Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.6 / Issue 33 / December 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Traditional Healthcare Practice for Common Ailments of Children in Pudukkottai District, TamilNadu – A survey

Sambath G[1], Komalavalli N[2*] and Ravikumar R[3]

[1]Block Resource Teacher, Block Resource Centre, Thiruvadanai -623407, Ramanathapuram, Tamil Nadu, India.
[2]Associate Professor, PG and Research Department of Botany, Alagappa Govt. Arts College, Karaikudi – 620 020.
[3]Assistant Professor, PG and Research Department of Botany, Jamal Mohamed College (Autonomous), Tiruchirappalli – 620 020, Tamil Nadu, India.

Received: 28 Sep 2015 Revised: 24 Oct 2015 Accepted: 28 Nov 2015

***Address for correspondence**
Komalavalli N
Associate Professor
PG and Research Department of Botany,
Alagappa Govt. Arts College,
Karaikudi – 620 020, Tamil Nadu, India.
Email: nkomalavalli@gmail.com

ABSTRACT

The present study deals with the herbal remedies used for curing common day-to-day ailments of children. The information is based on real experiences of the common rural people including 26 rural women elders, 13 rural men elders, 13 herbalist and 13 traditional healers with a total of 65 informants aged between 30 - 85 years living in and around 13 development blocks namely Alangudi, Aranthangi, Arimalam, Avadaiyarkoil, Gandarvakottai, Karambakkudi, Kottaipattinam, Kulathur, Illuppur, Manamelkudi, Ponnamaravathy, Pudukkottai and Thirumayam of Pudukkottai district. The study reveals that a total of 97 plants belonging to 55families have been successfully used as a promising remedy for curing various ailments of children.

Keywords: Children Ailments, Health care, Herbal remedies, Pudukkottai, Traditional practice.

INTRODUCTION

The herbal medicine practice for curing common ailments, promotion of health and happiness of mankind have been practised by all classes or societies of people in India and abroad since time immemorial. People move into the future

rapidly nowadays, creating new methods and means for the betterment in everyday life outdating many of the technologies used in the past by replacing modern ideas and science. Thus Indigenous medicines are replaced by allopathic/ modern system of medicine. But it is important to remember the wisdom of the past since they still serve a purpose. From this perspective, old fashioned herbal remedies and recipes become an indispensable living example of history. High treatment cost, temporary relief by masking symptoms, harmful reactions and side effects of modern medicines made the people presently to look towards the traditional systems of medicine owing to its ability of fixing underlying problem of ailment, safe, inexpensive, curative effects of healing herbs and hopefully provide good health. Children are very susceptible to infectious diseases caused by virus, bacteria, fungi and mycoplasma when compared to grownups because of their very low diseases resistant power. As the immune system of children is not well developed they are always at high risk of getting some kind of ailment. The common ailments of children are Stomach ache, Fever, Catarrh, Vomiting, Nausea, Constipation, Cough and Cold, Dyspepsia, Pneumonia, Wheezing, Worms, Dysentery, Convulsion, Teething, Measles, Mumps, Eye Flu etc. During last 40 years, many botanists have been engaged in collecting the data on medicinal uses of plants from different parts of the world and several research papers has been published about the curing properties of the medicinal plants by primitive societies [1]. But there is hardly any publication specifically on the herbal remedies for the childhood ailments in India. A perusal of the available literature reveals that information on the comprehensive survey, documentation and enumeration of child care remedies used by the people of Pudukkottai District, Tamil Nadu is meagre[2, 3, 4].

The art of herbal treatment has very deep roots in Tamil culture. Even today in most of the rural areas people are depending on herbal drug systems for primary health care. The rural aged people are the storehouses of information and knowledge on the multiple uses of plants. However, such traditional knowledge is rapidly disappearing. There is an urgent need to document this knowledge, as otherwise it will be lost forever. The knowledge of the use of natural plant products amongst our people is truly phenomenal. Hence, the present study deals with the first-hand information of the traditional medicinal claims referring to treat child care ailments in the district of Pudukkottai in Tamilnadu. So far no systematic medical ethno survey has been made in this area. Moreover, information on the usage of herbal remedies by the people of Pudukkottai district has not been documented. In light of this, the present study was initiated with an aim to survey and document the knowledge of Pudukkottai district people about various herbal practices used to cure common child care ailments.

MATERIALS AND METHODS

Study area

Pudukkottai District was carved out of Tiruchirappalli and Thanjavur districts in January 1974 with an area cover of 4663 Sq. Km. and a coast line of 39 Kms. The district is located between 78.25' and 79.15' of the East of Longitude and between 9.50' and 10.40' of the North of Latitude and bounded by Tiruchirappalli district in the North and West, Sivaganga district in the South, Bay of Bengal in the East and Thanjavur district in the North East. It is predominantly an agricultural oriented district. Pudukkottai district is predominantly an agricultural oriented district where district's agricultural production depends mainly on the rainfall due to the prevailing dry and hot climate. The normal annual rainfall of Pudukkottai district is 922.8 mm out of which 52.2 mm is received in winter, 124.6 mm is received in hot weather period, 351.9 mm is received during South West Monsoon and 394.1 mm is received in North East Monsoon. The climate is continental with the temperature varies from 19° C to 38° C. The altitude is about 87.78 meters (288 feet). Pudukkottai district is comprised of 9 taluks, 13 development blocks (Alangudi, Aranthangi,Arimalam, Avadaiyarkoil, Gandarvakottai, Karambakkudi, Kottaipattinam, Kulathur, Illuppur,Manamelkudi, Ponnamaravathy, Pudukkottaiand Thirumayam) and 764 revenue villages [Fig. 1].

