalignant lesions to regress both in animals and in humans. The present review focuses primarily on in vivo and human dietary studies of various berry fruits and discusses whether regular dietary intake of berries can prevent cancer initiation and delay in humans or ameliorate patients' cancer status. A berry is a small, pulpy and often edible fruit. Typically berries are juicy, rounded brightly coloured, sweet, sour or tart, and do not have a stone or pit, although many seeds may be present. Common examples are strawberries, raspberries, blueberries, blackberries, red currants, white currants and black currants. In Britain, soft fruit is a horticultural term for such fruits. In common usage the term berry differs from the scientific definition of a fruit produced from the ovary of a single flower in which the outer layer of ovary wall develops into an edible fleshy portion known as pericarp



**Niharika Patnaik and Preetha Bhadra****Keywords:**Berries, soft fruit, micronutrients, anthocyanins, cancer, heart disease.

INTRODUCTION

Wherever humans have lived, berries have been a part of their diet. Most of these have never developed beyond local markets but some have become globally important crops. Berries are considered soft fruits and include botanically different types, of fruits such as black berries, blue berries, strawberries, cranberries, gooseberries and currants. These fruits are used in desserts and also in processing. They are canned, frozen, or made into jams, jellies and preserves and are also used in beverages and icecream. Production figures for all berries are not available. They are produced mainly in the United States, European countries, North America, grey China. New Zealand is the largest producer of kiwi fruit. Followed by Italy, Japan, France, United States and Chile. Germany, Japan are the major importers, with high per capita consumption. Black berries are native to North America. They have strong and erect stems. Black berries vary in colour ranging from dark red to reddish black. Brison, Rosborough Womack, Cheyenne, and Hull Thornless are some of the Cultivars of blackberries. Commercial blue berries - both wild i.e. lowbush and cultivated i.e. highbush are all native to North America. The high bush varieties were introduced into Europe during the 1930s. Blueberries are usually prostrate shrubs that can vary in size from 10 centimetres (4 inches) to 4 metres (13 feet) in height.

Morphology of Berries

- A Berry is a simple fruit having seeds and fleshy pulp, known as pericarp from the ovary of a single flower.
- The ovary can be inferior or superior. It is indehiscent.
- It does not have a special line of weakness along which it splits to release the seeds when ripe.

Theory

Berry, is derived from a single ovary of an individual flower. The middle and inner layers of the fruit wall often are not distinct from each other. Together with drupes and pomes, berries are one of the main types of fleshy fruits. There are two specific types of berries that characterize certain taxonomic groups. The leathery rinded berry of citrus fruits is called a hesperidie elongated tough skinned fruits of the family cucurbitaceae, including watermelons, cucumbers and gourds are a type of berry referred to as pepos. Any small fleshy fruit is popularly called a berry. especially if it is edible.

Chemical Composition of Berries

The bioactive compounds in berries contain mainly phenolic compounds i.e. Phenolic acids, flavonoids such as anthocyanins and flavonols and tanins and ascorbic acid. Blackberry fruit contains high level of anthocyanins and other phenolic compounds, mainly flavonols and ellagitannins, which contributes to its high antioxidant capacity and other biological activities. Blackberry phenolic composition and concentrations are known to be influenced by genetics, growing conditions and maturation. Cyanidin 3-glucoside is the predominant anthocyanin in black berries, but they also contain cyanidin 3-deoxyglucoside thought to be unique to black berries. The blueberry fruit were harvested from 3 commercial berry plantations and from one natural habitat in 2003. The anthocyanin content ranged from 125-405 mg 100 g⁻¹ of fresh berry in different species and cultivars. Half high bush blue berry North blue had the largest berry size but ascorbic acid and anthocyanin contents were low. Grape berry contains the phenols that is anthocyanins, flavonols, flavones, chalcones, stilbenes. Sugars present in grape berry are glucose and fructose. Nitrogenous compounds present in grape berry are Amino acids, peptides and proteins. Minerals present in grape berry are copper, potassium, manganese and organic acids present in grape berry are Tartaric acid, mallic acid, citric acid, succinic acid and fumaric acid

Phytochemicals in Berries

Common berry fruits including strawberry, blackberry, raspberry, and blue berries have long been appreciated for their dessert like quality. Berry fruits have appealing colors imparted by anthocyanin pigments that range from red



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to purple to black, in addition to unique tastes and aromatic notes. They contain important micronutrients such as vitamin C and folic acid are considered excellent sources of dietary fibre. Bioactive properties called phytochemicals afford protection against chronic diseases, berry fruits are a rich source of phytochemicals in particular phenolic compounds, which are reported to have a range of potential anticancer and anti heart disease. Berries contain high level of a diversity of phytochemicals known as phenolics including flavonoids, proanthocyanidins, ellagitannins and gallotannins, stilbenoids and phenolic acid. Although berry phenolics are potent in vitro antioxidants, they exert in vivo biological activities beyond antioxidation and can have complementary and overlapping mechanism of action. Berry phenolics can impart preventive benefits through the regulation of enzymes important in metabolizing xenobiotics and carcinogens, by modulating nuclear receptors, gene expression and sub-cellular signaling of proliferation and apoptosis, and by acting indirectly through antioxidant actions that protect DNA from damage. This overview examines the impact of consumption of natural berry bioactive compounds on human health and disease prevention.

Antioxidant Activities

Berries are the part of traditional diet contributing to intake of flavonoids and other phenolic compounds. The antioxidant effect of berry phenolics is strongly dependent on the choice of berry raw material, as the antioxidant activity differs between the different phenolic constituents, including anthocyanins, ellagitannins, and proanthocyanidins. In foods, the antioxidant effect is also influenced by the structure of food. Tannin-containing berries exhibit antimicrobial properties against pathogenic bacteria, thus offering many new applications for food industry. Much of the interest in berry has focused on cranberries and both cultivated and wild blueberries, although also other berries including black currants, cloudberry, lingonberry, and red raspberries possess promising bioactivities that may have relevance to human health. Antioxidant activity of berry, in addition to other mechanisms, may contribute to human health, but the possible relationship remains yet to be scientifically substantiated.

Benefits of Berries

Transporters of essential antioxidants: They are wealthy in cancer prevention agents like anthocyanins, ellagic acid, and resveratrol. These cell reinforcements limit free radical injury in the body. Cancer prevention agent properties of berries might give assurance against contamination harm. Blueberries, blackberries and raspberries contain the biggest number of cell reinforcements than some other organic products.

It Makes our brain sharp: Consuming this astounding organic product is really great for our cerebrum wellbeing. This delightful organic product can postpone mental hindrances by around over two years. It has flavonoids, specifically anthocyanins that further develop cerebrum learning and memory focuses. In ladies, eating berries reinforces rationale and memory.

Can balance diabetes: Can Manage as well as Balance Diabetes. Notwithstanding tasting sweet, they are great for individuals with diabetes. Any diabetic individual can eat berries with practically no concern as the organic product has a low glycemic record. High fiber content can forestall diabetes beginning in individuals. They contain normal sugar that is alright for diabetic individuals to eat. They might assist with glucose control.

Berries can reduce inflammation: Berries have strong anti-inflammatory characteristics.

Manages bad cholesterol levels: This amazing natural product can assist with forestalling harm of LDL cholesterol, therefore lessening the gamble of coronary illness. The organic product additionally may lessen aggravation and cholesterol levels in the heart, consequently forestalling any gamble of stopped up corridors. Eating berries may fundamentally decrease the gamble of atherosclerosis.



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Berries can keep our Arteries Healthy:In many experiments, conducted berries have been shown to enhance the arterial function of people suffering from metabolic syndromes. Blueberries can reduce arterial stiffness.

Prevents Urinary Tract Infections:Berries, especially Cranberries, can reduce the risk of urinary tract infections (UTIs). The fruit prevents E. Coli from binding to the bladder or urinary tract wall, thereby decreasing the chance of infection.

What is cancer?

Cancer is a group of disease involving abnormal cell growth with the potential to spread other parts of the body. These stand out from harmless cancers, which don't spread. Potential signs and side effects incorporate a knot, strange dying, delayed hack, unexplained weight reduction, and an adjustment of gut movements. While these side effects demonstrate disease, they can likewise have other causes. Over 100 sorts of malignant growths influence people. The gamble of fostering specific malignant growths can be decreased by not smoking, keeping a solid weight, restricting liquor eating a lot of vegetables, natural products, and entire grains, immunization against specific irresistible infections, restricting utilization of handled meat and red meat, and restricting openness to coordinate sunlight. Early identification through screening is helpful for cervical and colorectal cancer. The advantages of evaluating for bosom malignant growth are controversial. Cancer is frequently treated with a mix of radiation treatment, medical procedure, chemotherapy and designated therapy. Pain and side effect the board are a significant piece of care. Palliative care is especially significant in individuals with cutting edge disease. The chance of endurance relies upon the sort of malignant growth and degree of illness toward the beginning of treatment. [10] In youngsters under 15 at conclusion, the five-year endurance rate in the created world is on normal 80%. For malignant growth in the United States, the normal five-year endurance rate is 66%.

Benefits of Berries for Cancer

Among bright organic products, berries and their inferred items order a staggering and quickly developing collection of logical information to help their capacity to forestall, delay and possibly treat specific kinds of human diseases. Considering that the overall rate of disease is quickly expanding, intercession with staples, like berries and berry details, give an alluring system to malignant growth anticipation. In the same way as other different natural products, berries contain miniature and macronutrients like nutrients, minerals, and fiber. Notwithstanding, berries additionally contain a large number of plant auxiliary metabolites (phytochemicals) that display a different exhibit of synthetic constructions. It has become clear that numerous berry constituents, through added substance, correlative, and additionally synergistic cooperations, show chemopreventive impacts better than any single part alone. Mounting proof reports an assortment of medical advantages of berry natural products that are typically ascribed to their non-nutritive bioactive mixtures, basically phenolic substances like flavonoids or. Despite the fact that it is as yet hazy which specific constituents are liable for the drawn out medical advantages, apparently entire berry utilization by and large presents an enemy of oxidant and calming insurance to people. With respect to disease, studies have detailed gainful impacts of berries or their constituents including weakening of aggravation, hindrance of angiogenesis, security from DNA harm, as well as consequences for apoptosis or multiplication paces of threatening cells. Berries broaden impacts on the expansion paces of both premalignant and dangerous cells. Their impact on premalignant cells is significant for their capacity to make premalignant sores relapse both in creatures and in people. The current audit centers basically around in vivo and human dietary investigations of different berry products of the soil whether normal dietary admit for malignant growth commencement and postpone movement in people or improve patients' disease status.

CONCLUSION

Berries are rich sources of essential micronutrients, particularly vitamin C and folic acid. They also contain numerous phytochemicals. These have diverse effects *in-vitro* which suggest potential health benefits. However, until more is known about the absorption and metabolic fate of berry anthocyanins and flavonols in vivo, it would be unwise to



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ascribe additional health promoting properties to berries beyond those recognized for fruit and vegetables in general. However, in populations with habitually low intakes of plant-based foods, locally grown fresh or frozen berries are an underused and potentially valuable dietary resource.

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Neem as a Biopesticide

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ABSTRACT

The biological effects of a neem-based biopesticide, containing 4.5% azadirachtin, were assessed against the Asian citrus psyllid, *DiaphorinacitriKuwayama*, a recently introduced insect pest and potential disease vector of citrus in the United States. Over the concentration range 11-180 ppm azadirachtin, no mortality of adult psyllids was observed when exposed to treated plants. Adult psyllids demonstrated a small but significant repellent effect from treated plants in a choice experiment, but showed no preference to oviposit on treated or untreated plants. Psyllid nymphs were susceptible to azadirachtin at very low concentrations and activity perhaps was due to developmental inhibition. At a concentration of 22.5 ppm azadirachtin, ecdysis was not observed past 4 days after treatment and all nymphs were dead within 7 hydys. The densities of psyllid nymphs on treated plants exposed to a greenhouse population were significantly reduced by concentrations as low as 10 ppm azadirachtin. Over the range of concentrations used in these experiments, the product caused no phytotoxicity to tender foliage of either citrus or orange jasmine plants. Field trials are warranted to determine suitability of neem-based biopesticides for inclusion in citrus integrated pest management programs.

INTRODUCTION

Neem plant is considered the most useful traditional plant in India. The various properties of different parts of the neem tree are used mainly as insecticide, fertilizer, manure, soil conditioner, urea coating agent, fumigant, etc. In the recent era the major challenge is to increase food production and safeguard food from pest without harming the environment. Since the last decades, pesticides have become an integral component in sustainable agriculture and



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modern cultural practices. The use of chemical pesticides and fertilizers are ineliminable. The natural pesticides from *Azadirachtaindica* are considered less harmful, biodegradable, least persistent, less toxic to the non-target organism and also economic. Fruitful results of the application of formulated neem-based products in agriculture will provide a cost-effective technology to the farming community. Neem (*Azadirachtaindica*) is commonly called 'Indian Lilac' and belongs to the family Meliaceae, subfamily Meloideae and tribe Melieae. It is an evergreen, tall, fast-growing tree having height of 25m and 2.5m in girth which has an attractive crown of deep green foliage and honey-scented flowers. Neem is the most versatile, multifarious tree of tropics, with immense potential. It possesses maximum useful non-wood products (leaves, bark, flowers, fruits, seed, gum, oil and neem cake) than any other tree species.

Neem as a biopesticide

Various results obtained globally have shown that neem and its allelochemicals have variety of effects on pests. More than 140 active principals have been identified to date that occur in different parts of the tree. The most important components identified have been the tetranortriterpenoids, the azadirachtins. These occur at concentrations of 0.1 to 0.9 per cent in the seed core and it has been established that 30 to 60 g azadirachtin per hectare suffice to combat and repel the key pests of various crops. It seems that approximately 20 to 30 kg of neem seeds are required per hectare if 2 g of azadirachtin per kg of seed is obtained. This will incur a cost in the range of US \$ 1 to 60, although in most countries the range may narrow down to \$ 5 to 20. Neem has been shown to control key pests in varied ways. It has a high level of efficacy, low risk of pest resistance due to different mode-of-action, specific effects on pests, safety for non-target organisms, biodegradable nature and is easily obtained from a renewable source. It is only in the past decade that the pest control potential of neem, which does not kill pests like neurotoxins but affects their behaviour and physiology, has been recognized. Though subtle, neem's effects such as repellency, feeding and oviposition deterrence, growth inhibition, mating disruption, chemo-sterilization, etc. (Schmutterer, 1995, 2002) are now considered far more desirable than a quick knock-down in integrated pest management programs as they reduce the risk of exposing pests natural enemies to poisoned food or starvation.

In spite of high selectivity, neem derivatives affect ca. 400 to 500 species of insects belonging to Blattodea, Caelifera, Coleoptera, Dermaptera, Diptera, Ensifera, Hetroptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera, Phasmida, Phthiraptera, Siphonoptera, Thysanoptera, on species of ostracod and several species of mites. Neem preparations also act as nematocides against endoparasitic species of Meloidogyne and Globodera, ectoparasite species of Hoplolaimus and Tylenchorhynchus and semiendoparasitic species of Rotylenchus and Pratylenchus nematodes (Musabyimana and Saxena, 1999). Similarly as a fungicide neem products are effective against a number of fungal pathogens. Water snails as vectors of diseases such as Meliniascabra (schistosomiasis) and phytophagous land-snails in greenhouses and horticulture are killed by neem preparations (West and Mordue, 1992). The neem products also control many acarines of Tetranychus genus, bacterial plant pathogens and animal and plant viruses (Mansour et al., 1987; Hunter and Ullman, 1992; Schmutterer, 1995).

Usage of neem in crop protection

The extent to which neem is used as a biopesticide in the FCT-Abuja and Niger State in 2006 is shown in Table 1. Among the urban farmers, only an average of 11.77% of them used neem as a biopesticide. This percentage increased by 5.42% among the rural farmers. The highest percentage of neem users among the agro-allied personnel/researchers was from State (20.00%). On the whole, the highest group of users of neem as a biopesticide was the rural dwellers (17.19%) with the highest percentage (22.50%) from Western zone of the FCT. It is relatively cheap and easily available. Its complex mixture of active ingredients which function differently on various parts of the insects life cycle and physiology makes it difficult for pests to develop resistance to it. It is systemic, thereby protecting the plant from within. This has resulted in wheat, barley, rice, sugar cane, tomatoes, cotton etc being protected from damaging insects for up to ten (10) weeks. It parades a wide spectrum of pesticidal activity. Insects controlled by neem include migratory locust, army worms, whitefly and even head lice. The pathogens it controls include Meloidogyne root-knot nematode, Rhizoctonia root-rot fungus and Rice stunt virus (Anonymous,



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1992; Anjorin et al., 2004). It is found to be safe to beneficial organisms such as earthworms. Khalid and Shad (2002) specifically reported that their toxic effect is normally of an ephemeral nature disappearing within 14 - 21 days.

Neem-based insecticides

Commercially available neem formulations [Azatrol (1.2% azadirachtin), Triple Action Neem Oil (70% neem oil) and Pure Neem Oil (100% neem oil)] were obtained from Pbi/Gordon Corporation, Kansas City, Missouri, USA; Southern Agricultural Insecticides Inc., Palmetto, Florida, USA; and Dyna-Gro, San Pablo, California, USA, respectively. The formulated products were screened at recommended application concentrations of 31.5 ml/l for Azatrol, 7.5 ml/l for Triple Action Neem Oil, and 7.5 ml/l for Pure Neem Oil to evaluate repellent, antifeedant, and toxic effects on *M. persicae* aphids.

Source of raw material

Personal interview indicated that few of the neem users (two out of ten) personally owned their neem tree. Majority of the users (seven out of ten) obtained their neem materials from semi-wild trees on communal land. While one out of ten users paid labourers for raw materials. This was more common among the agro-allied personnel (50.00%). There was no report of government established neem plantation in the FCT inspite of its economic importance. Majority of the farmers (eight out of ten) used neem for medicinal purpose. This is followed by its use for protecting farm produce in the store (66.67%). The purpose of use of neem for the agro-allied personnel was mainly experimental. The least purpose which neem products is put is for soil drenching (19.05%) on the crop field. The highest percentage of the neem users (66.67%) mostly from the rural areas reported that they use neem because it was in line with their tradition. Next to this reason was that neem materials are cheap and easy to prepare and that it is effective (61.90%). The effectiveness indicated by 50.00% of the agro-allied personnel neem users was only confirmed in the laboratory and not on to field.

Unlike vitamins, polyphenols are not essential components of human diet. Nevertheless, they are consumed on daily basis due to their ubiquitous presence in fruits and vegetables. Many researches have shown that polyphenols and/or polyphenol-rich foods have an important role in health preservation due to antioxidant properties [15, 16, 24]. The antioxidant activity of cocoa and chocolate was shown to be correlated with their catechin and procyanidin contents. Antioxidant properties of polyphenols highly depend on the arrangement of functional groups around the nuclear structure. Free radical scavenging capacity is primarily attributed to hydroxyl groups, and aglycones are more potent antioxidant than their responding glycosides. Polyphenols can act as proton donor-scavenging radicals [27], inhibitors of enzymes that increase oxidative stress, chelate metals, bind carbohydrates, and proteins [26]. These properties enable them to act as anticarcinogenic, anti-inflammatory, antihepatotoxic, antibacterial, antiviral, and anti-allergenic compounds.

This is supported by research of Hollenberg et al. [31], who established relationship between high consumption of cocoa beverages and very low blood pressure levels, reduced frequency of myocardial infarction, stroke, diabetes mellitus, and cancer in Kuna Indians residing in archipelago on the Caribbean Coast of Panama, unlike Kuna Indians residing on Mainland. Another study, conducted on elderly men free of chronic diseases in Zutphen, Netherlands, showed that consumption of cocoa reduced blood pressure and decreased risk of cardiovascular and all-cause death by 45–50%. Grassi et al. observed decrease of blood pressure by short-term administration of dark chocolate in healthy [33] and glucose-intolerant, hypertensive subjects [34]. However, they investigated only 15 subjects per research and these findings should be taken with reserve. Djoussé et al. [35, 36] associated frequent consumption of dark chocolate with lower prevalence of cardiovascular diseases in men and women independently of traditional risk factors estimated based on health questionnaire. This association was perceived both in smokers and nonsmokers, as well as in subjects under and above 60 years of age. The research included large number of examinees, but data about consumption of chocolate were self-reported and there was no differentiation between dark and milk chocolate.



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Hot cocoa beverage was proven to successfully reduce LDL cholesterol, increase HDL cholesterol, and suppress LDL oxidation in research of Baba et al. [43]. Atherosclerotic cholesterol profile (cholesterol : HDL ratio) in patients with diabetes was improved after 8-week chocolate consumption without affecting weight, inflammatory markers, insulin control, or glycaemic control. In addition to lowering blood pressure levels, cocoa polyphenols might be involved in cholesterol control. Waterhouse et al. (1996) reported polyphenols from chocolate inhibited LDL oxidation by 75%, compared to 37–65% of red wine (adopted from [16]). In addition, Vinson et al. [39] reported that dark chocolate had higher quality of phenol antioxidants expressed as IC50 for LDL + VLDL oxidation compared to red wine and black tea, with high lipoprotein bound antioxidant activity, which is very important in prevention of heart diseases. A survey implemented by a group of experts showed that in the case of similar absorption, about 50 g of dark chocolate should be eaten to provide equivalent flavonoids to about 200 mL of red wine, which has been shown to reduce heart attack risk for an average adult. Flavanol-rich cocoa increases blood flow to key areas of brain increasing blood oxygenation level-dependent response to cognitive task switching paradigm in healthy young people [54] and could be useful in treatment of cerebrovascular flow (CBF) dementia [55], Alzheimer's disease [56], and stroke [57]. Chandranayagam et al. [58] reported that tannin-rich chocolate can be considered as functional food which effectively antagonizes adverse effects of arsenic intoxication. However, this research was conducted on Sprague Dawley rats and should yet be confirmed by research on humans.

Challenges and improvement of neem Biopesticide utilisation

Establishment of quality standards for neem products involves numerous interwoven practical, scientific and legal issues. There has been lack of coordinated research directed towards the development and validation of neem bioassays through the demonstration of correlation between in vitro and field pesticidal efficacy. Infrastructures such as specialized research laboratory and storage facilities for neem raw materials and products are inadequate. Thus it has been very difficult to analyse the quality and quantity of active ingredients in local neem or increase the potency lifespan of neem pesticide. There is need for collaboration with the Government agencies such as National Agency for Food and Drug Administration (NAFDAC), Standard Organization of Nigeria (SON) and the farmers. Standard formula of extraction technique of crude, semi-purified or purified neempesticidal products is lacking but should be originated and introduced in order to produce a more consistent botanical pesticide in the area under review. Proper identification of neem products used as biopesticide is imperative. This requires the use of validated methods that will reliably discriminate the presence of substitutes, contaminants, or and adulterants. There is need for a written macroscopic and microscopic descriptions and Thin Layer Chromatography, HPLC (High Performance Liquid Chromatography) and NMR (Nuclear Magnetic Resonance) facilities for characterizing and confirming the identity of neem powder or extracts from different parts of the plant or different geographical locations. Other emerging scientifically superior and high techniques for ensuring identity are capillary electrophoresis (CE) and DNA analysis. Reference materials or authenticated standards are an essential prerequisite for any type of scientific assessment of botanical pesticide in order to obtain accurate identity and purity testing. However, potential issues in the selection and use of standards which might be natural active ingredient or synthetic prototype are instability, special handling or storage requirements and their shelf-life. Economics might be an important factor, as the cost of these standards is often prohibitive for routine analysis.

Applications

In addition to its medical applications, neem has aroused interest in many other areas. In the cosmetics and hygiene sector, neem is used in the composition of face masks, lotions, sunscreens, soaps, and toothpastes. Products derived from neem can contribute to sustainable development and the resolution of pest control problems in agriculture. These products benefit from the natural properties of neem as a powerful insect growth regulator (IGR) that also affects many other organisms (such as nematodes and fungi) and can act as a plant fertilizer. The use of neem in agriculture is not a new practice. In India, the traditional farming system employed neem extracts for pest management and to supply nutrients to plants. Scientific research has shown that neem is safe for workers, with no handling risks, and can be used throughout the entire crop production cycle. Neem has proven used as a fertilizer, with the organic and inorganic compounds present in the plant material acting to improve soil quality and enhance



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the quality and quantity of crops. The waste remaining after extraction of the oil from neem seeds (neem seed cake) can be used as a biofertilizer, providing the macronutrients essential for plant growth.

Nitrogen is one of the main nutrients required by plants for their development, and urea is the main source of nitrogen fertilizer used worldwide to supply the nitrogen demand of crops. The control of urea hydrolysis and nitrification is one of the principal strategies employed to avoid nitrogen losses in agriculture. Neem has demonstrated activity as a nitrification inhibitor, helping to slow the bacterial activity that is responsible for denitrification, hence decreasing the loss of urea from the soil. Due to their compositional complexity, neem-based products can act as anti-feedants, growth regulators, sterilants, anti-oviposition, insecticide, antifungal and repellents. Other factors that have stimulated the use of neem-based products for pest control in agriculture are ecological and toxicological aspects (low toxicity to non-target organisms), as well as economic aspects.

These features of neem support its contribution to organic agricultural production systems that are more sustainable and do not generate chemical residues (plants and crops are grown without the use of any agrochemicals). This method also helps to maintain soil productivity, ensuring longer production times. Organic agriculture can be a viable alternative production method for farmers, but there are numerous challenges to overcome. A key to success is to be open to new approaches, and in this respect neem products can effectively contribute to organic agriculture, being used as organic pesticides and as soilfertilizers. In addition, growing concerns about conventional agriculture and the demand for products that do not generate waste justify increased adoption of the use of biopesticides by farmers, which contributes to the growth of organic agriculturefertilizers. In addition, growing concerns about conventional agriculture and the demand for products that do not generate waste justify increased adoption of the use of biopesticides by farmers, which contributes to the growth of organic agriculture.

Commercial products derived from Neem

Beyond all the possible pesticides and pharmaceuticals, neem provides many useful and valuable commonplace materials. For instance, oil extracted from the seeds goes into soaps, waxes and lubricants, as well as into fuels for lighting and heating. The solid residue left after the oil is removed from the kernels is employed as a fertilizer and soil amendment. In addition, wood from the trees is valued for construction, cabinetry, and fuel. The bark is tapped for gum and extracted for tannins and dental-care products. The leaves are sometimes used for emergency livestock feed and the profuse flowers are a prized source of honey. Neem oil works in a number of different ways. The oil forms a coating on the insect's body, blocking the breathing openings and suffocating the insect. It also has a repellent effect on certain insects and mites. Neem oil prevents the germination and penetration of some fungal spores. In one study, researchers discovered that one percent neem oil treatment was effective in managing powdery mildew on hydrangeas, lilacs and phlox. More than 60 insect pests may be affected by azadirachtin including aphids, beetles, caterpillars, lace bugs, leafhoppers, leafminers, mealybugs, psyllids, thrips and whiteflies. Due to its insect growth regulating properties, it is most effective against the immature stages of insects. For example, the immature larvae of many species in the Lepidoptera family (moths and butterflies) are particularly sensitive to azadirachtin. Neem products may be registered for use on certain fruits, herbs and vegetables in addition to ornaments. For edible crops, some neem-based products may be used up to the day of harvest.

Benefits of neem as biopesticide

1. Neem Pesticide is a natural product, absolutely nontoxic, 100% biodegradable and eco-friendly.
2. It is suited for mixing with other synthetic pesticides and in fact, enhances their action.
3. None or a lesser quantity of synthetic pesticides needs to be used, thereby reducing the environmental load.
4. Several synthetic pesticides being single chemical compounds cause easy development of resistant species of pests. Neem consists of several compounds; hence development of resistance is impossible.
5. Neem does not destroy natural predators and parasites of pests, thereby allowing these natural enemies to keep a check on the pest population.



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6. Neem also has systemic action and seedlings can absorb and accumulate the neem compounds to make the whole plant pest resistant.
7. Neem has a broad spectrum of action active on more than 200 species of pests.
8. Neem is harmless to non-target and beneficial organisms like pollinators, honey bees, mammals and other vertebrates.

CONCLUSION

The use of plant secondary metabolites synthesized by some plant species as part of their natural self-defense against stored product insect pests seems to be an excellent alternative. Botanical pesticides (essential oils, flavonoids, alkaloids, glycosides, esters, and fatty acids) have various chemical properties and modes of action and affect insects in different ways, namely, repellents, feeding deterrents/antifeedants, toxicants, growth retardants, chemosterilants, and attractants. Thus, it is preferable to use botanical insecticides instead of synthetic insecticides and these botanical insecticides are recognized by organic crop producers in industrialized countries. It is also important to raise awareness among farmers to use the botanical products as pesticides. In some areas in particular, high attention is now paid to raise awareness especially in developing countries where long-term projects are being designed and aimed at educating growers about the basic skills of manufacturing botanical pesticides. Based on the number of scientific studies focused on the research of plant substances with insecticidal effects, it seems that commercial botanical insecticides should occupy an important position in the market. However, a better understanding of their mode of action, effects, and information related to regulatory issues is needed for their adoption. The scientific standardization may help further to raise their profile among the public and policymakers and hence enable them to realize their contributions to sustainability. Therefore, it is recommended to use botanical insecticides and conducting research to find new sources of botanical insecticides is very crucial.

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Ginger in Nutraceuticals

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ABSTRACT

Immunity boosters are products which claim to be able to support our immune system so we aren't as likely to get sick. Additionally, it also suggests that if we do get sick, taking the supplements will make our illness pass faster. Ginger root is one of the healthiest and a great immune booster. Ginger root comes from the *Zingiberofficinale* plant, and it has been used in Chinese and Indian medicine for thousands of years. People typically use fresh or dried ginger in cooking or as herbal tea, and some take ginger supplements for their possible health benefits. Sometimes Ginger helps relieve nausea and vomiting and aid digestion. Antioxidants and other nutrients in ginger root may help prevent or treat arthritis, inflammation, and various types of infection and also reduce the risk of diabetes, cancer, and other health problems.

INTRODUCTION

Ginger originated from Maritime Southeast Asia. It is a true cultigen and does not exist in its wild state. The most ancient evidence of its domestication is among the Austronesian peoples where it was among several species of ginger cultivated and exploited since ancient times. They cultivated other gingers including turmeric (*Curcuma longa*), white turmeric (*Curcuma zedoaria*), and bitter ginger (*Zingiberzerumbet*). The rhizomes and the leaves were used to enhance flavour in food or eaten directly. The leaves were also used to weave mats. Aside from these uses, ginger had religious significance among Austronesians, being used in rituals for healing and for asking protection from spirits. It Contains gingerol, which has powerful medicinal properties. It is used to treat many forms of nausea,





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especially morning sickness. It May help with weight loss and also with osteoarthritis. It may drastically lower blood sugars and improve heart disease risk factor. Anticancer activities of ginger against colorectal cancer have been well documented (S Prasad and A K Tyagi, 2015). Numerous *in vitro* studies showed that ginger and its active components inhibit growth and proliferation of colorectal cancer cells (S Prasad and A K Tyagi, 2015). In a study, 6-gingerol inhibited growth of colon cancer HCT116 cells. The suppression of tumours growth was found to be linked with the inhibition of leukotriene A4 hydrolase activity, which was further confirmed by *in silico* approach (S Prasad and A K Tyagi, 2015). Besides these, various other mechanisms were reported to be involved in 6-gingerol-induced cell growth inhibition and apoptosis in human colorectal cancer cells. These include protein degradation as well as downregulation of cyclin D1, NAG-1 beta-catenin, PKCepsilon, and GSK-3 β pathways (S Prasad and A K Tyagi, 2015). Radhakrishnan *et al.*, reported that the anticancer activity of 6-gingerol could be associated with the inhibition of ERK1/2/JNK/AP-1 pathway and whole ginger extract also prevents the primary stage of colon carcinogenesis. Administration of ginger extract to the mice pretreated with carcinogen 1,2-dimethylhydrazine (DMH) inhibited the levels of fecal bile acids, neutral sterols, tissue cholesterol, HMG CoA reductase, free fatty acids, triglycerides, phospholipase A, and phospholipase C. Thus, ginger supplementation reduced the risk of colon cancer markedly by virtue of its hypolipidemic and antioxidative effects (Manju V *et al.*, 2006). Ginger extract not only inhibits carcinogenesis of colorectal cancer cells but also enhances the anticancer effects of chemotherapeutic drug 5-fluorouracil (Manju V *et al.*, 2006).

History of Ginger

Europe saw ginger for the first time in the 1st century when the ancient Romans traded with the India. When the Rome fell, Europe forgot about ginger until Marco Polo brought it again from his travel to the East. The fast development of human society and therefore the improvement of standard life, cancer as a growing threat, is that the second leading non contagious sickness of death globally next solely to upset. Malignant increase is stated to be an essential purpose for dying, and there had been kind of 9.6 million times of dying in 2018. A few exam works have exhibited those regular items. As of late, ginger has been commonly explored for its anticancer houses in opposition to numerous malignant increase types, like bosom, cervical, colorectal, and prostate disorder. The viable structures of interest consist of the restraint of multiplication and the popularity of apoptosis in malignant increase. The cytotoxic influences and hidden structures of ginger in prostate malignancy had been assessed each *in vivo* and *in vitro*. The anticancer additives on the whole consist of the enlistment of apoptosis and the restraint of the enlargement of malignancy cells.

Ginger is belongs to the Zingiberaceae family, the rhizome is the part which is normally used as a spice. It's often called ginger root or simply ginger. Ginger can be used in different form or state like raw, dried, powdered or as oil. It's a very common ingredient in cooking recipes as a spice and flavouring agent. It's sometimes added to processed foods and decorative. Ginger is a flowering plant that which is fast found in Southeast Asia. It's known as the healthiest spices. In a Meta investigation taking a goose at numerous phytochemical impacts on colon malady, 2 specific examinations utilizing mice noticed [6]-gingerol intensifies instigated programmed cell death in malignant growth cells by meddling with the mitochondrial film (Tsai Y *et al.*, 2020). There have been likewise noticed instruments connected with the disturbance of G1 stage super molecules to prevent the propagation of malignant growth cells that is in addition a related advantage of alternative vital malignant neoplasm studies. The elemental system by which chemical irritant phytochemicals follow abreast of disease cells is by all accounts protein interruption (Tsai Y *et al.*, 2020). Many Years before British surgeon Dr. James Lind discovered that lime could prevent scurvy; fifth-century Chinese sailors were using ginger's vitamin C nutritive value for the same purpose on long voyages (Milne I., 2012). The cultural outlook on aphrodisiacs in the seventeenth century was another factor in the reduction of its usage as a therapeutic agent.

Ginger

Ginger is burned-thru worldwide as zest, seasoning specialist, embellish, medication, and food additive and is carried out each new, in a brand-new glue, or dry, in a dry powder. The heady perfume of ginger is entering into and





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sweet-smelling. Ginger is called as "Adrak" (neighbourhood name) with inside the subcontinent like India and Pakistan and is a crucial element of numerous dishes.

Nutritional Composition of Ginger

Chemical Composition

The estimated chemical composition of ginger was: moisture (15 ± 0.033)%, fiber (17 ± 0.03)%, ash (6.5 ± 0.001)%, protein (5.2 ± 0.1)%, fat (8.0 ± 0.003)% and carbohydrates (48.3 ± 1.16)%. Ginger essential oil was collected by steam distillation process. The physicochemical analysis of the oil was carried out, and the acid value was found to be 2.9, saponification value 25, ester value 30.22, free fatty acids 1.45 g oleic acid/100 g oil and the refractive index 1.5060% at 27°C (Sulieman AE *et al.*, 2013). The inhibitory effect of ginger oil was detected for growth of six microorganisms: the bacteria *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurim*, the mould *Aspergillus niger*, *Aspergillus flavus* and the yeast *Sacharomyces cerevisiae*. The results indicated that ginger oil has a potent antimicrobial activity against all tested organisms (Liu Q *et al.*, 2017). The highest antibacterial activity was detected against E.coli, where 23 and 30mm were the inhibition zone diameters at the lower and his higher oil concentrations, respectively. The higher antimicrobial activity, among all tested organisms was found against moulds where complete inhibition (100%) was recorded (Liu Q *et al.*, 2017).

Benefits

Ginger may have anti-inflammatory, antibacterial, and antiviral properties. Below are some of ginger's potential medicinal uses.

Reducing gas and improving digestion

According to Bodagh MN *et al.*, 2018, several studies have investigated ginger's effects on the gasses that form in the intestinal tract during digestion. This research indicates that enzymes in ginger can help break up and expel this gas, providing relief from any discomfort. In addition, the research shows that ginger may help increase movement through the digestive tract, suggesting that it may relieve or prevent constipation. Ginger also appears to have beneficial effects on the enzyme pancreatic lipase, which aids digestion in the small intestine.

Relieving nausea

Anh HN *et al.*, 2020 indicates that ginger can help alleviate morning sickness and relieve nausea following cancer treatment. Lete I *et al.*, 2016 review suggests that the odour-producing principles gingerols and shogaols are effective in preventing nausea and vomiting. However, the amounts of those compounds can vary, depending on the form of ginger. The researchers determined that dried ginger, followed by fresh ginger and powdered ginger tea had the highest concentrations of gingerol. One study that the review analysed included 576 adult cancer patients. The scientists found that doses of 0.5 grams (g) and 1.0 g were most effective at reducing nausea. Of the seven studies analysed, five showed gingers to be beneficial, while two found no beneficial outcomes. The authors of the review suggest that the mixed results may stem from differences in the forms and preparations of ginger. They also called for further studies in humans, in order to fully understand the effects of ginger on nausea and other gastrointestinal issues.

Supporting the immune system

Many people use ginger to help recover from a cold or the flu. However, the evidence supporting this use is mostly anecdotal. In an older study from Chang SJ *et al.*, 2013, researchers investigated the effects of fresh and dried ginger on one respiratory virus in human cells. The results suggest that fresh ginger may help protect the respiratory system, while dried ginger did not have the same impact. A large cross-sectional study from Chang SJ *et al.*, 2013 suggested that daily ginger consumption may support the immune system. This may protect against chronic disease and support recovery from other illnesses, such as the common cold or flu. A small (Mahassni SH *et al.*, 2019) study on the effects of ginger extract on smokers and non-smokers found that daily consumption of ginger extract





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was associated with a stronger antibody response in non-smokers. However, confirming ginger's effects on the immune system will require further research.

Reducing inflammation

Bartels EM *et al.*, 2015 review concluded that taking ginger by mouth is “modestly efficacious and reasonably safe” for treating inflammation caused by osteoarthritis. However, the authors noted that the studies in their meta-analysis were small and may not represent the general population. Meanwhile, a (Inserra P *et al.*, 2017) review of 16 clinical trials determined that the phytochemical properties in ginger may combat inflammation. These authors also called for further research into the most effective dosages and types of ginger extract.

Relieving pain

Ginger may ease Trusted Source pain through anti-inflammatory and analgesic effects of its gingerol compounds. A (Chen CX *et al.*, 2016) review concluded that ginger may specifically help reduce dysmenorrhea – pain right before or during a period. However, the authors acknowledge that the studies they had reviewed were often small or of poor quality. Fully exploring a connection between ginger consumption and pain relief will require more research.

Supporting cardiovascular health

There is some evidence that ginger extract may help prevent cardiovascular disease. A (Wang Y *et al.*, 2016) study Trusted Source of 4,628 people found that daily ginger consumption may protect, diabetes, hyperlipidaemia, cerebrovascular disease, and fatty liver disease, among other chronic conditions. The authors concluded that ginger may have potential as a preventive therapy. against coronary heart disease, high blood pressure. Determining whether ginger may support treatment for those with cardiovascular disease will require further research. Meanwhile, a small (Chen CX *et al.*, 2016) study found that ginger extract helped reduce the occurrence of heart abnormalities in rats with diabetes. The authors noted that this reduction may stem, in part, from the antioxidant properties of the extract.

Lowering cancer risk

Ginger does not provide protein or other nutrients, but it is an excellent source of antioxidants. Research Trusted Source has shown that, for this reason, ginger can reduce various types of oxidative stress. Oxidative stress can happen when too many Trusted Source free radicals build up in the body. Free radicals are toxic substances produced by metabolism and other factors. When they build up in the body, free radicals can cause cellular damage, which can lead to conditions such as rheumatoid arthritis, heart attack, chronic inflammation, and cancer. Dietary antioxidants can help the body get rid of free radicals. A (S Prasad and A K Tyagi, 2015) review suggests that ginger may be effective against certain cancers of the gastrointestinal system, including colorectal cancer, gastric cancer, pancreatic cancer, and liver cancer. The review concludes that ginger may inhibit the growth of cancer cells in certain types of cancer or contribute to the death of cancer cells in other types.

Nutrition and dosage

Ginger is a good source of antioxidants, but it does not provide many vitamins, minerals, or calories. As the Department of Agriculture notes, 2 teaspoons of ginger provide only 4 calories Trusted Source and no significant amount of any nutrient. Most of the research on ginger has looked at dosages of between 250 milligrams (mg) and 1 g, taken between one and four times each day. The Food and Drug Administration (FDA) considers ginger root to be generally safe with an approved daily intake recommendation of up to 4g (Modi M *et al.*, 2021).

Risks

The FDA considers ginger to be safe in the diet, but it does not guarantee or regulate its use as a medicine or supplement. Researchers (Bode AM *et al.*, 2020) have not investigated many of the compounds in ginger. Also, scientific evidence does not support some claims about ginger's healing qualities. Before adding more ginger to the diet or taking a ginger supplement, consult a healthcare professional. Some supplements can interact with medications or cause other health complications (Bode AM *et al.*, 2020).





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Biological Activity of Gingerol

In a pre-medical meta-exam of gingerol intensifies anticancer, calming, opposed to contagious, most cancers prevention agent, neuroprotective and gastroprotective residences had been accounted for, which don't forget reads for vitro and A couple in-vivo examines have recommended that gingerols inspire sound glucose guiding principle for diabetics. Numerous examinations were across the influences of gingerols on a huge scope of malignant growths which include leukaemia, prostate, bosom, skin, ovarian, lung, pancreatic and there has now no longer been a number of medical attempting bent on note gingerols physiological leads to human. Whereas a huge wide range of the substance additives related to the influences of gingerols on cells were completely contemplated, few were in an exceedingly medical setting. This can be because of the very smart quality in normal phytochemicals and also the absence of viability in analysis. Most flavourers healthful drug, that embody gingerols, are below the constraints of the Food and Drug Administration withinside the u. s. and take a glance at techniques have currently not control the maximum amount as research which has pale the motivation in phytochemical research. Herbal medicine is untested for fine affirmation, energy and adequacy in medical settings because of a insufficiency of finance in Jap medical research. Most exploration on [6]-Gingerol has been on each mouse subjects (in-vivo) or on delicate human tissue (in-vitro) and is maybe applied in an exceedingly whereas to talk some capability programs for multi-goal infectious.

CONCLUSION

Regardless of advances in the therapy of bosom disease, there stays a necessity to conquer helpful opposition and create novel therapies for metastatic bosom malignancy. Ongoing investigations have exhibited that BCSCs might be answerable for opposition. Crosstalk between the essential tumor and the stroma or microenvironment is allegedly conceivably answerable for the relocation and obtrusive nature of metastatic bosom disease. To address these worries, consideration should be centred around intensifies that explicitly influence different subatomic targets related with undifferentiated cells and the metastatic tumour micro-environment. Phytochemicals have demonstrated viable at focusing on various flagging pathways and BCSCs in bosom malignancy.

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Garlic as Functional Food - Review

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ABSTRACT

Garlic (*Allium sativum* L.) botanically a member of the Lillaceae family and *Allium* genus, is considered to be rich in medicinal properties. The undamaged bulbs contain allin which will be enzymatically converted to allicin during cutting. Allicin, is the major bioactive compound found in garlic followed by other organosulfur compounds. Preparation of garlic extractions can be done in liquid form and solid form. The composition and bioactivity of the extraction depend on the strain, age, method of preparation and consumption method. When extracted and isolated, the bioactive compounds of garlic show a wide range of beneficial health effects to treat various infectious diseases, non-communicable diseases as well as metabolic and genetic disorders. An overview is provided on the numerous clinical and experimental investigations done on the reduction of LDL cholesterol level, cardiovascular disease, blood glucose levels by garlic derivative compounds. Furthermore, anti-carcinogenic effect, anti-microbial effect and antioxidant property of garlic are also reviewed.