Data collection

The present study adhered to the research guidelines and ethnobotanical surveys conducted were carried out with the full consent of all participants with further verbal agreement and understanding that the research shall not be used for commercial purposes, but to serve as enlightenment on the diversity of medicinal plants used in the management of natal diseases in Pudukkottai district. The field survey was conducted in different localities of Pudukkottai district for 12 months from June 2014 to May 2015. The information regarding medicinal uses of plants, used to cure various common ailments of children, was collected personally from women respondents, herbalists and traditional healers through structured questionnaire, interviews and discussions [5]. The questionnaire items included each healer's age, their experience of school education and medicinal plant(s) used for a particular disease were recorded. In the case of herbalist healers his/her age at the first practice of herbal therapy was also noted. For the medicinal plants, which were used by the healers and households, their vernacular names in Tamil were recorded. Respondents were selected using snowball technique (6).The present study has been investigated on only single plant species and their effective utilization as paediatric drugs are being taken into account, however the combination of herbal drugs are not included. This is the unique feature of the herbal treatments, that one plant species can cure a particular disease and also can cure a number of other ailments in combinations with other plant species and, with different dosages, form and mode of administration. The information on plants were documented as follows: the local name of the plant, life form, parts used, method of preparation, mode of administration, other uses and references for the uses. Such studies have also been reported elsewhere [7, 8].

Identification and preservation of medicinal plant specimens

Standard method was followed with regard to collection of plant materials, drying, preparation and preservation of plant specimens [9]. Specimens of collected plants are preserved in the Botany department herbarium and they were botanically identified by one of the authors (KV) with the help of Flora of Tamil Nadu Carnatic[10] and An Excursion flora of Central Tamil Nadu[11].Further characterization of the plants and their previous usage was established by a literature search [12, 13, 14].

RESULTS AND DISCUSSION

Twenty six (26) rural women elders, 13 rural men elders, 13 herbalist and 13 traditional healers with a total of 65 informants aged between 30 -85 years were participated in the study.85% of the informants are more than 50 years old which implies that the use of herbal remedy in the child care practice is becoming endangered in the study area. The informants were 40 females and 25 males, the participation of females in the use of herbal remedy for the management of children disease may be attributed to the fact that women have immense knowledge of medicinal plants because they are generally responsible for the upkeep of the home and families [15]. A similar result was recorded in a survey conducted on the plants used for the natal care in the study area of Kerala [5]. The profile of medicinal plants used in child care practices is presented in Table 1. Ninety seven (97) plant species from 55 families were identified as being used to treat children ailments. The most represented family was Apiaceae and Lamiaceae with seven (7) species followed by Euphorbiaceae with six species (6), Zingiberaceae with five (5) species, Papilionaceae with four (4) species, Acanthaceae, Asclepiadiaceae, Liliaceae, Piperaceaeand Rubiaceae with three (3) species each. The Asteraceae, Poaceae, Malvaceae, Menispermaceae, Rutaceae, Solanaceae, Sapotaceaeand Verbenaceaewere represented by two (2) species each, the other familiesAltinginaceae, Araceae, Arecaceae, Bombacaceae, Caesalpiniaceae, Capparidaceae, Caricaceae, Combretaceae, Convolvulaceae,Cucurbitaceae, Dipterocarpaceae, Gentianaceae, Haemodoraceae, Iridaceae ,Lecythidaceae, Magnoliaceae, Meliaceae, Mimosaceae, Moraceae, Moringaceae, Myrsinaceae, Myrtaceae, Pinaceae, Plantaginaceae, Polygonaceae, Punicaceae, Ranunculaceae, Santalaceae, Scrophulariaceae, Sterculiaceae, Styracaceae, Thymelaeaceae, Tiliaceae, Violaceae, Vitaceae and Zygophyllaceae had one (1) species each associated with natal care. This is an indication of phyto therapy usage for natal care by the people. The use of traditional remedies is a cultural practice of elder people till

date and they are in belief that the potency of herbs are easily accessible and affordable to the economically poor people. Various plant parts were used in the child care practices. The leaf was widely used followed by fruit, root, bark and the whole plant. Other plant parts such as the twigs, fruit rind, seeds, gum and resins were also used, but in meagre level (Table 1). The preference for the use of leaves in treatment might due to easy availability and freshness of the material. The potency of leaf in treatment was also reported by earlier workers [16, 17]. The indigenous method of preparation of remedies includes mostly juices, decoction, infusions, extract, paste and powder. The mode of administration of herbal medicine is either orally or as external applications. The frequent use of decoction could be attributed to the fact that it is easy and simple to administer the herbal drug to children. Moreover, it creates a close interaction between the herbal drug and the causative agent associated with natal care there by giving a maximum efficacy of cure to the natal diseases. Similar research has been carried out on plants used for child care [3, 4, 5, 16].

CONCLUSION

This study has contributed immensely to the preservation of indigenous knowledge. The survey will be helpful in biodiversity conservation and future phytomedicine research.

ACKNOWLEDGMENTS

This research was done with the support of 65 informants spread over the length and breadth of 13 taluks of Pudukkottai district. We acknowledge their participation in documenting their knowledge.

REFERENCES

1. Singh SP, Tripathi S and Shukla RS (2003). Ethnomedicinal heritage for Bio-prospecting and Drug development in North-Eastern States of India.*Journal of Economic and Taxonomic Botany* **26**: 384-395.
2. Ignachimuthu S, SankaraSivaraman K and Kesavan L (1998). Medico-ethnobotanical surveyamong Kanikkartribals of Mundanthurai Sanctuary, Western Ghats, India.*Fitoterapia*. 69: 409-414.
3. Ayyanar M and Ignacimuthu S (2005a). Traditional knowledge of Kanitribals in Kouthalai of Tirunelveli hills, Tamil Nadu, India. *Journal of Ethnopharmacology*.**102**: 246-255.
4. Ayyanar M and Ignacimuthu S (2005b). Medicinal plants used by the tribals of Tirunelveli hills, Tamil Nadu to treat poisonous bits and skin diseases. *Indian Journal of Traditional knowledge*.**4**: 229-236.
5. Rasiyabeegum and Nayar TS (2011). Plants used for natal health care in folk medicine of Kerala, India. *Indian Journal of Traditional knowledge*.**10**: 523-527.
6. Mariano Martinez Espinosa, Isanete G. C. Bieski, DomingosTabajara de Oliveira Martins (2012), Probability sampling design in ethnobotanical surveys of medicinal plants. RevistaBrasileira de Farmacognosia Brazilian Journal of Pharmacognosy 22(6): 1362-1367.
7. Shanmugam S, Rajendran K, Suresh K (2012). Traditional uses of medicinal plants among the rural people in Sivagangai district of Tamil Nadu, Southern India. Asian Pacific Journal of Tropical Biomedicine, S429-S434.
8. Nadeem, M., Z.K. Shinwari and M. Qaiser. 2013. Screening of Folk Remedies by Genus Artemisia Based on Ethnomedicinal Surveys and Traditional Knowledge of Native Communities Of Pakistan. Pak. J. Bot., 45(S1): 111-117.
9. Jain, S.K and R.R. Rao. 1976. A handbook of field and herbarium methods. Today and Tomorrow Printers and Publishers, New Delhi.
10. Matthew KM. Flora of Tamil Nadu Carnatic: Rapinat Herbarium, St. Joseph's College, Tiruchirapalli, Tamil Nadu; 1983.
11. Matthew KM. An Excursion Flora of Central Tamil Nadu: Oxford and IBH Publishing Co Pvt Ltd, New Delhi; 1991.

12. Chopra R N, Nayar S L and Chopra I C, Glossary of Indian Medicinal Plants, New Delhi. 1956.
13. Nadkarni A K, The Indian MateriaMedica, Vol. I – II (Third Edition) Popular Book Department, Bombay, 1976.
14. Jain S K, Dictionary of Indian Folkmedicine and Ethno Plant Science. Deep Publication, 1991.
15. Zobolo, A.M and A. Mkabela. 2006. Traditional knowledge transfer of activities practised by woman to manage medicinal and food plant gardens. Afr. J. Ran. For. Sci. 23: 77-80.
16. Moshi, M.J., D.F. Otieno, P.K. Mbabazi and A. Weisheit. 2009. The Ethnomedicine of the Haya people of Bugabo ward, Kagera Region, north western Tanzania. J. Ethnobio. Ethnomed. 31: 5:24
17. Zainol, N.A., S.C.Voo, M.R. Sarmidi and R.A. Aziz. 2008. Profiling of Centellaasiatica (L.) Urban extract.Mal. J. Analytic. Sci., 12: 322-327.