Keywords: Garlic, infectious diseases, genetic disorders, anti-carcinogenic effect, anti-microbial

INTRODUCTION

Functional foods have begun a new era in food technology with the increasing global health threats as well as with the lack of nutritional food sources. According to European researches, food products can only be considered functional if together with the basic nutritional impact it has beneficial effects on one or more functions of the human organism thus either improving the general and physical conditions or/and decreasing the risk of the



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evolution of diseases There are numerous natural foods with functional properties. Garlic is claimed as one of the important element in daily diet which also acts as a functional food. Garlic is a widely grown crop around the world with a need of plenty of sunlight. It is used directly as a food or as a seasoning or as a spice. It has a pungent odor due to the odorous sulfur compounds. Plants of genus *Allium* are considered to have rich medicinal properties to prevent different non-communicable diseases, metabolic disorders as well as genetic disorders. From the genus *Allium*, the frequently used plant by humans is garlic (*Allium sativum* L.). Chemical constituents of garlic such as organosulfur compounds are responsible for the functional properties in garlic. The principle bioactive compound present in garlic is called "Allicin" which is in higher percentage in raw form of garlic. Different processing methods like crushing or chopping activate the allinase enzyme which will convert allin into its bioactive form allicin. Garlic can be prepared in solid form and liquid form. Solid form can be used as dry powder or soft heated mass. Liquid form could be prepared as a water formulation, oil or an extraction using solvents like alcohol However, the composition and the effect of its bioactivity depend on the garlic strain, age, storage condition, method of processing and consumption An animal experiment was done using normal rats which were treated orally and intraperitoneally with raw and boiled aqueous extracts of garlic for 4 weeks and their serum levels of glucose, cholesterol, and triglycerides were measured. It was found out that the raw garlic had a profound effect in reducing the glucose, cholesterol, and triglyceride levels, whereas boiled garlic had little effect in controlling these parameters bulbs have S-allyl-L-cysteine sulfoxide (allin) and γ -glutamyl cysteine derivatives as the main compounds while steam distilled oils are rich in sulfide family compounds. Dried garlic powder is rich in allin and diallyl disulfides. Macerates (ground garlic) are enriched extractions with sulfide family compounds, dithiines, and (E-Z)-ajoene compounds. And soaked, sliced, aged garlic extract in ethanol solution contains S-allyl-L-cysteine (SAC) and S-allyl mercaptocysteine Garlic is being used since ancient history for different therapeutic effects It is said that in early Olympics in Greece the athletes were fed garlic to increase their stamina levels . The ancient China and India have used garlic to help respiration and digestion as well as to treat parasitic infections Furthermore, it had been recommended to treat arthritis, toothache, chronic cough, constipation, parasitic infestation, snake and insect bites, gynecologic diseases, as well as in infectious diseases. COMPOSITION OF GARL elements (macro-and micronutrients-calcium,

Functionality Of Allicin And Other Active Components**Antimicrobial Activity**

It was reported that allicin showed antibiotic activity. Subramanyan *et al.* studied *in vitro* the effect of some of the more commonly used spices on intestinal bacteria in health and disease and reported that garlic was most potent in inhibiting the growth of some bacteria. Incorporation of garlic in the diet on caecal microflora of rats showed that garlic could considerably reduce the microflora in synthetic and stock diet fed animals. A significant decrease in caecal flora of rats was observed in rats fed poor rice diet supplemented with red gram dhal or butter milk along with garlic for 5 days. Nakagawa *et al.* reported the death and/or retarded growth in rats fed 5 ml of raw garlic extract per kg body weight. After feeding raw garlic extract for a period of 4 weeks to albino rats there was a decrease in total *Streptococci*, *Coliforms*, *Lactobacilli*, aerobes, and anaerobes. The effect on aerobes and anaerobes was found equally pronounced. None of the above changes were observed after feeding boiled garlic extract, instead stimulated the growth of certain intestinal bacteria, such as *Streptococci* and *Coliforms*. When garlic extract containing 8 μ M of allicin was administered intragastrically to albino rats, a maximum of 0.4 μ M in the intestine and 2.4 μ M in caecum was detected after a period of 4 and 6 hr, respectively.[49] About 50–60% reduction in microflora was observed in intestine after 4 hours of administration, but no such change was observed in caecum even after 6 hours. However, a 5-fold decrease in microflora was seen in caecum only at the end of 8 hours. Shashikanth *et al.* pointed that the gradual decrease in allicin content while passing through the gut is perhaps due to reducing agents in the gut, natural instability of allicin, antagonism by food materials and absorption in the intestine. Generally, the aerobes were more susceptible to allicin concentration in the gut than the anaerobes.

Sharma *et al.* demonstrated *in vivo* antibacterial property of *Allium sativum* water extracts on gram negative and gram positive flora of the gastro-intestinal tract of chicks indicating its effectiveness in inhibiting both types of flora. Garlic



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extract also inhibited some bacterial flora, which were resistant to some of the antibiotics used. Kumar and Sharma further indicated the inhibitory effect of garlic on enterotoxigenic *Escherichia coli*. The anti-microbial activity of a range of garlic products including dried garlic powder produced by different methods, commercial garlic products, and garlic oil was determined against a range of selected bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Bacillus cereus*, and a mixed lactic culture consisting of *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Streptococcus thermophilus*). Generally fresh garlic produced the greatest inhibition followed by freeze-dried powder. The results showed that both drying temperature and time had major effects on retaining the active components responsible for the inhibition of microbial growth. Allicin showed antibacterial effect against *Helicobacter pylori*. *Helicobacter pylori* is considered to be one of the main reasons of gastric and duodenal ulcers as well as gastric cancer. *In vitro* and *in vivo* research proved that *Helicobacter pylori*, resistant to many antibiotics, was sensitive to garlic extract in relatively low concentrations. Sivam *et al.* investigated the antimicrobial activity of aqueous extract of garlic against *H. pylori* and found that minimum inhibitory concentration of the extract was 40 µg allicin/ml. Cellini *et al.* tested 16 clinical isolates of *H. pylori* and showed the concentration of garlic extract required to inhibit the bacterial growth was between 2 and 5 mg/ml. The inhibitory concentration of garlic reported in the above studies is achievable in the stomach by consuming a medium size clove of garlic. Interestingly, *H. Pylori* can survive and grow in the acidic environment of stomach by producing abundant amounts of enzyme urease, which hydrolyzes urea present in gastric juice. The ammonia, generated from this reaction, produced a local alkaline microenvironment, thereby protecting the bacterium against the hostile acidic conditions.[56] Juskiewicz *et al.* found allicin or garlic extract inhibited the urease attributed to the reaction of allicin with SH-group. Thiol reagents (L-cysteine, 2-mercaptoethanol, glutathione, dithiothreitol) strongly protect the enzyme from the loss of enzyme activity, while urea and boric acid showed weaker protection.

Bakri and Douglas studied the inhibitory effect of garlic extract with allicin on oral bacteria including 13 gram-positive and 6 gram-negative types of bacteria, and one type of fungi. The garlic extract 57.1% (w/v) containing 220 µg/ml allicin inhibited the growth and killed most of the organisms tested. In general, the minimal inhibitory concentrations for Gram-negative strains varied from 0.4–6.87 µg/ml which were lower than those for Gram-positive strains (13.8–55.0 µg/ml) tested. Time-kill curves for *Streptococcus mutans* and *Porphyromonas gingivalis* showed that killing of the later started almost immediately, whereas there was a delay before *S. mutans* was killed. The garlic extract also inhibited the trypsin-like and total protease activity of *P. gingivalis* by 92.7% and 94.88%, respectively.

Garlic extracts showed to be antifungal and antiviral and antiprotozoal activity. Cu²⁺ showed a dose dependent fungicidal activity against *Saccharomyces cerevisiae* cells, and its lethal effect was extremely enhanced in the presence of allicin. Allicin influenced the mode of cell surface localization or the related function of AHP1 as a defense against phospholipids peroxidation by the external action of Cu²⁺. There are several physiological processes in microorganisms which were affected by allicin, such as lipid biosynthesis RNA synthesis, lowering of lipids in mammals, and aggregation of platelets. Allicin was shown to be a specific inhibitor of acetyl-CoA synthetases from plants, yeast, and mammals. The bacterial acetyl-CoA-forming system, consisting of acetate kinase and phosphotransacetylase was inhibited. It was found to be specific to the enzymes of the fatty acid synthesis sequence. Allicin reacts very rapidly with free thiol groups, via thiol-disulphide exchange, therefore it is thought that its main mechanism of antimicrobial action is through interaction with thiol-containing enzymes, including cysteine proteases and alcohol dehydrogenases.

Anticancer Activity

Alliin itself did not show any inhibition of tumor cell growth indicating that the antiproliferative effects of garlic was due to breakdown products of alliin. Allicin plays a major role in the antiproliferative effect of water-soluble garlic preparations and this effect may be attributed to the ability of allicin to transiently deplete the intracellular glutathione (GSH) level. The extent of the decrease in GSH levels correlated well with the growth inhibitory activity of allicin. The antiproliferative effect of garlic is also clear. Allyl sulphur compounds are important antitumorigenic agents and diallyl disulfide reduced the size and the number of preneoplastic foci in rats liver induced by AFB1.



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The protection of garlic against cancer aroused from several mechanisms including the blockage of nitrosamines formation and bioactivation. These substances are much suspected to be carcinogens and influence the cancer risk in humans. Dion *et al.* reported that their formations were retarded by S-allylcysteine. Garlic also decreased the bioactivation of carcinogens. Cytochrome P4502E1 is a hepatic phase I enzyme implicated in the metabolism of nitrosamine, and others carcinogens, and its activity is modulated by organo-sulfur compounds such as diallyl disulfide. Organo-sulfur from garlic blocked the bioactivation and carcinogenicity of non-nitrosamines such as aflatoxin B1 (AFB1), a cancer agent for the liver. Phase I enzymes CYP1A1, 1A2, 2B1, and 3A4 involved in carcinogen bioactivation showed a modified activities after diets supplementation in rats with garlic or several sulfur compound such as diallyl disulfide. The induction of phase II enzyme detoxication such as glutathione-S-transferase (GST), quinone reductase (QR), and uridine diphosphoglucuronate glucuronosyltransferase (UGT) by OSC, such as diallyl disulfide was demonstrated and similar effects were observed with garlic consumption. Garlic intake can modify the risk of colon cancer to women because diallyl disulfide is an effective inhibitor of the growth of neoplastic CMT-13 cells and of N-acetyltransferase activity in human colon adenocarcinoma cell line. Furthermore, diallyl disulfide showed to be an effective inhibitor for the promotion phase of 9,10-dimethyl-1,2-benzanthracene induced skin tumors in the mouse.

Apoptosis, or programmed cell death, is a genetically controlled process, where by the cell actively participates in its own destruction in response to environmental or developmental cues. Apoptosis is morphologically characterized by membrane blebbing, cytoplasmic, nuclear and chromatin condensation, and DNA fragmentation. Allicin showed effects on DNA processing, RNA synthesis, signal transduction and apoptosis. Certain effects of allicin are mediated through nitric oxide formation. Oommen *et al.* studied the chemo-preventive action of allicin on the growth of cancer cells of murine and human origin by cell viability assay. They found that allicin inhibited the proliferation of cancer cells and induced apoptosis with typical features such as apoptotic bodies, DNA fragmentation, activation of caspases and poly (ADP-ribose) polymerase cleavage, thus these effects of allicin account partly for the anticarcinogenic properties of garlic. Park *et al.* showed that allicin induced apoptosis of the cells through caspase-independent apoptosis pathway, which was accompanied by the mitochondrial release of AIF and protein kinase A (PKA) appeared to play an important role in the caspase-independent apoptosis.

Shalinsky *et al.* studied the effects of allicin on other terminal prostaglandin biosynthetic enzymes, such as isomerase for PGE₂ and on cyclooxygenase activity. They found that allicin selectively inhibited the GSH dependent PGH₂ to PGE₂ isomerase in the adenocarcinoma cell line. Macrophages play an important role in host defenses against tumors by killing them and produce secretory products, which resulted in the protection against bacterial, virus infection and malignant cell growth. Allicin is an efficient immunomodulator of macrophage secretory and cellular activities; it showed a differential effect on production of cytokines and cytotoxic molecules. It altered cellular functions of macrophages to kill tumor cells and produce various molecules, such as NO, H₂O₂, TNF- α , IL-1 (interleukin-1) and IL-6 after treated with various doses (1, 10, 100 ng/ml) for 20 hours.

Allicin and its corresponding sulfite inhibited the proliferation and induced apoptosis of several human non-leukaemia malignant cells including breast, bladder, colorectal, hepatic, prostate cancer, lymphoma, and skin tumor cell lines. Ajoene (which has greater chemical stability) showed to inhibit proliferation and induced apoptosis of human leukaemia CD34-negative cells including HL-60, U937, HEL and OCIM-I. More significantly, ajoene showed to induce 30% apoptosis in myeloblasts from a chronic myeloid leukaemia patient in blastic crisis. Acute myeloid leukaemia, is a heterogeneous malignant disease, has a major impact on resistance to chemotherapy and relapse. Ajoene significantly enhanced the inhibitory effect of the two chemotherapeutic drugs, cytarabine and fludarabine on bcl-2-expression in KG1 cells. The two key antileukaemia biological actions of ajoene were the inhibition of proliferation and the induction of apoptosis. Studies showed the anti-proliferation activity of ajoene to be associated with a block in the G2/M phase of cell cycle in human myeloid leukaemia cells. The apoptosis inducing activity of ajoene is via the mitochondria dependent caspase cascade through a significant reduction of the anti-apoptotic bcl-2 that results in release of cytochrome c and the activation of caspase-3. Since acute myeloid leukaemia (AML) is a heterogeneous malignant disease in which disease progression at the level of CD34-positive cells has major impact



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on resistance to chemotherapy and release and the inability to undergo apoptosis is a crucial mechanism of multi-drug resistance in AML patients.

Antioxidant Activity

It was reported that soybean oil increased the stability of garlic flavor while cooking garlic with an oil-water mixture and garlic flavor acted as an inhibitor of soybean oil oxidation. Kim et al. studied the antioxidant activity of garlic homogenates blended with distilled water and different amounts of oil. Antioxidant activity of garlic aroma concentrate in the oil layer was found strong. Diallyl disulfide was found to be most stable compared to other two sulfur compounds disulfide and diallyl trisulfide when analyzed by HPLC. It was found that the effectiveness and strength of diallyl disulfide as an antioxidant could be increased by addition of α -tocopherol and L-ascorbyl palmitate in a lard system.

In vitro allicin acts by reacting with free thiol containing enzymes, serving as an efficient antioxidant by trapping of radicals. Allicin was found to scavenge hydroxyl radicals and to inhibit superoxide production by phorbol ester-activated human granulocytes. Allicin showed the modification of SH-dependent activities, an additional therapeutic property, and inhibitory effect on NO formation. Ajoene also showed the inhibitory effect of NO formation. The inflammatory environment in human atherosclerotic lesions resulted in an expression of the inducible form of nitric oxide synthase and subsequently in the formation of peroxynitrite. Peroxynitrite is a potent oxidant, which is formed when the synthesis of large amounts of NO coincides with superoxide production. Peroxynitrite is able to initiate LDL oxidation and to promote platelet aggregation and thus aggravates the atherogenic process. Peroxynitrite formation, however, occurred only if NO is produced in high enough concentrations to compete endogenous superoxide dismutase for superoxide. In D-galactosamine/lipopolysaccharide induced hepatitis rats, a significant increase of lipid peroxidation and decreased liver antioxidant enzyme levels were observed. Pretreatment with allicin prevented these alterations, thus provided hepatoprotective effect. Allicin showed antigenotoxic effect against methyl methanesulphonate induced genotoxic damage.

Reducing Cardiovascular Diseases

Cardiovascular diseases include a great number of factors such as high cholesterol, hypertension and increased platelet aggregation. The role of garlic in the reduction of cardiovascular diseases was historically proved but contradictory clinical studies emerged from different methodologies. The hypotensive effect is one of the earliest established properties of garlic preparations and was later confirmed in animals and in humans. The health benefit observed varies with the garlic products tested, i.e., garlic powder, aged garlic extract, garlic oil, and garlic oil macerate, with the state of cooked-, raw-, fried-, or boiled-garlic and with the percentage of active components. The contradictory results may be due to several factors and the lack of knowledge about the substances present is certainly one of them. Garlic reduced blood pressure, antioxidant, inhibited platelet aggregation, and reduced blood glucose. Allicin altered the lipid profile in hyperlipidemic rabbits. Synthetic preparation of allicin was found to lower blood pressure, insulin, and triglycerides levels in fructose-fed rats. Similar beneficial effect of allicin and enalapril (hypertensive drug) on blood pressure, insulin, and triglycerides were observed in fructose-induced hyperinsulinemic, hyperlipidemic and hypertensive rats, thus it reinforces the trend toward combining the nonpharmacologic approach with drug therapy. It also reduced the gain in weight of the groups fed fructose with allicin.

Atherosclerosis is one of the major risk factor in the development of hypertension and cardiovascular diseases. For centuries, garlic has been used for treating high blood pressure in China and Japan. Furthermore, garlic has been found to act as an antihypertensive agent and has been officially recognized for the treatment of hypertension by the Japanese Food and Drug Administration. In hypercholesterolemic rabbits garlic elicited a significant reduction of the hypercholesterolemia in a way comparable to gemfibrozil while serum triglycerides values remain unchanged after garlic treatment. Ali *et al.* studied the effect of garlic powder (0.6% allicin) on the serum cholesterol, triglyceride, glucose, protein, and systolic blood pressure in rats fed with a high cholesterol diet. They found significant reduction in serum cholesterol, triglycerides, and blood pressure level, thus providing that garlic has beneficial functions.



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Effect on two risk factors for atherosclerosis: hyperlipidemia and hypertension. However, no changes were observed in the serum glucose and protein in all of the rats. Allicin showed significant vasodilator activity in the pulmonary vascular bed of rat when tone is increased experimentally. However, diallyl disulfide and allyl mercaptan, metabolites of allicin, did not possess this vasodilator action. Although the mechanism by which allicin induces vasodilatation is uncertain, the results of the investigation of Kaye *et al.* suggest that this garlic derivative may be useful in the treatment of pulmonary hypertensive disorders, including primary pulmonary hypertension and chronic obstructive pulmonary disease. Allicin also showed vasodilator activity in the pulmonary vascular bed of cat and rat. When baseline tone in the pulmonary vascular bed of cat was raised, intralobar injections of allicin produced dose-related decrease in pulmonary arterial pressure without changing left arterial pressure. Allicin also decreased systemic arterial pressure in a dose-related manner. The pulmonary vasodilator responses to allicin were independent of the synthesis of endothelial derived relaxing factor or the activation of soluble guanylate cyclase. Other allicin analogs should be developed for clinical use because of allicin's vasodilator efficacy and apparent lack of toxicity. A modest dose (14 mg of allicin) was shown to cause decrease in diastolic blood pressure in severely hypertensive patients.

Inflammatory diseases such as atherosclerosis lead to coronary thrombosis. The platelet aggregation contributes to minimize the atherosclerosis, and it was reduced by allicin and two others thiosulphinates from onion. The antiplatelet activity in onion was significantly positively correlated with high sulfur level. Garlic powder also inhibited the platelet aggregation. Lawson *et al.* reported the variation of the antiplatelet activity of different garlic preparation, cloves or commercial products. These variations are due to the different nature of organo-sulfur compound present. Extracts of garlic was found to inhibit human platelet aggregation in vitro. The inhibition of human platelet aggregation did not involve an effect on cyclooxygenase thromboxane synthase activity, or on adenosine 3', 5'-cyclic monophosphate levels. Miron *et al.* synthesized allylmercaptocaptopril (CPSSA) by reacting captopril (antihypertensive drug, antitension converting enzyme inhibitor) with pure allicin and studied their effects on fructose induced hypertensive groups of rats. They found that CPSSA decreased blood pressure and reduced triglycerides, whereas it found no effect on the insulin serum level. This new stable compound combines the beneficial properties of captopril and allicin and is a potential candidate for antihypertensive drug therapy. In rats, treatment with low dose of CPSSA (5 mg/kg day) lowered SBP but did not improve any further measured parameter, while treatment with a higher dose (50 mg/kg day) significantly decreased blood pressure, triglycerides, and homocysteine concentrations.

Garlic decreases certain diseases caused by immune dysfunction. Thus, aged garlic extract presents an immunomodulation effect. The immunomodulatory effect of garlic or garlic constituents shows a modulation of cytokine production as mediator of inflammation. The nuclear factor-KB (NF-KB) is a central transcription factor and has a central role in the expression of genes that control immune response. NF-KB is strongly involved in the activation and regulation of key molecules associated with inflammatory diseases and cancer. It increases the expression of the genes of some cytokines. The inhibition of NF-KB by garlic products was indirectly controlled by a modulation of pro- and anti-inflammatory cytokines. Epithelial cells have an important role in intestinal inflammation and allicin showed anti-inflammatory properties. Allicin inhibited spontaneous and TNF- α induced secretion of pro-inflammatory cytokines and chemokines from intestinal epithelial cells. Josling found that an allicin containing supplement can prevent attack by common cold virus. One hundred forty six volunteers were randomized to receive a placebo or an allicin-containing garlic supplement, one capsule daily, over a 12-week period. The active treatment group had significantly fewer colds than the placebo group. The placebo group, in contrast, recorded significantly more days challenged virally and a significantly longer duration of symptoms.

Effect on Protein and Fat Profile

Augusti and Mathew showed decreased serum protein levels in rats administered raw garlic extract. Raw garlic extract showed decreased total serum proteins as well as serum globulin when fed albino rats for 4 weeks, whereas boiled garlic extracts did not show any effects. The long term feeding (4 weeks) of raw and boiled garlic extract to albino rats resulted weight loss. Extracts of garlic were also reported to possess hypoglycemic and



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hypocholesteremic properties. Allicin also reduced serum cholesterol and triglyceride levels, as well as atherosclerotic plaque formation, prevented platelet aggregation and decreased blood pressure.

Bioavailability Of Allicin

Figure 2lf allicin or an allicin containing substance is ingested, it would be exposed to the acidic conditions of the stomach and then to the neutral conditions of the intestines. For these reasons, Freeman and Koderastudied the relative stability of allicin in blood, different solvents [ethyl acetate, methanol, water (pH 1.2 and 7.5)], simulated gastric fluid (SGF, pH 1.2), and simulated intestinal fluid (SIF, pH 7.5). At 37°C, allicin decomposed rapidly in methanol and ethyl acetate, however, it was more stable in protic polar methanol than in aprotic polar ethyl acetate. Approximately 90% of allicin remained after incubation at 37°C for 5 hours in water at pH 1.2 and 7.5. After 1 day at pH 1.2, about 80% of allicin remained, and at pH 7.5, about 62% of allicin remained. Interestingly, allicin did not appear to generate its normal transformation products at these pH values since an increase in the concentration of diallyl disulfide, ajoene, and dithiin was not observed. Therefore, gastric or intestinal pH may not be a significant factor affecting allicin availability or decomposition in the body during the digestive period. There was likely to inactivation of allinase, the enzyme that catalyzes the conversion of alliin to allicin at \leq pH 3.

Food substances are normally present in the gastrointestinal tract, thus interaction of allicin with cow's milk was also examined by Freeman and Koder. After exposure of milk for 1 hour, almost all allicin was recovered. Only traces of allicin could be detected after it was incubated in blood for 5 minutes. Allicin was more reactive to the blood cell fraction than to the plasma fraction. No allicin was detected after 3 minutes when it was incubated in blood cell fraction, while in the plasma fraction, the concentration of allicin decreased gradually and the half-life of allicin was estimated to be about 50 minutes. A rapid change in color of red blood cells to a dark color was observed after addition of allicin, which may be due to the rapid oxidization of iron in hemoglobin. Concurrent with the disappearance of allicin, the presence of diallyl disulfide was observed in the mixture of allicin and blood. Diallyl disulfide showed stability in blood. The concentration of diallyl disulfide remained unchanged after 1 hour.

Similar results were also observed in case of liver cell. After incubation for 3 minutes in liver homogenate, a decrease of 90% initial allicin was observed and the decrease of allicin was 99% after 6 minutes. On the other hand, it was observed that the metabolism of diallyl disulfide in mice reached a maximal concentration in the liver after 90 minutes of ip injection. Although allicin disappeared rapidly in the body after absorption, diallyl disulfide may be absorbed and delivered to organs. Egen-Schwind et al.[146] investigated pharmacokinetics of vinylthiins (transformation products of allicin) after oral administration of 27 mg 1,3-vinylthiin and 9 mg of 1,2-vinylthiin to rats. In serum, kidney, and fat tissue, both vinylthiins were detected over a period of 24 hours, whereas in liver only 1,3-vinylthiin was found. 1,3-vinylthiin seems to be less lipophilic and was rapidly eliminated from serum, kidney, and fat tissue, whereas 1,2-vinylthiin is more lipophilic and showed a tendency to accumulate in fat tissue. Allicin, the precursor of vinylthiins, is metabolized more rapidly in liver homogenate than the vinylthiins. Allicin is assumed to be the active component of garlic *in vivo*, but not *in vitro*. Allicin cannot be detected in the blood or urine after the ingestion of raw garlic or pure allicin within 1 to 24 hours after ingestion of 25 g raw garlic (~90 mg allicin).

It was reported that allicin can be produced by human liver microsomes from diallyl disulfide. These suggested that despite its rapid disappearance from the bloodstream, allicin can be reformed in the process of interconversion of its metabolites and as a result, act intracellularly. Agarwal suggested that metabolites of allicin, rather than the agent itself, are responsible for this wide range of beneficial health effects. Several such metabolites are S-allylmercaptocysteine, diallyl sulfide, diallyl disulfide, diallyl trisulfide, ajoene, and S-allylcysteine. The pharmacokinetic studies of S-allyl cysteine demonstrated rapid absorption and almost 100% bioavailability after oral administration. In addition, since both safety and effectiveness of S-allyl cysteine have been reported, this compound appeared to play an important role in garlic's medicinal effects.



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Freeman and Kodera also studied the allicin content and allicin-producing potential of commercial garlic preparations. No allicin was observed in all commercial products. Although no product on the market contains a detectable amount of allicin (< 1 ppm) some garlic powder products claim to be able to generate certain amount of allicin, so-called "allicin potential" or "allicin yield." Variable allicin potentials from 0.64–4.64 g/kg were observed in different brands of products, as well as among different lots of the same brand. After 1 hour at 37°C under SIF conditions, about 62% (2.86 g/kg) of the amount of allicin produced with water was observed, and after 1 hour under SGF condition, only 4% (0.19 g/kg) could be found. This low level of allicin production under SGF condition may be due to hydrolysis of alliinase by acid and enzyme pepsin. Under simulated digestive conditions (sequential combination of SGF and SIF), only about 1% of allicin was observed. The small amount of allicin observed is not due to its decomposition during the incubation period because it was shown to be stable at gastric and intestinal pH for 2 to 3 hours. At pH 6.8 commercial products released very fast highly soluble compounds, amino acids, and dipeptides. Within less than 5 minutes nearly 100% of these compounds were released and allicin was formed. Alliin was very fast released from the powder and immediately metabolized to allicin (100%) within the pH range of 3.5–8.4. At pH 2, alliin was completely released within a minutes without further degradation to allicin. Higher pH values seem to stabilize the allicin, whereas lower pH values accelerated its degradation. However, sulfides and dithiines could not be detected in the release media within 30 minutes.

Arnault *et al.* analyzed alliin, allicin, and storage stability of 11 European products and found the majority of the products present a very different composition from what was claimed by the companies. Only one product possessed the legal status of a drug. For that product, values were found as claimed on the package. It was known that alliin and allicin content decreased in correlation to their storage time. This can be explained by the residual activity of the alliinase. Their results clearly showed the instability of products when stored under ambient conditions for 18 months. The best products remained activity of 70–90%, whereas most of the products showed below 50% value. The BET monolayer value of freeze-dried garlic, which is considered as most stability of dried products during storage, was found 5.7 g/100 g sample at 20°C. However, Arnault *et al.* recommended residual water of less than 3 g/100 g sample (i.e., 3%), and a complete water vapor tight packaging material is needed for keeping their functionality. In addition, high packing of the tablet and organic film coating could also improve the stability up to 24 months.

The certitude that garlic provides beneficial effects on health lead the industry to market some garlic market products for human consumption in the last decades. There are 2 major types of market garlic products on the European pharmaceutical and food supplement market and a third one on the American market: (a) the oil macerates of fresh garlic, formulated commonly in soft gelatin capsules; (b) the dry powder products of fresh garlic formulated either as sugar or film coated tablets; and (c) dry powder products of aged garlic formulated either as sugar or film coated tablets. It is the task of the pharmaceutical science to formulate each active principle individually in such a way that the active principle is fully available under the physiological condition of the application. The appearances and characteristics of medicinal products are significantly influenced and controlled by the formulation technology (galenic) and the analytical technology. Both determine the ability to manufacture quality/stability, efficacy, and consumer acceptance of the final medicinal product.

Although not all of the active ingredients are known, ample research suggested that several biological components likely contribute to the observed beneficial effects of garlic. Other active organosulfur compounds found in garlic derived from allicin are diallyl disulfide, methyl allyl trisulfide, and ajoene. Allylic and allenic thiosulfinates are of biological interest due to their similarity to allicin. Braverman *et al.* developed a convenient preparation method of mixed allyl allenic and bis-allylic thiosulfinates. Being a chemically reactive compound and hence rather unstable, allicin rapidly transforms into a number of derivatives found in aged garlic and various crushed garlic preparations. For garlic extract, whole or sliced garlic cloves are soaked in an extracting solution (e.g., purified water and diluted alcohol) for varying amount of time. After separation of the solution the extract is generally concentrated and used. Powdered forms of the extract are also available. The extract, especially aged, contains mainly the water soluble constituents in garlic and a small amount of oil-soluble compounds. The extract is characterized by water soluble



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sulfur containing compounds, including S-allyl cysteine (SAC) and S-allyl mercaptocysteine. S-allyl cysteine can be used for standardization because it is bioavailable and can be detected in the plasma, liver and kidney after oral intake. The bioavailability of SAC was 103% in mice, 98.2% in rats, and 87.2% in dogs. S-acetyl SAC was identified as a metabolite of SAC in the urine of dogs and humans. Other metabolites of garlic constituents, such as N-acetyl-S-(2-carboxypropyl)-cysteine, N-acetyl-cystein and hexahydrohippuric acid, were detected in human urine after ingestion of garlic. This suggested that SAC could be transformed by N-acetyltransferase. SAC and its metabolite(s) are possible compliance markers for clinical studies involving garlic.

Allyl mercaptan and diallyl disulfide were the first compounds identified as components that produce the strong odor detectable after ingestion of garlic. Allicin, perfused into isolated rat livers, showed a remarkable first-pass effect and was metabolized as diallyl disulfide and allyl mercaptan, whereas ajoenes and vinyl-dithiines were recovered in the effluent. The inhibition ability of platelet aggregation on the basis of ajoene and dithiin content may be inadequate because other compounds may act synergistically or independently to bring the effect. For example, aged garlic, which contains neither ajoene nor dithiin, significantly reduced platelet aggregation and adhesion in two double-blind, placebo-controlled clinical studies. As a result, Amagase et al. pointed that the concentration of various compounds and their effects in vitro may not determine effectiveness. Preclinical or preferably clinical studies are required to confirm or refute the effectiveness of a product in question, whatever its chemical composition. Aged garlic extract is aged for up to 20 months. During the aging process, the odorous, harsh, and irritating compounds of garlic are converted naturally into stable and safe sulfur compounds. Further the safety of aged garlic has been confirmed by various toxicological studies.

Allicin rapidly disappears from the circulation after intravenous injection suggesting that it is transformed into secondary products. A variety of biological effects of allicin are attributed both to its SH-modifying and its antioxidant activity, which also found in model systems. Allicin can easily permeate cell membranes of phospholipids bilayers, carry out its activity intracellularly and interacted with SH groups. Fast diffusion and permeation of allicin across human red blood cell membranes was also demonstrated. Allicin did not induce leakage, fusion or aggregation of membrane. The high permeability of allicin through membranes may greatly enhance the intracellular interaction with thiols. It is important to find which by-product of allicin is the active species that modulates extra- and intra-cellularly processes. It was found S-allylmercaptocysteine (CSSA) as the product of the reaction of cysteine with allicin. It is one of the active ingredients of aged garlic extract. The antiproliferative activity of CSSA on different cell lines was demonstrated, whereas S-allylcysteine showed no such effect. CSSA also revealed antioxidant activity and decreasing ocular pressure activities. Allicin elevated intracellular Ca^{2+} concentration in rabbit nonpigmented ciliary epithelial cells by allicin. The reduced glutathione most abundant non-protein thiol in mammalian systems has potential to interact with allicin. Rabinkov et al. studied the formation of S-allylmercaptogluthathione (GSSA) upon interaction of allicin with glutathione. The potential of GSSA to serve as vehicles for the prolonged action of allicin was demonstrated by its SH-modifying properties and high antioxidant activity. It showed reduction of OH radical reactions, and lowered the production of lipid peroxides. Thus it has a role in the biological activity of allicin and its derivatives. Though individual compounds, such as S-allyl cysteine showed activity, however Koch emphasized that the activity of various sulfur compounds could not alone be responsible for the benefits of garlic and fixation on a single group of components can lead to mistakes and wrong conclusions. Overall, the active principles in garlic was not fully characterized and it is assumed that the bioavailability of these sulfur containing compounds will play an important role in determining the biological response to various garlic preparations.

Safety And Toxicity

The effectiveness of garlic may be prevention rather than therapy, thus it may need long-term supplementation. Long-term use of supplements raises issues about toxicity. Although garlic has been used safely in cooking as a popular condiment or flavoring and used traditionally for medicinal purposes, it is commonly known that excessive consumption of garlic can cause problems. Recently Amagase *et al.* reviewed the safety of garlic preparation. Garlic produces odor on breath and skin and occasional allergic reactions. Other adverse effects associated with garlic are:



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stomach disorders and diarrhea, decrease of serum protein and calcium, anemia, bronchial asthma, contact dermatitis, inhibition of spermatogenesis [and damage of intestinal lining and the stomach. The following results were reported by Imada: (i) allicin is one of the major irritants in raw garlic; (ii) oil soluble sulfur compounds are more toxic than water soluble compounds; and (iii) when garlic is extracted in water for a certain period, its toxicity is greatly reduced. A number of toxicological and clinical studies of aged garlic showed no adverse effects. The safety of aged garlic was established in the following studies: acute and substance toxicity tests, chronic toxicity test, mutagenicity tests, general toxicity tests, teratogenicity tests, toxicity test conducted by the FDA, and clinical studies conducted on > 1000 subjects.

Effect Of Garlic On Lipid Metabolism

There are four types of lipoproteins such as chylomicrons, high-density lipoproteins (HDL), low density lipoproteins (LDL) and triglycerides. A clinical investigation was done on the blood lipid profiles of 23 volunteer humans subjected with high blood cholesterol (>5.98 mmol/L). The subjects were treated with garlic extract supplementation for 4 months and the change in their blood lipid profiles were analyzed before and after treatment. Thus it was found out that their serum total cholesterol, low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL) cholesterol and triglyceride levels were significantly lower after the treatment of extract. But an increased level of high-density lipoprotein cholesterol (HDL) level was observed after the extract use. A study using mice has proved that the lyophilized garlic supplementation at 2% and 5% levels on a diet containing 1% cholesterol or 15% lard, decreases the low density lipoprotein (LDL) while increasing the high density lipoprotein cholesterol (HDL) and resulting in a similar effect on lipid metabolism as at 2% and 5% levels. It was further identified that the liver weight, total liver lipid and cholesterol levels were increased in rats fed with the cholesterol diet but a supplementation of garlic decreased those parameters by about 30%. Aged garlic extract reduces cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl-CoA reductase and is additive with statins in its action. Thus, garlic inhibits enzymes involved in lipid synthesis, decrease platelet aggregation, prevent lipid peroxidation of oxidized erythrocytes and LDL, increase antioxidant status, and inhibit angiotension-converting enzyme. The effect of allicin in preventing atherosclerosis and hypercholesterolemia was investigated using male ICR mice. They were given oral administration of allicin with doses of 5, 10, or 20 mg/kg body weight, daily for 12 weeks. A decrease in daily food consumption was also noted in most of the treated animals. Meanwhile, allicin showed a favorable effect in reducing blood cholesterol, triglycerides, and glucose levels and caused a significant decrease in lowering the hepatic cholesterol storage.

Effect Of Garlic On Cardiovascular Diseases

Cardiovascular disease has been a major non-communicable disease in recent history. Allicin which is the main bioactive compound in garlic has the beneficial effects to the cardiovascular system. From the pharmacokinetic studies, allicin is known to be hydrophobic and can be readily absorbed through the cell membrane without inducing any damage to the phospholipid bilayer and then rapidly metabolized to exert pharmacological effects that are important to the cardiovascular system. It was found to provide cardio-protective effects by inducing vasorelaxation and alleviating various pathological conditions of CVD, including cardiac hypertrophy, angiogenesis, platelet aggregation, hyperlipidemia and hyperglycemia. Cardiovascular diseases are resulted from high cholesterol, high homocysteine, hypertension and inflammation. This will also increase the risk of dementia and result in Alzheimer disease. Oxidative damage is a major factor in cardiovascular disease and dementia, diseases whose risk increases with age. Garlic, extracted and aged to form antioxidant-rich aged garlic extract (AGE or Kyolic), may help reduce the risk of these diseases. A study investigated that allicin, the principle active ingredient in garlic has the antioxidant properties which help to prevent the oxidative stress induced cell injury or cell death. The antioxidant capacities of allicin were measured by using 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay and hydrogen peroxide (H₂O₂)-induced cell damage on H₉C₂ cardiomyoblasts. It is believed that the protective effect of allicin on H₉C₂ cells could inhibit intracellular ROS production instead of scavenging extracellular H₂O₂ or free radicals. For the observed protective effect on H₉C₂ cells, allicin might also be effective in reducing free radical-induced myocardial cell death in ischemic condition.



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Effect of garlic on blood glucose level is an area which is still being studied by scientists. The beneficial effect of garlic on diabetes mellitus is mainly attributed to the presence of volatile sulfur compounds, such as alliin, allicin, diallyl disulfide, diallyl trisulfide, diallyl sulfide, S-allyl cysteine, ajoene and allyl mercaptan. Garlic extracts have been reported to be effective in reducing insulin resistance. Aged garlic extract (AGE) decreases homocysteine, lowers blood pressure, and increases microcirculation, which is important in diabetes. Few studies have shown its positive effect on reducing the blood glucose levels of diabetes mellitus patients. A study was conducted on fasting blood glucose levels of diabetes patients for 12 weeks provided with a metformin and a garlic treatment. It was found out that the reduction in blood glucose was more substantial in the metformin with garlic treatment than the metformin treatment alone. Diabetes type 2 patients with hyperlipidemia had shown a successful decrease in LDL cholesterol levels and increase in HDL cholesterol levels in females when treated with "gsarsin", a derivative of garlic present in Arabian countries.

Anti-Carcinogenic Effect Of Garlic

Garlic is considered to be a food with great anti carcinogenic properties. Garlic acts as an anti-carcinogen through different mechanisms including the scavenging of radicals, increasing glutathione levels, increasing the activities of enzymes such as glutathione S-transferase, catalase, inhibition of cytochrome p4502E1, DNA repair mechanisms, prevention of chromosomal damage etc. Anti-proliferative action of garlic compounds to retard the tumor growth has been studied using the epidemiologic studies and animal experiments. Mice transplanted with mammary tumor cells were given supplements of protein extractions from garlic bulb and a significant decrease in the size of mouse mammary tumor was observed. Different studies show that garlic rich in allicin compound inhibits proliferation of human mammary endometrial and colon cancer cells. Linoleic acid is considered to be an enhancer of breast cancer risk. It was examined that the garlic compound increases the effect of eicosapentaenoic acid, a breast cancer suppressor while decreases the effect of linoleic acid. Organosulfur compounds in garlic such as allyl-sulfides are responsible to decrease the risk of hormone responsive cancers. It has been proposed that the sulfhydryl-group hydrophobic portion of proteins, as well as estrogen receptors with cysteine residues in hormone-binding, could be a target of inhibition from organosulfur compounds of garlic, therefore prevents the hormone responsive cancers. Several experiments were done on the ability of garlic and its organic allyl sulfur components inhibit the cancer process. It was revealed that the water soluble S- allyl cysteine was effective in reducing the risk of chemically induced tumors in animal models but had no effect on established tumors. However, oil-soluble compounds such as diallyl disulfide are effective in reducing the proliferation of neoplasms.

CONCLUSION

The main active chemical constituent in garlic is allicin. Allicin together with other organosulfur compounds have been tested positively for their antioxidant property, anti-carcinogenic property and antimicrobial property as well as their successful prevention of cardiovascular diseases, diabetes mellitus, high blood cholesterol (LDL, triglycerides, VLDL) levels.

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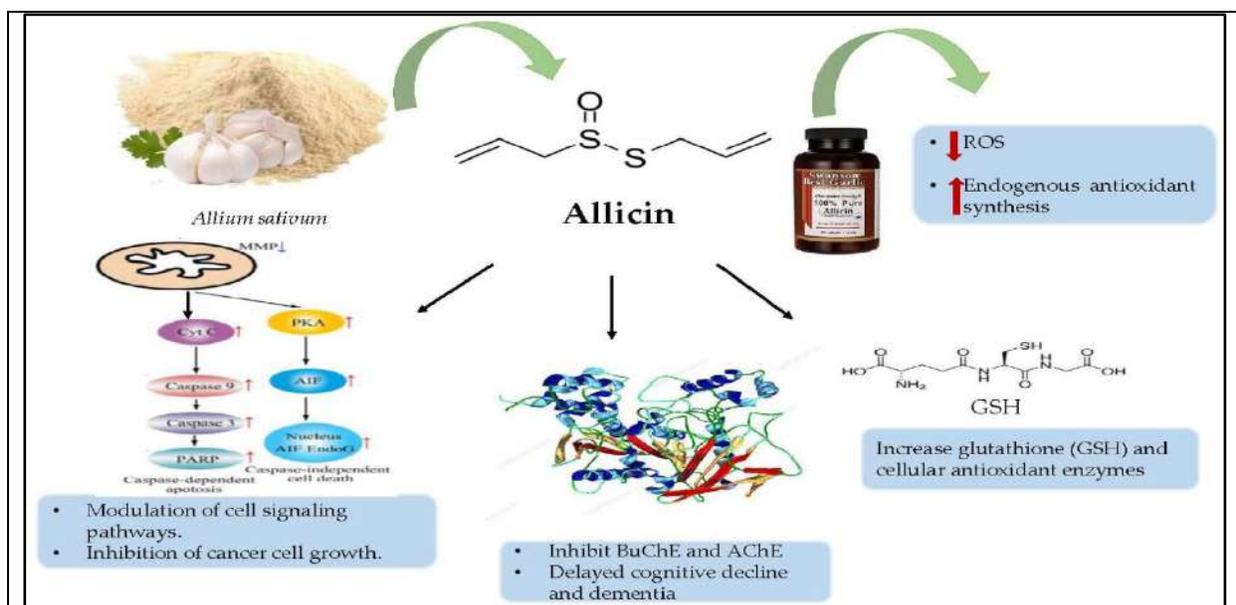
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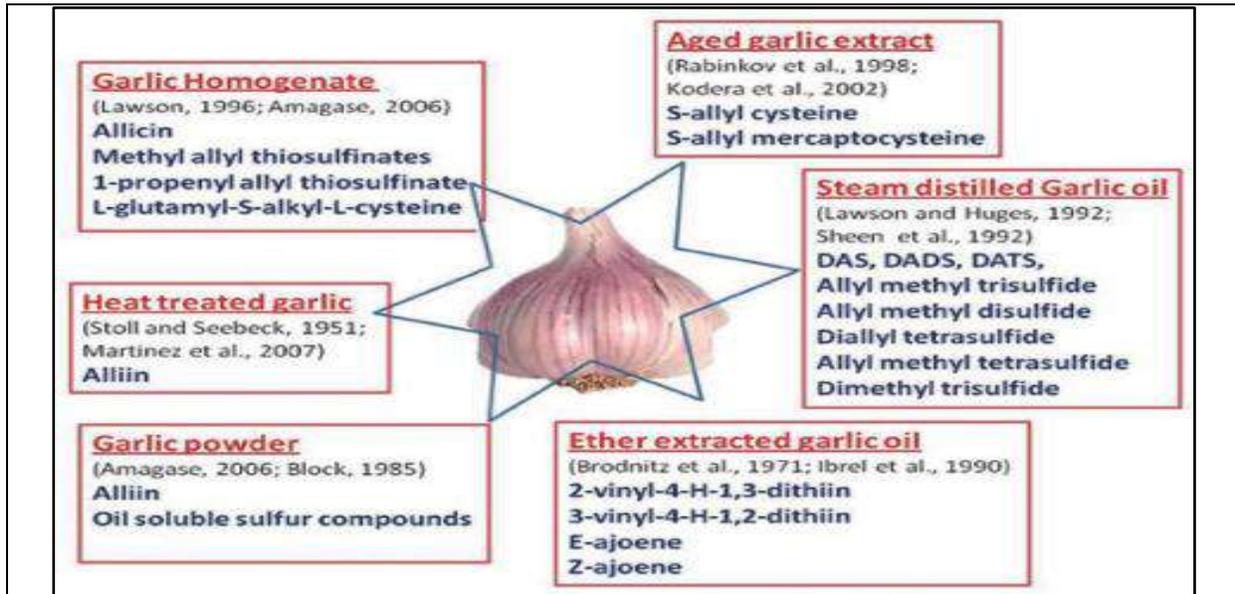


Figure 1 Improving Immune Function

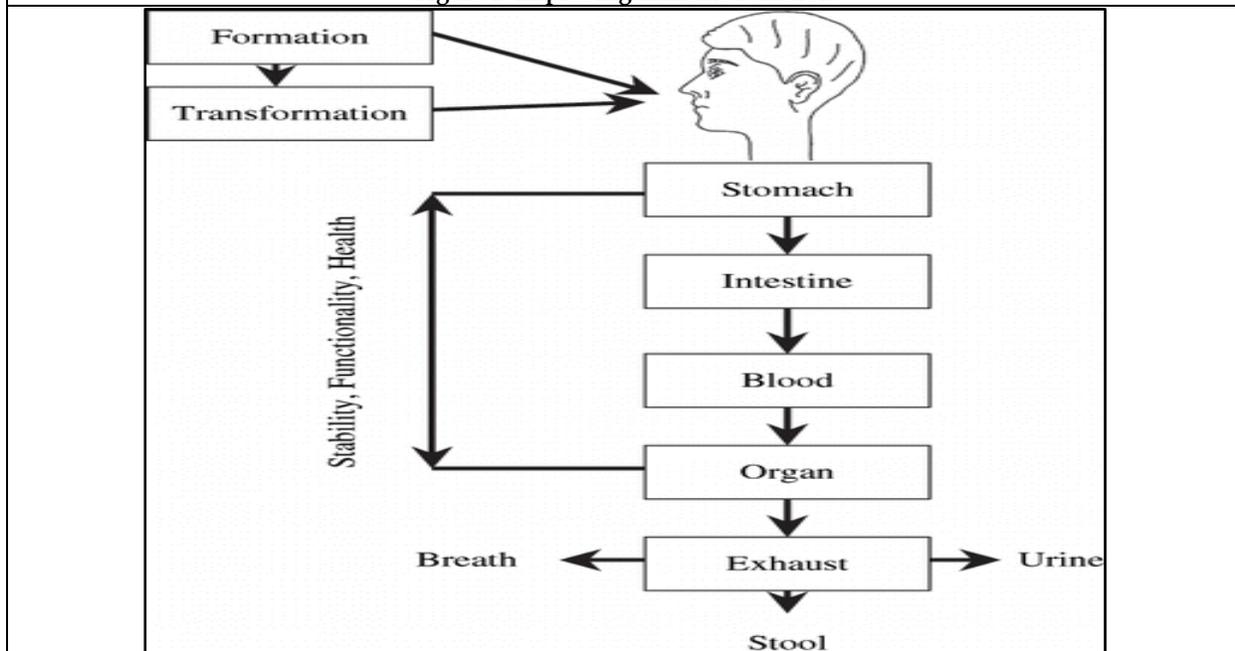


Figure 2 Cycle of active components in body.





Benefits of Tulshi a Review

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ABSTRACT

Tulsi (*Ocimum sanctum* L.) Plants are being used as medication more and more over the world since they have little or no adverse effects. In Hindu religious beliefs, Tulsi, often known as "Queen of Herbs," is a significant epitome of God. Tulsi has a complicated makeup and was traditionally used by Hindus, but it is currently utilised by a large number of people due to its tremendous healing properties. The holy basil or tulsi cure many more disease and cancer also added in the list. Scientists may not discovery or research any medicinal value of tulsi recovery cancer but the phytochemical constituents are required to almost all cure the dangerous disease like lung cancer, liver cancer, breast cancer and stomach cancer. The largest cause of worldwide disease and mortality is lifestyle-related chronic illnesses, many of which may be treated with Ayurvedic treatment, which emphasises good lifestyle choices and frequent ingestion of soothing herbs. Tulsi is one of the best herbs used in Ayurveda and has been proven to have positive benefits. Tulsi can manage physical, physiological, metabolic, and mental stress through an unique mix of pharmacological activities, according to a large body of research.

INTRODUCTION

Taxonomy of tulsi
Kingdom- Plantae
Order - Lamiales





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Family -Lamiaceae

Genus- Ocimum

Species - sanctum

Holy basil (*Ocimum sanctum* L.), often known as tulsi, is native to the Indian mainland and is revered for its healing properties in Ayurvedic and Siddha medicine. Numerous in vitro, animal, and human studies show that tulsi has a variety of healing properties, including There are apoptogenic, antimicrobial, relaxing, cardioprotective, and immunomodulatory effects, but no studies have been done to yet. There are no precise assessments of human research on the clinical efficacy and safety of tulsi. Basil has antimicrobial activity throughout a broad spectrum, including activity against a variety of human and animal diseases. It's also been suggested as a hand sanitizer, mouthwash, water purifier, wound healer and Food preservation.

Tulsi is used in a variety of ayurvedic medications, which empowers Indian farmers by selling herbs and creates a disease-free society by employing tulsi as medicine. In Hindu religious beliefs, "Queen of herbs" is a significant epitome of God. Tulsi has a complicated constitution and was traditionally used by Hindus, but now that its tremendous healing properties have been recognised, it is being utilised by a growing number of people. Tulsi has been shown to prevent organs and tissues from physicochemical stress caused by the industrial toxins and contaminants, as well as physical stress caused by long - term physical effort, ischemia, physical constraint, and exposure to coldness and extreme noise. The use of the plants as medicine is slowly and gradually increasing across the world as they have minor or no side effect (Jordan et al., 2010). In Nepal, more than 2300 plant species are used as traditional medicine (Rokaya et al., 2013). Tulsi (*Ocimum sanctum* Linn.) is queen of herb. Because of the many therapeutic benefits it provides, the Tulsi plant is extremely important to humanity.

Tulsi leaves are often used in Ayurvedic prescription preparation. It has been shown to extend one's life span. The extricates obtained from the herb are commonly used to treat a variety of ailments, such as the flu. A common cold, irritability, gastrointestinal illness, coronary artery disease, headaches, stomach problems, kidney stones, heart problems, and a few more. In China tulsi was used first the natural herbal preparations as medicines have therapeutic uses. In the old literatures tulsi has described 4000-5000 B.C (Subhash et al., 2016). Tulsi often known as the "Goddess of Herbs" and the "Matchless One," is one of the purest and most revered herbs used for most medicinal and healing purposes. The herb is slowly but steadily making its way into Ayurvedic illness therapy. Tulsi leaves are widely used for their recuperative properties. It is a tonic for the visual receptors, and as a result, it aids with memory enhancement. The leaves of Tulsi plant are amazingly valuable during the blustery season, when infections like jungle fever and dengue defraud the nation. Heat up the delicate leaves of Tulsi tea and offer it to the patient.

History of Tulsi

Holy basil is a fragrant shrub. It is supposed to have originated in central and northern India and currently flourishes all over Asia. The tulsi plant, also known as basil, which means "matchless one" in Hindu, is a perennial with a mild lemon aroma and purple-pink blossoms. Rama tulsi is white and green and Shyamtulsi, is deep pinkish-purple are the two most prevalent varieties of tulsi. Krishna tulsi and Vanatulsi are two further varieties of tulsi. For culinary purposes, at than 40 distinct basil types are produced across the world. It is the genus that includes all forms of basil. Tulsi is an upright, several more branched subshrub with hairy stems that grows 30–60 cm (12–24 in) tall. Green or purple leaves are simple, petioled, and have an oblong cutting up to 5 cm (2 in) long with a little toothed border. They are intensely fragrant and have a decussate product is suitable. On elongated racemes, purple blooms are arranged in tight whorls. Various biodiversity isolates of basil from the Indian subcontinent now have DNA barcodes. A team of researchers used chloroplast genome sequences to perform a large-scale phylogeographical investigation of this species and discovered that it is native to North-Central India. Tulsi is largely used herb plant to prepare medicine all over the world and the value of such plant is too expensive.





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Tulsi

Tulsi is one of the 60 species of genus *Ocimum*, which we Indians have been praising and cultivating in our homes for centuries. In Sanskrit, the culture in which our Vedas are written, it is called as Tulasi. It has an aromatic smell and used for health benefit.

Nutritional Composition of Tulsi

Chemical Composition

Various experimental investigations have demonstrated tulsi's capacity to protect against by the harmful effects of different toxicants. These studies attest to the ability of tulsi to prevent liver, kidney and brain injury by protecting against the genetic, immune and cellular damage caused by pesticides, pharmaceuticals and industrial chemicals (Marc et al., 2014). Tulsi, often known as Holy Basil, is a medicinal herb native to India. Tulsi is utilised all around the world for health problems, antioxidants, and other purposes due to its therapeutic properties. Its chemical components include the following: Eugenol, Carvacrol, Cineole, Limatrol, Sterols, Vitamins A and C, Iron, and Zinc. Oleic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, and beta caryophyllene are some of the phytochemical elements of tulsi (about 8 percent). Tulsi essential oil is mostly composed of eugenol (70%), elemene (11.0%), caryophyllene (8%), and germacrene (2%), with the remainder consisting of different trace chemicals, predominantly terpenes.

Phytochemical Composition

Phytochemical analysis was performed on the test sample to determine the presence of phytoconstituents elements. Alkaloids and tannins, Cardiac, saponins and flavonoids, and terpenoids were all tested using phytochemicals. Main chemical composition are oleic acid, Ursolic acid, Beta caryophyllene, Eugenol, carvacrol, linalool and Rosmarinic acid.

Bioactive compound of Tulsi

Mainly 4 types of bioactive compound are present in tulsi such as garlic acid, cinnamic acid, catechol and p-coumaric acid are found in methanol extract. Methanol was determined to be the best extraction phase for recovering bioactive components with higher antioxidant capacity among the solutions employed.

Tulsi as an Anti-Oxidant

The antioxidant capacity of white and red holy basil leaves (*Ocimum sanctum* Linn., white and red varieties) from fresh markets in Chiang Mai was estimated by three different methods; ferric reducing antioxidant power (FRAP) assay, improved ABTS radical cation decolorization assay, and DPPH free radical scavenging activity; together with their total phenolic contents. Water and 95% ethanol ratios of extraction solvents were also studied, and it was found that 57 and 76% ethanol solvents were more suitable in this study. All three antioxidant capacity assays showed that red holy basil was higher in anti oxidant capacity than white holy basil ($p < 0.05$). ABTS values were higher than FRAP and DPPH values, respectively, because of their different mechanisms, but their significant correlation ($p < 0.01$) expressed their similar trends. In addition, correlation between results of all antioxidant capacity and total phenolic content was found ($p < 0.01$). ABTS values were highly correlated into the results of total phenolic content than were FRAP and DPPH values (Wangcharoen and Morasuk et al., 2007). Elements in holy water have antioxidant action. One of the main reasons for its pharmacological properties is tulsi. Holy basil extracts include phenolic chemicals such as eugenol, cirsilineol, isothymusin, isothymonin, and rosmarinic acid. Tulsi demonstrated protective effects against copper sulphate toxicity in rats. Copper sulphate caused the development of free hydroxyl radicals and subsequent increased lipid peroxidation and led to rises in levels of antioxidant enzymes such as superoxide dismutase and catalase. Administration of tulsi restored the various parameters to near normal values (Subash and Pradeep et al., 2016).

Anti-Bacterial properties

The antibacterial substances found in this unique plant include carvacrol and terpene. B-caryophyllene, a sesquiterpene, serves the same effect. This naturally occurring ingredient in Tulsi is an FDA-approved food





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ingredient. It aids in the protection of the body against bacteria that cause sickness. Anti-inflammatory - In addition to being an antioxidants, rosmarinic acid is a strong part of the antioxidant.

Use in Antiarthritic

Tulsi fixed oil's anti - arthritic efficacy was tested in rats with formaldehyde-induced arthritic. The diameter of the injured paw was greatly decreased by the fixed oil. The arthritic symptoms in rats improved significantly after 10 days of intraperitoneal treatment of the fixed oil.

Anti-cancer activity

The anticancer activity of tulsi has been proved and cited by several investigators [24]. The alcoholic extract of leaves of tulsi has a modulatory influence on carcinogen metabolizing enzymes such as cytochrome P 450, cytochrome b5, aryl hydrocarbon hydroxylase and glutathione transferase (GST), which are important in detoxification of carcinogens and mutagens. The anticancer activity of tulsi has been reported against human fibro-sarcoma cells culture, wherein AIE of this drug induced cytotoxicity at 50 mg/ml and above. Morphologically, the cells showed shrunken cytoplasm and condensed nuclei. The DNA was found to be fragmented on observant on in agar tulsi gel electrophoresis. It significantly decreased the incidence of benzo (a) pyrine induced neoplasia of fore stomach of mice and 3'-methyl-4-dimethylaminoazobenzene induced hepatomas in rats. The AIE of the leaves of tulsi was shown to have an inhibitory effect on chemically induced skin papillomas in mice (Subhash and Pradeep et al.,2016).

Cancer

This plant has been shown to have disease-fighting effects. As a result, nearly every Indian home has a Basil plant in their backyard, where the plant's leaves may be utilised to treat and cure ailments such as cancer. Scientists from all around the world have been studying this plant in recent years to learn enough about its anti-cancer effects. The specific effect of this herb in reducing malignant activity within the body has been successfully understood via investigations. The several modes of action of Tulsi in the treatment of cancer. The capacity of Holy Basil to block an enzyme called COX-2, which induces an inflammatory response from the body and causes carcinogenic activity, is its primary function in cancer prevention. Holy basil lowers pain and inflammation by inhibiting the function of this enzyme.

Several animal and experimental studies have shown tulsi can repair and replace cells that have been harmed by radiation and oxidation, suggesting its enormous potential for eradicating pre-cancerous lesions. The primary ingredient in tulsi leaves, eugenol, prevents cancer cells from dividing and migrating. It also stops cancer cells from infecting surrounding structures and triggers apoptosis. By decreasing cell growth, holy basil is helpful in controlling the growth and progression of breast cancer. It also inhibits angiogenesis, the formation of new blood vessels within the cancerous tumour. As a result, cancer cells do not receive the oxygen and nourishment they require to survive, ending in their demise. Holy basil, dubbed "the finest medicine of nature" by the Ayurvedic medical school, is a potent natural healer. Eating of its leaves on a daily basis can help people avoid cancer and keep healthy. By inducing the death of cancer cells, the usage of Holy Tulsi for treatment for cancer helps speed up the patient's recovery.

Skin Cancer

Tulsi leaf alcoholic extract used topically protected against drug induced skin cancer. In mouse model of skin cancer treatment with an alcoholic extract of basil leaves reduced the number of tumours caused by a variety of skin carcinogens.

Lung cancer

Lung cancer is the most dangerous disease in this era. Tulsi leaves are best for cure lung cancer. Tulsi alcoholic extraction inhibited lung cancer cell growth and caused cell demish in human lung cancer cell. Tulsi phytochemicals, induced as carnolic acid, rosmarinic acid and lutelin, have been shown to suppress lung cancer growth.





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Breast Cancer

Holi basil extract has been demonstrated in cell experiments to avoid the growth of breast cancer as well as an increase in inflammation levels. Human breast cancer cells were demonstrated to be inhibited by carnosic acid and rosmarinic acid.

Liver Cancer

In response to cancer causing toxins and drug exposures, tulsi leaves alcohol extraction saved liver cells from cell injury and boosted antioxidants activity. Tulsi phytochemicals apigenin, luteolin, carnosic acid and rosmarinic acid had further anti cancer actions in liver cells.

Stomach Cancer

Basil caused cell death in chemically produced stomach cancer but not in healthy stomach tissue. Tulsi leaf extract with 70% alcohol decreased chemical induced stomach cancer. Tulsi phytochemicals such as luteolic, sitosterol, ursolic acid and apigenin have been found in cell culture research to suppress the growth and destroy stomach tumours.

Extraction of Tulsi

Tulsi leaves were collected from courtyards and the local market. A biologist and a seeking to take verified the validity of the specimens. The leaves were removed from the stem, rinsed with clean water, and dried until they were dry enough to grind. In an electric grinder, dried leaves were ground individually until a homogeneous powder was formed. The powder produced using the "cold extraction technique" was used to make ethanolic extract. 250 grammes of powder from Tulsi were macerated in 100 percent ethanol for three days. To get a clean filtrate, the ethanol decoction was filtered with Whatman 1 filter paper. The resulting filter was reduced to a solid of Tulsi extract at a low temp of 60°C. Approximately 18 g of solid residue was recovered from 250 g of Tulsi powder treated in 1 L of ethanol. To produce a 10% volume of extract, one gramme of this isolate was diluted in 10 ml of dmf. Similarly, by diluting Tulsi extract with suitable proportions of solvent, concentrations of 0.5 %, 1 %, 2 %, and 5 % were achieved.

Super Critical CO2 Extraction

Extraction of eugenol from dried tulsi leaves powder (*Ocimum sanctum* Linn.) of 'West Bengal origin' (Eastern India), Ram tulsi variety, was carried out using supercritical carbon dioxide (SC-CO₂) extraction. The optimized parameters for highest yield of eugenol [2.96 mg (g dry tulsi leaves)⁻¹] were 20 g of tulsi leaves powder (dp = 0.42 mm) extracted at 50 °C, 200 bar and 90 min extraction time at a constant flow rate of 2.5 L min⁻¹ of gaseous CO₂. Statistical analyses revealed that only extraction pressure showed significantly effect on the yield of eugenol (Dipan and pobir et al.,2017) The compact bed of tulsi leaves was also characterised during stable and unstable extraction stages, and empirical relationships between dimensionless Reynolds, Sherwood, and Schmidt numbers were derived. Leaf mucilage hampered the collection of eugenol from basil leaves, resulting in poor correlations.

Soxhlet Technique

The dried powder of Tulsi (50 g) was placed in the thimble of Soxhlet apparatus. 500 ml of distilled water was used as a solvent. The extraction was continued till clear solvent was seen in the thimble. The extract was concentrated using Rota vapor. Then the extract was dried in a digital water bath till a dark green residue was obtained. The percentage yield of the extract was calculated using the following formula

$$\text{Percentage yield} = \frac{\text{Final weight of the dried extract}}{\text{Initial weight of the powder}} \times 100$$

The percentage yield was 7 % W/W. The extracts were kept in the refrigerator till further use (Prashant et al.,2020).





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The leaf samples were dried at a temperature of about 40-45°C. 40 g of fine powder of plant material was placed in a small size of muslin cloth bag in a Soxhlet apparatus (A.V. and R.M. et al., 2020).

Isolation of Components from Tulsi

Eugenol

Eugenol is an allyl filament guaiacol, a chemical compound belonging to the allylbenzene class. It's an oily liquid with a colourless to pale yellow hue that's made from essential oils including clove, nutmeg, cinnamon, basil, and bay leaf. It's found in clove bud oil in quantities of 80–90%. Eugenol has a clove-like fragrance that is pleasant and spicy. *Eugenia caryophyllata*, the previous Linnean taxonomy word for cloves, is the source of the name. *Syzygium aromaticum* is the current accepted name.

Biological Activity of Tulsi

A pale yellow tinted fixed oil is found in the seeds of *Ocimum sanctum* L. (Labiatae; often known as 'Tulsi'). Due to combined suppression of arachidonate metabolism and antihistaminic action, the oil has anti-inflammatory properties. The pituitary-adrenal axis has little effect on anti-inflammatory action. Prostacyclin inhibition and glancingly acting analgesic action give the oil antipyretic properties. The oil has been found to be effective against formaldehyde or adjuvant induced arthritis and turpentine oil induced joint edema in animals. Lipoxigenase inhibitory, histamine antagonistic and antisecretory activities of the oil contribute towards anti-ulcer activity.

Over view

The holy basil plant is endemic to India. It's a frequent ingredient in traditional Indian medicine. Because of its spicy flavour, holy basil is also known as hot basil. Holy basil contains compounds that are considered to reduce pain and edema. They may also help diabetics decrease their blood sugar levels. Antioxidant properties of holy basil mostly used to treat anxiety, stress, excessive cholesterol, and a variety of other ailments.

CONCLUSION

Tulsi is a highly significant holy basil for a longer and more tranquil life because of all of these therapeutic elements. It has a strong traditional importance in both Hindu and non-Hindu communities. This little shrub is unquestionably rich in therapeutic potential. Tulsi has been dubbed the "Queen of Herbs." Tulsi protects against illnesses and decreases stress, improves stamina and endurance, and improves the body's effective utilisation of oxygen, according to a recent scientific review. It boosts the immune system, lowers inflammation, and protects against infection. Radiation damage is reduced; ageing factors are reduced; the heart is supported. Antibiotic, antiviral, and antifungal properties are found in the lungs and liver. Characteristics; improves the efficiency of a variety of other treatments; and delivers a plentiful supply of oxidants and other nutrients additional vitamins and minerals. Tulsi on the menu, as well as in drugs, may surely assist in the protection or degradation of many health situations, and it merits more medical evaluation.

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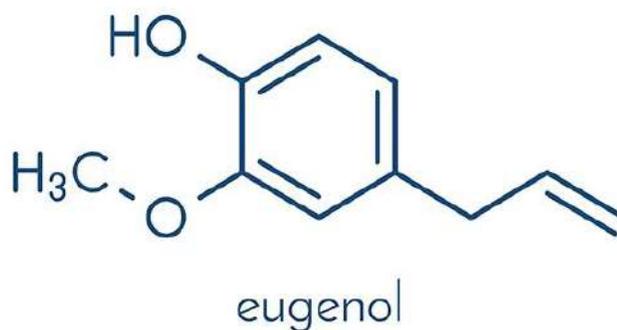
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Role of Ginger in Immune Booster

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ABSTRACT

Spice ginger is obtained from the underground stems or rhizomes of *Zingiber officinale*, a herbaceous tropical perennial belonging to the family Zingiberaceae. The chapter begins with a description of the ginger plant and its chemical structure before going on to discuss production, varieties and cultivars. Products of ginger rhizomes – fresh ginger, preserved ginger, dry ginger, ginger powder, ginger oil, ginger oleoresin and ginger paste – are detailed. The main uses and functional properties of ginger, culinary and medicinal (both traditional and modern) are outlined before the chapter finishes with a look at quality specifications, organic ginger and some biotechnology studies. Immunity boosters are products which claim to be able to support your immune system so you aren't as likely to get sick. Additionally, they typically also suggest that if you do get sick, taking the supplements will make your illness pass faster. Ginger root comes from the *Zingiber officinale* plant, and it has been used in Chinese and Indian medicine for thousandsof years . People typically use fresh or dried ginger in cooking or herbal tea, and some take ginger supplements for their possible health benefits. Ginger may help relieve nausea and vomiting and aid digestion. Antioxidants and other nutrients in ginger root may help prevent or treat arthritis, inflammation, and various types of infection. Ginger may also reduce the risk of diabetes, cancer, and other health problems.

Keywords:Ginger, immune boosters, chemical constituents

INTRODUCTION

Ginger originated from Maritime Southeast Asia . It is a true cultigen and does not exist in its wild state. The most ancient evidence of its domestication is among the Austronesianpeoples where it was among several species

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of ginger cultivated and exploited since ancient times . They cultivated other gingers including turmeric (*Curcuma longa*), white turmeric (*Curcuma zedoaria*), and bitter ginger (*Zingiber zerumb et*) . The rhizomes and the leaves were used to flavour food or eaten directly. The leaves were also used to weave mats. Aside from these uses, ginger had religious significance among Austronesians, being used in rituals for healing and for asking protection from spirits. It Contains gingerol, which has powerful medicinal properties. It Can treat many forms of nausea, especially morning sickness. It May help with weight loss. It Can help with osteoarthritis. It May drastically lower blood sugars and improve heart disease risk factor Anticancer activities of ginger against colorectal cancer have been well documented. Numerous *in vitro* studies showed that ginger and its active components inhibit growth and proliferation of colorectal cancer cells. In a study, 6-gingerol inhibited growth of colon cancer HCT116 cells. The suppression of tumor growth was found to be linked with the inhibition of leukotriene A4 hydrolase activity, which was further confirmed by *in silico* approach. Besides these, various other mechanisms were reported to be involved in 6-gingerol-induced cell growth inhibition and apoptosis in human colorectal cancer cells. These include protein degradation as well as downregulation of cyclin D1, NAG-1 beta-catenin, PKCepsilon , an GSK-3 β pathways . Radhakrishnan et al. reported that the anticancer activity of 6-gingerol could be associated with the inhibition of ERK1/2/JNK/AP-1 pathway . Whole ginger extract also prevents the primary stage of colon carcinogenesis. Administration of ginger extract to the mice pretreated with carcinogen 1,2-dimethylhydrazine (DMH) inhibited the levels of fecal bile acids, neutral sterols, tissue cholesterol, HMG CoA reductase, free fatty acids, triglycerides, phospholipase A, and phospholipase C. Thus, ginger supplementation reduced the risk of colon cancer markedly by virtue of its hypolipidemic and antioxidative effects. Ginger extract not only inhibits carcinogenesis of colorectal cancer cells but also enhances the anticancer effects of chemotherapeutic drug 5-fluorouracil.

History of ginger

Europe saw ginger for the first time in the 1st century when the ancient Romans traded with the India. When the Rome fell, Europe forgot about ginger until Marco Polo brought it again from his travel to the East .The fast development of human society and therefore the improvement of standard life, cancer as a growing threat, is that the second leading non contagious sickness of death globally next solely to upset. Malignant increase is stated to be a essential purpose for dying, and there had been kind of 9.6 million times of dying in 2018. A few exam works have exhibited that regular items. As of late, ginger has been commonly explored for its anticancer houses in opposition to numerous malignant increase types, like bosom, cervical, colorectal, and prostate disorder. The viable structures of interest consist of the restraint of multiplication and the popularity of apoptosis in malignant increase. The cytotoxic influences and hidden structures of ginger in prostate malignancy had been assessed each in vivo and in vitro. The anticancer additives on the whole consist of the enlistment of apoptosis and the restraint of the enlargement of malignancy cells. It is belongs to the Zingiberaceae family, The rhizome is the part which is normally used as a spice. It's often called ginger root or simply ginger.

Ginger can be used in different form or state like raw, dried, powdered or as an oil. It's a very common ingredient in cooking recipes as a spice and flavouring agent. It's sometimes added to processed foods and decorative. Here are 11 health benefits of ginger that are discovered and supported by scientific researchers. Ginger is a flowering plant that which is fast found in Southeast Asia. It's known as the healthiest spices Effect of Ginger in Colon Cancer. On the planet in between all other spices. In a meta investigation taking a goose at numerous phytochemical impacts on colon malady, 2 specific examinations utilizing mice noticed [6]-gingerol intensifies instigated programmed cell death in malignant growth cells by meddling with the mitochondrial film. there have been likewise noticed instruments connected with the disturbance of G1 stage super molecules to prevent the propagation of malignant growth cells that is in addition a related advantage of alternative vital malignant neoplasm studies. the elemental system by which chemical irritant phytochemicals follow abreast of disease cells is by all accounts protein interruption. Many Years before British surgeon Dr. James Lind discovered that lime could prevent scurvy; fifth-century Chinese sailors were using ginger's vitamin C nutritive value for the same purpose on long voyages. The cultural outlook on aphrodisiacs in the seventeenth century was another factor in the reduction of its usage as a therapeutic agent.



**Madhusmita Panda and Preetha Bhadra****Ginger**

Ginger is burned-thru worldwide as zest, seasoning specialist, embellish, medication, and food additive and is carried out each new, in a brand-new glue, or dry, in a dry powder. New ginger can be fill in for dried ground ginger, albeit the forms of new and dried ginger are quite unique. The heady perfume of ginger is entering into and sweet-smelling. Ginger is called as "Adrak" (neighbourhood name) withinside the subcontinent like India and Pakistan and is a crucial element of numerous dishes.

Nutritional Composition of Ginger**Chemical Composition**

The chemical composition and the antimicrobial characteristics of ginger and ginger oil, respectively. The estimated chemical composition of ginger was: moisture (15 ±0.033)%, fiber (17± 0.03)%, ash (6.5±0.001)%, protein (5.2±0.1)I%, fat (8.0± 0.003)% and carbohydrates (48.3 ±1.16)%. Ginger essential oil was collected by steam distillation process. The physicochemical analysis of the oil was carried out, and the acid value was found to be 2.9, saponification value 25, ester value 30.22, free fatty acids 1.45 g oleic acid/100 g oil and the refractive index 1.5060% at 27 deg C. The inhibitory effect of ginger oil was detected for growth of six microorganisms: the bacteria *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurim*, the mould *Aspergillusniger*, *Aspergillusflavus* and the yeast *Sacharomycescerevisiae*. The results indicated that ginger oil has a potent antimicrobial activity against all tested organisms. The highest antibacterial activity was detected against E.coli, where 23 and 30mm were the inhibition zone diameters at the lower and his higher oil concentrations, respectively. The higher antimicrobial activity, among all tested organisms was found against moulds where complete inhibition (100%) was recorded.

Benefits

Ginger may have anti-inflammatory, antibacterial, and antiviral properties. Below are some of ginger's potential medicinal uses.

Reducing gas and improving digestion

According to a 2018 review Trusted Source, several studies have investigated ginger's effects on the gasses that form in the intestinal tract during digestion. This research indicates that enzymes in ginger can help break up and expel this gas, providing relief from any discomfort. In addition, the research shows that ginger may help increase movement through the digestive tract, suggesting that it may relieve or prevent constipation. Ginger also appears to have beneficial effects Trusted Source on the enzyme pancreatic lipase, which aids digestion in the small intestine.

Relieving nausea

A 2020 review indicates that ginger can help alleviate morning sickness and relieve nausea following cancer treatment. A 2016 review suggests that the odour-producing principles gingerols and shogaols are effective in preventing nausea and vomiting. However, the amounts of those compounds can vary, depending on the form of ginger. The researchers determined that dried ginger, followed by fresh ginger and powdered ginger tea had the highest concentrations of gingerol. One study that the review analysed included 576 adult cancer patients. The scientists found that doses of 0.5 grams (g) and 1.0 g were most effective at reducing nausea. Of the seven studies analysed, five showed gingers to be beneficial, while two found no beneficial outcomes. The authors of the review suggest that the mixed results may stem from differences in the forms and preparations of ginger. They also called for further studies in humans, in order to fully understand the effects of ginger on nausea and other gastrointestinal issues.

Supporting the immune system

Many people use ginger to help recover from a cold or the flu. However, the evidence supporting this use is mostly anecdotal. In an older study from 2013 Trusted Source, researchers investigated the effects of fresh and dried ginger on one respiratory virus in human cells. The results suggest that fresh ginger may help protect the respiratory system, while dried ginger did not have the same impact. A large cross-sectional study from 2017 Trusted



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Source suggested that daily ginger consumption may support the immune system. This may protect against chronic disease and support recovery from other illnesses, such as the common cold or flu. A small 2019 study on the effects of ginger extract on smokers and non-smokers found that daily consumption of ginger extract was associated with a stronger antibody response in non-smokers. However, confirming ginger's effects on the immune system will require further research.

Reducing inflammation

A 2015 review concluded that taking ginger by mouth is “modestly efficacious and reasonably safe” for treating inflammation caused by osteoarthritis. However, the authors noted that the studies in their meta-analysis were small and may not represent the general population. Meanwhile, a 2017 review of 16 clinical trials determined that the phytochemical properties in ginger may combat inflammation. These authors also called for further research into the most effective dosages and types of ginger extract.

Relieving pain

Ginger may ease Trusted Source pain through anti-inflammatory and analgesic effects of its gingerol compounds. A 2016 review concluded that ginger may specifically help reduce dysmenorrhea – pain right before or during a period. However, the authors acknowledge that the studies they had reviewed were often small or of poor quality. Fully exploring a connection between ginger consumption and pain relief will require more research.

Supporting cardiovascular health

There is some evidence that ginger extract may help prevent cardiovascular disease. A 2017 study Trusted Source of 4,628 people found that daily ginger consumption may protect, diabetes, hyperlipidaemia, cerebrovascular disease, and fatty liver disease, among other chronic conditions. The authors concluded that ginger may have potential as a preventive therapy. against coronary heart disease, high blood pressure. Determining whether ginger may support treatment for those with cardiovascular disease will require further research. Meanwhile, a small 2016 study Trusted Source found that ginger extract helped reduce the occurrence of heart abnormalities in rats with diabetes. The authors noted that this reduction may stem, in part, from the antioxidant properties of the extract.

Lowering cancer risk

Ginger does not provide protein or other nutrients, but it is an excellent source of antioxidants. Research Trusted Source has shown that, for this reason, ginger can reduce various types of oxidative stress. Oxidative stress can happen when too many Trusted Source free radicals build up in the body. Free radicals are toxic substances produced by metabolism and other factors. When they build up in the body, free radicals can cause cellular damage, which can lead to conditions such as rheumatoid arthritis, heart attack, chronic inflammation, and cancer. Dietary antioxidants can help the body get rid of free radicals. A 2015 review Trusted Source suggests that ginger may be effective against certain cancers of the gastrointestinal system, including colorectal cancer, gastric cancer, pancreatic cancer, and liver cancer. The review concludes that ginger may inhibit the growth of cancer cells in certain types of cancer or contribute to the death of cancer cells in other types.

Nutrition and dosage

Ginger is a good source of antioxidants, but it does not provide many vitamins, minerals, or calories. As the Department of Agriculture notes, 2 teaspoons of ginger provide only 4 calories Trusted Source and no significant amount of any nutrient. Most of the research on ginger has looked at dosages of between 250 milligrams (mg) and 1 g, taken between one and four times each day. The Food and Drug Administration (FDA) considers ginger root to be generally safe with an approved daily intake recommendation of up to 4 trusted Source.

Risks

The FDA considers ginger to be safe in the diet, but it does not guarantee or regulate its use as a medicine or supplement. Researchers Trusted Source have not investigated many of the compounds in ginger. Also, scientific evidence does not support some claims about ginger's healing qualities. Before adding more ginger to the diet or





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taking a ginger supplement, consult a healthcare professional. Some supplements can interact with medications or cause other health complications.

Biological Activity of Gingerol

In a pre-medical meta-exam of gingerol intensifies anticancer, calming, opposed to contagious, most cancers prevention agent, neuroprotective and gastroprotective residences had been accounted for, which don't forget reads for vitro and A couple in-vivo examines have recommended that gingerols inspire sound glucose guiding principle for diabetics. Numerous examinations were across the influences of gingerols on a huge scope of malignant growths which include leukaemia, prostate, bosom, skin, ovarian, lung, pancreatic and There has now no longer been a number of medical attempting bent on note gingerols physiological leads to human. whereas a huge wide range of the substance additives related to the influences of gingerols on cells were completely contemplated, few were in an exceedingly medical setting. this can be because of the very smart quality in normal phytochemicals and also the absence of viability in analysis. Most flavorer healthful drug, that embody gingerols, are below the constraints of the Food and Drug Administration withinside the u. s. and take a glance at techniques have currently not control the maximum amount as research which has pale the motivation in phytochemical research. Herbal medicine is untested for fine affirmation, energy and adequacy in medical settings because of a insufficiency of finance in Jap medical research. Most exploration on [6]-Gingerol has been on each mouse subjects (in-vivo) or on delicate human tissue (in-vitro) and is maybe applied in an exceedingly whereas to talk some capability programs for multi-goal infectious.

CONCLUSION

Regardless of advances in the therapy of bosom disease, there stays a necessity to conquer helpful opposition and create novel therapies for metastatic bosom malignancy. Ongoing investigations have exhibited that BCSCs might be answerable for opposition. Crosstalk between the essential tumor and the stroma or microenvironment is allegedly conceivably answerable for the relocation and obtrusive nature of metastatic bosom disease. To address these worries, consideration should be centred around intensifies that explicitly influence different subatomic targets related with undifferentiated cells and the metastatic tumour micro-environment. Phytochemicals have demonstrated viable at focusing on various flagging pathways and BCSCs in bosom malignancy.

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Clove as a Personalized Medicine

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ABSTRACT

Clove (*Syzygium aromaticum*) is a treasured spice that has been used for centuries as a food preservation and for a number of therapeutic purposes. Clove is an Indonesian native that is now grown in many parts of the world, especially in the Brazilian state of Bahia. This plant has a great deal of potential in the pharmacological, cosmetic, food, and agriculture sectors because it is one of the greatest sources of phenolic compounds like eugenol, eugenol acetate, and gallic acid. The most important publications on clove and eugenol's biological activities are included in this review. Clove has a higher antioxidant and antibacterial activity than many fruits, vegetables, and other spices, therefore it's worth noting. An innovative strategy for controlling malaria is the use of clove as a larvicidal agent.

Keywords:

INTRODUCTION

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Myrtales
Family: Myrtaceae
Genus: *Syzygium*
Species: *aromaticum*



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Spices like clove, mint, oregano, thyme, and cinnamon have been used as storage and medicinal plants for millennia, owing to their antioxidant and antibacterial properties. Many studies now show that spice plants have antibacterial, antifungal, antiviral, and anticarcinogenic effects. Cloves has gotten a lot of attention because of its powerful antioxidant and antibacterial properties that set it apart from other spices. Clove (*Syzygium aromaticum*), a spice made from the tiny reddish-brown buds of a tropical evergreen tree in the Myrtaceae family. Cloves are said to be indigenous to Indonesia's Moluccas, or Spice Islands, and were used in the early spice trade. Cloves have a strong perfume and a fiery, pungent flavour that is used to flavour a variety of foods, particularly meats and bread items, in Europe and the United States, the spice is a common flavouring agent in Festive season dishes such as mincemeat and wassail. Clove trees are commonly grown in coastal regions to highest altitudes of 200 metres above sea level. After four years of plantation, the production of flower buds, which is the tree's commercialised section, begins. Before flowering, flower buds are harvested throughout the maturation phase. The harvesting might be done manually or chemically, with the help of a natural phytohormone that releases ethylene in the vegetal tissue, resulting in early maturation.

History

Envoys from Java brought cloves to the Han-dynasty court in China as early as 200 BCE, which were used to fragrance the breath during listeners with the monarch. Cloves were also used to store, flavouring, and garnish food in Europe during the late Middle Ages. Clove production was most totally localized to Indonesia, and the Dutch eliminated cloves from all islands save Amboina and Ternate in the early 17th century in order to generate shortage and keep prices high. The French broke the Dutch monopoly by smuggling cloves from the East Indies to Indian Ocean islands and the New World in the latter part of the 18th century. Indonesia was the world's leading clove exporter in the early twenty-first century, followed by Sri Lanka, Madagascar & Tanzania.

Morphology

The clove plant is an evergreen that reaches a height of 8 to 12 metres (25 to 40 ft). It has tiny, simple, opposite leaves with glands. Typically, the plants are reproduced from seeds that are grown in shady regions. Flowering occurs in the fifth year, and a tree can produce up to 34 kg (75 pounds) of dry buds per year. In late summer and also in the winter, the buds are hand-picked then sun-dried. Cloves range in size from 13 to 19 mm in length (0.5 to 0.75 inch). This clove buds consists of 14 to 20% essential oil, the most important of this is eugenol, a fragrant oil. Cloves have a strong odour due to eugenol, which will be obtained by distillation to produce clove oil. This oil is a local anaesthetic for toothaches and is used to prepare microscopic slides for viewing. Eugenol is employed as a sweetener or intensifier, as well as in germicides, fragrances, and mouthwashes. It is also utilised in the manufacture of vanillin.

Chemical Composition

Clove is a significant source of phenolic components such as hidrobenzoic acids, flavonoid, hidroxyphenyl propens and hidroxicinamic acids in the form of flavonoids, hidrobenzoic acids, hidroxicinamic acids. Clove's major bioactive constituent is eugenol, produced in amounts varying from 9381.70 to 14650.00 mg per 100 g of freshly plant material. Eugenol consists of 72–90% of the essential oil obtained from cloves and is the chemical that gives cloves their distinctive perfume. In pressured water at 125 °C (257 °F), complete extraction takes 80 minutes. Ultrasound-assisted and microwave-assisted extraction technologies give faster extraction rates while using less energy. Some another main essential oil composition of clove oil is beta-caryophyllene, Acetyl eugenol, vanillin, crategolic acid, tannins such as bicornin, gallotannic acid, methyl salicylate (painkiller), flavonoids including kaempferol, eugenin, eugenitin and rhamnetin, triterpenoids like stigmasterol, oleanolic acid, and campesterol.

Eugenol

The metabolism of eugenol was studied in healthy male and female volunteers. In the liver, eugenol is converted to glucuronic acid or sulphate conjugate. Different CYP 450 enzymes partially metabolised methyl eugenol in the liver to reactive 2', 3'-(allylic) epoxide or 1' hydroxy-derivatives. In the urine, less than 0.1 percent of the eugenol dose was



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secreted in the unmetabolized form, whereas 95 percent of the dose was restored, with more than 99 percent constituted of phenolic conjugates and 50% as eugenol-glucuronide and sulphate. Eugenol conjugates as well as other metabolites (e.g., cis- and trans-isoeugenol, 4-hydroxy-3-methoxyphenyl-propane, 3-(4-hydroxy-3-methoxyphenyl)-propionic acid, 3-(4-hydroxy-3-methoxyphenyl)-propane-1, 2-diol, and 3-(4-hydroxy-3-methoxyphenyl)-propane-1, 2-oxide)

Personalised Medicine

Personalized medicine is a new field of medicine that uses a person's genetic profile to help guide decisions about illness prevention, diagnosis, and treatment. Personalized medicine is a new field of medicine that uses a person's genetic profile to help guide decisions about illness prevention, diagnosis, and treatment. Doctors can use a patient's genetic background to assist them choose the right medication or therapy for them and administer it at the right dose or regimen. The Human Genome Project's data is being used to enhance personalised treatment. Personalized medicine (PM) is the practise of designing a treatment to be as unique as the patient. The method is based on finding genetic, epigenomic, and medical data that helps us to gain a better understanding about how an individual's specific genome portfolio makes them sensitive to particular diseases. The Personalized Medicine Initiative is a long-term research project combining the National Institutes of Health (NIH) and a number of other research institutions. Its goal is to learn how a person's genetics, environment, and lifestyle can influence the best way to prevent or treat disease.

Effect Of Clove On Personalised Medicine

According to the National Nutrient Database of the United States Department of Agriculture, 2.1 grammes (g) or 1 teaspoon of ground cloves from Trusted Source contains:

- calorie count: 6 (kcal)
- Protein in the amount of 0.13 grammes (g)
- Total fat: 0.27 g
- Carbohydrate: 1.38 g
- fibre (0.7 g)

The same amount of ground cloves also contains 1.263 milligrams (mg) of manganese, which is equivalent to 63 percent of the Daily Value (DV) for manganese.

Biological Activities**Antioxidant Activity**

The US Department of Agriculture recently created a database with the polyphenol content and antioxidant activity of various foods in partnership with universities and private enterprises. (Perez-Jimenez et al). Identified the 100 highest dietary sources of polyphenols using this database. The primary phenolic components of 26 spices were identified and quantified using high-performance liquid chromatography, followed by the ABTS method for in vitro antioxidant activity study. The results revealed a strong link between polyphenol concentration and antioxidant activity. Clove (buds) was the spice with the most antioxidant activity and polyphenol content, as well as the highest levels of tetraethylammonium chloride and gallic acid. Phenolic acids (gallic acid), flavanol glucosides, phenolic volatile oils (eugenol, acetyl eugenol), and tannins were the most common phenolic chemicals discovered. The enormous potential of clove as a radical scavenger and a commercial source of polyphenols was underlined. When tested as metal quelants, superoxide radical capture, and DPPH radical scavenging, ethanol and aqueous extracts of clove and lavender at doses of 20, 40, and 60 g/mL showed inhibitions of up to 95%. The strong hydrogen donating capacity, metal chelating ability, and scavenging of free radicals, hydrogen peroxide, and superoxide activity of both extracts can be linked to their potent antioxidant activity.