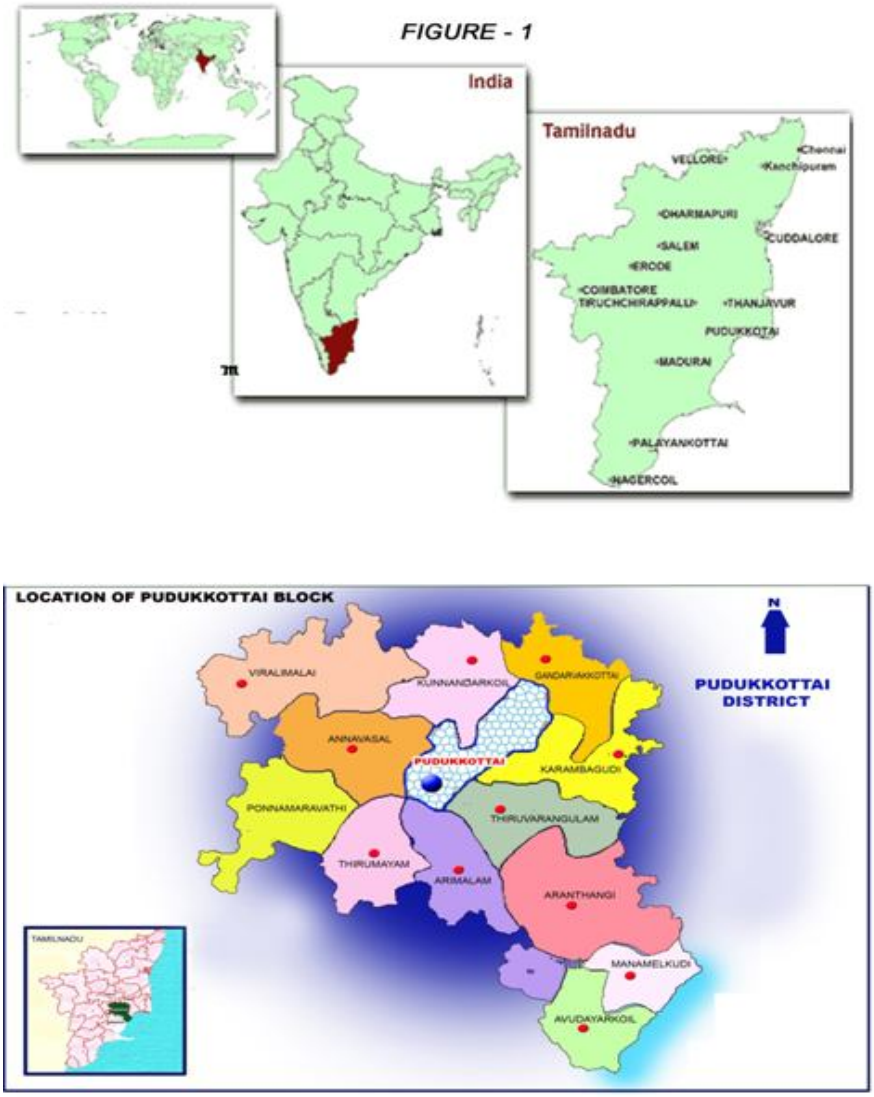


Fig.1.Study Area

Table: 1.Plant species used to cure common child care ailments by the people of Pudukkottai district

S.No.	Plant Name	Local name	Family	Part Used	Mode of Admin.	Ailments
1	*Abies spectabilis (D.Don) Mirb*	Talispatri	Pinaceae	Leaves	Juice	Fever during dentition
2	*Abrus precatorius L.*	Kundumani	Papilionaceae	Leaves	Leaf syrup	Pneumonia like cough
3	*Abutilon indicum G.Don*	Thutthi	Malvaceae	Seed	Seeds smoke	Anthelmintic
4	*Acacia catechu (L.f.) Willd.*	Karunkaali	Mimosaceae	Wood	Wood extract	Diarrhea, Hemorrhages cough
5	*Acalypha indica L.*	Kuppaimeni	Euphorbiaceae	Leaves	Juice&paste	Emetic, expectorant, chronic bronchitis, paste in skin diseases
6	Aconitum heterophyllum Wall. Ex Royle	Adivitayam	Ranunculaceae	Root	Powder	Coryza, cough, fever, vomiting
7	Acorus calamus L.	Vasambu	Araceae	Rhizome	Infusion	Dysentery, flatulence, speech & vocal problems, fever, cough
8	Adhatoda vasica Nees	Adathodai	Acanthaceae	Leaves	Juice	Chronic bronchitis, cough, anemia
9	Aegle marmelos Corr. Serr.	Vilvam	Rutaceae	Fruit	Pulp	Chronic diarrhea and dysentery
10	Allium cepa L.	Vengayam	Liliaceae	Bulb	Juice	Ear pain, stunted growth, heat stroke
11	Allium sativa L.	Poondu	Liliaceae	Bulb	Juice	Throat pain, whooping cough, typhoid fever
12	Achras zapota L	Shimai eluppai	Sapotaceae	Unripe fruit	Infusion	Dysentery, diarrhea, convulsion
13	Aloe vera (L.) Burm. F	Sotrukatralai	Liliaceae	Leaves	Juice	Anthelmintic, jaundice, liver disorders, burns
14	*Alpinia galanga (L.) Willd.*	Perarattai	Zingiberaceae	Rhizome	Powder	Whooping cough, bronchitis
15	*Altingia excelsa Noronha*	Neriyachichedi	Altinginaceae	Wood	Resin	Colic pain
16	*Andrographis paniculata Nees.*	Nilavembu	Acanthaceae	Leaves	Juice	Diarrhea, anthelmintic, fever, liver swelling
17	*Cymbopogon citratus (DC.) Stapf.*	Elumichaipullu	Poaceae	Leaves	Decoction	Stomach pain, Disphoric fever
18	*Cymbopogon nardus (L.) Rendle*	Mandapullu	Poaceae	Leaves	Decoction	Stomach pain
19	*Anisochilus carnosus (L.f.) Wall.*	Kattu Karporavalli	Lamiaceae	Leaves	Juice	Cough
20	*Anisomeles malabarica R. Br. Ex Sims.*	Erattai peimiratti	Lamiaceae	Leaves	Juice	Colitis, dyspepsia, fever (teething)
21	*Chamaemelum nobile (L.) All*	Vellachamandhi	Asteraceae	Flower	Infusion	Toothache, anthelmintic
22	*Neolamarckia cadamba (Roxb.) Bosser*	Kadampamaram	Rubiaceae	Fruit	Juice	Fever, gastric irritability
23	*Aquilaria agaliocha Roxb.*	Agil	Thymelaeaceae	Wood	Paste	Bronchitis, tonic