Antimicrobial Activity

Clove has been shown to have antibacterial properties against a variety of bacteria and fungus species. (Sofia .et .al) The aqueous extract of clove at 3 percent was the only sample that demonstrated complete bactericidal action against



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all three food-borne pathogens tested, *Escherichia coli* (*E. coli*), *Staphylococcus aureus*, and *Bacillus cereus*. Clove extract, at a concentration of 1%, had a strong inhibitory effect. Clove essential oil's antibacterial activity was evaluated against *E. coli* O157:H7, revealing the varying levels of inhibition. Similarly, formulations containing eugenol and carvacrol encapsulated in a non-ionic surfactant were tested against four strains of two important foodborne pathogens, *E. coli* O157:H7 and *Listeria monocitogenes*. The results support the use of eugenol to inhibit the growth of these microorganisms on food-contact surfaces.

Anti-Viral Activity

The antiviral activity of eugenin, a chemical isolated from *S. aromaticum*, was evaluated against herpes virus strains and shown to be effective at 5 g/mL. It was concluded that one of eugenin's main targets is viral DNA synthesis by inhibiting viral DNA polymerase.

Analgesic, Digestive And Hepatoprotective Activity

Clove's analgesic properties have been known since the thirteenth century. Clove has been shown to help with toothache, joint discomfort, and spasmodic pain (Cortes-Rojas *et al.* 2014). The activation of chloride and calcium channels in ganglion cells is required for analgesic action (Shields *et al.* 2004). The clove's pain-relieving action is also attributed to capsaicin agonist activity (Vriens *et al.* 2008). Clove also contains liver-protective properties. In the paracetamol-intoxicated liver injury, clove ethanol and aqueous extracts showed hepatoprotective effect. Clove extract reduced the activity of the enzymes AST and ALT (Milind and Deepa 2011).

Benefits Of Clove Sexually**1. Sex Hormone Production Is Boosted**

Because cloves contain manganese, they are thought to be highly useful for sex hormone synthesis. Cloves were found to help boost testosterone and prolactin secretion in a 2006 study.

2. Improves Sexual Health in General

Cloves are believed to increase sexual desire in males and hence improve their overall sexual health. Many studies have proven this time and time again. Cloves' potential advantages are also recognised in Ayurveda and Unani medicine. It's been used to treat sexual issues in males for a long time.

3. Stimulates Nerves More Effectively

Cloves include sterols and phenols, which have been shown in studies to increase neuronal activation. This also aids in enhancing male sexual responses.

4. STD Risk is Reduced

Pathogens and microorganisms such as bacteria and fungi cause sexually transmitted diseases (STDs). Cloves' antibacterial qualities have been shown in studies to help fight germs and prevent illnesses.

5. Cloves for Erectile Dysfunction (erectile dysfunction)

Cloves aid in the increase of testosterone levels in the body. As a result, the desired erection and sexual satisfaction can be obtained. It increases male libido.

6. Clove is used to inhibit early ejaculation.

Clove works by boosting blood flow to the genitals and stimulating nerves, avoiding premature ejaculation. For both parties, a longer ejaculation duration leads to increased sexual performance and better sex and closeness.

7. Clove is used to boost sperm motility and count.

Cloves are high in critical vitamins, flavonoids, alkaloids, carbs, proteins, and other nutrients that boost sperm count and motility.

Health Benefits**Oral Health**

Clove oil is being studied as a natural strategy to preserve dental health due to its influence on plaque, gingivitis, and bacteria in the mouth. Researchers from Trusted Source compared a herbal mouth rinse with clove, basil, and tea tree oil to an available commercially essential oil mouth rinse.



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Both mouthwashes were effective on plaque and gingivitis, implying that they may be useful in lowering oral inflammation and germs. The researchers also found that a clove-infused mouth rinse was more effective than a commercial mouthwash at reducing the number of harmful germs in the mouth.

Diabetes

- Clove oil is being studied as a natural method for preserving oral health because of its influence on plaque, gingivitis, and bacteria in the mouth.
- Researchers from Trusted Source tested the effectiveness of a herbal mouth rinse containing clove, basil, and tea tree oil to an available commercially essential oil mouth rinse.
- In a rat study, clove extracts and nigericin, a constituent of clove extract, was found to improve insulin resistance in muscle cells. Mice with diabetes who ate nigericin showed enhanced glucose tolerance, insulin production, and beta cell activity, as well as lower insulin resistance. Another experiment with animals. Trusted Source looked at the effects of clove bud powder on laboratory markers in a rat model of diabetes. They found out many herbs and spices are high in antioxidants, which are molecules that help to prevent cancer by lowering cell damage. According to Today's Dietitian, "only 1/2 teaspoon of ground clove is estimated to provide further antioxidants than 1/2 cup of blueberries."
- In one laboratory study, clove extract was discovered to be able to stop the growth of many kinds of human cancer cells Trusted Source. Clove extract was also found to kill cancer cells in the colon.
- In the same study, the impact of clove extract on tumour formation in mice was studied. The clove extract-treated animals formed tumours at a considerably slower rate than the control group.
- According to Trusted Source, scientists investigated the impact of various preparations in another study.

Mosquito Repellent

Mosquito repellent is a product that is used to keep mosquitos at bay. According to preliminary study, putting clove oil or clove oil gel to the skin can keep mosquitos at bay for up to 5 hours.

Clove Water

- Clove water, consumed every morning, can strengthen your immune system and protect you from illnesses and flu. Clove water is high in vitamins and minerals that might help to improve your health and immunity. Manganese, vitamin k, vitamin c, calcium, and magnesium are all abundant in it.
- Every morning, drink clove water to strengthen your immunity system and protect yourself from illnesses and flu. Clove water is high in vitamins and minerals that really can help you stay healthy and strong. Manganese, vitamin k, vitamin c, calcium, and magnesium are all abundant in this vegetable.
- Clove is high in antioxidants, which can aid in the reduction of oxidative stress. Clove contains antibacterial characteristics that can help prevent infections and hazardous microbes.
- Cloves are beneficial to both men and women's sexual health. It has anti-oxidant, anti-microbial, anti-fungal, anti-viral, anti-nociceptive, immunomodulatory, and anti-carcinogenic properties and aids in the improvement of libido, neural stimulation, sperm count, motility, and general sexual health.
- Clove is a powerful herb that aids in the removal of pimples and acne. It has antibacterial properties. It can be used to treat cuts, infections, and even body aches. It also eliminates bacteria on the skin and helps to clear it up.

CONCLUSION

Based on the data presented, it is possible to infer that clove is a fascinating plant with tremendous potential as a food preservative and a rich source of antioxidant chemicals. Its biological activity has been proven, implying the production of medical goods for humans and animals, and confirming why this plant has been used for ages. The important investigations on the biological activity of clove (*S. aromaticum*) and eugenol are included in this overview. Clove has more antioxidant and antibacterial activity than many fruits, vegetables, and other spices, therefore it's worth paying attention to. Dengue fever is a severe health problem in Brazil and other tropical nations,

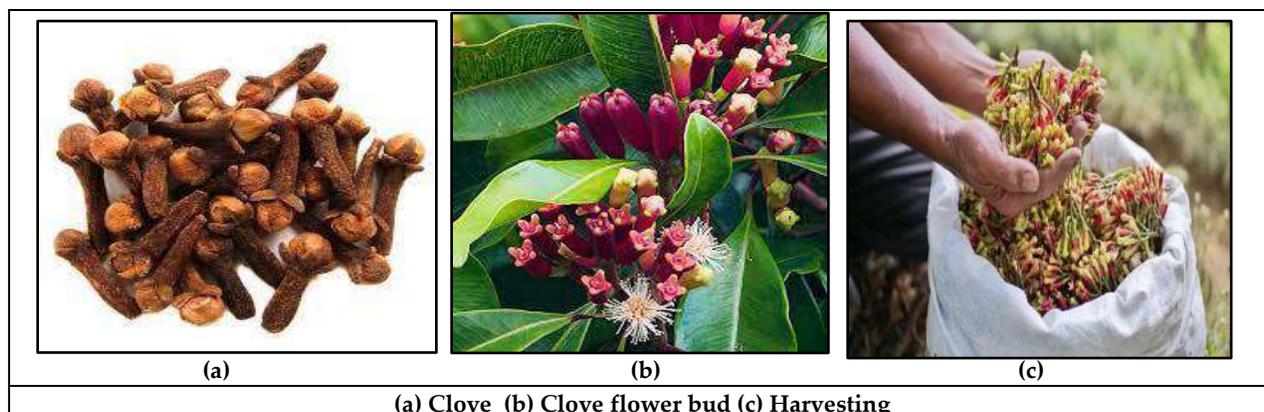


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and a new application of clove as a larvicidal agent is an intriguing technique to combat it. Studies on pharmacokinetics and toxicology were also mentioned. The several research evaluated in this article validate clove's traditional use as a food preservative and medicinal plant, emphasising the plant's importance in a variety of uses. This review comprises the most important papers on clove and eugenol's biological activity. Based on the data presented, it is possible to infer that clove is a fascinating plant with tremendous potential as a food preservative and a rich source of antioxidant chemicals. Its biological activity has been proven, implying the production of medical goods for humans and animals, and confirming why this plant has been used for ages.

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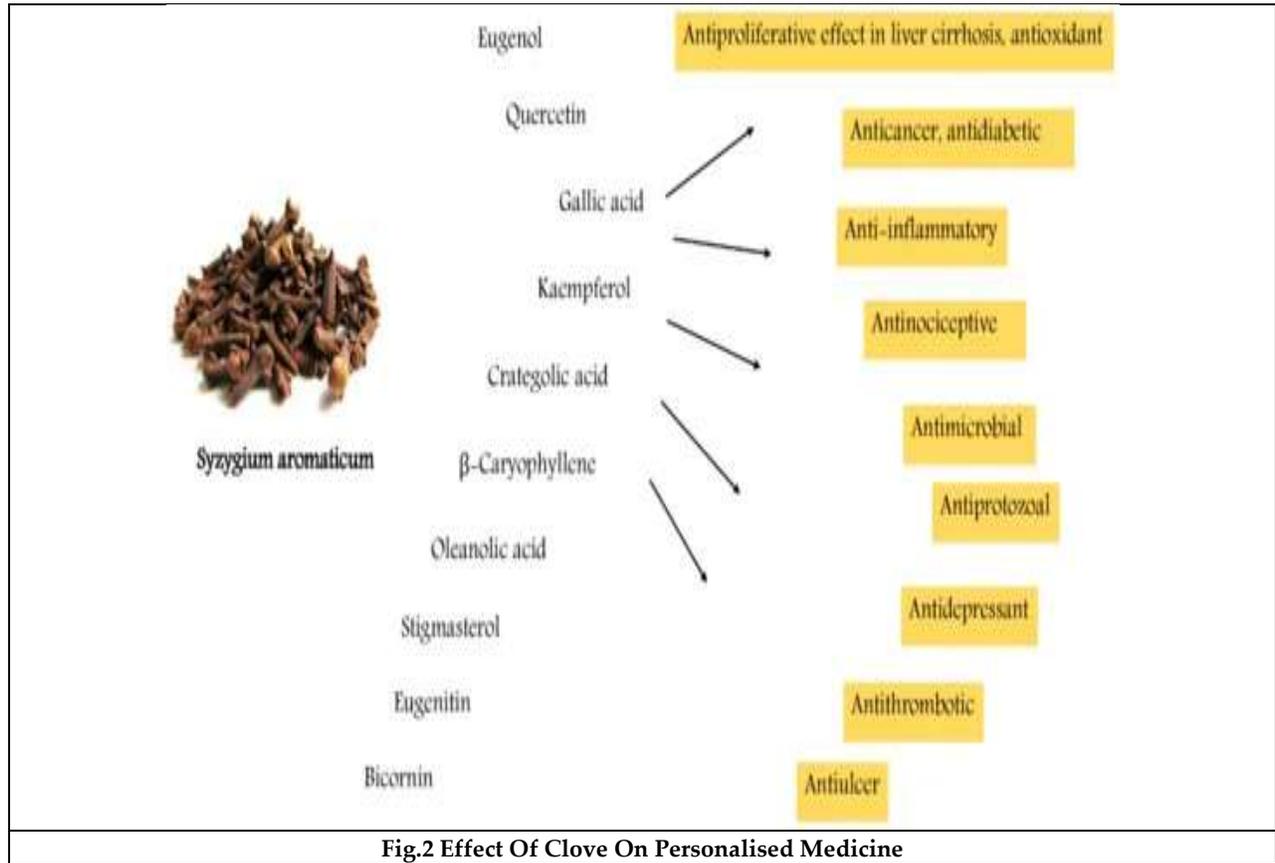


Fig.2 Effect Of Clove On Personalised Medicine





Review of Citrus Lemon- Applications in the Modern Pharmaceutical, Food and Cosmetic Industries, and Biotechnological Studies

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ABSTRACT

This review presents important botanical, chemical and pharmacological characteristics of *Citrus limon* (lemon)—a species with valuable pharmaceutical, cosmetic and culinary (healthy food) properties. A short description of the genus *Citrus* is followed by information on the chemical composition, metabolomic studies and biological activities of the main raw materials obtained from *C. limon* (fruit extract, juice, essential oil). The valuable biological activity of *C. limon* is determined by its high content of phenolic compounds, mainly flavonoids (e.g., diosmin, hesperidin, limocitrin) and phenolic acids (e.g., ferulic, synapic, p-hydroxybenzoic acids). The essential oil is rich in bioactive monoterpenoids such as D-limonene, β -pinene, γ -terpinene. Recently scientifically proven therapeutic activities of *C. limon* include anti-inflammatory, antimicrobial, anticancer and antiparasitic activities. The review pays particular attention, with references to published scientific research, to the use of *C. limon* in the food industry and cosmetology. It also addresses the safety of use and potential phototoxicity of the raw materials. Lastly, the review emphasizes the significance of biotechnological studies on *C. limon*.

Keywords: lemon, chemical composition, biological activity, cosmetic applications, phototoxicity, biotechnological studies





INTRODUCTION

Citrus limon (L.) Burm. f. is a tree with evergreen leaves and yellow edible fruits from the family Rutaceae. In some languages, *C. limon* is known as lemon (English), Zitronen (German), le citron (French), limón (Spanish). The main raw material of *C. limon* is the fruit, particularly the essential oil and juice obtained from it. The *C. limon* fruit stands out as having well-known nutritional properties, but it is worth remarking that its valuable biological activities are underestimated in modern phytotherapy and cosmetology. *C. limon* fruit juice (lemon juice) has traditionally been used as a remedy for scurvy before the discovery of vitamin C. This common use of *C. limon*, known since ancient times, has nowadays been supported by numerous scientific studies. Other uses for lemon juice, known from traditional medicine, include treatment of high blood pressure, the common cold, and irregular menstruation. Moreover, the essential oil of *C. limon* is a known remedy for coughs. In Romanian traditional medicine, *C. limon* essential oil was administered on sugar for suppressing coughs. Aside from being rich in vitamin C, which assists in warding off infections, the juice is traditionally used to treat scurvy, sore throats, fevers, rheumatism, high blood pressure, and chest pain.

In Trinidad, a mixture of lemon juice with alcohol or coconut oil has been used to treat fever, coughs in the common cold, and high blood pressure. Moreover, the juice or grated skin, mixed with molasses, has been used to remove excess water from the body, and the juice mixed with olive oil has been administered for womb infection and kidney stones. According to Indian traditional medicine, *C. limon* juice can induce menstruation; the recommended dose for this is two teaspoons consumed twice a day. Currently, valuable scientific publications focus on the ever wider pharmacological actions of *C. limon* fruit extract, juice and essential oil. They include studies of, for example, antibacterial, antifungal, anti-inflammatory, anticancer, hepatoregenerating and cardioprotective activities. The pharmacological potential of *C. limon* is determined by its rich chemical composition. The most important group of secondary metabolites in the fruit includes flavonoids and also other compounds, such as phenolic acids, coumarins, carboxylic acids, amino acids and vitamins. The main compounds of essential oil are monoterpenoids, especially D-limonene. These valuable chemical components are the reason for the important position of *C. limon* in the food and cosmetics industries. The aim of this overview is a systematic review of scientific works and in-depth analyses of the latest investigations and promotions related to *C. limon* as a valuable plant species, important in pharmacy, cosmetology and the food industry. Additionally, relevant biotechnological investigations are presented.

The Genus Citrus

The genus Citrus is one of the most important taxonomic subunits of the family Rutaceae. Fruits produced by the species belonging to this genus are called 'citrus' in colloquial language, or citrus fruits. Citrus fruits are commonly known for their valuable nutritional, pharmaceutical and cosmetic properties. The genus Citrus includes evergreen plants, shrubs or trees (from 3 to 15 m tall). Their leaves are leathery, ovoid or elliptical in shape. Some of them have spikes. The flowers grow individually in leaf axils. Each flower has five petals, white or reddish. The fruit is a hesperidium berry. The species belonging to the genus Citrus occurs naturally in areas with a warm and mild climate, mainly in the Mediterranean region. They are usually sensitive to frost.

One of the best known and most used species of the genus Citrus is the lemon— *Citrus limon* (L.) Burm. f. (Latin synonyms: *C. × limonia*, *C. limonum*). Other important species included in this taxonomic unit are: *Citrus aurantium* ssp. *aurantium*—bitter orange, *Citrus sinensis*—Chinese orange, *Citrus reticulata*—mandarin, Citrus paradise—grapefruit, Citrus bergamia—bergamot orange, Citrus medica—citron, and many others. A team of scientists from the University of California (Oakland, California, USA) analyzed the origin of several species of the genus Citrus, including *C. limon*. They found that *C. limon* was a plant that had formed as a result of the combination of two species— *C. aurantium* and *C. medica*. In the studies of scientists from Southwest University of China (Chongqing, China), the metabolite profiles of *C. limon*, *C. aurantium* and *C. medica* were evaluated using gas chromatography–mass spectrometry (GC-MS) and the partial least squares discriminant analysis (PLS-DA) score plot. They proved that *C. limon* has a smaller distance between *C. aurantium* and *C. medica* in comparison with other Citrus species. These studies demonstrated that *C. limon* was likely a hybrid of *C. medica* and *C. aurantium*, as previously suspected.



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Botanical classification of the species of the genus *Citrus* is very difficult due to the frequent formation of hybrids and the introduction of numerous cultivars through cross-pollination. Hybrids are produced to obtain fruit with valuable organoleptic and industrial properties, including seedless fruit, high juiciness, and the required taste. For older varieties, hybrids and cultivars, the latest molecular techniques are often needed to identify them. *C. limon*, like many other prolific citrus species, gives rise to numerous varieties, cultivars.

Chemical Composition of *C. limon*

The chemical composition of *C. limon* fruit is well known. It has not only been determined for the whole fruit but also separately for the pericarp, juice, pomace, and essential oil. The compositions of the leaves and the fatty oil extracted from *C. limon* seeds are also known. Due to the large number of *C. limon* varieties, cultivars and hybrids, various research centres undertake the task of analyzing the chemical composition of the raw materials obtained from them. The most important group of bioactive compounds in both *C. limon* fruit and its juice, determining their biological activity, are flavonoids such as: flavonones—eriodictyol, hesperidin, hesperetin, naringin; flavones—apigenin, diosmin; flavonols—quercetin; and their derivatives. In the whole fruit, other flavonoids are additionally detected: flavonols—limocitrin and spinacetin, and flavones—orientin and vitexin. Some flavonoids, such as neohesperidin, naringin and hesperidin, are characteristic for *C. limon* fruit. In comparison to another *Citrus* species, *C. limon* has the highest content of eriocitrin

Biological Activity of *C. limon* Raw Materials**Anticancer Activity**

C. limon nanovesicles have been isolated from the fruit juice using the ultracentrifugation method and purification on a 30% sucrose gradient, using an in vitro approach. The study showed that isolated nanovesicles (20 µg/mL) inhibited cancer cell proliferation in different tumour cell lines, by activating a TRAIL-mediated apoptotic cell death. Furthermore, *C. limon* nanovesicles suppress chronic myeloid leukemia (CML) tumour growth in vivo by specifically reaching the tumour site and by activating TRAIL-mediated apoptotic cell processes

Antioxidant Activity

It has been shown that the antioxidant activity of the flavonoids from *C. limon*—hesperidin and hesperetin—was not only limited to their radical scavenging activity but also augmented the antioxidant cellular defences via the ERK/Nrf2 signalling pathway. In addition, vitamin C prevents the formation of free radicals and protects DNA from mutations. Studies have also shown a reduction in lipid peroxidation in seizures and status epilepticus was induced by pilocarpine in adult rats.

Anti-Inflammatory Activity

Various in vitro and in vivo studies have been conducted to evaluate hesperidin metabolites, or their synthetic derivatives, at their effectiveness in reducing inflammatory targets including NF-κB, iNOS, and COX-2, and the markers of chronic inflammation. The essential oil from *C. limon* (30 or 10 mg/kg p.o.) exhibited anti-inflammatory effects in mice under formalin test by reducing cell migration, cytokine production and protein extravasation induced by carrageenan. These effects were also obtained with similar amounts of pure D-limonene. The anti-inflammatory effect of *C. limon* essential oil is probably due to the high concentration of D-limonene

Limon in the Food Industry

Due to the rich chemical composition of *C. limon* fruit and other lemon-derived raw materials, they have applications in the food industry and in food processing. The lemon fruit is used mainly as a fresh fruit, but it is also processed to make juices, jams, jellies, molasses, etc. Fresh lemon fruit can be kept for several months, maintaining their levels of juice, vitamins, minerals, fibre, and carbohydrates. The vitamin C (ascorbic acid) content in lemon fruits and juices decreases during storage and industrial processing. The factors lowering this content are: oxygen, heat, light, time, storage temperature and storage duration. To prevent the reduction in the ascorbic acid levels and antioxidant capacity of both the lemon fruit and lemon juice, they should be kept at 0–5 °C and protected from water loss by



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proper packaging, with high relative humidity during distribution. Under such conditions, lemon products show a good retention of vitamin C and antioxidant capacity. *C. limon* peel is rich in pectin, which is used in a wide range of food industrial processes as a gelling agent, including the production of jams and jellies, and as thickener, texturizer, emulsifier and stabilizer in dairy products. Due to its jellifying properties, the pectin is also used in pharmaceutical, dental and cosmetic formulations. Lemon juice is used as an ingredient in beverages, particularly lemonade and soft drinks, and in other foods, such as salad dressings, sauces, and baked products. Lemon juice is a natural flavouring and preservative, and it is also used to add an acidic, or sour, taste to foods and soft drinks. *C. limon* is the most suitable, being free from pesticide residues, raw material for enhancing the flavour of liqueurs, e.g., “limoncello”, the traditional liqueur of Sicily. It is made by the maceration of lemon peel in ethanol, water and sugar. Currently, the essential oil from lemon, i.e., pure isolated linalol and citral, are used mainly as a flavouring and natural preservative due to their functional properties (antimicrobial, antifungal, etc.). In particular, they are often used to extend the short shelf-life of seafood products and in the production of some types of cheese because they significantly reduces populations of microorganisms, especially those from the family Enterobacteriaceae.

Cosmetological Applications

C. limon fruit extracts and essential oil, as well as the active compounds isolated from these raw materials, have become the object of numerous scientific studies aimed at proving the possibility of their use in cosmetology. Lemon-derived products have long been credited with having a positive effect on acne-prone skin that is easily affected by sunburn or mycosis. In this regard, traditional uses of this raw materials are known in various parts of the world. In Tanzania, the fruit juice of *C. limon* is mixed with egg albumin, honey and cucumber, and applied to the skin every day at night to smooth the facial skin and treat acne. Juice from freshly squeezed fruit of *C. limon* mixed with olive oil is used as a natural remedy for the treatment of hair and scalp disorders in the West Bank in Palestine. Currently, knowledge of the cosmetic activity of *C. limon* is constantly expanding. *C. limon* essential oil shows antibiotic and flavouring properties, and for this reason it is used in formulations of shampoos, toothpaste, disinfectants, topical ointments and other cosmetics. Scientific studies have shown a significant antioxidant effect of *C. limon* fruit extracts, which is the reason they are recommended for use in anti-ageing cosmetics. The use of different carriers for *C. limon* extracts (e.g., hyalurosomes, glycerosomes) in cosmetics production technology contributes to an even greater inhibition of oxidative stress in skin-building structures, including keratinocytes and fibroblast. In addition, vitamin C from *C. limon* is used as an ingredient in specialized dermocosmetics. Its external use increases collagen production, which makes the skin smoother and more tense. It is used in anti-aging products, to reduce shallow wrinkles, and as a synergistic antioxidant in combination with vitamin E.

Plant Biotechnological Studies on *C. limon*

Plant biotechnology creates opportunities for the potential use of plant in vitro cultures in the pharmaceutical, cosmetics and food industries. In vitro cultures can be a good alternative to plants growing in vivo. Plant biotechnology enables control and optimization of the conditions for conducting in vitro cultures to increase the accumulation of active compounds. It facilitates, among other things, optimization of the culture medium, including the concentration of plant growth and development regulators, the use of elicitors (stressors), the selection of highly productive cell lines and genetic transformations. In vitro cultures can also be used in plant propagation (micro-propagation process). *C. limon* cultures in vitro have thus far been the subject of research concerned with the development of micropropagation protocols. They have focused on the selection of plant growth regulators (PGRs) that induced shoot and root production in in vitro cultures. In 2012, biotechnological research on the micropropagation of *C. limon* was performed by Goswami et al. from SKN Rajasthan Agricultural University in India. Shoot cultures were propagated from plant nodes on a Murashige and Skoog (MS) medium containing different types and concentrations of PGRs. The maximum number of shoots and shoot regenerations was observed at a low level of 6-benzyladenine (BA) –0.1 mg/L, or kinetin –0.5 mg/L. Shoot proliferation was also observed in combinations of PGRs such as BA and 1-naphthaleneacetic acid in concentrations of 0.1 mg/L each. With an increase in BA concentration in MS medium, shoot proliferation decreased. Regenerated shoots showed root induction on MS basal medium or on MS medium containing 1.0 mg/L of indole-3-butyric acid. Another biotechnological study on *C.*





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limon was carried out in the Department of Citriculture in Murcia (Spain). The researchers studied organogenesis and made histological characterization of mature nodal explants of two important cultivars of *C. limon*—‘Verna 51’ and ‘Fino 49’. The highest number of buds per regenerating explant was obtained on the MS medium in comparison with the Woody plant medium. The presence of 1–3 mg/L BA, in combination with 1 mg/L of 1-gibberellic acid (GA) in the culture medium, was essential for the development of adventitious buds. The lowest extent of organogenesis was observed when BA was used in the medium without GA.

CONCLUSION

The presented review proves that *C. limon* is a very attractive object of different scientific studies. The *C. limon* fruit is a raw material that can be used in different forms, e.g., extracts, juice and essential oil. The rich chemical composition of this species determines a wide range of its biological activity and its being recommended for use in phytopharmacology. The studies have focused on the essential oil and its main active compound—D-limonene. Extracts from *C. limon* fruits are rich in flavonoids such as naringenin and hesperetin. Current pharmacological studies have confirmed the health-promoting activities of *C. limon*, especially its anti-cancer and antioxidant properties. *C. limon* also finds increasing application in cosmetology and food production. There has been some biotechnological research aimed at developing effective in vitro micropropagation protocols for *C. limon*.

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Turmeric as Immune Booster

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ABSTRACT

The plant *Curcuma longa*, has been used for centuries in traditional Indian and Chinese medicine to treat a variety of ailments, including jaundice and hepatic disorders, rheumatism, anorexia, diabetic wounds, and menstrual problems. Turmeric is a brilliant yellow spice made from the rhizomes of *Curcuma longa* Linn, an Indian, Chinese, and Indonesian plant. *Curcuma longa* is a perennial herb in the Zingiberaceae (ginger) family that has pulpy, orange tuberous roots or rhizomes and oblong pointed leaves with funnel-shaped yellow flowers. Curcumin, the active ingredient in turmeric, is responsible for the majority of its therapeutic properties. Curcumin is the one of the best source to boost the immunity. Curcumin's anti-inflammatory and antioxidant properties, as well as its ability to modulate the expression of transcription factors, cell cycle proteins, and signal transducing kinases, have prompted mechanism-based research into Curcumin's potential to prevent and treat cancer and inflammatory diseases.

Keywords: Turmeric, therapeutic, immunity, cancer

INTRODUCTION

Turmeric is a herbaceous perennial plant that grows up to 1 m (3 ft - 3 in) tall.. Turmeric powder, in addition to orange, cylindrical, and scented. Its culinary appeal, has long been used in traditional Indian (Ayurvedic) and Chinese medicine for medical purposes, notably as an anti-inflammatory agent. In chemical analysis, turmeric paper is employed as an indicator for acidity and alkalinity. In acidic and neutral solutions, the paper is yellow, but in alkaline solutions, it turns brown to reddish-brown, with a pH transition between 7.4 and 9.2. On the basis of their



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long-standing traditional usage, the European Medicines Agency decided in 2019 that turmeric herbal teas or other forms taken by mouth might be used to alleviate minor digestive disorders such as sensations of fullness and gas. Curcumin is a diarylheptanoid that belongs to the category of curcuminoids, which are phenolic pigments that give turmeric its yellow hue. There's also an orange-colored volatile oil in there. Turmeric (*Curcuma longa*), sometimes known as "Indian saffron" because to its vivid yellow color, is a spice plant native to the Indian subcontinent and Southeast Asia and a member of the ginger family (Zingerberaceae) with a scientific history dating back over two centuries. India is the world's largest producer of turmeric, which has been used as an Ayurvedic cure and flavoring ingredient since antiquity. Turmeric derived from powdered dry root has varying quantities of volatile and nonvolatile oils, proteins, lipids, minerals, and carbs depending on its origin and growing circumstances. Curcuminoids are a group of three compounds that make up commercially accessible curcumin. Curcumin is the most common (60–70%), followed by demethoxycurcumin (20–27%), and bisdemethoxycurcumin (10–12%). Curcumin's therapeutic usage has been limited by a number of factors, including its poor pharmacokinetic/pharmacodynamic features, chemical instability, low performance in several in vitro and in vivo illness models, and its hazardous profile in specific experimental conditions. Curcumin is a diarylheptanoid that belongs to the category of curcuminoids, which are phenolic pigments that give turmeric its yellow hue. There's also an orange-colored volatile oil in there. Curcumin is a brilliant yellow substance that is generated by *Curcuma longa* plants. It's the main curcuminoid in turmeric (*Curcuma longa*), which belongs to the Zingerberaceae ginger family. Curcumin was recently suggested to be part of a group of chemicals known for interfering with biological tests known as pan assay interference substances (PAINS). Different formulations, administration changes, and the development of nanotechnology-based delivery systems have all aided in overcoming the critical pharmaceutical issues associated with curcumin pharmacokinetics in order to improve therapeutic efficacy and provide new hope for a clinical application of this natural compound. Curcumin has been shown to be useful in the prevention and treatment of a variety of human illnesses, including cancer, cardiovascular, inflammatory, metabolic, neurological, and skin disorders, in both preclinical and clinical studies. One of the most researched features of curcumin is its anti-inflammatory profile, which may be beneficial in both acute and chronic inflammation. Curcumin's immunomodulatory properties are due to its interactions with a variety of immunomodulators, including not only cellular components like dendritic cells, macrophages, and both B and T lymphocytes, but also molecular components involved in inflammatory processes like cytokines and various transcription factors, as well as their downstream signalling pathways. Curcumin has been discovered to disrupt the immunostimulatory activity of dendritic cells (DCs) as well as interfere with the development of myeloid DCs. These effects have been linked to the inhibition of MAPK (Mitogen-Activated Protein Kinase) activation and NF- κ B (nuclear factor kappa B) translocation, as well as the suppression of CD80 and CD86 expression, two co-working membrane proteins that provide stimulatory signals required for T cell activation, and the impairment in pro-inflammatory cytokine production (IL-12). Curcumin supplementation in rabbit diets (2, 4 and 6 g/kg) considerably boosted blood levels of IgG and IgM, indicating that curcumin can help with immunological functions as well.

Turmeric As Immune Booster

Our bodies' natural defense against disease-causing bacteria and viruses is immunity. It can significantly reduce the chances of becoming ill. Summer is approaching, and the change in weather is causing our immune system to become slightly weakened. People are only becoming infected with the widespread coronavirus and other pandemics as a result of their weakened immunity. Turmeric contains curcumin, which has anti-inflammatory, antiseptic, and antibacterial properties. Turmeric aids in the strengthening of our immune systems; the main life-saving ingredient in turmeric is about 3-5 percent Curcumin, a phyto-derivative with healing properties. However, because of the low percentage levels in standardised turmeric powder, it may be difficult to reap all of the benefits by simply taking turmeric in small doses, necessitating the use of supplements.

It is very common for people to catch a common cold or flu as the weather changes from cold to warm. Turmeric aids in the natural cleansing of the respiratory tract, aids in the fight against infection, and relieves individuals from the direct effects of colds and flu due to its anti-inflammatory properties. Due to lowered immunity, people with



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bronchial problems such as Sinusitis, Sinus, and others face a slew of issues. Curcumin can assist in dealing with such issues and strengthening the immune system so that problems with breathing do not arise. Symptoms include bronchial asthma, nasal and airway congestion due to inflammation, cough, cold, and, on rare occasions, shortness of breath, affecting children, adults, and the elderly. Inflammation narrows the airways, making breathing difficult. This is frequently linked to an over reactive immune system, which results in chronic inflammation and free radical damage to lung tissue. Curcumin reduces inflammation, relieves congestion, and pain, which helps us breathe better. Curcumin aids in the improvement of immunity and the prevention of viral replication. Curcumin inhibits a number of inflammatory molecules that are responsible for virus-caused damage. It relieves symptoms and helps to reduce them. Reduces the virus's replication, exhibiting all antiviral properties. As a result, curcumin lowers viral load.

Consumers have become more conscious and aware of their well-being in recent years. As a result, consumers pay closer attention to foods that contain specific ingredients that have the potential to affect their health and physiological functions. Functional foods are successfully formulated using nutraceuticals and bioactive compounds to meet the nutritional and physiological needs of consumers using this approach. Many bioactive compounds, such as polyphenols, phytosterols, vitamins, and minerals, have been reported as ingredients in the production of functional food in the last 20 years. Curcumin, a bioactive compound discovered and extracted from *Curcuma longa* plants, is also regarded as an important bioactive compound. This polyphenolic compound's potential health benefits have also been clinically demonstrated. Curcumin was used in the development of functional foods in its free or encapsulated form based on its suitability. During the development of functional foods, curcumin is used as a natural ingredient with a distinct color and flavor profile, as well as potential health benefits. Because of the immunomodulatory properties of curcumin, which have already been discussed previously by some researchers, such functional foods can help people fight the COVID-19 virus if they are recommended for oral consumption. Curcumin has been shown to be effective in the prevention and treatment of a variety of human diseases, including cancer, cardiovascular, inflammatory, metabolic, neurological, and skin diseases, in both preclinical and clinical studies. One of the most studied properties of curcumin is its anti-inflammatory profile, which may be beneficial in both acute and chronic inflammation. Curcumin's immunomodulatory properties are due to its interactions with a variety of immunomodulators, including not only cellular components like dendritic cells, macrophages, and both B and T lymphocytes, but also molecular components involved in inflammatory processes like cytokines and various transcription factors, as well as their downstream signalling pathways.

Curcumin has been discovered to inhibit the immunostimulatory function of dendritic cells (DCs) as well as interfere with the maturation of myeloid DCs. These effects have been linked to the inhibition of MAPK (Mitogen-Activated Protein Kinase) activation and NF- κ B (nuclear factor kappa B) translocation, as well as the suppression of CD80 and CD86 expression, two co-working membrane proteins that provide stimulatory signals required for T cell activation, and the impairment in pro-inflammatory cytokine production (IL-12). Curcumin supplementation in rabbit diets (2, 4 and 6 g/kg) significantly increased serum levels of IgG and IgM, implying that curcumin can improve immune functions as well. The JAK/STAT (Janus Kinase/signal transducers and activators of transcription) signalling pathway modulates a wide range of cytokines and growth factors involved in cell proliferation, differentiation, cell migration, and apoptosis, and are directly involved in cellular homeostasis and immune responses. Curcumin concentrations of 20 to 50 μ M have been shown to inhibit STAT3 phosphorylation in a variety of cell types in vitro. This finding is in line with Liu et al's findings' on Curcumin's ability to modulate the STAT3 pathway in a mouse model of colitis induced by dextran sulphate sodium (DSS) [63]. Curcumin (50 mg/kg) treatment resulted in a significant improvement in the disease activity index and histological injury score when compared to the control group. In addition, myeloperoxidase activity (MPO), a marker of leukocyte infiltration, and STAT3 phosphorylation were significantly reduced. Following STAT3's decreased DNA-binding activity, the expression of IL-1 and TNF- α was significantly reduced after curcumin treatment. Curcumin at low concentrations (7.5 μ M) has been found to induce an anti-inflammatory profile in DCs in vitro, enhancing STAT3 phosphorylation and activity, implying a biphasic effect of curcumin on STAT3 modulation depending on the range of curcumin concentrations.



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This finding has also been made when curcumin is combined with opioids, the drugs of choice for the relief of acute and chronic pain as well as the reduction of opioid tolerance. Curcumin, in particular, appears to be relatively safe to take as a single high dose orally, but its effect on morphine tolerance has been suggested to be biphasic, so it should be used with caution.

Suppressor of Cytokine Signalling proteins (SOCS), which are involved in the regulation of proinflammatory proteins and cytokines production, block the JAK/STAT signalling pathway. Curcumin potently inhibited lipopolysaccharide (LPS)-induced expression of IL-6, TNF, and prostaglandin-endoperoxide synthase 2 mRNA in murine RAW 264.7 macrophages by preventing SOCS1 and 3 inhibitions, according to Guimares *et al.* Curcumin also inhibited LPS-induced p38 MAPK activation by reducing its phosphorylation and nuclear translocation, highlighting the significance of this molecular pathway in inflammatory processes. These findings support the ability of pure curcumin to suppress class I histone deacetylases and thus increase the expression of SOCS1 and SOCS3 proteins in primary myeloproliferative neoplasms cells (especially HDAC8 activity). Aside from JAK/STAT, NF- κ B, a transcription factor that regulates the inflammatory response and immune system homeostasis, is another important molecular pathway involved in inflammation. NF- κ B has been shown to regulate the expression of more than 400 genes involved in inflammation and other chronic diseases, as well as control the expression of inflammatory mediators such as COX-2, inducible nitric oxide synthase (iNOS), and interleukins. Curcumin's ability to modulate cytokine levels has been linked to its inhibition of the NF- κ B signalling pathway. Curcumin inhibited pancreatic leucocyte infiltration and preserved insulin-expressing cells in type 1 diabetes, a T cell-mediated autoimmune disease in which the IS destroys pancreatic cells. These effects have been linked to reduced NF- κ B activation in TCR-stimulated NOD lymphocytes and an impairment of dendritic cell T cell stimulatory function, resulting in lower proinflammatory cytokine and nitric oxide (NO) secretion as well as antigen-presenting cell activity. Cianciulli *et al.* investigated the role of NF- κ B and iNOS in anti-inflammatory curcumin effects in BV-2 murine microglial cells, a specialised population of macrophages found in the central nervous system. Curcumin inhibited the release of NO and pro-inflammatory cytokines induced by LPS, as well as iNOS expression and NF- κ B activation. These anti-inflammatory effects have been shown to be mediated by iNOS, COX-2, HO-1, MAPK, and NF- κ B, implying that curcumin influences microglial cells through modulation of NF- κ B activity and thus plays an important role in the attenuation of inflammatory responses in the central nervous system. The activation of TLRs is required for the induction of NF- κ B in particular. TLR4 is the most studied member of the TLR family, and its critical role in immune system response regulation is well understood, as TLR4 receptor agonists have been approved as vaccine adjuvant. TLR4 activation causes MyD88 (myeloid differentiation factor) to be recruited, resulting in NF- κ B induction. Curcumin administration after TBI in mice resulted in less functional impairment, brain oedema, and reduced neuronal cell death, as well as a general reduction in microglia/macrophage activation, according to Zhu *et al.* Curcumin, in particular, normalized the LPS-induced up regulation of TLR4, MyD88, and NF- κ B in C57BL/6 mice with an induced TBI both in vivo and in vitro in a co-culture system of microglia and neurons. Curcumin also reduced the release of proinflammatory cytokines TNF-, IL-1, and IL-6 in rats after spinal cord injury (SCI) [78]. Curcumin also inhibited the inflammatory signalling pathways TLR4 and NF- κ B, reducing SCI-induced hind limb locomotion deficits, spinal cord oedema, and apoptosis Urdzikova *et al.* observed similar effects in a rat model of SCI, where curcumin, administered both intraperitoneally and in situ, reduced glial scar formation by lowering MIP1, IL-2, and Regulated on Activation, Normal T cell Expressed and Secreted (RANTES) production and NF- κ B activity. MIP1 and RANTES are members of the CC chemokine family, also known as CCL3 and CCL5, and are involved in the inflammatory response as well as immune cell recruitment and activation.

Curcumin has been shown in other studies to reduce the release of these (and other) chemokines, demonstrating the compound's ability to modulate the chemotaxis process in the immune response. Curcumin's anti-inflammatory properties have also been used to boost the efficacy of already approved antimicrobial agents through synergistic effects. Curcumin protected BALB/c mice from Klebsiella pneumoniae-induced lung inflammation, according to Bansal *et al.* In this study, mice given curcumin alone or in combination with augmentin had a significant reduction in neutrophil influx into the lungs, as well as a reduction in NO production, MPO activity, and TNF- levels. Curcumin's antiinflammatory properties were tested in an in vitro human macrophage model against



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Mycobacterium tuberculosis (MTB) infection and found to be partially mediated by both NF- κ B inhibition and caspase 3 activation. Overall, these findings suggest that curcumin may modulate molecular pathways involved in inflammation and immune response, implying that it could be used as a supplement or nutritional approach.

CONCLUSION

Medical plant active components have traditionally been a valuable source of clinical medicines due to their chemical variety, which is frequently connected with multi-pharmacological activity. Their qualities and health advantages have been extensively acknowledged in traditional medicine since ancient times. Due to constraints such as a lack of standardization of active components, qualitative and quantitative fluctuations in preparations, and a lack of rigorous effectiveness testing, the immunomodulatory effects of herbal treatments have been highlighted. Curcumin also has a number of drawbacks, including poor pharmacokinetic/pharmacodynamic properties, chemical instability, and a PAINS character. Even though it is acknowledged that microbial end toxins might change the parameters and response of the immune system, no suitable microbial contamination control techniques have been followed in many studies undertaken to investigate the influence of natural extracts on the immune system. Curcumin's therapeutic application is limited by its poor bioavailability and low solubility, despite its multi-target action and safety at larger dosages. The efficacy of a highly bioavailable version of curcumin in a natural turmeric matrix to treat the clinical symptoms of an autoimmune, inflammatory condition was investigated.

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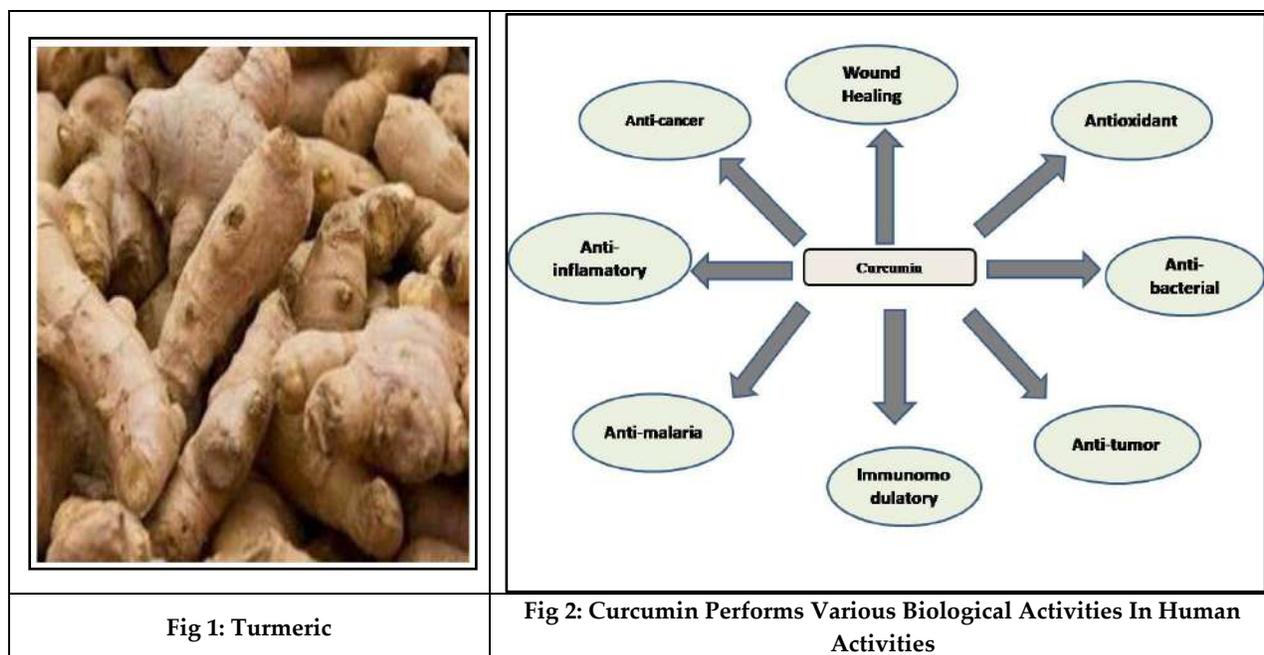
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Furunculosis

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ABSTRACT

Furunculosis is a serious, septicemic, bacterial disease found principally in salmonid fishes. but it may also occur in goldfish and other cyprinids. The common name of the disease is derived from the presence of “blisters” or furuncles on the surface of chronically infected salmonids (Snieszko and Bullock 1975). However, this sign is not diagnostic of this disease inasmuch as it may be encountered in fish infected with other pathogens. It should be pointed out that, in acute cases of furunculosis, the furuncles may not be present.

Keywords: Causative agent, Sign of infection, Diagnosis, Methods of control.

INTRODUCTION

The disease is caused by a Gram-negative bacterium, *Aeromonas salmonicida* described by Griffittin *et al.* (1953). It has recently been demonstrated (Paterson *et al.* 1980) that the ulcer disease attributed to *Hemophilus piscium* is, in fact, caused by a strain of *A. salmonicida*. Numerous reports in the literature describe the epizootiology and control of the disease (McCraw 1952; Herman 1968; and Bullock *et al.* 1971). Furunculosis is found worldwide with few exceptions and causes disease in many species of cold water and warm water fishes. In trout hatcheries in North America, it accounts for a high percentage of the fish losses attributable to infectious diseases. Furunculosis is highly contagious disease that affects fish of all ages. The disease is most commonly found in hatcheries where hatchery related stress factors provide a favourable environment for furunculosis to grow.





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Causative Agent

Aeromonas salmonicida is considered to be an obligate pathogen of fish the organism may survive for days or weeks in water but cannot persist indefinitely in the absence of carrier fish.

Sign Of Infection

Clinically-infected fingerlings will usually exhibit hemorrhages at the base of fins and erosion of the pectoral fins. Bloody or hemorrhagic vents and petechial hemorrhages on the ventral surface are frequently observed. In chronically infected adults, typical "furuncles" or blisters on the skin containing an amorphous yellow substance and blood may be present. This is rarely seen in small or fingerling fish since an acute infection frequently causes massive bacteremia and death before gross lesions develop (Snieszko and Bullock 1975).

Diagnosis

Positive diagnosis of furunculosis depends upon isolation and identification of the causative agent, *A. salmonicida*. The organism is typically a Gram-negative, non-motile rod that ferments selected carbohydrates, produces cytochrome oxidase, and produces a water-soluble brown pigment on several types of isolation agar. Care must be exercised, however, in the identification of non-motile cytochrome oxidase-positive, Gram-negative rods since a number of atypical and achromogenic variants have been reported (Elliot and Shotts 1980a; Paterson *et al.* 1980) in several species of fish. If atypical *A. salmonicida*, such as that encountered in ulcerative disease of goldfish is suspected, enriched isolation media may be required. Elliot and Shotts (1980a) reported that either chocolate agar or tryptic soy agar plus 5% defibrinated sheep's blood was required for adequate growth of isolates. Isolates from suspect variants of *A. salmonicida* should be given sufficient culture time to allow for those strains which slowly produce a brown, water soluble pigment to do so (Elliot and Shotts 1980b). In such cases, or when rapid identification is needed, the fluorescent antibody technique (FAT) may be used (McDaniel 1979). It is generally accepted that asymptomatic carriers are very difficult to detect. If it is necessary to establish the absence of carriers in a potential brood stock population, the use of serum agglutination techniques or corticosteroid techniques described by Bullock and Stuckey (1975) might be employed.

Modes Of Transmission

Transmission generally occurs as a result of contact with diseased or carrier fish, but can occur through water passed from one pond or raceway to another. Contaminated clothing or equipment may also transfer the disease from one culture unit to another. The possibility also exists that fish-eating birds may transfer the disease either by contact or by dropping infected fish into an uninfected pond (Snieszko and Bullock 1975). If eggs from carrier brood stocks are not disinfected prior to incubation, the organisms may be transferred on the surface of the eggs (Wood 1974). Japanese investigators have conducted studies that indicate *A. salmonicida* is not an invasive pathogen. According to their work, infection occurs experimentally only when the pathogen is ingested or has access to external injuries on the fish (Sakai 1979).

Incubation Period

The incubation period for acute cases of furunculosis is probably from 2-4 d. However, in chronic cases, particularly at lower temperatures, the period may be extended by several weeks (Groberg *et al.* 1978). Furunculosis is usually seasonal with the highest incidence of disease during the midsummer months of July and August. Incidence is related to temperature; the disease is most prevalent in the range from 12.8°C (55°F) to 21.1°C (70°F). At low temperatures, chronic furunculosis has been observed in landlocked salmon cultured in the Adirondack Mountains of New York at water temperatures of 0.5°C (33°F) to 1.6°C (35°F).

Prevention

A basic step in the prevention of serious communicable fish diseases is the adherence to a sound program of hatchery inspections and a disease classification system. As a minimum, all lots of fish in a hatchery should be inspected at least once per year for the presence of disease. Utilizing the data generated from these inspections,



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transfers of suspect or known carrier fish from hatchery to hatchery should be avoided. All eggs from susceptible species should be routinely disinfected using organic iodine compounds at 100 ppm of active iodine for 10 min (Amend 1974) on water hardened eggs. The hatchery water supply should be kept free of fish. Barriers should be provided to prevent the introduction of potential wild carrier fish into the hatchery. Resistant strains of fish should be utilized as a disease management tool where appropriate. If eggs must be imported from outside of the hatchery system, insist that only eggs supplied from inspected and certified furunculosis-free sources be used.

Antibiotics Used Treatment Of Disease

Epizootics of the disease may be treated through the addition of drugs to the fish feed. Terramycin (oxytetracycline) should be added to feed at the rate of 3.0g/100 lb fish, administered daily for 10 d to affected fish. Sulfamerazine should be administered at the rate of 5-10 g/100 lb fish and fed for 10 or 15 consecutive days .

CONCLUSION

Furunculosis research encompasses the contribution of workers in a number of different discipline. Research without money is impossible. Therefore the amount of research funding available and the research targets at which it is directed will remain important. For some years research funding for furunculosis studies has been related to the importance of the disease in commercial salmon farming. The recent development and application of oil-based vaccines has had a major impact on the way in which furunculosis is perceived by the industry. The idea that is currently dominate, with in this industry, is that furunculosis is under control and that the loses it currently causes to commercial farmers are acceptable.

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The Versatile Importance of Intercropping System in Agriculture: A Review

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ABSTRACT

Cropping system is of great importance than managing the crops individually in a system. The modern agricultural practices based on sequential cropping systems are supply driven technologies that greatly depend on exogenous supply of essential inputs. On the other hand, intercropping systems are ecologically sound, less demanding to external inputs and address agricultural sustainability. The intercropping systems have enough potential to address the issues related to sustainable agriculture such as ecofriendly nature, diversified, more effective in resources utilization and maximization of crops productivity. An Attempt has been made in the article to highlight the multiple benefits of an intercropping system.

Keywords: Intercropping system, multiple benefits, sustainable agriculture, ecofriendly agriculture

INTRODUCTION

The growth of crops is a natural process in which critical biotic factors such as water, sunlight and carbon dioxide hold special objectives. The inclusive effect of these critical elements constitutes the growth of crops. There are different systems in which crops are cultivated to obtain the desired yield (Laik *et al.*, 2021; Maitra *et al.*, 2001a). The impact and importance of different cropping systems vary as per the climatic conditions of the region. Agriculture has its own practice of cropping system in fertile lands that focuses both on enhancement and achievement of crop yields (Maitra and Shankar, 2019; Maitra and Ray, 2019; Maitra, 2020a). Intercropping is a system of cultivating two or more crop in the same piece of land at the same time. The variability in inter-cropping can result in higher yields than crops grown as sole crops (Usmani *et al.*, 2012). The combined cultivation of two or more crops has a lot of enchanting effects on the final yield and efficiency outputs (Maitra *et al.*, 2021). Intercropping has to be a wider part of adaptability with greater implementation strategies. Maitra *et al.* (2001b) found that the net returns were greater when castor was intercropped with cluster bean and cucumber. The motivation to farmers about intercropping has



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to be focused to extreme levels for popularizing the system of intercropping. In intercropping, two or more crop species can be cultivated simultaneously as they act as companion part of the crop cycle and show both inter and intra relationship nature with the agro-ecosystem (Maitra *et al.*, 2020).

The combination of crops in an intercropping system enables a greater benefit to the agricultural productivity in crop fields. This practice of cropping insures the farmers income when implemented with accurate strategies. The biological and physiological benefits to the soil can be better enhanced with the practices of intercropping. Panda *et al.* (2021) found that rice + groundnut or rice + green gram intercropping systems yielded more than pure stands of the individual crops. A balance in the soil structure, soil fertility and nutrient availability in the soil conditions, focus for the shift of mono cropping to intercropping. The shout for soil conservation can be lauded with the practice of intercropping. The maximum yield was produced in rice-wheat-green manuring intercropping system than sole cropping of rice and wheat (Lithourgidis *et al.*, 2011). The multifaceted benefits are observed in the intercropping system than pure stands of individual crops (Sarath Kumar and Maitra, 2020; Maitra and Gitari, 2020; Panda *et al.*, 2021). The resource-poor farmers prefer intercropping system for their food and nutritional security under sub-optimal conditions (Maitra *et al.*, 1999; Sarkar *et al.*, 2000; Maitra *et al.*, 2018).

Farmers in India are less influenced with the beneficial effects of intercropping, hence a move or approach towards it is the prime factor for the future. There are different types of intercropping systems that are commonly accepted in different nations (Ofori *et al.*, 1987). The sustainability in food requirements can be fulfilled only with the adoption of intercropping strategy as a major plan. Willey (1979) reported highest rice average productivity in rice-potato + onion, mustard + black gram intercropping system. The focus of mono cropping and dependence on profit should be now altered with the practices of intercropping. In intercropping, two or more crop species are grown at the same time as they complement the crop cycle and interact among themselves and agro-ecosystem (Manasa *et al.*, 2018). The beneficial effects on soil, water and other available resources have to be represented in a very detailed view to highlight the importance of intercropping. Intercropping needs a specific planning to grow crops. Nasri, (2014) revealed that rice-potato-onion intercropping + maize relay cropping produced the highest mean yield. The land utilization ratio should be focused carefully to obtain a good efficiency of land and available resources. Intercropping of cereals with shade providing plantation also allows additional yield and highest profit followed by better land use efficiencies (Ahmed *et al.*, 2018). Intercropping involves growing of shallow-rooted crops with deep-rooted crops, tall crops with a short plant, shade-loving plants with light-requiring plants, early-maturing crops with late-maturing crops, etc... thereby creating an interesting arena of cultivation and an profit orienting path for the farmers. Singh, (2008) revealed that rice-potato-green gram sequence was found the most efficient for production with better employment generation and monetary return and water-use efficiency.

The biological activity of different microbes in the soil gets a positive growth branch as intercropping involves the usage of legume crops. The nutrient exhausting percentage from the soil gets recovered due to the N fixing ability of legume crops. The essential micro and macro nutrients can be maintained in the field when intercropping system of farming is practiced over a longer period of time. Intercropping systems describe different competition features under different crop communities, including competitive effects, intensity of competition, and outcome of competition (Raza *et al.*, 2019). The ratio of sowing crops gets an appreciated modification in intercropping farming method and with this the profit of farming also gets doubled. To develop an intercropping system, many elements must be considered both before and during the growing period (Maitra, 2018). The cost of cultivation also gets divided among three crop which was spent only on one crop cultivation in mono cropping system. The share of quantity increases which directly decreases the input expenditure in the farm. The profit from three crops usually varies with the market demand thereby providing the farmer with a good income. Africa implemented certain projects on legume cultivations as an intercrop or in rotation to reduce the use of foreign inputs and improve soil fertility (Sanginga and Woomer, 2009; Palai *et al.*, 2021; Jena *et al.*, 2022). There has been an additive benefit of intercropping like it helps in maintain the stability in resource utilization and providing with an aggregate output. The strategy of weed control and weed suppressing is a critical factor to avoid a competitive effect between crop but the burden of allied management decreases to a maximum extent. The alertness on competition effect between two



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crops should always be avoided to get the maximum resource utilization efficiency and receive a better crop yield. Bhadoria *et al.*, (2002) reported that intercropping systems expressed higher production efficiency, better water-use efficiency and energy intensiveness in economic terms.

The profit from cultivation is always the prime objective of any farmer. The cultivation strategy has always been an old age and traditional process that has led to diminishing effects in crop fields. The trend of mono cropping needs a sheer shift to enhance the profitability of farming. India is prone to natural calamities all around the year; thereby the dependence on single cropping system has been a demotivating plan. Intercropping is more productive than traditional cereal-based cropping methods (Ahmed *et al.*, 2018). When a single crop is cultivated in the field then the consumption and utilization of available resources is higher than the system of intercropping. The efficiency also gets decreased with the current trend of monotonous cropping plans. The efficiency index of cropping increases with intercropping system. Farmers remain vulnerable to different problematic conditions in mono cropping system whereas the percentage of dependence decreases in case of intercropping system. Li *et al.*, (2021) reported that grain yields in intercropped systems were 22% greater than monocultures and had greater year-to-year stability. The yield benefits of intercropping also increased with time thereby suggesting that intercropping may increase soil fertility via observed increases in soil organic matter, total nitrogen and macro-aggregates.

The profit quotients and terminologies get summed up with intercropping system of farming. Intercropping allows the economic and yield advantage choice for farmers. Mandal *et al.* (2014) also observed that rice – potato – green gram and rice-onion sequences produced higher production efficiency, higher land-use efficiency and better employment-generation efficiency due to intensification of this system. The knowledge of intercropping enables productive advantages to the crop grower. The importance of different crops can be realized. The pattern of crop sowing also gets modified and improved with intercropping. Maitra(2020b) reported that the highest net return of Rs 96,581/ha/year was produced with rice-garlic-maize intercropping which was at par with that of rice-potato-onion + maize relay cropping (Rs 92,837 /ha/year).

CONCLUSION

The dependence on yield and profit gets decreased by higher percentages with cultivation of different number of crop simultaneously. Kruallee *et al.* (2021) found that the intercropping sequences like rice-maize- significantly produced higher rice-equivalent yield and gross returns. The net returns and benefit: cost ratio were also higher in rice-maize- sunflower intercropping sequences. This proximity cultivation helps in enhancing the income generation from crop fields. The income to expenditure ratio upholds a positive shift and climb for farmers. Intercropping enables a mutual exchange of resources that fulfills the nutrient demands of companion crops. Panda *et al.*, (2021) also reported that the returns per Re invested were the highest for rice-field pea-sesame system followed by rice-maize-cowpea and rice-maize-green gram systems. The reduced use of chemicals and fertilizers also acts as a boon to the soil conditions in the farm land. Gitari *et al.* (2020) revealed that intercropping is more profitable than sole cropping in Kenya. The rice-potato-Japanese mint and rice-potato-onion crop sequences are more productive and economically viable as they also fetched more net returns per unit area for the time invested. This also acts as an economic benefit to the farmers and thereby, increasing their profit from farming.

There are several advantages of intercropping system such as greater utilization of resources, yield enhancement, monetary advantages and superior ecosystem services over sole cropping. The functional diversity created in an intercropping system is also beneficial for crop protection, soil fertility improvement and diversification of agriculture. The article suggests the adoption of intercropping system to harness the multifaceted benefits of intercropping system under the changing climatic conditions for agricultural sustainability.





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AquaCrop and SIMDualKc Crop Models for Irrigation Scheduling – A Review

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ABSTRACT

It is most important to assess the yield estimation, by determining the precise amount of water required for evaporation from soil (E) and transpiration from the plants (T). The accurate and user friendly method is most desirable for estimation of Evapotranspiration (ET). This study aims to augment the information on two important ET models in terms of feasibility and accuracy. AquaCrop and SIMDualKc models used to estimate accurate ET of large number of crops. These models are able to predict the yield for different irrigation scenario to determine the irrigation schedule. Different aspects of the models are discussed in the manuscript.

Keywords: crop models, Aqua Crop, simulation,

INTRODUCTION

The crop development models are necessary to evaluate the response of crop yield and WUE to different irrigation levels under limiting water conditions (Lee et al., 2009). The generalization and simplification of the real systems are defined as simulation models (Zaman and Maitra, 2017). Crop models encompass huge components and processes interacting with a range of agricultural inputs. The crop models are useful for scientific studies mainly to understand the results of field experiments and as agronomic research tools for research knowledge purpose. Extensive and field experiments involve high expenditure with huge treatments can be estimated using a well-established model. Optimum management strategy such as date of planting, selection of cultivar, soil nutrients, water, and pesticides usage, can be estimated through well-established simulation models for making crop-related decisions. Other benefits, such as planning and policy analysis, can benefit from modeling as well. The modeling approach can be distinguished as scientific and engineering based on the purpose and objectives of the crop model. The scientific method is mainly focusing on the crop behavior, crop physiology, and crop responses to environmental changes.





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This approach is working based on proven laws and system functioning theories. The engineering approach attempts to advise farmers with better management practices and provide better predictions for policymakers. This approach is combinations of well-established theories and proved empirical relationships. The present study aims to gather information on two crop models i.e. AquaCrop and SIMDualKc Models normally used to estimate the Evaporation (E) and Transpiration (T) as individual components of evapotranspiration (ET). The current study also discusses the model applicability and its limitations.

AquaCrop Model

The AquaCrop is the recently introduced international crop model which mainly focuses on crop growth driven by water and this model developed by FAO (Steduto et al., 2009, Raes et al., 2009). AquaCrop model can simulate attainable yields of the major crops in different irrigation scenarios. This model predicts effective strategies to manage water resource for agriculture. The AquaCrop model is based on an engineering approach aiming to simulate achievable crop biomass and marketable yield with respect to the water applied. Models recreate the processes in the real system and estimate variables at regular time interval in the simulation. AquaCrop model can suggest irrigation recommendations to achieve higher WUE by evaluating water management techniques (Ines et al., 2001). However, AquaCrop model requires calibration and validation for individual crops with local soils and climate. AquaCrop requires less numbers of parameters to simulate and give accurate output with less rigorous procedures as compared to other crop models, The model considers crop growth relation to water in which transpiration is likely to convert into biomass. Whereas other models such as CropSyst and WOFOST are based on radiation and carbon drove modules respectively (Todorovic et al., 2009).

AquaCrop model effectively separates the i) Crop evapotranspiration (ET_c) into evaporation component (E) and crop transpiration component (T) and ii) yield (Y) into biomass (B) and harvest index (HI). The model replaces the simulation of leaf area index (LAI) with the simulation of canopy cover (CC) (Duchemin et al., 2008). Therefore the CC must be monitored at regular interval at the field. The data required to run the AquaCrop model are crop, type of soil, irrigation management and initial soil water condition as input files (Raes et al., 2009). There are 23 locations and cultivar dependent parameters stored as user-predefined parameters. Equation for response of yield to water applied was developed by Doorenbos and Kassam (1986) and is given by Equation 2.1.

$$\left(\frac{Y_x - Y_a}{Y_x}\right) = K_y \left(\frac{ET_x - ET_a}{ET_x}\right) \quad \text{--- (2.1)}$$

where Y_x and Y_a are the maximum and actual yield; ET_x and ET_a are the maximum and actual evapotranspiration, respectively and K_y is the crop yield response factor. The separation of ET into T and E helps to quantify non-productive consumptive use of water (E), especially during incomplete canopy cover. This separation led to the conceptual equation at the core of the AquaCrop growth engine (Equation 2.2)

$$B = WP \cdot \sum \left(\frac{T_a}{ET_0}\right) \quad \text{---(2.2)}$$

where WP is water productivity, which is constant for a given climatic condition (Steduto et al., 2007), T_a is the actual transpiration. The relationship is used seasonally for equation 2.1, while for equation 2.2 the relationship considers daily time steps (Steduto, 2003).

AquaCrop model mainly emphasizes on irrigation, also includes fertility of the soil, developmental of crops, and water productivity. The crop adjustment requires compensating for different stresses and attainable yield. The effect of pests and disease attack are not considered in the model. AquaCrop incorporates procedures to estimates infiltration of water, deep percolation, evaporation rate, transpiration rate, biomass production, and yield formation. The model simulates water content in the crop root zone depth and computes net irrigation requirement of the crop (Raes et al., 2009). AquaCrop model simulates the accurate output of soil moisture, biomass, and yield (Hsiao et al.,



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2009, Heng et al., 2009, Araya et al., 2010, Paredes et al., 2014, Tavakoli et al., 2015, Toumi et al., 2016). AquaCrop can also be used to simulate different crop sequences by analyzing future climate scenarios.

SIMDualKc model

The crop evapotranspiration (ET_c) estimated by the dual K_c approach more accurate compares to single K_c approach (Allen et al., 2005). The dual K_c methodology was applied first time on cotton in a study comparing with several methods used to estimate ET_c in Turkey (Liu and Pereira, 2000), later it was applied to the maize crop in the North China Plain. Other studies conducted on cotton by Tolk and Howell et al. (2001), who confirmed the accuracy of dual K_c approach and also advocated its usefulness in deficit irrigation. Er-Raki et al. (2007) found accurate dual K_c estimates for full cover crops like wheat in a Mediterranean environment. Few studies reported appropriateness of using this approach in orchards crops, especially for partial cover crops (Goodwin et al., 2006; PaÁo et al., 2006). The procedure to compute E and T by the dual K_c approach as suggested by FAO-56 works well under drip irrigation in which water is applied on a particular place of the soil surface from where it spreads radially while infiltrating down into the soil. Above studies demand suitable applications to adopt the dual K_c approach for various irrigation scenarios.

There are many models such as Shuttleworth-Wallace, ENWATBAL, Cupid-DPEVAP, SWEAT, TSEB, and HYDRUS-1D available for estimating E and T separately for ET. Above listed models are complex and need huge input parameters, which will be difficult to measure on regular basis. The SIMDualKc is used to compute ET_c and to schedule irrigation with dual crop coefficient approach (Allen et al., 1998; Allen and Tasumi, 2005) by computing daily soil water balance parameters at the field scale. This model can be used for frequent interval irrigations and for limited cover crops. Rosa et al. (2012) suggested the calibration and validation procedure.

SIMDualKc model was developed to operate alone, it includes all the computational procedures which can be integrated with other irrigation models (i.e., WINSAREG) to adopt the dual K_c approach. However, SIMDualKc model may be used for real-time irrigation scheduling and also for simulating the soil water content in different depth of soil. The SIMDualKc model was developed using three tiers architecture. Thus, three different components, i.e., a graphical user interface (GUI), a mathematical model, and a database included in the model.

The study showed that the SIMDualKc and AquaCrop models performed well with their simulation of ET and T under full irrigation conditions. Under the deficit irrigation scenario, the ET and T components simulated by the AquaCrop model were much wider to the actual measurement when compared with the results simulated by the SIMDualKc model. For the simulated E, however, both SIMDualKc and AquaCrop models generated data that were distant from the actual measurements taken under full or deficit irrigation conditions using plastic film-mulch, although when the SIMDualKc model simulated E data, it came closer to the measurement than did the AquaCrop model. Some researchers compared both these AquaCrop and SIMDualKc models (Paredes *et al.*, 2015, Pereira *et al.*, 2015), for estimating crop water requirement, standardize irrigation schedule and compute soil water balance components. However, these researches were carried out particularly for Mediterranean climatic conditions.

CONCLUSION

The AquaCrop model worked on biomass and the yield relationship with the ET. The SIMDualKc model estimates the ET and provides the information on water balance. Both the models estimate E and T components separately which may help to estimate water requirement for mulched conditions. These models help to select the appropriate water management option for local weather conditions. The ET values generated from the AquaCrop model are less accurate in comparison to SIMDualKc model. However we can choose the models based on the input data availability.





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Role of Biofertilizers in Nutrient Management of Cereals

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ABSTRACT

In developing countries agriculture plays an important role in countries economy. The current alarming rate of reducing soil fertility and soil degradation has become a dilemma for future agriculture sustainability and productivity. The chemical deposition to the soil in the form of synthetic fertilizers has become a huge pollution factor in agriculture. But on the other hand, without proper nutrient management the productivity of the crops cannot be attained. In this regards biofertilizers can be an alternative for chemical fertilizers. Biofertilizers are the bio inoculants of bacteria, fungi and other microorganisms which are present in the soil and can supply nutrients to the plants through fixation or mobilization of primary and secondary nutrients. Biofertilizers consists of living cells, which are applied to soil, seed or seedlings for maximizing nutrient availability and nutrient uptake from soil. Use of biofertilizers has currently became a cost effective and eco-friendly alternative to chemical fertilizers. Substantial development has been achieved recently in development of effective biofertilizers for different crops.

Keywords: Biofertilizer, Nitrogen, phosphorous, Sulphur, Yield, Nutrient.

INTRODUCTION

Population of India is increasing in a regular manner and for providing food to the growing population we have to increase the productivity of crop. As a result, farmers are using more chemical fertilizer to increase productivity which ultimately affected the soil quality leads to several agricultural problems (Duary *et al.*, 2021). Nitrogen which has a large importance in mineral nutrients for plants which influences the growth and development of plants along with that yield and protein content of the grains also increases. But more usage of the chemical fertilisers has a negative effect on soil health and climate therefore the sustainable soil fertility and crop yield can be achieved through integrated and a well-balanced application of biological, chemical, and organic fertilisers can plays a significant function. Microorganisms present in the biofertilizers serves as alternate towards the fertilizers and it is less cost compared with chemical fertilizer (Ramya *et al.*, 2020). Biofertilizers are the compounds, containing special

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beneficial cells of different efficient microorganism and it also helps to increase the availability of various plant nutrients in the rhizosphere zone by their activity (Mohammadi *et al.*,2012). In other words, the substance which consists of useful microorganisms is known as biofertilizer (Maitra *et al.*,2021). Application of biofertilizer helps to promote the plant growth by increasing available nutrients in the soil (Yadav *et al.*,2019). It being the low cost, effective and environmentally friendly and also improves the crop productivity. Bacteria that fix nitrogen, such as azospirillum and fungi which improve the growth of plant by uptake of relatively immobile nutrients such as p, Zn, cu etc. and also plays a key function in the biological control of root pathogen and has greater resistance towards the water stress (Gill *et al.*,2016). Microorganisms interact with each other and shows the synergistic effect in producing even good result as compare to separate application(Maitra *et al.*, 2021). Azatobacter is a non-symbiotic bio fertiliser that delivers roughly 20-25 kg nitrogen per hectare to crops such as wheat, maize, cotton, and other crops that are grown in favourable conditions. Phosphorous solubilizing bacteria increases the yield up to 20 percent (Patra *et al.*,2019).In these regards biofertilizers can play a major role in nutrient balance in soil and there by maintaining soil health.

Types of biofertilizers

Nitrogen-fixing microbes

The nitrogen fixing microorganisms are mostly belong to the family Rhizobiaceae. These family consists of genes such as *Rhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Sinorhizobium*. These genes are best known for symbiotic nitrogen fixing and they function from the plant root nodules in most of the leguminous crops (Mishra and Dadhick, 2010; Palai *et al.*, 2021). *Rhizobium* present in the root nodules, fixes atmospheric nitrogen in leguminous plants. These organisms use nitrogenase enzyme for fixing atmospheric nitrogen in to the soil.

Phosphorus solubilizing micro-organisms

Phosphorus is a macronutrient whose scarcity severely restricts plant growth and productivity. The majority of the time, phosphorus is present in high concentrations in the soil as phosphate, which can be in either an organic or inorganic form (Itelima *et al.*, 2018). The majority of inorganic phosphate is immobilised in insoluble salts, so only a small fraction of it is available to the biosphere in the soil solution. Phosphorus solubilization involves local acidification or alkalization, as seen in some *Pseudomonas*, *Cyanobacteria*, and *Bacillus* species which are isolated from plant rhizosphere.

Potassium solubilizing microbes

Potassium is an important macronutrient that regulates the activities of many enzymes, including amylases (starch-degrading enzymes), which are involved in the coordination of root-shoot ratio. Potassium deficiency causes poor root development, increased susceptibility to pathogens, and decreased plant growth and yield (Chand *et al.*, 2014). In different studies, a large number of potassium solubilizing microorganisms have been found in the soil. Some bacteria, such as *Bacillus mucilaginous*, *Azotobacter chroococcum*, and *Rhizobium* spp., have been reported to increase maize, chili, cotton, sugarcane, sorghum, and wheat productivity through potassium solubilization (Fasusi *et al.*, 2021). Inoculation of wheat plants with a potassium-solubilizing strain of *Bacillus edaphicus* resulted in a significant increase in root and shoot growth when compared to uninoculated plants (Basuet *et al.*, 2018).

Sulphur oxidizing microbes

Sulphur is an essential secondary nutrient that plants require in large amounts. Sulphur is a part of amino acids like cysteine, cystine, and methionine, as well as enzymes like superoxide dismutase, ascorbate peroxidase, dehydroascorbate reductase, monodehydroascorbate reductase and glutathione reductase (Etesami *et al.*, 2017). Plants with sulphur deficiency have a reduced nitrogen metabolism, which results in chlorosis, a low lipid percentage, and reduced plant growth and yield. Sulphur exists in two forms in the soil: organic and inorganic, though the inorganic form (i.e., SO₄²⁻) is primarily absorbed by plants. Sulphur-oxidizing microbes belonging to the genera *Xanthobacter*, *Alcaligenes*, *Bacillus*, and *Pseudomonas* can convert organic sulphur into inorganic sulphur forms (Sangeeth *et al.*,2012; Saha *et al.*, 2018). Some recent studies are recommending that, the application of sulphur-oxidizing microorganisms in the formulation of biofertilizer for maize, wheat, onion, oats, ginger, grape, garlic, and



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cauliflower under alkaline soil conditions can improve the yields and potassium uptake of crops (Pourbabae et al., 2020).

Functions of biofertilizers in crop production

Soil microbes are important in controlling the dynamics of organic matter decomposition and the availability of plant nutrients like N, P, K and S. Microbial inoculants are widely acknowledged as an important component of integrated nutrient management, which leads to sustainable agriculture (Williams et al., 2016). Furthermore, microbial inoculants can be used as a cost-effective way to boost crop productivity, reduce fertiliser usage, and maintains soil nutrient balance by maintaining productivity and quality and promoting nutritionally supplied plants with lower production costs. Nitrogen-fixing microorganisms help to increase yield by converting atmospheric nitrogen into organic forms that plants can use. Rhizobia and legumes are mutually associated, and nitrogen fixation takes place in the bacterium's root or stem nodules. Rhizobium inoculation improves root nodulation, plant growth, and grain yield by 10-15% in cultivated conditions when compared to a crop that has not been inoculated (Singh, 2006). Annual legumes have been reported to fix 35-270 kg of nitrogen per hectare per year. Alcaligenes, Azospirillum, Bacillus, Herbaspirillum, Klebsiella, Pseudomonas, and Rhizobium species are likely candidates for biological N fixation in rice. Rhizobium enters the root hairs, multiplies, and forms root nodules due to its resistance to extreme temperatures. According to multiple studies, bio-fertilizers play a vital role in improving soil fertility, crop yield, and final yield. Furthermore, their use in soil improves soil biota while reducing the use of chemical fertilisers. Bio fertilisers increase the availability of nutrients that promote the development of biological activities in soils, which facilitates plant health (Saint Pierre et al., 2008). This is aided by the addition of balanced nutrients that provide food and growth for microorganisms, as well as the presence of beneficial soil worms. Root growth and organic matter in the soil are improved as a result of good soil structure. The application of bio fertilisers has a significant impact on mycorrhizal development, which in turn is responsible for the soil's high phosphorus content.

Role of biofertilizers in nutrient management

Biofertilizer enhances the plant growth and quality of plants and it also increases the yield by different types like nitrogen fixation, mobilization of phosphorous, solubilisation of k and micro nutrient solubilisation, it also promotes the plant growth and reduces the soil organic matter depletion in the soil (Silpa et al., 2021). It maintains good ecological balance in the soil.

Role of biofertilizers on growth, yield and nutrient uptake

Bio fertilizers have been used to contribute to soil fertility in a positive way, allowing farmers to increase their output without negatively impacting the environment, soil or water. Bacteria play an important role in nitrogen mobilisation and phosphorus solubilisation for improved plant growth by fixing nitrogen and phosphorus. (Sadaf et al., 2017). According to the study, Azotobacter inoculation resulted in a 72.03 percent increase in plant growth, dry matter accumulation and nitrogen uptake over the control at 80 DAS, which was comparable to the addition of 20 kg N ha⁻¹ alone (Parewa et al., 2014). In terms of altering the nitrogen content in straw and grain, Azotobacter alone and 20 kg N ha⁻¹ were statistically equal (Tarafdar and Rao 1997). Vessey, (2006) stated that the yields of straw, grain, and total yield was increased by inoculation of PSB alone and enhanced phosphorus uptake by around 37.97, percent above the control. Ritika and Utpal, (2014) reported that inoculating wheat with a 1:1 mixture of Azotobacter and Azospirillum increased nitrogen utilisation efficiency. Wheat grain nitrogen content increased as nitrogen application rates increased. Similarly kedar et.al In wheat, the treatment containing 168 kg N ha⁻¹ + cow dung + Azotobacter had the maximum N uptake (23.2 mg plant⁻¹), while the control had the lowest (11.03 mg plant⁻¹). The High as well as quality and stable yield can be attained by applying the proper combination of organic and inorganic fertilizers (Guttieri et al., 2005). We can understand by several studies that the integrated nutrient management improves the yield and the yield traits of the cereal crops (Zaidi et al., 2005). The combination of chemical fertilizer and bio inoculants shows effective use in availability of nutrients in cereals than the solitary application of chemical fertilizer (Suri et al., 2010). Similarly through the combination of organic and inorganic fertilizer gives more yield than the solitary application of chemical fertilizer. (Nosheen et al., 2021). Through several developed research works it is found that plant height, weight of 1000 grains, LAI, and yield of cereals like maize is increased by combined



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application of *Azotobacter* with 60kg N\ha of inorganic fertilizer gives better results than the sole application of each fertilizer (Singh *et al.*,2016). Through combined application of fertilizers like 50% of NPK with poultry manure and fertilizers (*Azospirillum* +phosphorous solubilising bacteria) will give better results like taller plants (183.1 cm) and higher LAI (3.47) (Maçik *et al.*, 2020).

Advantages of biofertilizers

Bio fertiliser increases crop yield by 20-30%, replaces chemical nitrogen and phosphorus by 25%, and stimulates plant growth. As a result, it can be used in addition to chemical fertilisers. It is less expensive than chemical fertilisers and has low manufacturing costs, particularly when N and P are used (Ramya *et al.*, 2020). Organic fertilisers have been shown to increase soil biodiversity and long-term productivity, as well as serve as a large carbon sink. It greatly enhances the number of soil organisms like fungal mycorrhiza, which aid plants in nutrient absorption. Biofertilizers increases soil porosity, water holding capacity, and secretes growth-promoting substances. It promotes seed germination, increases soil fertility and nutrient use efficiency there by resulting in higher crop yields.

Limitations in application of biofertilizers

The availability of biofertilizers are not much when compared with organic manures and chemical fertilizers. They are susceptible to biotic and abiotic stress and those biofertilizers which are performed well under lab conditions may not work under field conditions. Also, biofertilizers acts as slow supplier than compared with synthetic fertilizers. Biofertilizers contains living microbial cells with a short shelf life, and their storage and transportation necessarily imply extra caution and care which results in raising the cost of the fertilizers and their unavailability to remote locations. The farmers are also not aware of the dosage and application of biofertilizers due to their inexperience and more preference to chemical fertilizers.

CONCLUSION

Biofertilizers can created a greater impact on soil quality as well as soil fertility which results in increasing crop productivity without any risks to the water and soil environment. Biofertilizer can provide ecologically sound and economically attractive source for providing nutrient to the plant. They act as a supplementary, renewable and eco-friendly plant nutrient source. Biofertilizer will help to minimize the utilization of injudicious use of chemical fertilizer in growing world and also reduce the risks associated with the excessive use of inorganic chemical fertilizers. Therefore, biofertilizers can play a vital role in modern day crop production and there by emphasizes significant importance of biological inoculants in upcoming years.

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Table 1. Biofertilizer recommendation for crops

Biofertilizer	Recommended crops
Rhizobium	Pulses, fodders , oil seeds,
Azospirillum	Rice, millets, maize, sorghum, sugarcane.
Azatobactor	Rice, vegetables, sunflower, mustard, cotton, wheat, flowers.
Azolla	Submerged rice
Blue green algae	Rice in submerged condition
Phosphorous solubilising microorganisms	Suitable for all types of crops

(Rajesha and Ray, 2020).

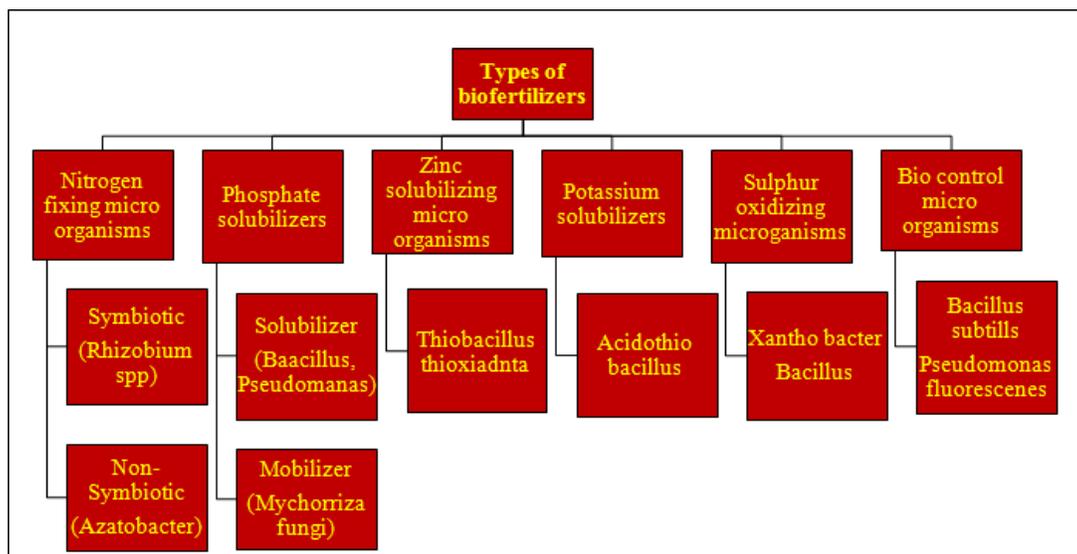


Figure 1. Types of biofertilizers





Integrated Nutrient Management in Rice (*Oryza sativa* L.): A Critical Review

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ABSTRACT

Rice (*Oryza sativa* L.) is one of the most important staple food crops in both India and the world. In India, rice covers one-fourth of total cropped area and more than 40 per cent of food grain production. The high yielding cultivars require more nutrients and generally these are supplied through inorganic fertilizers which results in deterioration of soil fertility. As rice is the major nutrient draining crop, there will be a huge deficit in the soil nutrients in rice based cropping system. To overcome the problem and maintain soil fertility, there is need for integration of nutrients from organic and inorganic sources which can help in obtaining good crop yields as well as the production sustainability. The combined use of inorganic fertilizers along with organic sources like FYM and vermicompost can improve the soil health and also helps in proper growth and productivity of rice. The Integrated Nutrient Management (INM) is extremely essential in rice production. Use of chemical fertilizers along with organic manures has been found promising in rice cultivation by arresting the declining trend in soil-health and productivity through the correction of marginal deficiencies of some primary, secondary and micro-nutrients.

Keywords: INM, Rice, FYM, Vermicompost, Inorganic fertilizers

INTRODUCTION

Globally rice (*Oryza sativa* L.) is one most important staple cereal after wheat and maize in India, the total cultivated area under rice (43.8 M ha), production (163.7 mt) and productivity of 2.78 t/ha (Agriculture Statistics at a Glance, 2018). In India, rice covers one-fourth of total cropped area and more than 40 per cent of food grain production. The HYV rice generally required more amount of nutrients with organic and inorganic which improve soil fertility (Imade *et al.*, 2017; Pattanayak *et al.*, 2022). Production sustainability in rice farming can be achieved by maintaining and management of soil fertility with INM. The INM supplies nutrients to crop and improves soil health (Mohanarao *et al.*, 2017). In rice based cropping system, continuous cultivation of rice with only application of primary nutrients through chemicals imposes depletion of soil fertility and multi-nutrient deficiency leading to poor productivity



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(Imade *et al.*, 2017; Yin *et al.*, 2018). Intensive agriculture with high yielding varieties cause heavy removal of nutrients from the soil and non-judicious use of primary nutrients degrades soil health (Singh and Maiti, 2016; Harikesh *et al.*, 2017; Mohanta *et al.*, 2021). The adoption of integrated nutrient management is essential to produce sustainable yields and crops of global standards in terms of quantity and quality (Shankar *et al.*, 2020).

In Odisha like other states of India, rice is cultivated as main staple crop. Improper management of nutrients has led with excess nutrients to nutritional imbalances in the soil, although other nutrients have been decreased (Maurya *et al.*, 2019). The declining trend of rice productivity can be traced to inappropriate and inefficient use of nutrients. The Integrated Nutrient Management (INM) is extremely essential in rice production (Upadhyay *et al.*, 2011). Manures like FYM, vermicompost, poultry manure and green manure can be substituted with the inorganic fertilizers for balanced and continuous supplement of nutrients for the crop (Pandit *et al.*, 2020). Nitrogen is an important primary nutrient that promotes growth and development, and also affects other nutrient availability. Nitrogen is one of the critical inputs in the irrigated ecosystem which limits rice productivity. The processing of 15 to 20 kg of grain requires around 1 kg of nitrogen, but the efficiency of nitrogen usage in India is very poor (Zhang *et al.*, 2012).

While fertiliser usage promises increased productivity. On the other hand indiscriminate and imbalanced usages of fertilizers affect productivity, soil health, and the environment. Agriculture is also focussed on establishing environmentally sustainable resource management activities. The farmers use many organic sources of nutrients that have varying nutrient levels (Sahu and Chaubey 2020; Mangaraj *et al.*, 2022). Therefore, the various sources of organic manures need to be tested for facilitating productivity in order to standardise the recommendation to the farmers. Nutrient use efficiency (NUE) is a critically important concept in the evaluation of crop production systems. It can be greatly impacted by fertilizer management as well as by soil- and plant-water management (Fixen *et al.*, 2014; Das *et al.*, 2021) (Kakraliya *et al.*, 2017). Significant efforts have been made to economise the use of fertilizers in field crops through application of bio-fertilizer, vermicompost and farmyard manure (FYM) (Malika *et al.*, 2015; Sairam *et al.*, 2020a, b; Maitra *et al.*, 2022) in rice production. The article addresses some of the Sustainable Development Goals (SDGs) such as SDG 1 (no poverty), SDG 2 (zero hunger), SDG 3 (good health and wellbeing) and SDG 15 (life on land) (UN, 2021).