24	*Balanites roxburghii Planch.*	Nanchundanpattai	Zygophyllaceae	Leaves, Fruit	Decoction	Anthelmintic
25	*Barlaria prionities L.*	Manjadecember	Acanthaceae	Leaves	Juice	Catarrhal fever
26	*Barringtonia acutangula (L.) Gaertn*	Perukadambu	Lecythidaceae	Seed	Powder	Catarrhal vomiting
27	*Cadaba trifoliata (Roxb.) Wight & Arn.*	Viluthi	Capparidaceae	Leaves	Decoction	Anti- inflammatory, anthelmintic
28	*Calotropis gigantea (L.) Dryand.*	Velerrukku	Asclepiadaceae	Leaves	Juice	Ear ache, convulsion
29	*Carica papaya L.*	Pappali	Caricaceae	Unripe fruit	Raw	Indigestion, dyspepsia, flatulence
30	*Chamaecrista desvauxii var. mol lissima (Benth.) H.S.Irwin & Barneby.*	Nilaavarai	Caesalpiniaceae	Leaves	Decoction	Purgative, colitis
31	*Centella asiatica L.*	Vallarai	Apiaceae	Leaves	Infusion	Mental retardation, tonic, leprosies
32	*Clitoria ternatea L.*	Sanguppoo	Papilionaceae	Seed	Powder	Colic, Constipation
33	*Cocculus cordifolius DC.*	Seendilkodi	Menispermaceae	Root	Decoction	Fever, abnormal heart beating
34	*Cocculus villosus DL.*	Sirikattukkodi	Menispermaceae	Root	Powder	Stomach pain
35	*Plectranthus amboinicus (Lour.) Spreng.*	Karpooravall	Lamiaceae	Leaves	Juice	Chronic cough, asthma
36	*Corchorus capsularis L.*	Piratti-kirai	Tiliaceae	Leaves	Decoction	Anthelmintic
37	*Coriandrum sativum L.*	Kotthamalli	Apiaceae	Fruit	Powder	Indigestion, colic, flatulence
38	*Crocus sativus L.*	Kungumapoo	Iridaceae	Dried stigma style	Decoction	Catarrhal affections, Pneumonia, asthma, to keep body warm
39	*Curcuma amada Roxb.*	Maaingi	Zingiberaceae	Rhizome	Paste	Skin diseases
40	*Curcuma longa Auctnon.*	Manjal	Zingiberaceae	Rhizome	Powder	Skin diseases, cuts, wounds, allergies, inflammation, pain
41	*Curcuma zedoaria (Christm.) Roscoe*	Kasthurimanjal	Zingiberaceae	Rhizome	Juice	Anthelmintic
42	*Pergularia daemia (Forssk.) Chiov.*	Veliparuthi	Asclepiadaceae	Leaves	Juice	Anthelmintic
43	*Embelia ribes Burm.F.*	Kattukkodi	Myrsinaceae	Seed	Powder	Dyspepsia, flatulence
44	*Ceiba pentandra (L.) Gaertn.*	Ilavamaram	Bombacaceae	Wood gum	Powder	Cooling laxative
45	*Eclipta prostrata L.*	Karisilanganni	Asteraceae	Leaves	Juice	Skin infection, liver disorders
46	*Erythrina indica Lam.*	Mullumurungai	Papilionaceae	Leaves	Juice	Cough, cold, anemia
47	*Euphorbia hirta L.*	Ammanpacharasi	Euphorbiaceae	Plant	Extract	Chronic dysentery
48	*Euphorbia neriifolia L.*	Kattaralikalli	Euphorbiaceae	Root	Powder	Eczema, skin infection
49	*Ferula asafoetida L.*	Perunkayam	Apiaceae	Root resin	Powder	Dyspepsia, flatulence, whooping, bronchitis cough,anthelmintic
50	*Ficus religiosa L.*	Arasamaram	Moraceae	Root	Juice	Aphthous sores
51	*Foeniculum vulgare Miller.*	Venthayam	Apiaceae	Seed	Extract	Colic flatulence

52	*Geniosporum prostratum (L.) Benth.*	Kattuthumba	Lamiaceae	Leaves	Juice	Cough, Colitis
53	*Hibiscus rosa-sinensis L.*	Sembaruthi	Malvaceae	Root	Decoction	Fever
54	*Helicteres isora L.*	Valampurikaai	Sterculiaceae	Root, Fruit	Decoction	Diarrhea, Chronic dysentery, Colic pain
55	*Hemidesmus indicus R. Br.*	Nannari	Asclepiadaceae	Root	Infusion	Chronic cough, diarrhea
56	*Bacopa monnieri (L.) Wettst.*	Brami	Plantaginaceae	Leaves	Poultice	Pneumonia, acute bronchitis, Cough
57	*Illicium verum Hook.*	Annasipoo	Magnoliaceae	Fruit	Infusion	Carminative
58	*Indigofera tinctoria L.*	Avuri	Papilionaceae	Branches	Juice	Apathies of mouth
59	*Operculina turpethum (L.)Silva Man*	Shivadaiver	Convolvulaceae	Root bark	Powder	Stomach disorders
60	*Leucas aspera Link.*	Thumbai	Lamiaceae	Leaves	Juice	Diarrhea
61	*Lippia nodiflora A. Rich.*	Poduthalai	Verbenaceae	Leaves	Infusion	Indigestion, Dysuria
62	*Mallotus philippensis (Lam.) Müll.Arg.*	Chenduramaram	Euphorbiaceae	Fruit	Powder	Anthelmintic, jaundice, Liver disorders
63	*Melia azadirach L.*	Malaivembu	Meliaceae	Leaves	Paste	Skin diseases, malaria
64	*Mimusops elangi L.*	Iluppamaram	Sapotaceae	Bark	Decoction	Throat infections, tooth ache, Whooping cough
65	*Momordica charantia L.*	Pavakkai	Cucurbitaceae	Leaves	Juice	Nausea, Emetic
66	*Morinda tinctoria Roxb.*	Manjanatthi	Rubiaceae	Leaves	Decoction	Dysentery, diarrhea
67	*Moringa oleifera Lam.*	Murungai	Moringaceae	Leaves	Infusion	Headache, Restlessness
68	*Mussaenda frondosa L.*	Vellailaichedi	Rubiaceae	Stem	Powder	Cough& cold
69	*Ocimum americanum L.*	Kanjakkorai	Lamiaceae	Leaves	Juice	Cold, bronchitis, fever
70	*Ocimum sanctum L.*	Thulasi	Lamiaceae	Leaves seed	Juice, powder	Cold, bronchitis, fever malaria, diarrhea
71	*Papaver somniferum L.*	Milagu	Papaveraceae	Unripe fruit	Sap	Diarrhea, dysentery, sleeplessness crying
72	*Anethum graveolens L.*	Satakuppai	Apiaceae	Fruit	Oil	Flatulence, colic & abdominal pain
73	*Phoenix dactylifera L.*	Echamaram	Arecaceae	Fruit	Pulp	Small pox, Feverish convulsion
74	*Phyllanthus amarus S & Th.*	Keezhaneli	Euphorbiaceae	Whole plant	Decoction	Liver disorders, jaundice, Inflammation of internal organs

75	*Picrorrhiza kurrooa Benth.*	Kattukurani	Scrophulariaceae	Rhizome	Powder	Whooping cough, Fever, malaria
76	*Pimpinella anisum L.*	Sombu	Apiaceae	Seeds	Powder	Indigestion, flatulence, asthma
77	*Piper betel L.*	Vetrilai	Piperaceae	Leaves	Extract	Inflammation of throat, laryngitis, Mouth ulcers
78	*Piper longum L.*	Tippili	Piperaceae	Fruit	Powder	Inflammation of throat, laryngitis, coolant, excessive salivation
79	*Piper nigrum L.*	Milagu	Piperaceae	Fruit	Decoction	Fever, cold & cough, jaundice
80	*Psidium guajava L.*	Koiya	Myrtaceae	Leave	Powder	Mouth ulcer, indigestion
81	*Trachyspermum ammi (L.)Spra gue*	Omam	Apiaceae	Fruit	Extract	Stomachache, indigestion, colitis
82	*Punica granatum L*	Madulai	Punicaceae	Fruit rind, Root	Decoction	Tuberculosis, anemia, cough
83	*Quisqualis indica L.*	Rangoonmalli	Combretaceae	Seed	Powder	Anthelmintic
84	*Rheum emodi Wall.*	Iravalchinna kilangu	Polygonaceae	Rhizome	Powder	Chronic dysentery
85	*Ricinus communis L.*	Amanakku	Euphorbiaceae	Seed	Oil	Constipation, oil massage in shunted growth
86	*Ruta graveolens L.*	Arooda	Rutaceae	Whole plant	Juice	Expectorant, antispasmodic, anthelmintic
87	*Santalum album L.*	Santhanam	Santalaceae	Wood	Oil, Powder	Skin infection, coolant fever
88	*Sanseviria roxburghiana Sc & Sc*	Marul	Haemodoraceae	Tender shoots	Juice	Clears viscid phlegm
89	*Shorea robusta Gaertn.*	Kungiliyam	Dipterocarpaceae	Wood	Resin	Dysentery
90	*Solanum trilobatum L.*	Thuthuvalai	Solanaceae	Leaves	Infusion	Cough, acute bronchitis
91	*Solanum xanthocarpum Sch.& WendI.*	Kandakatthiri	Solanaceae	Fruit	Powder	Cough, inflammation of liver
92	*Styrax benzoin Dryand*	Sambirani	Styracaceae	Bark	Gum	Jaundice, liver disorders
93	*Swertia chirata Ham.*	Sirattukuchi	Gentianaceae	Leaves	Juice	Fever
94	*Viola odorata L.*	Orital vuthathamarai	Violaceae	Flower	Syrup	Demulcent
95	*Vitex negundo L.*	Nochi	Verbenaceae	Leaves	Juice	Cough, Fever, Tonic
96	*Vitis vinifera L.*	Thiratchai	Vitaceae	Fruit	Juice	Anemia
97	*Zingiber officinale Rasc.*	Ingi	Zingiberaceae	Rhizome	Juice, Powder	Cough, Difficult breathing, Throat infections, tonic

Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.6 / Issue 33 / December 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Diallel Analysis and Heterotic Effects for Yield and Fibre Quality Traits in Upland Cotton

K.Senthil Kumar[1], K.Ashokkumar[1,2*] and R.Ravikesavan[1]

[1]Center of Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore-3, Tamil Nadu, India.