Effect of integrated nutrient management on growth parameters of rice**Plant height**

Application of 50% NPK along with 8 t ha⁻¹ of vermicompost resulted in more plant height (103.6 cm) at Agronomy field of Sher-e-Bangla Agricultural University, Dhaka as reported by Hasanuzzaman *et al.* (2010). Accordingly, Choudhary and Suri (2014) reported that the integration of nitrogen, phosphorous and potassium @ 90:45:45 kg/ha with 5 t/ha of FYM significantly resulted in higher magnitude of growth in terms of plant height (120 cm). Maruthupandi *et al.* (2017) on this experiment conducted at Panaimarathur village by Tamil Nadu Agricultural University revealed that the combined fertilizer treatments of fu RDF with 5 t/ha of vermicompost gave maximum plant height of 90.3 cm as compared to other fertility treatments. Apon *et al.* (2018) at Nagaland University, Medziphema and observed that maximum plant height (137 cm) was obtained with the treatment applying 75% RDF + 5 t ha⁻¹ FYM. The experiment conducted at Annamalai University Experimental Farm, Annamalai nagar showed taller plants (106 cm) with 75% of recommended dose of nitrogen as inorganic fertilizer and 25% vermicompost (Rao *et al.* 2019).

Number of tillers m⁻²

According to Hasanuzzaman *et al.* (2010) the application of 50% NPK + 8 t/ha of vermicompost resulted in 386 tillers m⁻² at Dhaka. Choudhary and Suri (2014) reported that combined application NPK at 90:45:45 kg/ha with 5 t/ha of FYM significantly resulted in higher magnitude of growth in terms of tillers m⁻² (396). The maximum No. of tillers m⁻² (389) was obtained with application of 3/4 RDF + 1/4 sunhemp under the experiment conducted by Kumar *et al.* (2017) at Crop Research Station, Uttar Pradesh. The number of tillers obtained maximum (160 m⁻²) with the treatment having 75% RDF + 5 t ha⁻¹ FYM during the research work conducted in sandy loam soils of Nagaland University,



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Medziphema by Apon *et al.* (2018). The experiment conducted at Annamalai University Experimental farm, Annamalainagar by Rao *et al.* (2019) showed that 75% of recommended dose of nitrogen as inorganic and 25% vermicompost gave a tiller number of 317 m⁻².

Dry matter accumulation

The experimental report given by Baishya *et al.* (2014) in case of dry matter stated that the maximum results was obtained by application of vermicompost of 1 t/ha followed by 2.5 t/ha of poultry manures and 5t/ha of FYM. Kumar *et al.* (2017) while conducting research at Crop Research Station, Ghaghraghat, Bahraich, U.P. reported that dry matter accumulation was 1613 g m⁻¹ which was observed in the treatment containing 75% RDF + 25% sunhemp. The dry matter of 1395 g m⁻² was obtained by application of 75% of recommended dose of nitrogen as inorganic and 25% vermicompost during the research work at Annamalai University Experimental farm, Annamalainagar by Rao *et al.* (2019). Shankar *et al.* (2020) while conducting an experiment at farmers' field at Binuria village of Birbhum, West Bengal found that INM practices exerted positive and significant effect on dry matter accumulation (DMA) of summer rice. The maximum DMA was recorded in crop receiving 75% RDN through chemical fertilizers and 25% of applied through FYM and poultry manure.

Leaf area index

Hasanuzzaman *et al.* (2010) stated that the application of 50% N+P+K along with 8t/ha of vermicompost showed the leaf area index of 5.1 under the experiment carried out at SBAU, Dhaka. Banerjee and Pal (2012) stated that LAI increased gradually up to 60 DAT and there after declined up to harvesting of the crop. At 60 DAT hybrid rice produced higher LAI (5.5) which indicated senescence of leaves during later stages. Maximum LAI was obtained with 100% RDF and followed by the treatment receiving 75% RDF+25 RD of N as FYM. Gautam *et al.* (2013) reported the leaf area index (4.11) in hybrid rice Arize-6129 increased significantly with integrated application of FYM and chemical fertilizer at 1:3 ratio. Zayed *et al.* (2013) reported that application of 2/3 of the RDN with different types of organic manures, @ 7 t ha⁻¹ and 5 t ha⁻¹ respectively, resulting in higher leaf area index of rice under saline soil conditions. Mondal *et al.* (2015) observed that application of 100% RDN along with 25 % of mustard oil cake resulted in highest LAI of (4.98) when the experiment was conducted at the Institute of Agriculture Visva-Bharati, Sriniketan, West Bengal. Shankar *et al.* (2020) observed that the maximum leaf area of the rice was obtained with the application of 75% RDN+25% FYM with a value of 4.77 under the study conducted at Birbhum, West Bengal.

Crop growth rate and net assimilation rate

Banerjee and Pal (2012) reported that CGR increased gradually up to 75 DAT and then decreased till harvest of crop due to senescence of the leaves and also the highest CGR was found in in FYM integrated with chemical fertilizers. The application of 10 t ha⁻¹ FYM along with 60kg ha⁻¹ of nitrogen obtained the maximum CGR of 33.3 g m⁻²d⁻¹ under the experiment conducted by Tadesse *et al.* (2013) at Fogera plains, in north-western Ethiopia. The same treatment also resulted in the maximum NAR of 8.63 g m⁻²d⁻¹. The net assimilation rate (NAR) was found maximum during 60-90 DAT and it was observed in the treatments having combination of organic and inorganic inputs stated by Harish *et al.* (2017). Mondal *et al.* (2015) under the lateritic and sandy-loam soil conditions observed that application of 75% RDN along with 25 % of mustard oil cake showed highest crop growth rate (CGR) of 26.9 g m⁻²d⁻¹ when the experiment was conducted at the Institute of Agriculture Visva-Bharati, Sriniketan, West Bengal.

Effect of integrated nutrient management on yield components of rice

Choudhary and Suri (2014) reported that NPK at 90:45:45 kg ha⁻¹ and FYM 5 t ha⁻¹ significantly resulted in higher magnitude of yield attributing characters like number of panicle⁻² (274.1), panicle length (22.7 cm), number of field grains panicle⁻¹ (100) and 1000 grain weight (22.59g). Dissanayake *et al.* (2014) mentioned on his experiment conducted at Rajarata University of Sri Lanka that the yield attributes like panicle length, filled grains per panicle was higher in INM compared to other treatments irrespective of season and variety. According to Kumar *et al.* (2014) the application of RDF (125%) with vermicompost (5t/ha) enhances the yield attributes of rice. Shankar *et al.* (2020) while conducted an experiment at Birbhum district of West Bengal under the red and lateritic soil revealed that test





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weight of rice grain did not vary significantly among the different nutrient management treatments. Sharma *et al.* (2017) carried out an experiment at Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad and revealed that the panicle length (25.65 cm) was found maximum with the integration of 50% of RDF and 50% of farm yard manure and vermicompost. further the foliar application seaweed extract in the plant as well as soil application of vermicompost of 5t/ha along with Azophos @ 25 kg ha⁻¹ + Azolla @ 10 kg ha⁻¹ applied at 15 days interval which result better in context of number of filled grains panicles⁻¹ (83.64) Ganguly *et al.* (2019). Under the lateritic and sandy-loam soil conditions at Visva-Bharati, Sriniketan, West Bengal, Mondal *et al.* (2018) observed that the test weight of the rice was not varied significantly under different nutrient management practices. The maximum no of panicles m⁻² was noticed (346 m⁻²) with the treatment having 75% RDN+25% poultry manure and also in proportion with 75% RDN+25% FYM under the experiment conducted by Shankar *et al.* (2020).

Effect of integrated nutrient management on yield of rice

The highest grain yields (5.3 and 5.5 t ha⁻¹ respectively) were achieved with 100% RDF (only chemicals) which were at par with other treatments with RDF supplied through FYM and green leaf manuring along with inorganic fertilizer combination (Banerjee and Pal 2012). Gill and Walia (2014) reported that the application of FYM 5 t ha⁻¹ in addition to 100% RDF significantly increased grain yield (6.35 t ha⁻¹), straw yield (9.2 t ha⁻¹) and harvest index (40.8%) than 100% RDF (5.77, 7.92 t ha⁻¹ and 39.8% respectively) and 80% RDF + 3 t FYM ha⁻¹ (5.76 t ha⁻¹, 7.83t ha⁻¹ and 39.7% respectively). Shekara *et al.* (2011) found significant interaction effect of grain and straw yield between fertilizer level and organic sources of nutrients applied at varied N levels in rice hybrid under SRI. The application of ¾ of RDF along with nitrogen(vermicompost) @ 45 kg/ha resulted in maximum grain yield (6.81 t ha⁻¹) which was closely followed by 100% RDF + 45 or 30 kg N ha⁻¹ through vermicompost or FYM (6.75, 6.68 and 6.61, 6.5 t ha⁻¹) respectively. Baishya *et al.* (2015) in their study in Nagaland under sandy loam soil conditions noticed that the treatment combination of 100% RDF along with 2.5 t ha⁻¹vermicompost resulted in highest grain yield that might be due sources of organic manure. Sannathimmappa *et al.* (2015) conclude that application of NPK (100: 50: 50 kg NPK) + 10 tons FYM ha⁻¹ gave maximum straw yield of 8.21 t ha⁻¹. Jeyajothi and Durairaj (2016) in their research conducted research trial, TNAU, Killikulam obtained a grain yield of 6.4t ha⁻¹ when the treatment given was FYM + Azophosmet +100 % N and P. The maximum yield of rice was observed as 6.05 t ha⁻¹ with the integrated nutrient management treatments of 100% RDF + Vermicompost 5 t ha⁻¹ when the crop was raised under the low wet land fields of Tamil Nadu Agricultural University at Coimbatore as reported by Maruthupandi *et al.* (2017). The maximum grain yield was observed as 3.6 t ha⁻¹ with the treatment receiving 50% of RDF along with 50% of farm yard manure and vermicompost under the silty loam soil conditions of Faizabad, U.P. (Sharma *et al.*, 2017). But the maximum straw yield and higher harvest index were recorded in the treatment received 50% recommended NPK through chemical fertilizers and 50% recommended N through FYM with 5 kg zinc ha⁻¹ Singh *et al.* (2018). Kumari *et al.* (2010) reported that the B:C ratio of rice crop was found to be maximum in the treatment having 100% RDF i.e., 1.44 under the experiment taken at Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur Bihar. Shankar *et al.*, (2020) in an experiment at Binuria village of Birbhum, West Bengal noted highest straw yield with the application of 100% RDF alone and it was at par with 75% RDN+25% FYM and 75% RDN+25% poultry manure.

Effect of integrated nutrient management on nutrient content and uptake of rice

Rani and Sukumari (2013) also observed that higher total N, P, K, Fe, Mn and Zn uptake by medicinal rice (Njavara) was recorded under integrated nutrient sources than the individual organic and inorganic. Zayed *et al.* (2013) also proved that application of two-thirds of the RDN plus some organic manures, either FYM or rice straw compost at a rate of 7 t ha⁻¹ and 5 t ha⁻¹ respectively, resulting in higher plant nitrogen and phosphorus content even under saline soil conditions of rice field. Ranjitha *et al.* (2013) found that 125 per cent RDF + 5 t ha⁻¹vermicompost increased P and K uptake in grain and straw over control. Kumar *et al.* (2014) proved that application of organic and inorganic sources of nutrient with 100% RDF + 5 t ha⁻¹vermicompost remarkably increased N uptake by grain (54.6 kg ha⁻¹) and straw (38.5 kg ha⁻¹), P uptake by grain (8.6 kg ha⁻¹) and straw (9.6 kg ha⁻¹) and K uptake by grain (19.2 kg ha⁻¹) and straw (110.4) over control. Singh *et al.* (2014) reported that significantly higher uptake of N in grain and straw with INM as compared to RDF. Highest uptake of 66.7 kg ha⁻¹ and 64.9 kg ha⁻¹ in grain and 85.8 and 85.3 kg ha⁻¹ N in



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straw were observed with application of 10t ha⁻¹ FYM+50% RDN+RD P and K as compared to RDF (59.1 and 57.1 kg ha⁻¹ N in grain and 76.2 and 77.8 kg ha⁻¹ in straw) during 2009-10 and 2010-11. Kumar *et al.* (2018) reported that the incorporation of organic manures likely FYM and vermicompost along with chemical fertilizers favoured in better root growth with resulted in high uptake of N (109.21 kg ha⁻¹) P (16.41 kg ha⁻¹) and K (102.36 kg ha⁻¹) under the experiment conducted at University of Agricultural and Horticultural Sciences, Shivamogga. Sahu *et al.* (2017) in their research at red and lateritic soils of West Bengal denoted that the uptake of N, P and K was more in the treatments having the combination of organic manures and fertilizers. The uptake of N and K was more in grain whereas the uptake of K was more in straw.

Effect of integrated nutrient management on availability of nutrients

Integrated nutrient management with 50% each of organic and inorganic nutrients resulted in higher available N (278 kg/ha), P (12.7 kg/ha) and K (281 kg/ha) in an experiment carried out by Dubey *et al.* (2014) at Jawaharlal Nehru Krishi Viswa Vidyalaya, Jabalpur. Harikesh *et al.* (2017) conducted research at Narendra Deva University; Faziabad conveyed that the application of 100% recommended doses of nutrients through various organic sources namely, FYM and vermicompost along with integration of chemical fertilizers enhanced available nitrogen content in soil after harvest of crop by 15.25% than application of inorganic fertilizers and organic manures alone. Kumar *et al.* (2017) while conducting research at Crop Research Station, Ghaghraghat, Bahraich, U.P. reported that maximum available N, P and K of 252, 17.5 and 282 kg ha⁻¹ respectively were noted in the treatment containing 75% RDF + 25% sunhemp. Patra *et al.* (2017) in his experiment conducted at ICR farm of the Assam Agricultural University, Jorhat revealed that application of 25% RD of NP + 100% K + enriched compost @ 2 t ha⁻¹ have improved the available soil nitrogen by 251 kg ha⁻¹. While carrying an experiment at University of Agricultural And Horticultural Sciences, Shivamogga in red clay loam soil by Kumar *et al.* (2018), they found that there is no significant difference in available nitrogen in post-harvest soil under different nutrient management treatments whereas the maximum P and K were obtained with integrated nutrient management with replacement of 1/3 of chemicals by vermicompost.

Effect of integrated nutrient management on nutrient use efficiency

Quanbao *et al.* (2007) mentioned that apparent recovery efficiency was maxing with less application of inorganic nitrogen than compared with integrated apply. Artacho *et al.* (2009) stated that the nutrient harvesting index increased with increasing level of nitrogen fertilizer application. Mandana *et al.* (2011) reported that the maximum agronomic use efficiency and physiological nutrient use efficiency were more when 90 kg ha⁻¹ of nitrogen was applied to the crop the values were 69 and 49.18 respectively. Tayefe *et al.* (2011) stated that PNUE of all was decreased with increasing N application with inorganic fertilizers and it was increased with integration of organic manures and chemicals as the results found in an experiment conducted at Rice Research Institute, Rasht, Guilan, Iran. The agronomic nutrient use efficiency was maximum with application of integrated nutrient management. The lower the inorganic inputs the higher the agronomic use efficiency as found under the study by Tayefe *et al.* (2011) at Rice Research Institute, Rasht, Guilan, Iran. Ramalakshmi *et al.* (2012) stated that the nitrogen use efficiency was higher with application of 50% inorganic fertilizers integrated with 50% organic manures than that of 75% inorganics with 25% organic manures.

Effect of integrated nutrient management on economics of summer rice

Samaint (2015) reported that the cost of cultivation was lowest with lower inorganic level (N100P22 K41.6) only where as it was highest when inorganic nutrient level was combined with FYM 10 t ha⁻¹. The highest net return (Rs. 50316/- ha⁻¹) was recorded with application of N100P22K41.6+FYM 10 t ha⁻¹. Sharma *et al.* (2017) concluded that high amount of cost of cultivation (Rs. 53590 ha⁻¹) was recorded under the treatment received 100% nutrients through organic manures (FYM, vermicompost and neemcake). As quantity of organic nutrients required more the cost increased in these treatments. Singh *et al.* (2018) reported that the benefit cost ratio was maximum in the treatments of 100% RDF with inorganic fertilizers as the quantity of inorganic fertilizers was less as compared with the cost of organic manures. Rahman *et al.* (2019) stated that the cost of cultivation involved in use of vermicompost was much higher and leading to higher cost of cultivation. Kumari *et al.* (2010) reported that the maximum net returns of rice





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crop was found in the treatment having 100% RDF i.e., Rs. 42410 ha⁻¹ under the experiment conducted at Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar. Further, they noted that gross returns (Rs. 84167 ha⁻¹) was maximum with the application of 75% RDF along with 5t ha⁻¹ FYM.

CONCLUSION

Integrated nutrient management practices can show a positive and favourable effect on improving almost all the growth characters, yield attributes, yield, nutrient content and nutrient uptake of rice. Application of manures and fertilizers in an appropriate dose can result in improving rice yield and there by maintaining soil fertility and productivity. Incorporation of manures like FYM, vermicompost, poultry manure and concentrated organic manures can replace the role of chemical fertilizers and thereby it is suggested that integrated approach for optimum management of nutrients in rice can be adopted for sustainable rice production.

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Implication of Plastic Mulch in Soil and Plant Nutrition - A Review

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ABSTRACT

Innovative cultivation practices are desired to transform of cropping system which will result in stretching agro-input resources manifolds to increase agricultural productivity both in quality and quantity. Plastic mulch is one of the most useful indirect agricultural inputs which modify the microclimate of crops. The use of polyethylene mulch in agriculture has gained a steep increase in the last couple of decades all over the world. This increase of use may be because polyethylene mulch increases soil temperature, crop yield, soil nutrient use efficiency, and chemical composition of fruits and vegetables and reduces weed growth, soil moisture loss, and pests and diseases. Many scientists and researchers have been conducted studies to know the effects of plastic mulch on soil and plant nutrition in different parts of the World. Therefore here it is attempted to compile all the scattered information related to effect of polyethylene mulch on soil, plant and crop development in a manuscript to assist researchers and extension personnel working in this area.

Keywords: Soil microbes, polyethylene, biodegradable mulch, soil temperature.

INTRODUCTION

Plastic materials are human made long-chain polymeric molecules. Plastic was invented in its solid form in the year 1935 by British chemists Fawcett and Gibson, and first manufactured in a sheet form in the year 1938 (Lamont, 2017). The excellent qualities of plastic such as processibility, chemical resistance, durability, flexibility and odor free attracts consumer to use extensively. Polyethylene films (PF) produced by the polymerization of ethylene under very high pressure. The half finished product comes in the form of pallets, which can be converted into flexible sheets film by an extrusion-blowing process.



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Some of the most commonly manufactured PFs are low-density polyethylene (LDPE), linear density polyethylene (LLDPE), high-density polyethylene (HDPE), and metallocene (Fleck-Arnold, 2000). The LDPE film has excellent physical properties of tensile strength and of resistance to tearing when exposed to strong wind. Its thickness can be significantly varied even maintaining good tensile strength. Several combinations of additives can be added to the PF to alter or improve the specific properties of the final product. These can include pigments for color, slip agents, anti-block agents, antioxidants, ultraviolet (UV) stabilizers, flame retardants, and photodegradable additives. The most popular low-density polyethylene (LDPE) film was the product of the pioneering research conducted by Dr. Emery Emmert in the 1950s at the University of Kentucky.

The practice of plastic mulch application in agriculture becomes a key management tool in many parts of the world. Wrapping soil around the upper surface of the plant root zone with a polyethylene film (PF) is called plastic mulching. PF modifies soil microclimate especially to increase soil temperature in agricultural production (Tripathy et al., 2022; Santosh and Maitra, 2022; Santosh et al., 2021; Anikwe et al., 2007). Increased soil temperature modifies the soil physico-chemical properties and promotes more rapid crop development and earlier yields (Stathakos et al., 2006; Ghouse Peera et al., 2020). Both researchers and farmers have widely recognized benefits of plastic mulching such as increase in soil porosity, water holding capacity, water savings and improvement of plant earliness (Santosh and Maitra, 2021; Stathakos et al., 2006; Zaman et al., 2017).

Poly mulch implication in soil physical properties

Using of plastic mulch on soil surface, modifies soil texture (loose, friable) and supply of adequate oxygen to plant roots to promote microbial activity. Application plastic mulch in sandy loam soil lowered the bulk density of soil and increases the concentration of nitrate which greatly influences the distribution of roots in soil (Tiwari et al., 2014). Anikwe et al. (2003) found increasing in bulk density which increases the mechanical resistance to growing roots entering the black soil under plastic mulch. Sali et al. (2003) did not found any changes in physical properties of soil covered with mulch in comparison to open soil surface. They also found that there is no significant change in bulk density of plastic mulch covered soil (1.40) in comparison to the soil not covered with the plastic mulch (1.43). By considering above points they concluded that the practice of plastic mulch cover on soil surface for short term will not cause soil compactness. Tiwari et al. (2014) conducted study for 10 years which revealed that application of plastic mulch for longer duration could reduce the bulk density (1.72gcm^{-3}) and may correspondingly increase in the porosity by 35.1% in comparison to non-mulch treatment. Van der et al. (2006) found a higher (0.72) aggregate stability index under plastic film mulch covered soil surface in comparison to the control (0.48). This may be due to less impact of rainfall on the breakdown of soil aggregates.

Poly mulch role in soil microbial growth

Application of plastic mulch on soil surface influenced the soil moisture content, soil temperature and vapour deficit and enhanced plant roots due to increased microbial activity inside the soil horizon. Plastic mulching can also decrease populations of soil invertebrates (Miñarro and Dapena, 2003), which may reduce top-down grazing pressures on soil microbes. A 28 year long period study conducted at Shenyang, China, found that the application of PF mulch on soil surface increases the relative abundances of soil bacteria such as Proteobacteria and Actinobacteria (Farmer et al., 2017). Other study conducted by Núñez-Zofío et al. (2011) reported decreasing quantity of *Phytophthora capsici* or increased mycotogenic fungi under plastic mulches (Munoz et al., 2015). Apart from the modification in microbial activity, plastic mulch also affects microbial functioning in the soil. Various studies conducted by different researchers (Mu et al., 2014; Zhang et al., 2015; Chen H. et al., 2017) reported increased microbial activity under plastic mulches. This report of variation in microbial activity may be due to the variation of temperature inside the plastic mulch due to different climatic conditions. During the winter season application of plastic mulch increases the soil temperature up to the optimum level which may be the reason of increased microbial activity. Whereas in summer soil temperature may increase beyond optimum level due to which activities of microbes decreasing (Moreno and Moreno, 2008).



**Santosh D T et al.,****Poly much role in plant nutrition**

Retention of the nutrients within the root zone and allow crop to utilize the nutrients efficiently is one of the major advantage of applying PF mulch on soil surface. The soil surface covered by PF mulch preserve greater amount of soluble minerals. The root zone temperature plays an important role in uptake of soil nutrients by plants (Baghour et al., 2002). Continuous moisture content, increased temperature and improved soil aeration play a vital role in completing nitrification through increased soil microbial population due to application of PF mulch. The process of organic matter mineralization in PF mulch covered soil is greater in comparison to soil with no mulch. Researchers found that PF mulch is durable and resistant material which guards the soil nutrients from leaching during the rainy season. The leaching of nitrate found maximum in the soil not covered with the mulch in comparison to the soil covered with the mulch (Romic et al., 2003). They also reported that nitrate-nitrogen leaching was reduced by 83% with soil covered with PF mulch in comparison to open soil condition. Hanada (1990) reported greatest quantity of nitrate in the soil covered with transparent PF mulch, followed by the black PF mulch, and lowest in non-mulch soil. Study conducted by Tiwari et al. (2014) reported higher organic carbon, organic matter, humic acid, microbial count, available potassium, and available phosphorous, total nitrogen content for the soil applied with PF mulch in comparison to the non-mulch soil. Hanada (1990) reported that the movement of water is directed upwards in soil under plastic mulch, which results in the accumulation of salts on the soil surface beneath the PF mulch. The electric conductivity of the soil was markedly higher under mulch. The content of exchangeable cations was also higher under transparent mulch during the middle stage of growth, while both transparent and black mulch gave higher cation content at the final growth stage. However, the pH value was found lower by 0.2-0.5 in soil under mulch compared to bare soil (pH 6.1), which indicates that anions such as nitrate, sulfate, and chloride also remain in the soil without being leached out. The number of nutrients (N, P, K, Ca, Mg) absorbed by the plant from the soil was 1.4-1.5 times higher in soil under mulch than in bare soil.

Kumar and Dey (2010) reported greater nutrient uptake (N: 13.80–16.90%, P: 23.00–38.12% and K: 17.50–20.80%) by strawberry crop under black PF mulch treatment in comparison to non-mulch treatment. Inflated root system and soil moisture regimes in plastic mulch covered soil surface influenced the uptake of P and K in strawberry crop (Vasane et al. 1997). Higher concentration of N and Mg found for tomato crop in a soil covered with the black plastic mulch in comparison to the soil not covered with the plastic mulch. Application of plastic mulch on soil surface results into increased phosphate uptake by crops due to the reasons that exhilarated surface root system, maintaining the soil moisture for longer time and restricting the fixation of applied phosphorous. Wien and Minotti (1993) reported, total yields and the shoot concentrations of N, P, K, Ca, Mg, Cu, and B were increased by plastic mulch in tomato crop. The concentration of N, Cu, and Mn was found higher in leaves of plants mulched with films of white/black, silver/black, and black colors compared to the leaves of plants from bare soil. Magnesium concentration reduced in leaves of plants mulched with plastics of black, silver/black, and aluminum color (Ruíz-Machuca, 2015). Partially contrast report made by Díaz-Pérez (2010), in which the accumulation of mineral nutrients in leaves and fruits of bell pepper was affected by the color of the plastic mulch during the spring but not in the fall season. Above statement indicates that the soil temperature plays an important role in the accumulation of nutrients in fruits and leaves. Tiwari et al. (2014) reported decreased available nitrogen content in the mulched soil compared to soil without mulch due to lack of exchange of oxygen for microorganisms and the restriction of nitrogen mineralization. They also found increased available phosphorous (14.91- 47.62%) and potassium (2.39- 26.9%) content in soil covered with plastic mulch.

Carbon dioxide (CO₂), given off by the roots or decomposition of organic matter in the soil, accumulates beneath the plastic mulch (Rubin Benjamin, 1984). It is then channeled through the perforations made at the time of planting and becomes concentrated around each plant. This small increase of CO₂ level around the stomata of foliage promotes growth by enhancing photosynthesis. Tiquia et al. (2002) reported that the CO₂ content in the soil under mulch was much higher than in non mulched areas, which means poor soil aeration and a detriment to tuber plant growth.





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CONCLUSION

The present review has covered the effects of plastic mulch on soil and plant nutrients. Change of soil physical properties like soil temperature, moisture content, bulk density due to application PF mulch elaborated. Reasons for enhancement in nutrient availability and microbial activity in the soil covered with plastic mulch were discussed.

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Saline Soil and it's Reclamation Process in India: A Review

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ABSTRACT

Saline soil is a current and constant growing problem in the whole world as well as in India. Many kinds of natural and also man-made happenings are the cause of the increase in saline soil. Because of an increase of saline soil area in India, productivity of many agricultural lands is decreasing. As a result, agricultural production in many areas is suffering heavily and also threatening to increase this problem in future in a high populated country. So, in this very hour, we need to reclaim those soils to boost the efficiency of those agricultural lands. Many methods are being developed to saline soil reclamation. The article focuses on the causes, distribution, impact and reclamation process of saline soil in India.

Keywords: Salinity, Reclamation, Saline soil, Leaching, Drainage

INTRODUCTION

Soil of all kind and natural water contains salt that is soluble. In the present context of climate change, the natural process of soil formation and anthropogenic activities have been altered causing further stress on the soil (Pramanick *et al.*, 2021). The salt amount in the root-zone can tell if the soil is affected by 'salt' or it's normal and when the amount of salt in the soil is 'upmost', then it effects the growth of plant immensely. The definition of saline soil can be given as a soil which has the saturated extract's conductivity which is more than 4dS/m (0.4 S/m or 4 mmhos/cm) with a sodium percentage that may be exchanged (ESP) lower than 15. Saline soil generally has a pH lower than 8.5. Previously as for the surface crust made by white salts, soils of this kind were named white alkali soils (Das, 2017). The main salts which dominate in saline soil are NaCl and Na₂SO₄ with plenty of cations which are soluble which we can tell in Na>Mg>Ca>K in this sequence. The scale of salinity in monsoon is 0.5 dS/m and in summer it is 50dS/m (Sabareshwari and Ramya, 2017). Most crops deliver less yield at this EC. Because of too much existence of salt, the osmotic potential grows and because of this, plants become unable to take up the moisture even so moisture is accessible to the plants. Normal agricultural operations are almost out of question in these antagonistic conditions. If soil salinity develops, it reduces the amount and quality of the produce, but also creates limitation of the crops that can be cultivated (Dhanushkodi and Subrahmaniyan, 2012). Estimations have been done and it is seen that globally 20% of entire cultivated 33% of total lands which are irrigated affected by high level of salinity. Additionally, the saline soil area is increasing 10% yearly because of diverse reasons which include less



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precipitation, high rate of evaporation from the surface, weathering process of native rocks, using saline water while giving irrigation and using inappropriate cultural behaviors. According to estimates, more over half of the agricultural fields will be succumbed under saline soil by the year 2050 (Shrivastava and Kumar, 2015). Out of the 17 SDGs suggested by UNDP, reclamation of saline soil has the potential to fulfill SDG 2, 3 and 15 (FAO, 2021).

Area and distribution of saline soils in India

In India, a state-level calculation has shown that 6.7m Ha soil in India is in the effect of salt affected soil among which 2.9mHa and 3.7mHa is being covered by saline and sodic soil (Mandal *et al.*, 2011). Among these areas, the costal areas of India are mostly affected by saline soil because of being near to the sea. Difficult soils like alkaline, saline, waterlogged, marshy and acid sulphate soils are seen in the costal regions, mostly through the deltas. Salinity in the costal areas are the main element accountable for bad yield of crops in about an area of 3.1 Mha (Arulmathi and Porkodi, 2020).

Causes of soil salinity

Coastal areas are most affected by saline soil. Direct invasion of sea water, intrusion through estuaries and salt's motion towards a high level from facile water level are the main reasons of salinity in soils which are affected by salt. Soils of coastal area have much salts mostly because of the existance of ground water table which is saline, facile depth and repeated saline water invasion in the areas which are low lying. The water from the ground which is affected by sea and saline water firth outstretchthe soil's top layer through capillary rise while seasons are dry, vaporizes from the ground in which case, The salt remains in the soil, causing it to become saline and barren for crop cultivation. So the salinity of the soil shows high variation of temporal and spacial which depends on the advancement, texture of the soil, effluent and other factors (Arulmathi and Porkodi, 2020). Bandyopadhyay *et al.* (1987) noticedthat saline soils of coastal areas are defined by clay loams including varying amounts of silt, sand.

The range of the conductivity of electricity spaned from 0.5 to 9.2 dS/m. Biswas *et al.* (1990) noticed that saline soils of coastal area normally underground water table which is shallow and extremely saline with uphill movement of the saline water during the dry seasons. Other than coastal areas, the reason for saline soil in the other parts of India are weathering of parent material where weathering of rock minerals which contain high salt, salts get released which are solvable. They are carried away from where they were created through surface or streams of groundwater. The areas which are arid, the thickness of the salts eventually grows till they start precipitating because of finite precipitation which is natural and leaching, high rates of evaporation and transpiration (Kumar and Sharma, 2020). Fossils salt accumulation is also responsible for saline soil in arid zone. Fossils salt can get diluted in under water depot which can be the reason of salinization (Bresler *et al.*, 1982). Substituting perennial vegetation with annual crops can be the cause of salinization of soil due to the procedure of saline seepage.

Changing the use of the land from forest to annual crops which supply food results in reduction of evaporation and itenhances leaching. The presence of less permeability or non-permeable subsurface layers can disrupt percolating water as it passesa journey across saline soils, which results in lateral seepage and salinization in low-lying locations (Doering and Sandoval, 1976). Secondary salinization, which affects land and water resources,is caused by non-selective irrigation with brackish and saline water, clogged drainage systems, increasing tables of water, and other issues (Rao *et al.*, 2014). In the absence of adequate soil-water-crop application, even giving irrigation with water of high-quality over a lengthy period of time may result in salinization (Dagar, 2005). Salts are brought to the surface at that place where they get precipitated at the time water gets evaporated (Rao *et al.*, 2014). Overflow water from canals is a major problem that adds to the increase of the water table and the evolvment of salinity along canal banks (Tewari *et al.*, 1997). Too much using of chemical fertilizers and soil amendmets can also lead to soil becoming saline (Kumar and Sharma, 2020).

Impact of saline soil

Under irrigation, soil salinity is a thorny problem. Soils are routinely saline in hot and arid places of the world, limiting agricultural possibilities. In this kind of areas, maximum crops grow with the help of irrigation and to



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increase the difficulties, improper management secondary salinization occurs as a result of irrigation, which influence 20% of irrigated land in the whole world (Glick *et al.*, 2007). Salinity of soil is the reason for serious restrictions for production of crops. Salinity of soil is the main reason for the constructional changes in soil and changes physical process of soil which affects the water and air flow in the soil (Oster and Jaywardane, 1998). Microbes osmotic and matric potentials, as well as their activities, are influenced at a large distance (Reitz and Haynes, 2003; Sardinha *et al.*, 2003). The pace of breakdown of organic substance slows down over time, resulting in nutrient release that affects plant development.

The actions of enzymes, the structure of the community of microbes in the soil, and salinity has an impact on all of their operations (Reitz and Haynes 2003; Vijayakumar *et al.*, 2013). It was revealed by Velayutham *et al.* (1999) that potential and resource of saline soil for different Agro-ecological zones of India. In saline soil, cumulation of salt and alkalinity in soil is more. Pre-eminence of lack of nutrients, toxicity, acid-sulphate in soils, bad filter ability of soil is seen (Arulmathi and Porkodi, 2020). Soil salinity impact adds low agricultural produce, less returns economically, erosion of soil (Hu and Schmidhalter, 2002). Soil salinity causes a complex interplay between biochemical, morphological, and physiological processes such as germination of seed, development of plant also intake of nutrient and water (Akbarimoghaddam *et al.*, 2011; Singh and Chatrath, 2001). Some components of saline soil like sodium, chlorine, boron have particular toxic reaction on plants. If sodium accumulates excessively in cell walls, it can cause death of cell and osmotic stress (Munns, 2002). Plants that are vulnerable to these elements can be harmed with a small amount of salt if there are enough noxious components in the soil.

As several plants do require salts as a source of nutrition, a high quantity of salt can disrupt the nutritional balance in plants or hinder the absorption of particular nutrients (Blaylock *et al.*, 1994). Photosynthesis is further hampered by soil salinity mostly by reducing the leaf area, number of chlorophyll, stomatal conductance and also decreases photosystem efficiency upto a lesser extent (Netondo *et al.*, 2004). These variables have a negative impact on development and growth of plant on a biochemical and physiological level (Munns and James, 2003), as well as on an atomic level (Tester and Davenport, 2003). Plants that thrive in a saline medium, it is critical to maintain the osmotic equilibrium. Turgidity will be lost if this balance is not maintained, cell dehydration, and eventually death of cell. On the contrary, adverse effects of salinity on plant development might be the result of a disruption in the availability of photosynthetic assimilates or hormones to the tissues in development (Ashraf, 2004). The negative impact of soil salinity can be credited to effects of cell cycle of salt stress and uniqueness. Salinity temporarily stops reduces the cell cycle by lowering production and action of cyclins and cyclin-dependent kinases, as a result, there are less cells in the meristem. and so growth is limited. Salinity has been shown to impair development and growth of plant, inhibit growth of seedling, germination of seed, enzyme (Seckin *et al.*, 2009), mitosis, protein synthesis, RNA, DNA, according to recent reports (Tabur and Demir, 2010; Javid *et al.*, 2011).

Reclamation of saline soil

Problems of soil salinity are many and last's long. It is increasing rapidly, with a calculation of 0.3-1.5 million ha of farmable land are getting affected by salinity every year. It is causing in reduction of production of crop by 20% per year. It is also reducing the capacity of production of another 20-60 million ha of farmable land. It is also reducing the diverseness of flora and increasing soil deterioration (Porcel *et al.*, 2012; FAO, 2015; Swallow and O'Sullivan, 2019). Annually, the cost of crop loss, including salt, is estimated to be over \$27 billion (Gies, 2017). If the issue of salinity is just not addressed, the saline lands will become uncultivable (FAO, 2017). For that reason, it is very much essential to provide solutions to this problem. To reduce this problem and increase the yield of crops, reclamation of saline soil must be done to favour growth of plant or to make the plant resistant and able to thrive in a salty climate (Maas, 1993). Lowering the salt level of the soil that is salty can help it be reclaimed, increasing the soil structure and reducing the pH (Yu *et al.*, 2010). A soil which is reclaimed must be able to control the concentration of salt in soil, keep up its porosity and be able to provide good conductive environment for transportation of water in the soil and encourage the plant's root growth and development. Soil reclamation can be done in many process. For



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implementing each process successfully and important point to consider is to provide proper drainage system in soil (Qadir *et al.*, 2000). The techniques for reclamation of saline soil are talked over as follows.

Flushing

In this technique, passing of water over the surface of the soil is used to force out the salt in the soil. This technique is only applicable when the salt has established an exterior coating over the surface of the soil. Yet this technique is not efficient and removes very small amount of salt (Abdel-Fattah, 2018).

Scraping

The gathered salt on the soil surface can be taken out through mechanical method. This method is very simple and inexpensive to reclaim saline soil in small area. It boosts the growth of the plant for a certain time as soil gathers repeatedly (Mary *et al.*, 2020).

Leaching

In this technique, soil of the upper layer is forced to move 45-60cm layer down which is well beneath the rootzone (Keren and Miyamoto, 1990; Qadir *et al.*, 2000). This technique needs water of good quality electrolyte concentration which is less which can drain through soil profile to help the salts dilute and move them down in the soil layer to stop extra salt escalation in the rootzone. The amount of water needed for reclamation of a soil can be fixed by the soil texture, salt amount present in the soil, amount of soil for reclamation and required level of salt in the rhizosphere and also the sort of plant that will be used when the restoration has been completed (Qadir *et al.*, 2000; Biswas and Biswas, 2014). When the soil's content of moisture is low and the groundwater table is deep, leaching is the preferred method (FAO). Leaching can be done in a variety of ways, including, intermittent ponding, continuous ponding, as well as sprinkling (Qadir *et al.*, 2000) continuous ponding is better suited to soils which have medium texture, and it allows for faster leaching. It is calculated that if the water depth is equal to the soil depth which needs to be reclaimed, above 70% of salt which is soluble is removed before leaching (Hoffman, 1980). Intermittent ponding can achieve leaching of equal level with one-third less amount of water than continuous ponding; although it takes more time (Hoffman, 1980; Qadir *et al.*, 2000) This method is better suited to soils which have fine texture, especially those in fields with a high water table or a tiled drainage system. It performs fairly good on soils which have fine texture, especially in places with tile drainage or a low water table. Dropping the water table enhances leaching efficiency (Talsma, 1967). Intermittent ponding can create cracks to occur on the soil surface when a surface seal forms, enabling water to enter (Abdel-Fattah, 2018). Sprinkling is a high-value, high-energy process that may be employed in areas that have not been prepared for ponded leaching (Shankar and Evelin, 2019).

Surface Drainage

The gathering and evacuation of water from the soil's surface is known as surface drainage. Low locations that collect water from neighbouring upper land and impermeable soils with limited ability to dispose of extra water through flow downward through the soil profile are two characteristics that favour the use of surface drainage (Mary *et al.*, 2020).

Sub-surface Drainage

It's a method for draining surplus salt from the root system into a collecting system (Gajja *et al.*, 2002). Deep open drains may be used to collect the salty water. Otherwise, subterranean silted pipe drains can recover salty water that has been leached and transport it to a drain collector. Not only may this approach decrease salinity by up to 50%, but also has the potential to lower the water table (Raju *et al.*, 2016). Patterns and intensities of cropping can be varied altered to increase the advantages of the subsurface drainage system as well as farmers' crop yields and revenues. The availability of qualified professional labour and infrastructural development are both necessary for subsurface drainage technology. However, active cooperation from both the government and farmers is required for it to be implemented successfully (Raju *et al.*, 2016). Farmers have proved that this strategy is technically practical, fiscally sustainable, and accepted by society (Chinnappa and Nagraj 2007; Tripathi 2011; Raju *et al.*, 2016).



**Souvik Sain et al.,****Green Manuring and Crop Residues**

Dhaicha (*Sesbania aculeata*), sunnhemp (*Crotalaria juncea*), barseem (*Trifolium alexandrinum*), sengi (*Melilotus parviflora*), and cowpea (*Vigna sinensis*) are some of the major crops used for green manuring (Maitra and Zaman, 2017; Maitra et al., 2018). They act as a source of immediately available nutrients during decomposition, as well as solubilizing calcium and neutralising high pH in alkali soils. Sulphur, sulphuric acid, lime sulphate, aluminium sulphate, iron sulphate sulphur, and other acids and acid formers that work as calcium mobilizers, such as iron sulphate, lime sulphate, sulphuric acid, sulphur, etc., assist pressmud from sugar factories and slag from iron factories, or acids and acid formers that work as calcium mobilizers, such as sulphuric acid (Mary et al., 2020).

Biomimicry

It's a revolutionary method for extracting effloresced salt from the soil surface that tries to emulate the capillary activity of vascular plants (Swallow and O'Sullivan, 2019). The scientists were able to reduce soil EC_e and over the span of 30 days, the concentration of salt increased by about tenfold. (from 8% to 0.8% and from 120 mS/cm to 14 mS/cm) by using this approach. Crystal blockers, to limit crystal formation and enhance evapotranspiration while stimulating dendritic crystal growth, chemicals such as ferrocyanides are sprayed on the soil surface. (Klaustermeier et al., 2017). In the soil, a capillary pump is created by dendritic crystals, allowing fluid of soil containing dissolved ions to travel quickly uphill (Sghaier and Prat, 2009). On the soil surface, salts effloresce as a result of evaporation and can be recovered. This technology is still in its infancy and has to be field tested before it can be utilised as an efficient desalinization tool to lower the salinity of the soil (Swallow and O'Sullivan, 2019).

Gypsum

The fineness of 0.59 mm gypsum was shown to be more efficient in the reclamation of salty and alkaline soils. It is harmless, less expensive, and the addition of calcium to reduce the proportion of exchangeable salt in soils leads to greater water permeability. The regular flow of water in the soil profile also aids in the reduction of salts the depth of the root zone (Mary et al., 2020).

Phyto-desalinization

It's a strategy for reclaiming saline soil using plants and their components. Cultivating salt-tolerant plants (halophytes) in a salty environment or adding organic matter such as organic manures, straw mulch, as well as other organic materials might help remove excess salt extracted from the root zone. It is a procedure that is safe, effective, cost-efficient, and ecologically friendly (Devi et al., 2015). Halophytes, the salty flora, may mate and thrive in salty environments with EC_e as high as 20 dS/m and produce progeny which is healthy (approximately 200 mM NaCl) (Flowers and Colmer 2008, 2015). Plants of such kind can indeed be perennials and annuals, monocotyledonous or dicotyledonous species, trees and shrubs, and they can be found across the angiosperm family (Rozema and Flowers 2008; Flowers and Colmer, 2008, 2015). Adaptation processes in halophytes include morphological, biochemical, and physiological adaptations including the ion adsorption of Na^+ in vacuoles, the activation of antioxidant mechanisms in these plants, which have a wide range of salt tolerance, and to combat osmotic stress, osmolytes are synthesised (Glenn et al., 1999; Breckle, 2002; Shevyakova et al., 2003; Flowers and Colmer 2008, 2015). Plants of such kind are capable of controlling Na^+ and Cl^- absorption and intrinsic concentrations while maintaining enough K^+ and Mg^{2+} proportions in the cytoplasm for cellular activities (Flowers and Colmer, 2015). As a result, plants like these may either collect salts, excrete, or exclude, and are divided into three categories: (1) salt accumulating, (2) salt secreting, and (3) salt excluding (Walter, 1961). Through anatomical alterations, halophytes that are salt-resistant can prevent salts out from the root system (Shankar and Evelin, 2019).