[*2]Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore-3, Tamil Nadu, India.

Received: 21 Sep 2015 Revised: 20 Oct 2015 Accepted: 29 Nov 2015

***Address for correspondence**
K.Ashokkumar
Center of Plant Breeding and Genetics,
Tamil Nadu Agricultural University,
Coimbatore-3, Tamil Nadu, India.
Email: biotech.ashok@gmail.com

ABSTRACT

An investigation was carried out to assess the mean performance and heterotic effect for yield and fibre quality traits of 42 hybrids generated from 7 parents in a diallel mating design Results revealed that parent BW 4-1 had the best mean performance for most of the traits. Hybrid TCH 1726 x BW4-1 was found to exhibit positive and significant relative heterosis for lint index, plant height, days to first boll bursting, number of bolls per plant, number of sympodial branches, seed cotton yield, 2.5 per cent span length and seed cotton yield. The hybrid TCH 1705 x Narasimha recorded positive and significant relative heterosis, and heterobeltiosis, for days to first boll bursting, number of bolls per plant, single plant yield. Additionally, hybrid KC 2 x MCU 3 recorded positive and significant relative heterosis, and heterbeltiosis for number of bolls per plant, and seed cotton yield. The heterosis along with *per se* performance of the hybrids gave an idea about the pratical utility of hybrid combinations for heterosis breeding.

Key words: Cotton, heterosis, seed cotton yield, fibre quality traits, diallel analysis

INTRODUCTION

Cotton (*Gossypiumhirsutum*L.) is an important fiber, crop and plays a vital role as a cash crop in many countries. It provides fibre for textile industry, cellulose from its lint, oil and protein rich meal from its seed (Ashokkumar and

Ravikesavan, 2011). In any breeding programme, the choice of best parents is an important step. However, cotton is highly amenable for both heterosis and recombination breeding. A considerable amount of heterosis has been reported in this crop (Marani, 1963; Singh, 1982; Ashokkumar and Ravikesavan, 2013; Ashokkumar et al., 2013). To exploit heterosis, the knowledge of selecting appropriate parents with good genetic potential is very essential. The parent should possess good combining ability and wide genetic diversity for various economic traits (Ashokkumar and Ravikesavan, 2011). Heterosis is useful in determining the most appropriate parents for specific traits (Khan et al., 2010). Development of hybrids as commercial varieties is increasingly becoming important. Cotton is highly amenable for both heterosis and recombination breeding. Heterosis has substantially remained as one of the significant developments in cotton breeding programs, (Singh, 1982; Chaudhari et al., 1992; Baloch et al., 2003; Baloch, 2004; Memon et al., 2005; Ganapathy and Nadarajan, 2008; Khan et al., 2010; Ashokkumar and Ravikesavan, 2013). Heterosis has substantially remained as one of the significant developments in cotton breeding programs, (Singh, 1982; Chaudhari et al., 1992; Baloch et al., 2003; Baloch, 2004; Memon et al., 2005; Ganapathy and Nadarajan, 2008; Khan et al., 2010; Ashokkumar and Ravikesavan, 2013).

The development of new cotton varieties with high yield and fibre quality is the primary objective of cotton breeders. The first step in a successful breeding program is the selection of appropriate parents. Diallel analysis provides a systematic approach for detection of appropriate parents and crosses in terms of investigated traits and it has been widely used by plant breeders in the selection of parents and crosses in the early generations (Marani, 1963; Green and Culp, 1990; Islam et al., 2001; Kiani et al., 2007; Karademir and Gencer, 2010; Senthilkumar et al., 2014). Several studies have been reported on yield and yield attributing traits, but little work has been reported on the genetics and heterosis of fibre quality traits in cotton breeding. A few reports in the literature (Rahman et al., 1993; Zhang et al., 2002; Basal and Turgut 2003; Preetha and Raveendran, 2008; Karademir et al., 2009; Karademir and Gencer, 2010; Karademir et al., 2011; Bolek et al., 2011; Ashokkumar and Ravikesavan, 2008; Ashokkumar et al., 2010; Ashokkumar and Ravikesavan, 2011; Ashokkumar et al., 2014) have determined that cotton genotypes differ in fibre quality traits. The estimates of mean performance and heterosis provided useful information with regard to the possibilities and extent of improvement in the yield and fibre characters of breeding material through selection.Therefore, the objective of the present study was to estimate genetic variation of parents and their hybrids and to estimate the effects of heterosis in F_1 cross combinations.

MATERIALS AND METHODS

Genetic material

The field experiment was conducted using seven parents, viz., Narasimha, TCH 1726, TCH 1705, KC2, MCU13, BW4-1 and MCU 3. All the seed materials were obtained from Department of Cotton, Tamil Nadu Agricultural University,Coimbatore, India. The commercial cultivars were cultivated in southern states of India.