Uses of salt stress tolerant plants

According to the United Nations Environment Program, salinity concerns affect around 20% of farmed area and 50% of farmland globally. Salt stress conditions on the ground reduce the caloric and nutritional potential of agricultural products (Deveshi Patel et al., 2020). Natural salinity exists in some regions, and salt stress resistant plants may be the best or only way to use these resources for food production. Using salt tolerance varieties helps handle the problem





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of soil salinity in a cost-effective manner. Plants can perhaps be biologically manipulated to control salinity (Thaker et al., 2021). As a result, a lot of effort is being put into developing salt-tolerant crop genotypes through plant breeding procedures that involve introducing into cultivated plants the genetic basis of wild species which are tolerant to salt (Pitman and Läuchli, 2002). However, there is a risk that making salt resistant genotypes more accessible will result in less effort being put towards recovering saline regions or preventing salinization. This will be unhelpful in the long run. Crops that are tolerant to salt will be able to utilise low-quality irrigation water more effectively. Because trees use a lot of water and therefore can decrease water tables, they could be used to soak some of the excess salt and prevent subsequent salinization (Niknam and McComb, 2000).

Crop varieties which are resistant to saline soil-

CONCLUSION

Salinity of soil is a huge problem in the world as well as in India. Many arid region and coastal areas are hugely suffering from this kind of soil problem and it is the need of the hour to reclaim these soils for better agricultural cultivation. A major problem in reclamation of these soils are availability of good quality water as well as these processes are costly and labour dependent and also many other factors are dependent. Leaching, for example, is expensive and requires water and a good system of drainage, in addition of lowering whole nitrogen-total carbon which is organic, activity of microbes, and total fertility of soil. For effective fertiliser application, it is vital to evaluate the nutrients and elements of saline soil. And accordingly we have to apply fertilizers. Also the use of organic manure, FYM etc. has shown to improve the soil texture which helps in leaching and other operations high success. Further researches must be conducted on this matter as it is a constantly growing problem and better solutions are needed.

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Table 1. The extent to which India's soils have been affected by salt (Mandalet al., 2011).

State	Sodic	Saline	Total
West Bengal	0	441.3	441.3
Tamil Nadu	354.8	13.2	368.0
Orissa	0	147.1	147.1
Uttar Pradesh	1347.0	22.0	1369.0
Andhra Pradesh	196.6	77.7	274.2
Rajasthan	179.4	195.6	375.0
Bihar	105.9	47.3	153.2
Andaman & Nicobar Islands	0	77.0	77.0
Haryana	183.4	49.2	232.6
Jammu & Kashmir	17.5	0	17.5
Gujarat	541.4	1680.6	2222.0
Karnataka	148.1	1.9	150.0
Madhya Pradesh	139.7	0	139.7
Punjab	151.7	0	151.7
Maharashtra	422.7	184.1	606.8
Kerala	0	20.0	20.0
Total	3788.2	2956.9	6745.1
		(Say 6.75 Mha)	

Table 2. Saline soil resistant crop varieties (Arulmathi and Porkodi, 2020)

Crops	Variety
Wheat	KRL 213, KRL 19, KRL 1-4, KRL 210
Indian Mustard	CS 52, CS 56, CS 54
Dhanicha	CSD-123 and CSD 137
Rice	CSR 27, CSR 10, CSR 23, CS 30(Basmati type), CSR-49, CSR 36, CSR 13, Coastal saline soil: Luna Suvarna (CR Dhan 403), Gangavathi Sona-05-01, Butnath (CSRC(S)5-2-2-5), Luna Sampad (CR Dhan 402), Luna Barial (CR Dhan 406), Sumati-CSRC, Luna Sankhi (CR Dhan 406), Sonamani
Chickpea (gram)	Karnal Chana 1





Crop Performance of Banana under Drip Irrigation and Plastic Mulch – A Review

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ABSTRACT

Banana is most preferable fruit in Indian diet. India ranks first in banana production and almost all the states of India are producing banana. Banana requires higher input such as water and fertilizer for its production. As Indian agriculture facing acute shortage of water, banana productivity was not able to reach desired level. Intervention of modern techniques such drip irrigation and plastic mulch effectively increased the production and productivity of banana in India. Drip irrigation reduces the water loss and increase the water use efficiency. Plastic mulch suppresses weeds and conserve soil moisture for longer time by reducing the evaporation loss. Current literature attempts to review the response of drip on the quantity and quality of banana. The study also aims to review the effectiveness of applying plastic mulch along with the drip and advise these techniques among the farmers to improve the yield and quality of produce.

Keywords: Water use efficiency, plastic mulch, drip irrigation, fertigation

INTRODUCTION

Drip irrigation is the most efficient irrigation method through which water is applied directly to the root zone of the plants using through plastic pipes and emitters (Dhawan, 2002; Zaman et al., 2017). The drip irrigation system operates with pressure ranging from 0.15 to 1.5 kg-cm⁻². High water application efficiency always found with drip irrigation due to a significant reduction of soil evaporation, surface runoff and reduced amount of deep percolation (Jiusheng et al. 2003). Including higher water use efficiency, drip irrigation system also has the advantage of the



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reduced cost of crop cultivation (fertilizers application, labor, tilling and weeding) in comparison to the conventional irrigation methods (Narayanamoorthy, 2003, 2005, 2009). This system can also apply a precise amount of soluble nutrients and pesticides to the crop, hence control over leaching is required, which is the threat to groundwater (Lamm and Camp 2007). Lack of field-oriented research and fewer farmers participation in technology development thus reduces dissemination and field adoption (Bhamoriya and Mathew, 2014).

Effect of drip irrigation on various vegetable crops such as cabbage (Tiwari et al., 2004), broccoli (Erdem et al. 2010), sugar beet (Kiyamazand Ertek, 2015), cucumber (Zhang et al., 2011), Brinjal (Aujla et al., 2007), potato (Wang et al., 2011) and tomato (Kuscu et al., 2014) has been studied and reported greater yield and WUE in comparison to surface irrigation methods. Experimental research findings reported the effect of drip irrigation on various fruit crops such as Guava (Singh et al., 2007), Sapota (Tiwari et al., 2014), Litchi (Tiwari et al., 2013) and Cashew (Tiwari et al., 2016). They reported greater fruit yield than to surface irrigation methods.

Singh et al. (2006) reported maximum water saving of 54.63 percent under drip irrigation at 60 kPa soil moisture level over surface irrigated treatments. Drip irrigation saves about 50% of the irrigation water used in conventional irrigation system (Bashour and Nimah, 2004). Manickasundaram et al. (2002) reported saving in irrigation water under drip scheduled at 50% of surface irrigation was 48.4% compared with that of the surface irrigation. Hassanli et al. (2009) reported higher water saving from using drip irrigation (5907 m³ ha⁻¹) and the minimum water saving was obtained using furrow (6822 m³ ha⁻¹) irrigation. Saving of 25% water under drip irrigation in comparison to the furrow irrigation reported by Aujla et al. (2007).

Ahmed et al. (2011) reported 471 m³ of water application for banana crop through drip irrigation system which is 273 m³ lesser than the water applied using surface irrigation (744 m³) for the growing same crop. Many field research studies results show 25% to 50% of water-saving due to drip irrigation in comparison to surface irrigation for the banana crop (Aujla et al., 2007). The field experiment was conducted on banana (cv. Cavendish) irrigated with drip irrigation results in better plant growth, early flowering, higher fruit yields, and increased WUE, compared with basin irrigation (Hegde and Srinivas, 1991). Tiwari et al. (1998) found daily water application of 18.6 L plant⁻¹ resulted in higher water use efficiency using drip irrigation and they also recommended the irrigation quantity for banana cultivation in sub-humid subtropical regions.

Experimental studies and field survey conducted by many researchers in different regions of India and reported significant water saving and WUE for different crops cultivated under drip irrigation when compared with those of conventional irrigation system (Narayanamoorthy, 2009). There is no moisture stress in drip irrigation. Therefore, the crop productivity was significantly higher in comparison to cultivated under conventional irrigation system (Shah and Keller, 2014). Narayanamoorthy (2009) conducted a study on banana crop using drip irrigation method and reported that 29% gain in productivity due to the drip irrigation method. Khalifa et al. (2012) obtained highest water productivity (1.4 and 1.40 kg m⁻³) with 120% of irrigation water requirement using drip irrigation and the lowest water productivity found with surface irrigation (0.30 kg m⁻³).

Influence of black plastic mulch on banana crop response

Soil mulching with plastic mulches influences the microclimate around plants and soil environment conditions. Plastic mulch positively influences the soil moisture content by reducing evaporation from the soil surface, improves infiltration, soil water content, decreases bulk density and improves soil water due to condensation of moisture from plastic film at night when temperature falls (Tiwari et al., 2014). Plastic mulch altering the microclimate of soil near the plant which gave additional advantages like the emergence of seed and root proliferation (Stigter et al., 2018). Plastic mulch also helps in suppressing weed population (Tripathy et al., 2022). The impact of mulch varies with soils, climate, the color of mulch used and the thickness of the plastic film. water-scarce LLDPE film is one of the widely used plastic mulch for many reasons such as early-season soil warming, reducing soil water evaporation and weed control. Black plastic mulch (BPM) improves the quality of absorbance of solar radiation ($\alpha_{mulch} = 0.96$; $\alpha_{soil} = 0.67$), which creates larger temperature difference between the soil below plastic mulch and no mulch. Due to the



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absorption of all shortwave radiations, the soil surface temperatures below black plastic mulch can reach up to 55 °C (Tarara, 2000). Soil temperature may increase from 3 to 5°C which may reach upto 15 cm beneath the mulch. Raised beds around the plant are always preferred while applying plastic mulches because of the warm soil surface quicker than flatbeds and provides better drainage condition. Raised beds with plastic mulches are always beneficial because they can improve drainage near the plant root system and explores the larger surface area under the mulch for more uniform warming. Raised beds also provide a strong holding of plastic mulches during installation due to which maximum heat transfer can take place within the soil. Due to raised bed the geometry soil along with mulch changes which significantly influences heat flux at the soil surface. Santosh and Maitra. (2022) reported high soil temperatures in raised beds along with plastic mulch in comparison to level soil covered with plastic mulch. An average increase in temperature from 2 to 4 °C has been reported by Santosh *et al.* (2021) for raised beds with plastic mulch.

Drip irrigation combined with BPM reduces evaporation from the soil and corresponding decrease in crop water requirements. Santosh *et al.* (2021) also suggested the BPM for drip irrigation to conserve the soil moisture. Higher soil moisture under plastic mulch with drip reduces the soil strength and improves the penetration of root and proliferation. The use of BPM along with drip irrigation results in a higher yield of crops like cabbage, cauliflower, sapota, watermelon, okra and capsicum (Tiwari *et al.*, 2014).

Performance of drip irrigation and plastic mulch on banana crop

A field trial was conducted by Agrawal and Agrawal (2005) on a sandy loam soil at Raipur (India) to study the response of banana crop under drip irrigation and plastic mulch. Results revealed that drip irrigation increased banana yield and conserved more water than surface irrigation. They also reported that banana yields were significantly higher with drip irrigation (83.8 t ha⁻¹) in comparison to basin irrigation (78.9 t ha⁻¹) mainly because of significant differences in bunch weights (27 and 25 kg, respectively). Paul *et al.* (2008) conducted an experiment to assess the viability of drip irrigation and BPM for the banana crop on sandy loam soil near the coastal area of Orissa state for two consecutive years (2006–08). The biometric and yield response of banana crop for three irrigation levels 100% irrigation volume (V), 80% irrigation volume (0.8V) and 60% irrigation volume (0.6V) using drip along with the black plastic mulch. The biometric and yield results obtained from ring basin irrigation with or without BPM were compared with drip irrigation treatments. The study resulted in higher plant height, early flowering, and enhancement in the yield under drip irrigation with BPM. The greater yield (70.97 t ha⁻¹) was recorded under 0.8V drip irrigation with BPM in comparison to other treatments. Further 33% higher yield, 49% more net seasonal income and highest benefit-cost ratio (2.24) were found with 0.8 V drip irrigation with BPM in comparison to ring basin irrigation. However, BPM without drip could improve the yield by 7%.

Mahmoud (2006) conducted the study with three different levels of irrigation through drip viz, 40%, 60% and 80% of ET_c and compared yield and quality parameters of the banana crop with surface irrigation. Irrigation level 40% (674 mm year⁻¹) in the main crop significantly improved plant height, bunch weight and fruit quality characters with a reduction in crop duration. Santosh and Tiwari (2019) conducted a study on the Cavendish variety of banana crop for different irrigation regimes under drip irrigation. They reported higher yield (46.83 t ha⁻¹) with the drip irrigation along with BPM. They reported the application of 8312 m³ water for the whole season for one hectare. The daily crop water requirement of banana under drip with black plastic mulch varies from 4 Litres to 18.6 Litres per plant for the whole crop season at different growth stages. The study also revealed that 60.2 percent increase in yield and about 61.0 percent higher seasonal income could be obtained by using drip irrigation with BPM in comparison to conventional basin method.

Ahmed *et al.* (2011) reported that different irrigation treatments through drip significantly increased plant height, plant girth, leaf area and the number of leaves at the time of flowering of the mother plant of banana cv. Grand Nain. The best biometric response of the banana crop was obtained with 120% of ET_c under drip irrigation. Sharma and Kispotta (2016) conducted a study and reported higher plant height (8.46%), the girth of the plant (17.24%), the average number of leaves (2.94%) by adopting drip irrigation and plastic mulch. They also found that drip irrigation





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reduces 28 and 32 days for fruit setting and harvesting, respectively. Pramanik and Patra (2016) recorded maximum plant height, girth, number of leaves, leaf length, leaf breadth, leaf area, and leaf area index (LAI) at the flowering stage for both the main and ratoon crops by irrigating at 0.7 ET_c using drip irrigation and plastic mulch. The results were significantly different from other levels of irrigation. Shashidhara *et al.* (2007) reported that drip irrigation reduces days to harvesting (398 days) in comparison to the conventional irrigation method (435 days).

Fandika *et al.* (2014) conducted an experiment in Shire Valley, Malawi, to study the effect of different crop evapotranspiration (ET_c) levels under drip irrigation and reported 60 t ha⁻¹ year⁻¹ banana yield due to the application of water with 100% ET_c. The increase in average bunch weight (5 to 20 kg) was reported when applying water from 10 % to 120% of ET. Salvin *et al.* (2000) reported the highest bunch weight (14.26 kg) and yield (44 t ha⁻¹) for Cavendish banana cv. under drip irrigation at 75% ET_c. The positive response of banana yield to the water application shows that irrigation water was essential for banana production which avoids critical soil stress, which was the most limiting non biological factor in banana crop production. Pramanik and Patra (2016) reported a significantly higher yield of both main crop (39.87 t ha⁻¹) and ratoon crop (36.85 t ha⁻¹) under drip irrigation compared to surface irrigation. Field studies were conducted by various researchers on banana crop using drip irrigation and plastic mulch and reported increased yield of 5.94% (Shashidhara *et al.*, 2007), 31% (Thadchayini and Thiruchelvam, 2005), 21.95% (Sharma and Kispotta, 2016), 32.5% (Pramanik *et al.*, 2016) by using drip system and plastic mulch. Maintenance of soil moisture content near field capacity in the active root zone of the banana crop during the entire growth period resulted in an increase of yield under drip irrigation. This was mainly because of low soil water tension, higher water, and nutrients uptake with a higher oxygen concentration in the root zone (Kumar *et al.*, 2007).

CONCLUSION

Banana demands greater water and nutrients in comparison to other fruit crops. Drip system reduces the irrigation water application loss and increases its use efficiency. Plastic mulch suppresses the weed and conserves the soil moisture. The combination of drip and plastic mulch increases the water use efficiency in banana. It was clear from the review that the biometric parameters of banana under drip and plastic mulch are significantly greater in comparison to the conventional irrigation methods. Better quality and higher yield of banana can be achieved by applying irrigation water using drip along with plastic mulch.

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An Overview of the Source-Sink Relationship

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ABSTRACT

In crop plants, the physiological basis of dry matter production is dependent on the source-sink concept, where the source is the potential capacity for photosynthesis and the sink is the potential capacity to utilize the photosynthetic products. This review focuses on the current understanding of source-sink relationship in plants, physiological processes involved in transferring photosynthates, the mechanics involved in alternative scenarios and how this relationship influences crop yield and quality of the produce. We represent the constraints associated in coordination between photosynthesis and yield under varying environmental conditions. We also highlight the possibility for manipulating source-sink dynamics to boost yields, as well as the importance of yield and nutritional quality resilience, which has implications for plant breeding techniques. A clear-cut understanding of source-sink relationship and developing well-modularized and highly mechanistic source-sink interaction models that can predict yield for various crops under various conditions, possess greater scope in improving overall agricultural productivity.

Key words: Source, sink, crop, physiological process, mechanism, constraint.



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INTRODUCTION

When a crop is cultivated in the field, the cultivator aims at maximizing production while maintaining quality. However, it is a rare occurrence where the crop reaches its maximum potential yield. Crop yield potential is defined as the yield of an adapted cultivar cultivated in a perfect environment with no biotic or abiotic stress (Evans and Fischer, 1999, Das *et al.*, 2021). This approach opens up a wider range of research where growers and researchers can assess the disparities in traditional production systems, such as yield sensitivity to stress that can be monitored and decreasing the yield gap between potential crop output and actual crop yield (Lobell *et al.*, 2009; Van Ittersum and Cassman, 2013; Dey *et al.*, 2021; Mangaraj *et al.*, 2021). There are several methods for minimising crop loss and increasing production potential. The source-sink relationship has been identified as a possible alternative to investigate in order to close the production gap and assure food security. While researching the parameters controlling the rate and direction of carbohydrates flow in Cotton, two British scientists named Mason and Maskell initially proposed the source-sink idea in 1928. (Mason and Maskell, 1928, Pramanick *et al.*, 2018). Different portions of the plant body are divided into two main components by this notion, with the source being the location of photosynthesis and the site being the site of food storage. Simply said, the source produces and exports the food, such as mature leaves and other green tissues, while the sink imports and consumes the food, such as fruits and tubers (Foyer and Paul, 2001, Dey *et al.*, 2021a). The source-sink interaction and how to use it as a weapon to increase yield potential has been extensively studied over the last few decades (Laik *et al.*, 2021; Pattanayak *et al.*, 2022). In summary, it is widely accepted that yield improvements can be achieved by adjusting photosynthetic rate and modifying the distribution of assimilates in different areas of the plant, based on known and hypothetical mechanisms involved in source-sink interaction. Although numerous studies have been conducted to better understand the source-sink interaction and its modification tactics, many parts of this domain remain unexplored. The basic source-sink idea, physiological processes engaged in this interaction, mechanics involved in various aspects, their limitations, and multidisciplinary efforts to alter source-sink activity to improve crop output are all discussed in this manuscript.

Basic idea of source-sink

Dry matter production determines grain output, which is influenced by the crop's prospective ability to photosynthesize and the capacity of spikelets to take photosynthates (Aye *et al.*, 2020). A photosynthetically active organ that provides food is the source. Sink, on the other hand, is the organ that stores and reserves food. This source is used for the plant's growth and development (Kozłowski, 1992, Singh *et al.*, 2017, Garai *et al.*, 2019). The source-sink connection determines whether photosynthates are stored as starch or delivered to the plant's growth portions. Carbon and nitrogen are the two most essential substrates in plants, and they can be classed as source and sink organs (Table 1). (Chang and Zhu, 2017).

The balance between organ assimilate demand for growth and maintenance and whole-plant assimilate supply via photosynthesis or reserve mobilisation is maintained by source and sink (Sadras & Denison, 2009). Plant growth and development are influenced by the ratio between organ assimilate demand and supply (Marcelis *et al.*, 2004). If the assimilate supply to demand ratio is discovered to be low due to abiotic restrictions or low incoming radiation, the plant's vegetative development is reduced, and as a result, dry matter production is reduced (Tardieu *et al.* 1999 in maize and sunflower, Lafarge *et al.* 2010 in rice, Pallas *et al.* 2011 in grapevine). To achieve a balanced ratio between source and sink, it is necessary to understand the flow and direction of assimilates from source to sink.

Physiological processes involved in assimilate transport from source to sink

Only a small percentage of the sugars generated in a plant are carried great distances through the phloem, regardless of the species or type of phloem loading. Sucrose is the primary carbon source in the phloem in all situations. Polyols (mostly sorbitol and mannitol) and raffinose oligosaccharides can also be detected in addition to sucrose. Polyols and raffinose are found in the phloem of several species (Rennie and Turgeon, 2009). A small number of taxa have been observed to transport hexose in the phloem (van Bel and Hess, 2008). According to many studies, mature leaves can





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transfer up to 80% of photosynthetic fixed carbon. Photosynthetic activity (carbon fixation), partitioning between starch synthesis in the chloroplast and triose-phosphates exported from the chloroplast for sucrose synthesis, and transitory storage of sucrose in the vacuole all influence the quantity of sucrose available for export from source leaves. s. During the early developmental phases, roots and young leaves are primary sinks, but tubers, fruit, and seeds become key sinks during the reproductive stages (Wardlaw, 1990, Pramanick *et al.*, 2020). SEs and phloem parenchyma cells have different membrane potential levels, which affects their ability to recover sucrose from the apoplast (Hafke *et al.*, 2005). Even in tree species where loading is symplastic in the collection phloem, SUTs are involved in sucrose transfer in the transport phloem (Turgeon, 2010b). The mechanisms for sucrose loading in the conducting cells of the phloem in herbaceous species have largely been reported in the context of active phloem loading (Fig.1). The sieve element-companion cell complex (SE/CC complex) has a greater solute content than the surrounding tissues due to active phloem loading.

Sucrose and polyol transporters have been found in various species as part of the mechanism of active phloem loading from the apoplastic region (Noiraud *et al.*, 2001b; Lalonde *et al.*, 2004; Sauer, 2007; Reinders *et al.*, 2012). By dissipating the proton gradient created by an H⁺/ATPase in the same cells, these transporters can concentrate sugars in the SE/CC complex. The recently found SWEET facilitators may regulate the release of sucrose in the apoplast in the region of the SE/CC complex (Chen *et al.*, 2012; Fig.1). Polymer trapping, in which sucrose is transformed to raffinose or bigger molecules by adding galactose to sucrose in intermediary cells, is the second process for active phloem loading (Rennie and Turgeon, 2009). Sugars migrate from cell to cell through a symplastic pathway in this scenario (Fig. 1). There are various evidence of passive phloem loading, at least in tree species, therefore active phloem loading may not be universal (Rennie and Turgeon, 2009; Turgeon, 2010b). The transit phloem transports sucrose from the source to the sink organs, while the release phloem delivers sucrose to the sink organs (Van Bel, 2003). Mass-flow, as proposed by Münch, is the most widely accepted theory for explaining solute transport in the phloem. Sugars can depart the phloem through either a symplastic or an apoplastic pathway in the release phloem, however the first steps are frequently symplastic (Fisher and Oparka, 1996; Patrick, 1997). Unloading routes, on the other hand, are dependent on the type of sink and its state of development (Fig . 1). During potato tuberization, a switch from apoplastic to symplastic unloading was seen (Viola *et al.*, 2001). In grape berry development, Zhang *et al.* (2006) revealed a change from symplastic to apoplastic unloading, whereas evidence for an apoplastic stage in sucrose and sorbitol unloading, requiring transporters, has been reported in apple and cherry fruit development (Gao *et al.*, 2003; Zhang *et al.*, 2004).

Mechanics of Source-Sink

Both the source and the sink can grow individually, or there may be a ratio between them that allows for growth. Three alternative scenarios can be visualised in crop plants:

- 1) The source might have a larger physical area than the sink;
- 2) The sink could have a larger size than the source; and
- 3) Both could be in dynamic equilibrium.

Condition 1: If the source exceeds the sink

Traditional types of various crop plants have more sources than sinks due to their leafy structure. Leafiness is reduced and sink size is enhanced by enhancing crop attributes. When the source is larger (fertilisation and cultural practises result in more foliage), the photosynthetic rate (Pr) increases and more photosynthates are produced; but, because the sink is small, some photosynthates are unable to exit the leaf, and translocation can be as low as 10%. (Tanaka and Fujita, 1974). Some photosynthates that leave the leaf collect in the leaves or stem again. Under such conditions, the leaves become twisted and coloured, and the leaf's Pr decreases, resulting in poor productivity. Cereals, pulses, and a variety of annuals, trees, and other plants exhibit this trait. Because of the soil-water system, weather conditions, and nutrient availability, there may be a shift in source-sink balance even within a crop or cultivar. At low N levels, cereals like rice and wheat generate ample leaves but weak panicles with unproductive tillers. In most cases, nutrient deficits (P, Zn, Mn) and toxicities (Fe, Al) result in enough leaves but low grain production. In each of these cases, the source is larger than the sink.



**Anurag Bera et al.,****Condition 2: If the sink exceeds the source**

The sink size (e.g., flower buds, fruit set, pegs, pods, and bolls) is bigger than the sources in fruit trees like mango, guava, citrus, vegetables like cucurbits, tomato, and pulses like pea, gramme, and cotton. The sinks, however, decline early because to a lack of assimilates. If fruit set can be raised by 1-2 percent in mango, guava, and citrus, crop yield can be increased by 50-100 percent. Breeding has expanded the sink size in cereals and some grain crops. Even though the sink size is adequate in those cases, grain production is low. Even in rice, increasing the Leaf Area Index (LAI) does not lead to higher grain output; instead, it approaches a plateau (Venkateswarlu, 1976). The distance between spikelets and grain widens as LAI rises. The actual leaf area may be greater than required in some cases, but the functional efficiencies are far lower. The functional capacities of these cultivars and crops can be improved with correct treatment.

Condition 3: An equilibrium maintained between source and sink

The sink in rice, primarily the number of spikelets produced per square centimetre of leaf, was projected to highlight the patterns between source and sink in physical terms in a study by Venkateswarlu and Maddulety in 1976. The number of spikelets created per square centimetre of leaf at various LAI values was calculated, and the possible production of spikelets at various LAI levels was determined based on those calculations. Actual spikelet counts at various LAI levels were used as controls. There were variances in length and genotype among the types. At 0 N level, the spikelet number per LAI was 8,500, implying that there may be 34,000 spikelets per roughly 4 LAI; however, only 23,000 spikelets were realised, implying that the sink size was smaller than the source at lower N levels. However, at 50 and 100 kg N, spikelet production increased proportionately with increasing LAI values (about 4-5), indicating a tendency for source-sink equilibrium.

Clearly, a larger source or a larger sink leads in lower productivity; despite full expression of the sink, the realisation of yield is lower in the former and later circumstances, respectively, due to smaller sink size and partial realisation of sink. Because the source is more susceptible to climatic, hydrological, soil, nutritional, and biotic stresses when both source and sink are in balance, there is a danger of lower and unstable yields. High spikelet number is connected with increased full grain number in a favourable habitat and climate. To realise the maximum sink size under field conditions, higher and more steady functional efficiency at moderate source size should be prioritised over big physical size.

Source-Sink constraints

Source or sink limitations, or both, are the most common causes of yield reduction or constraints. Understanding and monitoring metabolic indicators of source or sink limitation is an important aspect of global efforts to boost agricultural productivity in order to ensure future food security. Source constraints are mostly caused by a drop in LAI or a fall in NAR caused by defoliation caused by severe weather conditions, illnesses, or sucking insect assault, which limits the leaf area development and hence impacts the source size. Diminished source activity is also due to reduced light intensity on the plant canopy. Sink Limitations, on the other hand, are caused by floret sterility, insect damage, nutrient supply limitations, canopy temperature, hormonal variables, water logging, salt, and other causes. Unfavorable climatic conditions, such as heat stress, can impede both floret setting and sink activity. In certain wheat genotypes, changes in photoperiod sensitivity and fertilisation as a result of high temperatures impair anther and pollen viability, resulting in spikelet sterility (Ai Qing *et al.*, 2018, Prieto *et al.*, 2018, Hütsch *et al.*, 2019).

Effects of source-sink constraints on sesame yield and yield characteristics

The goal of this experiment was to see how source-sink limitations affected Sesame yield and yield characteristics. It was conducted from March to June 2015 in the Patuakhali Science and Technology University's experimental area of Agricultural Botany in Dumki, Patuakhali, Bangladesh. At the capsule development stage, source-sink manipulation treatments were applied by removing the specified source-sink organs using scissors (50 days after emergence). The experiment included five treatments, including a control group. M0 = Control, M1 = Lower empty leaves, lower empty branches, and top of the inflorescence were removed, M2 = Top of the inflorescence was removed, M3 = All



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branches and lower empty leaves were removed, and M4 = Lower empty branches were removed. Table 2 shows the findings of the experiment.

The number of capsule per plant

The number of capsule plant⁻¹ was significantly affected by source-sink modification. The control treatment produced the highest (17.83 capsule plant⁻¹) number of capsule plant⁻¹, which was statistically similar to the removal of lower leaves, lower empty branches, and top of the inflorescence (M1) (16.58 capsule plant⁻¹) and removal of top of the inflorescence (M2) (16.58 capsule plant⁻¹) (16.52 capsule plant⁻¹). The removal of all branches (M3) produced the lowest number of capsule plant⁻¹ (13.78 capsule plant⁻¹), which was statistically similar to the removal of lower leaves and lower empty branches (M4), which produced 14.63 capsule plant⁻¹. The number of capsules per plant was reduced by 22.71 percent when all branches were removed (M2). It's possible that the reduction is related to the removal of all branches.

Seeds per capsule

The source-sink modification has a considerable impact on the number of seeds per capsule. The removal of lower leaves, lower empty branches, and the apex of the inflorescence yielded the most seeds per capsule (57.13). (M1 manipulation). The apex of the inflorescence (M2 manipulation), all branches (M3 manipulation), and lower leaves and empty branches (M4 manipulation) all produced statistically equivalent seeds capsule⁻¹ (53.10, 50.78, and 52.05, respectively) and were ranked second. The control treatment had the smallest quantity of seeds per capsule (40.17).

1000 seed weight (g)

The weight of 1000 seeds was considerably affected by source-sink modification (Table 2). The removal of lower leaves, lower empty branches, and the top of the inflorescence (M1) resulted in the highest 1000 seed weight (2.92 g), which was statistically different from the other manipulation treatments. The removal of the top of the inflorescence (M2) resulted in the lowest 1000 seed weight (2.69 g), which was statistically similar to the removal of lower leaves and lower empty branches (M4) (2.55 g). The control treatment yielded the lowest 1000 seed weight (2.24 g). The removal of lower leaves, unproductive branches, and the top of the inflorescence increased dry matter accumulation in reproductive sinks, resulting in a greater 1000-seed weight due to reduced competition for dry matter assimilation.

Seed yield (kg ha⁻¹)

The seed output varied significantly depending on the source-sink modification techniques used. When the lower leaves, lower empty branches, and top of the inflorescence were removed (M1), the largest seed yield (1110.96 kg ha⁻¹) was recorded (Table 2). The removal of the inflorescence's top produced the second highest seed output (948.32 kg ha⁻¹) (M2). When all branches (M3) and lower leaves and empty branches (M4) were removed, a statistically equivalent yield was obtained (700.49 and 780.89 kg ha⁻¹, respectively). The minimum seed production in the control treatment was 645.57 kg ha⁻¹. The removal of lower leaves, lower empty branches, and the top of the inflorescence (M1 manipulation) increased seed yield by 71.77 percent; 46.88 percent due to the removal of the top of the inflorescence (M2); 8.52 percent due to the removal of all branches (M3); and 22.45 percent due to the removal of lower leaves and lower empty branches (M4). The elimination of relative sinks such as lower empty leaves and lower empty branches, as well as the apex of the inflorescence, enhanced the assimilate supply to the previously existent capsules. As a result, the number of seeds per capsule and the weight of 1000 seeds increased.

Harvest index

With source-sink adjustment, the harvest index was significantly raised. M1 manipulation showed the highest harvest index, which was significantly different from the other manipulation treatments. The removal of lower leaves, lower empty branches, and the top of the inflorescence resulted in the highest harvest index (37.34 percent) (M1). The lowest percentage, 30.95 percent, was seen in the control treatment (Table 2). In 2002 and 2003, researchers at the University of Massachusetts Agronomy Farm conducted an experiment to see how ambient and enhanced light conditions affected the quantity of seeds per pod of soybean. In this study, four soybean cultivars were used. Altona of maturity group 0 and Evans of maturity group I were the two cultivars taken for the experiment. Northrup



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King S19-V2 of maturity group I and Northup King S28-V8 of maturity group II, both Roundup Read, were the other two novel cultivars. On May 18, 2002, Altona and Northup King S19-V2 were planted. Evans and Northup King S28-V8, the other two cultivars, were planted on May 16, 2003. The experiment included five treatments, including a control group. CK – control plants (no manipulation), SP – removal of all but one pod from main axis nodes upon emergence, AP – removal of pods from alternate main axis nodes upon emergence, CL – removal of the central leaflet from each trifoliolate main axis leaf upon emergence, ALP – removal of the trifoliolate leaf and all pods from alternate main axis nodes upon emergence Fig. 2 to 5 show the results of the experiment.

When compared to changes in pod number per plant, seed number per pod responded less to light enrichment and source-sink treatments. In both light conditions, the SP and AP treatments did not raise the seed number per remaining pod for the two old cultivars, but it was slightly increased for the two new cultivars. Under ambient light, reducing the source in the CL treatment significantly reduced the seed number per pod in the old cultivars but not in the new cultivars. In comparison to the two old cultivars, the two new cultivars are able to maintain or increase the number of seeds per pod in their main axis under a limited source condition. Despite this, no significant differences between CL and check plants in all cultivars were found under light enrichment. In this enriched light environment, the ancient cultivars were able to compensate for the reduced leaf area by utilizing the additional light. The removal of the flag leaf also reduced grain yield per spike, number of grains per spike, and number of viable and non fertile spikelets in wheat by 10.1 percent, 8.4%, 9.2%, and 23.8 percent, respectively (Abdoli *et al.* 2013). In the instance of canola, once plants reached full blooming and pods formed in around 2/3 of the main raceme, 50 percent of pods and remaining flowers on raceme and branches were cut off with scissors, resulting in a 24 percent reduction in biomass and a 34 percent reduction in yield (Zhang *et al.* 2018).

Manipulation of Source-Sink

Plant resource partitioning and growth and development are controlled by a highly regulated balance between sources and sinks. Various methods of source sink modification exist:

Defoliation

The formation of assimilates by the leaves and the utilization of these assimilates in the developing seeds are the two most important processes that impact yield. The delay in the commencement of leaf senescence, suppression of chlorophyll breakdown, or a slower rate of senescence can all cause an increase in the green leaf area (Thomas and Howarth, 2000). Delaying leaf senescence is thought to boost photosynthetic assimilation capacity and, as a result, yield (Gregersen *et al.*, 2013). The source-sink ratio, which controls plant growth, development, and production, can be influenced by removing the apical bud (decapitation) and reducing sink strength through deflowering. Decapitation (decap) also maintains chloroplast integrity and reduces chlorophyll loss in crops like tomato and tobacco (Colbert and Beever, 1981). Decap has also been shown to boost plant growth and yield in some bean species (Tayo, 1982; Khan *et al.*, 2018). For example, in soyabean, decap was performed at 35 DAS when four nodes on the main stem had fully grown leaves beginning with the unifoliolate nodes, whereas R2LR was applied at 50 DAS when the plants had one open flower at one of the two topmost nodes on the main stem. During both experimental seasons, it was discovered that decap, R2LR, and their combination enhanced pod set, number of pods per plant, number of three- or more-seeded pods per plant, and seed yield per plant (Ibrahim *et al.*, 2021). During the first and second seasons, the percentage of pod sets increased by 18.8, 9.9, 24.9 percent and 28.9, 17.4, 32.5 percent in response to R2LR, decap, and R2LR+decap, respectively (Figure 6A). The number of pods produced per plant grew by 14.5, 45.7, 85.2 percent, and 11.7, 51.6, 77.5 percent, respectively (Figure 6B). Seed yield per plant increased significantly (114.9, 171.3, 129.1 percent and 61.6, 145.1, 136.6 percent, respectively) in response to R2LR, decap, and their combination throughout both experimental seasons (Figure 6C). Furthermore, decapping cowpeas at the fifth leaf stage boosted branching, flowering, pod set, yield, and harvest index (Argall and Stewart, 1984). As a result, reducing the competition for assimilates by removing racemes, i.e., lowering sink strength, may have helped to flower abortion decrease by raising the source-sink ratio. Decap, on the other hand, did not consistently improve seed yield, however it did increase the number of flowers, pod set, and number of 3- or more-seeded pods, according to Amuti (1983).



**Anurag Bera et al.,****By lowering the intensity of light**

Throughout the plant life cycle, light is one of the most important environmental factors that regulates plant physiology (Devlin *et al.*, 2007; Li and Kubota, 2009). Light influences stem elongation, branch emission, and leaf expansion throughout plant growth, determining plant design, and finally driving the transition to flowering, fruit setting, and seed production (Paik and Huq, 2019). Plant productivity is controlled not only by light quantity (photoperiod), but also by light quality (wavelength composition), which effects plant development and photomorphogenesis, as well as tissue composition (Ouzounis *et al.*, 2015; Carvalho *et al.*, 2011). Blue light, for example, affects stomatal opening, plant height, and chlorophyll biosynthesis, whereas far red light stimulates flowering in longday plants, and the red/far red ratio controls stem elongation and branching, leaf expansion, and reproduction (Zheng *et al.*, 2019). Changes in leaf anatomy, notably in palisade parenchyma, have been shown to be dependent on light spectrum-induced changes in leaf morphology, such as those in leaf thickness (Zheng and Van Labeke 2017).

Plants are subjected to a variety of abiotic stresses, with salinity and alkaline stress being the most limiting conditions for plant growth and development (Hasanuzzaman *et al.*, 2012; Martinez-Cuenca *et al.*, 2013). Red (peak 660 nm) and red/blue (1:1) (400–700 nm) light had a favourable effect on CO₂ assimilation under such stress situations. Blue/red light also boosted intrinsic WUE (Esmailizadeh *et al.*, 2020). It was also shown that altering the light spectrum can partially ameliorate the negative effects of salt and alkalinity stress on photosynthesis. These evidences demonstrate the significance of different wavelengths of the light spectrum, either individually or in combination in the production of morphological and physiological responses in plants (Folta and Childers 2008; Hogewoning *et al.*, 2011; Macedo *et al.*, 2011).

Employment of growth retardant

The unexpected behaviour of diverse crops is caused by excessive vegetative growth, inadequate bud development, flower shedding, and a growth imbalance between the source and sink (Ouzounidou *et al.*, 2010; Vistro *et al.*, 2017; Kintzios *et al.*, 2002). The key to improving biomass and grain yield is to extend the green canopy duration by delaying leaf senescence and increasing photosynthesis. According to Brar *et al.* (2020), application of mepiquat chloride (MC) in cotton, regardless of splits between 60 and 90 DAS, favoured source-sink relationships by reducing plant height and improving yield attributes, resulting in significantly higher seed cotton yield (SCY) when compared to control and de-topping (Table 3). Paclobutrazol changed the physiology of cashew trees by changing their size, canopy growth, internodal length, branching pattern, and overall ground covering (Mog *et al.*, 2019). Development retardants can thus be employed to harness morpho-physiological features for improved canopy growth and yield maximisation in a variety of plants (Sawan, 2013; Hussain *et al.*, 2022; Trethewey *et al.*, 2016).

Reducing inflorescence and flower production

When applying source-sink manipulation treatments on amaranthus at mid-flowering, it was discovered that removing all leaves had a significant negative impact on all parameters, with yield reductions ranging from –49 percent to –73 percent. In contrast, no significant yield drop was seen after 50 percent bloom removal and 50 percent defoliation (Schobesberger and Kaul, 2013). In leafy vegetables, we can deduce that source strength during flowering limits yield more than sink capacity (Andrade and Ferreira 1996; Borrás *et al.*, 2004; Gimplinger *et al.*, 2008). Water logging reduced grain weight by roughly 40% in wheat and barley in one experiment. Increasing the source sink ratio by cutting the spikes one week after anthesis resulted in a 40% increase in wheat grain weight and a 20% increase in barley grain weight (Becheran *et al.*, 2022). Furthermore, as documented in mungbean, deflowering changed the anatomical shape of the remaining racemes' rachises by stimulating the production of their conductive treachery components, resulting in a higher ability to feed the nutritional requirements of the growing pods (Modal *et al.*, 2011).





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CONCLUSION

Despite the fact that much effort has been put into studying the source–sink relationship, we are still a long way from fully comprehending source–sink interaction and even further from rational manipulation. In the current situation, when climate change and rising food demand are important concerns, we must investigate all aspects of the source-sink interaction in order to boost food production. To achieve this, we must develop well-modularized and highly mechanistic source-sink interaction models that can predict yield for various crops under various conditions, allowing us to manipulate resources to achieve higher yield.

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Table 1: Major source and sink organs differentiated in a plant

Substrates	Major source organs	Major sink organs
Carbon	Mature leaves (at least 75% or fully expanded), green fruits/seeds coat, green branches.	Root, tubers, elongating stem, developing fruits/seeds
Nitrogen	Root (inorganic N), Mature leaves, green tissues (organic N)	Tubers, fruits/seeds (coat), tender leaves, Elongating stem (organic N)

Table 2: The impact of source-sink constraints on sesame yield and yield characteristics (Howlader *et al.* 2018)

Treatments	Capsule plant ⁻¹ (no.)	Seed capsule ⁻¹ (no.)	1000 seed weight (g)	Seed Yield (kg ha ⁻¹)	Harvest index (%)
M0	17.83 a	40.17c	2.24d	645.57d	30.95d
M1	16.58a	57.13a	2.92a	1110.96a	37.34a
M2	16.52a	53.10b	2.69b	948.32b	34.94ab
M3	13.78b	50.78b	2.49c	700.49cd	32.20cd
M4	14.63b	52.05b	2.55bc	780.89c	34.44bc
LSD (p = 0.05)	0.52	1.59	0.056	35.68	0.851
CV (%)	7.16	6.45	6.45	8.23	6.39

Table 3: Bt cotton growth, yield, and yield contributing features under various growth retardant treatments

Treatments	Plant height (cm)	Bolls/ plant	Boll weight (g)	Seed Cotton Yield (kg/ha)
Control	110.2	27.6	2.77	1147
MC 50 g a.i./ha at 80 DAS	99.0	29.7	2.86	1298
MC 62.5 g a.i./ha at 80 DAS	98.5	30.1	2.96	1383
MC 75 ga.i./ha at 80 DAS	93.5	32.3	3.01	1499
Three time MC 25 g a.i./ha each at 60,75 and 90 DAS	86.3	35.7	3.30	1581
Twice MC 37.5 g a.i./ha each at 75 and 90 DAS	91.8	34.5	3.21	1532
Three time MC 25, 31.3 and 31.3 g a.i./ha at 60,75 and 90 DAS	87.0	36.5	3.39	1625
De-topping at 80 days	96.5	30.6	2.79	1309
LSD (p = 0.05)	11.3	4.47	0.38	233
CV (%)	8.04	9.47	8.59	11.14





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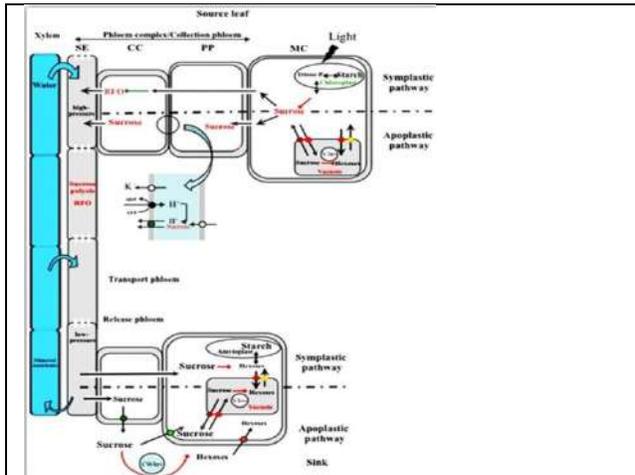


Fig. 1: Sugar transit from source to sink in symplastic and apoplastic active phloem loaders

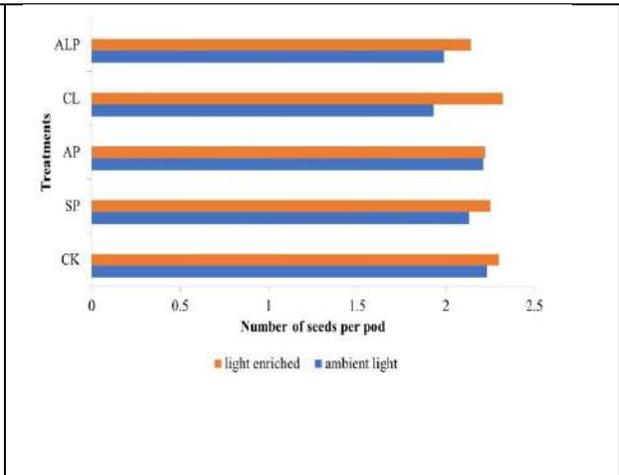


Fig.2: The total quantity of pods per plant of Altona is affected by ambient and enriched light conditions

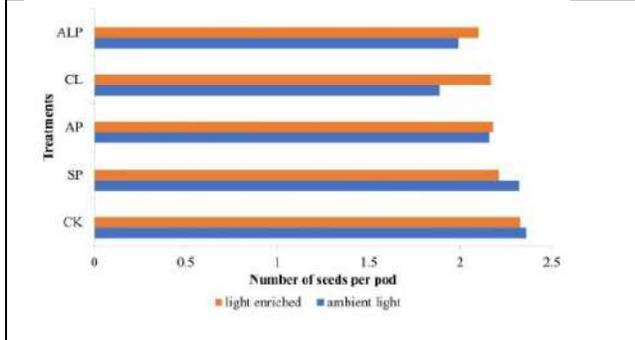


Fig. 3: The total quantity of pods per Evans plant is affected by ambient and enhanced light conditions

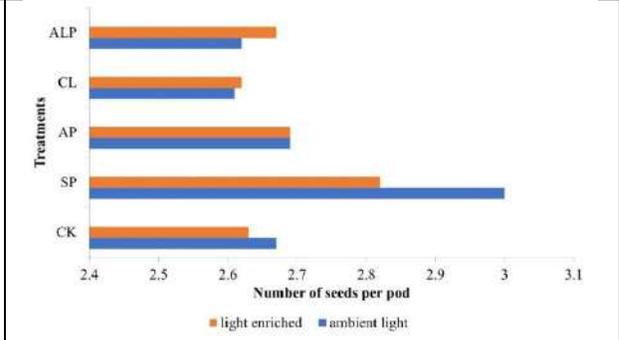


Fig. 4: Total number of pods per plant of S19-V2 under ambient and enhanced light conditions

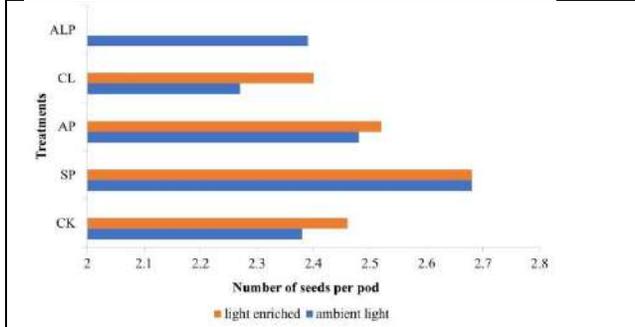


Fig. 5: Total number of pods per plant of S28-V8 under ambient and enhanced light conditions

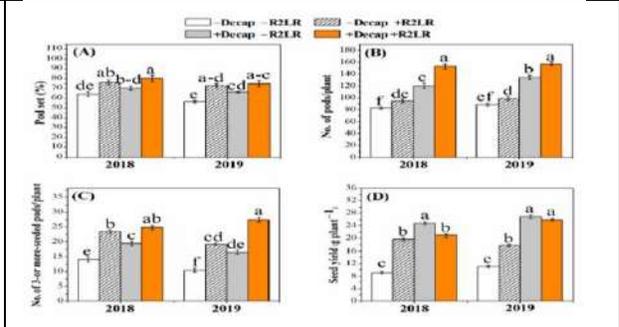


Fig. 6: Pod set (percentage, A), number of pods per plant (B), number of 3- or more-seeded pods per plant (C), seed yield per plant (D), and protein content (percentage, E) of soybean plants after decapitation and raceme removal treatments. -R2LR and +R2LR, respectively, no removal and removal of the two lowest racemes; -Decap and +Decap, respectively, no decapitation and decapitation





Estimation of Irrigation Scheduling and Irrigation Requirement for Maize (*Zea mays* L.) in Southern Odisha by using FAO CROPWAT8.0 Model

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ABSTRACT

Irrigation management is one of the key aspects in crop production. Proper scheduling of irrigation can result in higher water use efficiency and thereby conserving water resources. In south Odisha conditions during *rabi* the crops are raised through manual irrigation. During *rabi* maize is one of the important crops in this location and this crop requires huge amounts of water to complete its life cycle. In this regard proper irrigation scheduling can improve crop growth and increase productivity. Here, a simulation modelling study based on CROPWAT 8.0 model was carried out to estimate the water requirement and irrigation scheduling for maize in south Odisha conditions. The CROPWAT model predicted the daily, decadal as well as monthly crop water requirement at different growth stages of maize crop. The crop water requirement and actual irrigation requirement for maize crop was obtained as 452.7 mm and 402.7 mm. Considering the results provided by the model CROPWAT 8.0, it can be suggested that water requirement of maize can be calculated by adopting the model.

Keywords: Maize, Irrigation scheduling, Crop water requirement, CROPWAT

INTRODUCTION

Water is one of the important resources for agricultural crop production (Zaman *et al.*, 2017). In present days the concern for efficient use of water has increased due to increased population and reduction in water resources. Most of the fresh water resources are majorly used in agriculture. So in this case water use efficiency must be improved by various approaches and strategies for attaining agriculture sustainability (Surendran *et al.*, 2015). In India most of the agriculture is based on cereal based cropping. Rice, maize and wheat are the major cereals which consumes maximum water to complete their life cycle. Out of the major cereals grown in India maize is one of the important





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cereal crop grown in Odisha as well as in India for food and fodder (Maitra *et al.*, 2021). In *rabi* maize is mostly cultivated through manual irrigation as there is no scope for rainfall during *rabi* season. In this regards proper irrigation scheduling and calculating crop water requirement can enhance the water use efficiency and by saving water consumption. CROPWAT 8.0 is a type of modelling tool developed by Food and Agriculture Organization (FAO) which supports to perform calculations related to irrigation requirement and irrigation scheduling (Singh *et al.*, 2014). The main functions of CROPWAT 8.0 includes the calculation of reference evapotranspiration, crop water requirements and crop irrigation requirements along with irrigation schedules under various management conditions (Allen *et al.*, 1998). In this present study this modelling tool was used to calculate the irrigation requirement and irrigation scheduling of maize under red sandy loam soil conditions of South Odisha region.

MATERIALS AND METHODS

Study Location

The Southern Odisha is located between longitude 72°97' to 85°05' E and latitude 18°34' to 20°66' N. The meteorological data of gopalpur located at longitude 81°24.2' E to 84.2° E and latitude 17°4' N to 20°7' N was used for estimation of crop water requirement. The soil of the study area is mostly red sandy loam soils.

Model

CROPWAT 8.0 is an FAO-developed decision-support computer application that uses soil, crop, and climatic data to determine reference evapotranspiration (ET_0), crop water need, irrigation schedule, and irrigation water requirement. The programme provides general data for various crop characteristics, local climate conditions, and soil attributes and it aims to improve the irrigation schedules and the estimation of water supply for various crop patterns for both irrigated and rain-fed cropping systems. CROPWAT 8.0 can also be used to assess farmers' irrigation practices and to estimation the reference evapotranspiration, crop evapotranspiration, and irrigation water requirement.

Data used for estimation of ET_0

Monthly climatic data *viz.* minimum temperature, maximum temperature, relative humidity, wind velocity, duration of bright sunshine hours of Gopalpur located in studyarea was used (Table 1) for the estimation of reference evapotranspiration (ET_0) using Penman-Monteith formula by FAO CROPWAT 8.0.

Rainfall Data

Monthly rainfall data of Gopalpur was used for calculation of effective rainfall (Table 1). USDA soil conservation method is used in this software.

USDA Soil Conservation Service formula:

Case 1 : $P_{eff} = P_{mon} * (125 - 0.2 * P_{mon}) / 125$ (If $P_{mon} \leq 250$ mm)

Case 2: $P_{eff} = 125 + 0.1 * P_{mon}$ (If $P_{mon} > 250$ mm)

P_{eff} =Effective Precipitation

P_{mon} = Monthly Precipitation

Crop data

In Southern Odisha, during *rabi* season, the cultivation of crops generally begins after the harvest of kharif rice. In general, *rabi* maize, was sown around 15th of December and this crop takes 125 for maturity and harvesting. Duration and sowing date mentioned in the software helps in determine the phenophase of the crops. At each phenophase K_c value, rooting depth (m), critical depletion value and crop yield response factor were decided based on the duration of the crops. The yield response factor (K_y) is the ratio of relative yield reduction to relative evapotranspiration deficit that integrates the weather, crop and soil conditions that make crop yield less than its potential yield in the face of deficit evapo-transpiration also estimated in the model. The crop data used in this experiment were presented in Table 2.





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Soil data

Soil type in this area is a red sandy loam. The software needs some general soil data like total available soil moisture, maximum rooting depth, initial soil moisture depletion and initial available soil moisture and maximum rain infiltration rate. These information was obtained from FAO manual 56.

Crop Water Requirement

The crop water requirement for the given crop duration was computed using the input *viz.* climate, crop and soil data of the region. The crop evapotranspiration (ET_c) was calculated by the equation $ET_c = K_c \times ET_o$.

Where, K_c: crop coefficient,

ET_c: crop evapotranspiration

ET_o: reference evapotranspiration.

Difference between crop evapotranspiration and the effective rainfall determines the total irrigation requirement of the crop.

Irrigation Scheduling:

Irrigation scheduling determines when to irrigate and how much amount of water to be given to the crop at each irrigation. In this study, irrigation was scheduled at 50 per cent critical soil moisture depletion and irrigation was applied till the soil is refilled to field capacity at 70 per cent efficiency.

RESULTS AND DISCUSSION

Estimated Reference Evapo-Transpiration:

The reference evapo-transpiration was found to be maximum during the month of March (4.92 mm/day) and lowest was observed in the month of January (3.51 mm/day) (Table 1). This might be due to, wind velocity, sunshine hours in the month of March resulting in increased atmospheric demand for moisture (Bhat *et al.*, 2017; Todorovic *et al.*, 2013).

Crop Water Requirement

The value of daily or decadal crop water requirements for *rabi* maize, for Southern Odisha conditions was presented in the Table 3. The highest crop water requirement for maize was occurred in the midseason phase, during second decade of February (44.5 mm). While the lowest water requirement value was observed during initial phase. This might be due to less metabolic activities in the plants during initial days of crop growth while the plants require high water need during grand growth phase (Abirdew *et al.*, 2018; Roja *et al.* 2020). The crop water requirement estimated by CROPWAT 8.0 for the *rabi* maize in Southern Odisha was 410.0 mm. The actual irrigation requirement for *rabi* maize was computed after estimating the actual rainfall contribution in satisfying the part of water requirement. The computed irrigation requirements for *rabi* maize was 406.7mm.

Irrigation Scheduling

In Southern Odisha, under red sandy loam soils the irrigation scheduled at 50 per cent of critical depletion with a quantity of water sufficient to refill the soil to 100% field capacity, the maize required 10 irrigations during the growing period (Zhiming *et al.*, 2007). The net irrigation requirement of maize was 420.3 mm and gross irrigation requirement was 600.4 mm. Similar findings were reported by Roja *et al.* 2020 and Ramulu *et al.* (2010) in maize.

CONCLUSION

The analyzed data by FAO CROPWAT 8.0 model-based tool estimated that during *rabi* maize requires 406.7mm of irrigation in southern Odisha. The model suggests that maize requires 10 irrigations during the crop period. Further, the use of CROPWAT can assess the crop water requirement with a high level of accuracy and water needed for a





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particular crop and can suggest the crop rotation and crop pattern for the farmers. which when adopted will help in getting higher water productivity and gives greater scope in reducing the wastage of irrigation water.

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Table 1: Monthly meteorological data and estimated reference evapotranspiration (ET_o) using Penmen Monteith method

Month	Min Temp °C	Max Tem °C	Humi dity %	Wind km/day	Sun hours	Rad MJ/m ² /day	ET _o mm/day	Rainfall mm	Effective Rainfall mm
January	16.9	27.4	73	227	7.7	16.5	3.51	2.0	2.0
February	19.4	28.9	76	307	7.9	18.5	4.16	18.0	17.5
March	22.6	30.6	77	422	7.8	20.2	4.92	20.0	19.4
April	25.1	31.1	82	540	6.3	19.0	4.72	11.0	10.8





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May	26.8	32.1	84	609	4.8	17.2	4.52	35.0	33.0
June	26.9	32.2	83	412	3.9	15.7	4.26	149.0	113.5
July	26.1	30.8	85	415	3.1	14.4	3.75	194.0	133.8
August	26.1	31.1	84	347	3.8	15.2	3.88	239.0	147.6
September	25.8	31.6	82	288	4.2	15.2	3.92	189.0	131.8
October	23.9	31.1	79	251	5.6	15.7	3.98	145.0	111.4
November	19.5	29.3	71	249	7.7	16.8	4.12	60.0	54.2
December	16.6	27.5	71	235	7.9	16.1	3.61	6.0	5.9
Total	-	-	-	-	-	-	-	1068.0	780.9
Average	23.0	30.3	79	358	5.9	16.7	4.11	-	-

Source: <https://www.weather-atlas.com/en/india/Gopalpur-climate>

Table 2: Daily and Decadal Crop Water Requirement of maize in the study area

Crop Water Requirement														
Month	Dec	Dec	Jan	Jan	Jan	Feb	Feb	Feb	Mar	Mar	Mar	Apr	Apr	
Decade	2	3	1	2	3	1	2	2	3	1	2	3	1	
Stage	Init	Init	Deve	Deve	Deve	Mid	Mid	Mid	Mid	Late	Late	Late	Late	
Kc coeff	0.3	0.3	0.39	0.69	1.01	1.22	1.22	1.22	1.22	1.17	0.92	0.66	0.44	
ETc mm/day	1.08	1.07	1.37	2.41	3.76	4.82	5.10	5.40	5.79	5.88	4.55	3.17	2.06	
ETc mm/dec	6.5	11.8	13.7	24.1	41.4	48.2	51.0	43.2	57.9	58.8	50.0	31.7	16.5	454.8
Eff rain mm/dec	0.0	0.2	0.5	0.0	1.6	4.5	6.4	6.4	6.5	6.9	5.8	3.6	1.7	44.3
Irr. Req. mm/dec	6.5	11.6	13.1	24.1	39.8	43.7	44.5	36.8	51.4	51.9	44.2	28.1	14.4	410

Table 3: Irrigation schedules for Maize crop during the study period as per the CROPWAT model

Date	Day	Stage	Rain mm	Ka fraction	Eta %	Net Irrigation mm	Deficit mm	Loss mm	Gross Irrigation, mm	Flow L/s/ha
16 Dec	1	Init	0.0	1.00	100	18.2	0.0	0.0	26.0	1.50
2Jan	8	Init	0.0	1.00	100	27.3	0.0	0.0	39.0	0.27
18 Jan	17	Dev	0.0	1.00	100	37.9	0.0	0.0	54.2	0.39
29 Jan	29	Dev	0.0	1.00	100	42.6	0.0	0.0	60.8	0.64
9 Feb	40	Mid	0.0	1.00	100	47.7	0.0	0.0	68.2	0.72
19 Feb	51	Mid	0.0	1.00	100	44.0	0.0	0.0	62.9	0.73
1 Mar	63	Mid	0.0	1.00	100	47.5	0.0	0.0	67.8	0.78
10 Mar	74	Mid	0.0	1.00	100	45.4	0.0	0.0	64.8	0.83





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20 Mar	84	End	0.0	1.00	100	51.6	0.0	0.0	73.7	0.85
5 Apr	94	End	0.0	1.00	100	58.0	0.0	0.0	82.9	0.60
18 Apr	105	End	0.0	1.00	0	-	-	-	-	-

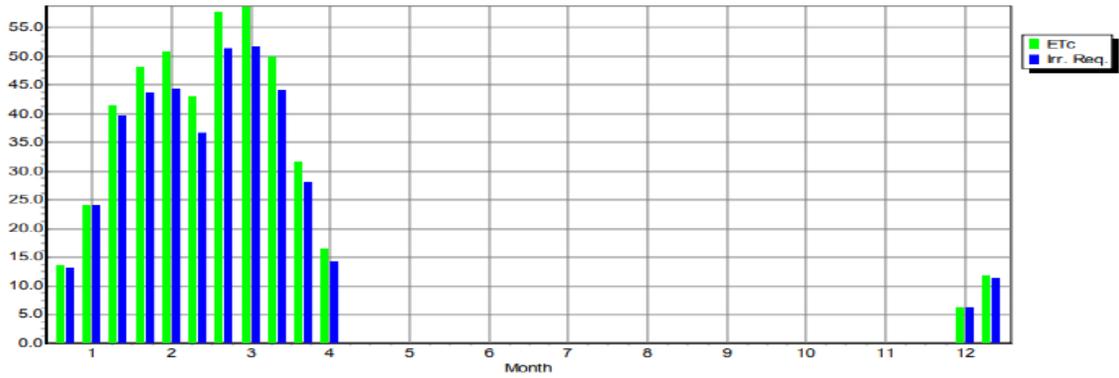


Fig 1. Estimation of crop evapotranspiration and irrigation requirement during crop period

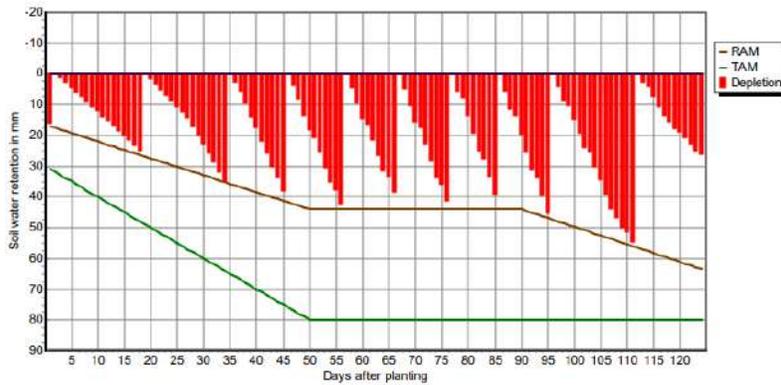


Figure 2. Irrigation scheduling of maize





Fenugreek and Lung Cancer

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ABSTRACT

Fenugreek (*Trigonella foenum-graceum*) though native to Southern Europe and Asia, it is used as a spice throughout the world. It also has many medicinal qualities. Other than that it is also used as a preservative, viscous and composting. Fenugreek is also imp for the evolution of lively, fresh and making of balanced products which is beneficial to health in our day to day life. To state the medicinal qualities are used to treat diabetes, preventing the development of cancer, inhibits reaction further by oxygen and studies the body's immune system. It contains a number of imp medicinal compounds such as volatile oils, alkaloids, flavonoids, saponins, fatty acids and rich source of polysaccharide galactomannan. The presence of phytochemical present in fenugreek helps in metabolites and antibacterial activity of the seed extracts of fenugreek against pathogenic bacteria like gram positive and gram negative. In this article this study was done to check out the potential toxic effect on cells by the fenugreek seeds to inhibit lung cancer cells which may prove useful for the care of cancer. This article is based upon to review the useful characteristics behaviour of fenugreek in nutraceuticals.

Keywords: Anticancer activity, Fenugreek, LC₄lv, Ligand, Lung cancer, Phytochemical



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INTRODUCTION

Fenugreek also known as *Trigonella foenum Graecum* which is happening yearly forage to recognise that it belongs to which family. Its spices name *f.graceum* meaning it undergoes in a pea family which consist of white flowers and trifoliate leaves which indicates that it is used to maintenance the crop. Previously fenugreek was suspects that it is indigenous to Europe and Africa lands but currently it was cultivated in every region as a spice in the globe. Especially in Mediterranean countries in Asia . India is one of the leading countries in the production of Fenugreek. Fenugreek is also reportedly grown in the region of Europe, Africa, USA as well as Australia. In ancient times Fenugreek is recorded as it is the former well studied healing plants. Its seed and leaves were a specially used traditionally in India , china , Egypt for their beneficial medicine effect such as reducing blood sugar, antibacterial, anti inflammatory and insulin tropic .the seeds are used both as whole as well grounded. The grounded powder is mainly used as flavouring agents for foods , curry powder, spice blend and tea. The medicinal values of Fenugreek is mainly attributed to its chemical composition. some of the major chemical components include proteins, dietary fibers, mucilaginous soluble fibre, fatty acids. The dietary fibers takes of the major part which is nearly 45 to 50 percentage of the composition. while proteins take up nearly 20 to 25 percent similarly the mucilaginous soluble fibre contributes 20 to 25 percent. Whereas the fatty acids take up 6 to 8 percentage. Then 2 to 5 percentage goes to steroidal saponins. Likewise some minor components such as alkaloids, free unnatural amino acids individual spirostanols and furastanols are identified to contribute some of the main biological effects.

Higher and sweet-smelling plants have been utilized generally in society prescriptions as well as to broaden the time span of usability of food varieties, showing restraint against microorganisms, organisms and yeasts. Organically dynamic mixtures from normal sources have continuously been an extraordinary interest for researchers dealing with irresistible diseases. Fenugreek (*Trigonella foenum-graecum*) is one of the world's most seasoned medicinal spices has a place with the family Fabaceae. The fenugreek seeds are rich in dietary fiber, that it can bring down glucose levels in diabetes. Fenugreek seed is generally utilized as a galactagogue that is regularly used to build milk supply in lactating ladies and fix bosom cancer. Fenugreek seed is valuable for tuberculosis, diabetes, atherosclerosis, obstruction, highcholesterol, hypertriglyceridemia and remotely it is utilized as a poultice for abscesses, bubbles, carbuncles, etc. Insulin is utilized to supplant fat and lessen the calories of food. It is reasonable for utilization by diabetes. The seeds of the fenugreek spice have harmful oils, and other bioactive constituents of the fenugreek seed incorporate unstable oils and alkaloids have been demonstrated to be harmful to bacteria, parasites and fungi. Ongoing pharmacological examination of the seed.

Fenugreek

Fenugreek is one of the broadly realized flavors utilized in the Indian curry making. The plant name of fenugreek is *Trigonella foenum-graecum* it has a place with the group of Fabaceae,

Genus :*Trigonella*,

Species: *T.foenum-graecum*

Order: Fabales

Domain -Eukaryote

Kingdom -Plantae

Phylum-Spermatophyte

Subphylum-Angiospermae

Class-Dicotyledonae

It found in the Mediterranean locale, southern Europe, and western Asia. Its seed and leaves are used for the making of curry. It is that the complimented flavours in human food. The seeds and fresh leaves of fenugreek are used in food what's more as in invigorating application that is the previous see of humanity's arrangement of encounters. It's been accustomed augmentation the flavoring and concealing, and moreover changes the energy of food materials. Seeds of fenugreek zing have remedial properties, for instance, hypocholesterolemic, lactation help, antibacterial,



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stomachic energizer, for anorexia, restorative medicine trained professional, galactagogue, hepatoprotective effect and anticancer. These accommodating physiological effects similarly as the antidiabetic and hypocholesterolemic effects of fenugreek are basically a direct result of the characteristic dietary fiber constituent that have promising nutraceutical regard. It's prominent for its fiber, gum, elective substance constituents and unusual substance. Dietary fiber of flavorer is concerning 25% that changes the energy of food. by and by it's used as food stabilizer, stick and emulsifying expert considering its high fiber, supermolecule and gum content. The protein of fenugreek is found to be a lot of dissolvable at alkalic hydrogen molecule center. Fenugreek has valuable effect on processing and also has the flexibility to change the food. It has been discovered to be a standout amongst other anti-microbial found in the every one of the flavour.

Phytochemicals Present In Fenugreek**Phytochemistry**

Fenugreek contains different compound constituents including steroidal saponin. Diosgenin part has been found in the smooth lacking creature of fenugreek. There are two furastanol glycosides, F-ring opened harbinger of diosgenin that have been represented in fenugreek moreover as hederagin glycosides. Alkaloids, for instance, trigocoumarin, nicotinic destructive, trimethylcoumarin and trigonelline are accessible in stem. The cement is a standing separated constituent of the seeds. There is about 28% glue; an eccentric oil; 2 alkaloids, for instance, trigonelline and Choline, 5% of a more grounded smelling, unforgiving fixed oil, 22% proteins and a yellow concealing substance are accessible in stem. Fenugreek contains 23–26% protein, 6–7% fat and 58% carbs of which about 25% is dietary fiber. Fenugreek is similarly a rich wellspring of iron, containing 33 mg/100 g dry weight.

Leaves

The leaves contain seven saponins, known as graecunins. These combinations are glycosides of diosgenin. Leaves contain about 86.1% sogginess, 4.4% protein, 0.9% fat, 1.5% minerals, 1.1% fiber, and 6% sugars. The mineral and supplements present in leaves fuse calcium, zinc iron, phosphorous, riboflavin, carotene, thiamine, niacin and supplement. It has been found that new leaves of fenugreek contain ascorbic destructive of about 220.97 mg per 100 g of leaves and β -carotene is accessible around 19 mg/100 g. On the contrary side, it was represented that 84.94% and 83.79% ascorbic destructive were reduced in sun and oven dried fenugreek leaves independently. New leaves are used as vegetables in the weight control plans. It was found that there was a predominant upkeep of enhancements in the leaves of fenugreek. The leaves of fenugreek should be taken care of in either in refrigeration conditions, or dried in oven, or brightened for eventually (around 5 min) and should be cooked in pressure cooker.

Seed

Fenugreek is known for its brilliantly serious, imperceptibly sweet seeds. The seeds are open in any design whether whole or ground structure is used to prepare various sustenances by and large curry powders, teas and flavor blend. Fenugreek seed has a central hard and yellow lacking organic entity which is enclosed by a corneous and generally huge layer of white and shady endosperm. Once-over of manufactured constituents is showed up in table under. The manufactured synthesis of fenugreek (like seeds, husk and cotyledons) showed that endosperm had the most raised (4.63 g/100 g) saponinand (43.8 g/100 g) protein content. As against this, husk contains higher total polyphenols. The concentrates of endosperm husk, and fenugreek seed at around 200 μ g center showed malignancy avoidance specialist activity 72%, 64%, and 56% independently by free-fanatic scavenging procedure. The seeds of fenugreek contain about 0.1–0.9% of diosgenin and are removed monetarily. The plan of diosgenin is showed up in Fig. 1. The plant tissue social orders from seeds of fenugreek when created under ideal conditions have been found to make as much as 2% diosgenin with more humble proportions of trigogenin and gitongenin. Seeds in like manner contain the saponin(fenugrin B). Fenugreek seeds have been found to contain a couple coumarin intensifies similarly as different alkaloids (e.g., trigonelline, gentianine, carpaine). The enormous proportion of trigonelline is debased to nicotinic destructive and related pyridines during stewing. The major bioactive blends in fenugreek seeds are acknowledged to be polyphenol compounds, for instance, rhaponticin ,isovitexin and Vanillin.



**Durgasi Mowlika et al.,****Nutraceutical Properties Of Fenugreek**

Fenugreek beneficially affects purifying the blood and as a diaphoretic it can welcome on a perspiration and to assist withdrawal from the body. Fenugreek has intense scent due to which it smelt on our body as sweat. Moreover fenugreek is familiar for its lymphatic purifying action however its indispensable job is that provide water to the cells to eliminate harmful materials, expired cells and the protein was caught by the body. Square of the lymphatic framework express helpless flow of liquid, liquid maintenance, torment, energy misfortune and sickness, anyplace in the body of an individual. Fenugreek keeps up bodily fluid states of the body, generally the lungs, by assisting with clearing blockage. It additionally goes about as a throat cleaning agent and bodily fluid dissolvable that likewise facilitates the inclination to hack. Savoring water which seeds of fenugreek have absorbed aides mellowing and dissolving, gathering and solidifying the majority of cell trash. Fenugreek should be utilized to alleviate some common and known diseases like cold, flu, asthma, sore throat, fever tuberculosis, bronchial grievances, pneumonia etc..

Cancer Prevention Agent

The fenugreek seed separate with methanol, ethanol, dichloromethane, CH₃CO, hexane and ethyl acetic acid derivation has an extremist searching movement. The revealed defensive impact of fenugreek, on lipid peroxidation and on enzymatic cell reinforcements. The general piece of fenugreek seeds, husk and cotyledons had the most noteworthy saponin and protein content. Conversely, husk had higher aggregate polyphenols. At 200 µg fixation, fenugreek seed, concentrates of husk and endosperm displayed 72%, 64%, and 56% cell reinforcement exercises individually by free-extremist searching action. From the examination it was shown that division of fenugreek seeds into husk and endosperm could have benefit of interaction practicality concerning earlier particular fractionation of bioactive parts for their powerful confinement. It contemplated the prophylaxis impact of fenugreek seeds on renal stone development in rodents. The fenugreek can be utilized in the treatment of patients with calcic urolithiasis. In an assessment it was seen that *Aegle marmelos* has the most raised phenolic content followed by fenugreek what's more, *Coriander sativum*; similarly the flavonoids substance are high in fenugreek followed by *C. sativum* and *A. marmelos*. Malignancy avoidance specialist property was checked by diminishing power, NBT analyze and H₂O₂ looking. *A. marmelos* showed the most significant reducing power followed by *C. sativum* and fenugreek anyway fenugreek showed the most essential superoxide and free fanatic looking followed by *C. sativum* and *A. marmelos* independently.

Anticancer

Malignant growth is specific main sources of death in general across the globe. The multitude detailed investigations have find the defensive impact on the seed of fenugreek to exploratory version of malignant growth utilizing or exploratory creatures. It showed that fenugreek seed separate essentially restrained 7, 12dimethylbenz(a) anthracene initiated mammary hyperplasia and decreases its frequency in rodents and exhorted that the counter bosom malignant growth defensive impacts of the fenugreek due to expanded apoptosis. Further, strong entire scatter concentrates of fenugreek appeared in the cytotoxicity of different human malignant growth by the lines of the cells, for example, IMR-32, a neuroblastoma cell line, and HT29, a disease cell line. A particular cytotoxic effect of fenugreek remove in vitro to a leading group of danger cell lines has been seen, tallying T-cell lymphoma. The improvement of MCF-7 cells, which is an estrogen receptor positive chest infection cell line, with ethanol concentrates of fenugreek, and reported that the ethanol concentrate of fenugreek lessened cell attainability and provoked early apoptotic changes, for instance, inversion of phosphatidyl serine and reduced mitochondrial film potential. Further, degradation of DNA into areas including results of approximately 180–200 base pair has furthermore been taken note. Cell cycle assessment revealed a sub-G1 apoptotic people close by cell cycle catch at G2/M stage in fenugreek separate treated cells including the work of fenugreek separate impelled apoptosis in its anticancer work. An eating routine containing fenugreek seed powder reduced colon tumor recurrence and hepatic lipid peroxidation in 1,2-dimethylhydrazine treated rodents and besides extended activities of catalase, superoxide dismutase, glutathione S-transferase and glutathione peroxidase in liver.



**Durgasi Mowlika et al.,****Fenugreek Against Lung Cancer**

Cellular breakdown in the lungs is the main source (represent 18%) of malignancy passing in the two people world-wide. The by and large 5-year endurance rate for all stages joined is frustrating (15%). The point of this examination is to assess the conceivable cytotoxic impacts of fenugreek on cellular breakdown in the lungs cell line and to decide its IC50 alone and in mix with cisplatin. What's more, to consider the effects of fenugreek on the outpouring of each of p53 and EGFR QU-DB cell breakdown in the lungs cells were refined in Eagle's MEM culture media upgraded with 5% FBS and against contamination specialists. The phones were developed in 96 well plate and the cytotoxic effects of each of cisplatin [25-0.195 $\mu\text{l/ml}$ (or $\mu\text{g/ml}$)] and fenugreek [300-1.1719 $\mu\text{l/ml}$ (each one μl is removed from 25 μg of dried seed)] was settled using impartial red take-up (NRU) test for 24, 48, and 72 hours in assessment with their relating control get-togethers. Joined effect of all of fenugreek and cisplatin was settled furthermore using NRU analyze. Cytotoxicity was additionally evaluated by trypan blue rejection test at IC50 of every specialist for 48 hours term. Immunocytochemistry measure was performed likewise to identify EGFR and p53 articulation. Cisplatin instigated a straightforwardly relative, portion ward and time-dependant cytotoxic impact with an IC50 of 8.5 $\mu\text{g/ml}$ and 7.3 $\mu\text{g/ml}$ after 48 hrs and 72 hrs of openness separately. Critical contrasts ($p < 0.05$) were seen in optic thickness of cisplatin bunch from that of the control for every single tried fixation. Fenugreek remove moreover impelled a clearly comparing, partition ward and time-dependant cytotoxic effect in investigates various roads in regards to 48 hrs and 72 hrs of transparency with an IC50 of 88.25 $\mu\text{l/ml}$ and 125 $\mu\text{l/ml}$ exclusively (every one μl is isolated from 25 μg of dried seed). While it conveys an advancement improving effect in 24 hrs receptiveness investigate. Colossal differences ($p < 0.05$) were found in optic thickness of fenugreek from that of the control at centralizations of 37.5 $\mu\text{l/ml}$ or more. Fenugreek makes an antagonistic movement when gotten together with cisplatin, mix document (CI) > 1.3 . Cisplatin especially on a very basic level ($p < 0.005$) extended EGFR verbalization at different obsessions. While fenugreek eliminate extraordinarily in a general sense ($p < 0.005$) diminished EGFR enunciation at 300 $\mu\text{l/ml}$ (each one μl is removed from 25 μg of dried seed). Cisplatin and fenugreek outstandingly basically ($p < 0.005$) lessened the surge of P53. Monotherapy of fenugreek anticancerly affect cell breakdown in the lungs cell line, yet a culpable effect on cisplatin when gotten together with it. Fenugreek may have an invaluable healing effect in lessening EGFR enunciation and reducing freak p53 verbalization. Further assessment is recommended to research the effect of fenugreek on other cell cycle proteins and to consider their feasible valuable supportive effects in vivo. Human cell breakdown in the lungs cell line "QU-DB" was purchased from public cell bank of Iran (NCBI), Pasteur Institute of Iran in Tehran/Iran. It is an immense cell carcinoma cell line. It was refined in DMEM + 10% fetal bull like serum (FBS) at the NCBI and was embraced in this assessment in Eagle's MEM + 5% FBS. Course of action of plants' and drugs' stock response for cytotoxic assessment Seeds of fenugreek were gotten from regular market and were perceived by the resources division of plant science directorate/Abugraib/Baghdad/Iraq. Quickly, crude plant seeds (fenugreek) were walloped, measured (2.5 g), macerated/homogenized and removed in 10 ml of all out ethanol for 7 days at 4 °C. The whole plan was by then centrifuged for 2 minutes at 5000 rpm. Each 1 ml of the supernatant was thusly debilitated to 10 ml with Hank's changed salt course of action (HBSS) + 5 mM HEPES, pre-adjusted to a pH of 7.4 with 0.1 N NaOH. The resultant plan was isolated through 0.45 micron and thereafter through 0.2 micron millipore channels. Cisplatin was used as an answer taken from the offered vial to intravenous implantation in an assembly of 1mg/ml (which contains sodium=30 mmol/L). Sodium chloride (SC) course of action (0.18% which contains sodium=30 mmol/L) was masterminded from sodium chloride game plan (0.9% which contains 150 mmol/L sodium).

Extraction of Fenugreek**MATERIALS AND METHODS****About the Chapter**

This section essentially addresses the data about the investigation done in the lab for example Extraction technique of the fenugreek seed, and the total strategy of docking of particle removed from the fenugreek with the lung protein LC_4lvt.





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Wet Lab Analysis

Phytochemical extraction is only the extraction of the bioactive segment present in the source for example fenugreek. There are a few compound technique which are can be ready for the extraction of bio supplements. The interaction is done in a few stage every one of the means are portrayed beneath.

Solution preparation

On the early phase we have our source fenugreek , we need to plan four arrangement of the example . The arrangement is made by drying the fenugreek seeds on sun to stay away from the overabundance dampness. At that point the seed are squashed and 4 arrangement are set up by utilizing water and ethanol, which will acted as our solvents. The four arrangement will resemble

1. 5g of fenugreek powder was taken in a dry funnel shaped cup then 50 mL of water added to it. After that the arrangement was took into account the mixing for the time being. At that point the arrangement was sifted.
 2. 5g of fenugreek powder was taken in a perfect and dry tapered jar 50 mL of ethanol added to it. After that the arrangement was took into account the blending for the time being. At that point the arrangement was sifted .
- As fenugreek is dry condition we need to check whether the arrangement made ought not be totally dry. Subsequent to setting up the arrangement we need to go for the subjective trial of fenugreek which will give an overall thought regarding what are the substance constituents present in the fenugreek.

Qualitative Analysis of Fenugreek

For the qualitative test of fenugreek we have followed the following protocol

Taninis

1mL of each concentrate was blended in with 2.0 mL of refined water and warmed on water shower the warmed combination at that point separated and 1mL of ferric chloride arrangement was added to the channel dim green arrangement demonstrate presence of tannin.

Flavonoids

1 mL of each concentrate was broken down in 1 mL of weakened arrangement of 0.2M arrangement of sodium hydroxide (NaOH) and 1.0 mL of 10% HCl arrangement was added . yellow arrangement that turns lackluster that demonstrates the presence of flavonoids.

Anthraquinones

1.0 mL of each concentrate was overflowed with 2.0 mL of 10% hydrochloric corrosive (HCl) answer for few moments in water shower. It was separated and permitted to cool. Equivalent volume of chloroform (CHCl₃) was added to channel. scarcely any drops of 10% NH₃ were added to combination and warmed. A rose pink tone demonstrate the presence of anthraquinones.

Saponins

1 mL of each concentrate added with 5mL of the refined water and afterward the test tube is shaken for 10-12 min the froth like air pocket shows up at the outside of the fluid . this show the presence of saponin in the concentrate.

Barfoed's test

For the Barfoed's test take 1 mL of each example in a test tubes add 2mL of Barfoed's reagent in each example then the example is set on the water shower . the shade of the example turn block red that demonstrates the presence of monosaccharides in the example.

Terpinoids or steroids

1.0mL of each concentrate was blended in with 2.0 mL of chloroform (CHCl₃) and 3.0 mL conc. Sulphuric corrosive was added cautiously to frame a layer .Redish earthy colored tone at the interface showed the presence of terpinoids.

Quantitative analysis of fenugreek

Here we have utilized different quantitative technique for the assurance of sum and their detachment of phytochemicals present in fenugreek. Here we have done some chromatographic strategy and protein assessment.





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Paper chromatography

Essentially paper chromatography is a method of chromatography to isolate diverse synthetic substances in the compound arrangement, similar to each chromatographic procedure this strategy is likewise comprise of two stages one is portable and another is fixed stage. Here the fixed stage is paper and the versatile stage is the arrangement. The fixed stage for example the paper is comprised of cellulose which has contain the – OH bunch. On the presence of the polar compound in the example it will get tie with that alongside some different powers are there like Vander divider powers , hydrogen holding and so forth these powers will hinder the polar atom and the non polar particle doesn't get influenced by such countless powers , so it will moves quicker on the paper surface.

Interaction

Step-1

A level line was drawn on the chromatographic paper surface about 1.5cm separated from the base layer.

Step-2

Mark a portion of the point on the line as the thing we are trying for. At that point add the example on the paper with the assistance of slim cylinder alongside the norm and imprint them with the letters in order of the or number. That will assist with distinguishing the particle last mentioned.

Step-3

At that point the paper is put noticeable all around close compartment of the alongside the reasonable arrangement. The dissolvable present in the case ought to must beneath than the checked line. At that point the crate should have been shut so that there will no air go through the compartment from an external perspective, to keep up the fume pressure.

Step-4

At that point after some time the arrangement is permitted to washed with KI answer for identify the checked lines.

Protein Estimation

Here we have done protein assessment by Lowry's Method. This technique is more powerful than any of the other strategy. The worth comes out from this technique is adequately delicate and thus to a great extent acknowledged.

Materials

1. **2% sodium carbonate in 0.1N sodium hydroxide (reagent A)** To plan reagent A , measure 2 g of sodium carbonate make it break down this in 100 mL of 0.1N of sodium hydroxide arrangement.
2. **0.5% copper sulfate in 1% potassium sodium tartrate (reagent B).** To plan reagent B , measure 0.5g of copper sulfate and 1g of potassium Tartrate , make it disintegrate this two synthetic substances in 100 mL of water.
3. **Alkaline Copper arrangement:** To make the antacid copper arrangement take 50 mL of An in measuring utencil and blend 1mL of B and denoted the recepticle as reagent C
4. **Folin-ciocalteau reagent:** Set up a combination of 100g sodium tungstate, 25g sodium molybdate 700mL water, 50mL of 85% phosphoric corrosive, and 100mL of moved HCl in 1.5L jar. Add 150g lithium sulfate, 50 mL water and a couple of drops of bromine water. At that point the combination is took into account the bubbling for 15min without condenser to eliminate abundance bromine cool the arrangement and weaken it to 1L at that point channel the arrangement.

Methodology

- Measure 500mg of the example and pound well with the pestle and mortar in 5-10mL support. Axis and utilize the supernatant adversary protein assessment.
- Take 5 spotless and dry test cylinder and measure 0.2, 0.4, 0.6, 0.8 and 1mL of the functioning norm into a progression of test tubes.
- Measure 0.1 mL and 0.2 mL of the example extricate in two other test tubes.
- Dilute each test cylinder to 1mL and in a clear test tube with 1mL water was taken
- Add 5mL of the reagent C in each test tube including the clear one which just loaded up with water . blended well all the test cylinder and make it rest for 10min.





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- Then add 0.5mL of reagent D, blended well and brood at room temperature in dull for 30 min. blue tone is created.
- Take the perusing at 600nm.
- Draw the standard diagram and compute the measure of protein in the example.

In silico analysis of Fenugreek(*Trigonella foenumgraecum*)against Lung cancer:

In-silico analysis has done using software and we further targeted some of the genes responsible for Lung cancer and pharmacophores from Fenugreek and did some in silico analysis. The software used for conducting this research are Biovia discovery studio, Open bible, Java, including some website like Corina Online , PubChem. Also ChemSketch is used to create and modify images of chemicals structures.

SUMMARY AND CONCLUSION

Fenugreek (*Trigonella foenumgraecum*) has been utilized as in Indian nourishment for such countless years. Studies in late year has shown that it has awesome potential against the malignancy . the phytochemical found in fenugreek can be utilized to therapy for different malignant growth related illnesses. There has been such countless examinations done on fenugreek on the therapy of malignant growth in the above investigation we have talk about the viability of the bioactive segment of the fenugreek towards the lung disease . In view of the above investigation fenugreek was discovered to be a decent helpful potential against the bosom malignant growth. It has entirely insignificant sugar measure of the sugar present I it so it tends to be treated against type – 2 diabetes like sicknesses . Preclinical examinations propose that it has great impact on disorderness like hypocholestromic, hypoglycemic, hypolipidic, antinociceptive, hepatoprotective and antimicrobial properties as we. As in this investigation it is found , the fenugreek has generally excellent potential against lung disease so different examinations on malignant growth like colon malignancy, lungs disease and so forth ought to be done to know the counter cancer-causing impact of the fenugreek quite well.

In this examination the lung disease protein which creating chemical i.e LC_4lvt has effectively tie with the fenugreek atom apigenin. The particle of apigenin are having the CID no. 71587693, 71587694, 71587694, 1.3E+08 and 57772203 has discovered to be no cancer-causing, mutagenic, tumorogenic, aggravation and opposite result o the human body. Ans additionally the discovered atom are have drug score , drug likeness, solvency, clogp and so forth in awesome locale. Which demonstrates that it will go about as a compelling medication against the lung disease. In-silico study recommend that , the particle of apigenin has effectively connected with the dynamic site of the LC_4lvt protein by the hydrogen holding, pi-pi association, customary covalent bonds, alkyl-pi cooperation and vanderwall powers . This show that the particle will ready to stop the reaction of the LC_4lvt protein which acting with the lung disease by bothering the quick cell division cycle of the disease cell . Further clinical investigations are required like in-vivo, in-vitro to demonstrate the viable standards of the fenugreek towards lung disease.

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<p>3.1(a)Structure of diosegenin</p>	<p>3.1(b) Structure of Rhaponticin</p>
<p>3.1(c)Structure of isovitexin</p>	<p>3.1(d) Structure of Vanillin</p>
<p>Fenugreek</p>	