Experimental design and field procedures

The cotton cultivars were evaluated in complete randomized block design (RBD) with three replications at Cotton Breeding Station, Tamil Nadu Agricultural University, Coimbatore, and Tamil Nadu in India. The seed of each parental genotype was sown in 20 rows of 6m length in crossing block with a spacing of 90 x 45 cm. Crosses were made between parents in a 7 x 7 full diallel mating design. The conventional hand emasculation and pollination method developed by Doak (1934) was followed. Emasculation of the flower buds was done in the ovule parent on the previous day evening. The entire staminal column with anthers was removed carefully along with corolla and bracts with the help of nail without any damage to ovary. Pollination was done on the next day morning by dusting the pollen grains on the stigma of ovule parent. Crossed bolls were collected separately and ginned to obtain F1 seeds. Seven parents and 42 hybrids were raised along with the standard check with three replications. The diallelanalysis was performed as model 1 and method 1 suggested by Griffing (1956).

Sampling, traits measurements and methods

For each genotype and its cross combinations, data were recorded on five randomly selected plants per replication for twelve characters namely, days to boll bursting, number of sympodia per plant, plant height at maturity (cm), number of bolls per plant, boll weight (g), lint index, seed index, ginning percent, single plant yield (g), 2.5% span length (mm), elongation percent and fiber fineness. Quality parameters were analyzed by High Volume Instrument (HVI). The expression of heterosiswas worked out for all the characters over mid parent, and better parent was estimated in theentire cross combinations under this study.The significance of heterosis was estimated by't' test using the formulae (Wynne et al., 1970).

't' value of relative heterosis (d_i) = $$\frac{\left(\overline{Fij}\right)-\left(\overline{MPij}\right)}{\sqrt{(3-2)\left(\sigma^2 e / r\right)}}$$

't' value of heterobeltiosis (d_{ii}) = $$\frac{\left(\overline{Fij}\right)-\left(\overline{BPij}\right)}{\sqrt{2\sigma^2 e / r}}$$

Where,

$\overline{Fij}$ = Mean of the ijth F_1 cross

$\overline{MPij}$ = Mean of mid parent value for ijth hybrid

$\overline{BPij}$ = Mean of better parent value for ijth hybrid

σ_e^2 = error variance

r = Number of replication

Statistical analysis was carried out by using the mean values over five sample plants (Indostat Statistical Software Package; Indostat Pvt. Ltd., Hyderabad, India).

RESULTS AND DISCUSSION

Analysis of variance showed highly significant differences among genotypes for all the traits, indicating the presence of sufficient variability among the genotypes assessed (Table 1). Significant differences in parents versus hybrids interaction provided adequacy for comparing the heterotic expression for all the characters except boll weight and micronaire. However, parents and hybrids showed significant differences between all the characters studied except single plant yield and bundle strength. Ashokkumar and Ravikesavan (2013) reported that all the characters were significant with parents and hybrids in upland cotton, and this is in agreement with our results.

Mean performance

The mean performance of seven parents and their 42 hybrids are presented (Table 2-5). Among the parents, plant height was ranged from 87.0 cm to 127.0 cm. The differences observed for mean plant height among cotton cultivars can be attributed tovariation in genetic makeup of crop plants. These results are supported by the findings of Anwaret al. (2002), Copur (2006) Ashokkumar et al. (2010) and Ashokkumar and Ravikesavan, (2011)who also noted significant differences among cultivars for plant height. Of the hybrids, the meanvalues ranged from 81.0. cm in BW4-1 x Narasimha to 136.0 cm in TCH 1726 x TCH 1705. For parents, highest number of sympodia per plant and lowest number of sympodia per plantwere produced by KC 2 (13) and TCH 1726 (17), respectively (Table 2). In hybrids, the weakest value of 13 and the greatest value of 19 were recorded with a mean value of 16. There was significant difference between the parents for the number of bolls per plant. The meanvalues for number of bolls per plants ranged from 21 in TCH 1726 to 27 in BW4-1. The hybridsdisplayed a variation from 21 bolls in MCU 13 x KC 2 to 34 bolls in KC 2 x TCH 1726 for the number of bolls per plant with a mean value of 25.4 bolls. The result indicates

the importance of hybrid KC 2 x TCH 1726 offering the scope of selection and can be utilized for the improvement of number of bolls per plant which will directly influence the seed cotton yield. In addition, the present results have been further supported by Soomro (2000), Baloch (2002),Chandio et al. (2002), Basbag and Gencer (2004), Soomro et al. (2008), and Ashokkumar andRavikesavan, (2011 and 2013).Boll weight is directly related to the seed cotton yield of cotton. An evaluation of data indicated that greatest boll weight was recorded in cultivar BW4-1(4.7 g), and the lowest boll weight was recorded Narasimha (4.1 g). Significant differences among parents for average boll weight also were reported (Hofs et al. 2006; Ashokkumar and Ravikesavan, 2011 and 2013). Mean boll weight among the hybrids ranged from 3.91g in Narasimha x Bw4-1 to 4.95 in MCU 13 x KC2. Boll weight was positively associated with seed cotton yield as reported (Rauf et al., 2005; Giteet al., 2006; Preetha and Raveendran, 2007; Ashokkumar and Ravikesavan, 2013). The ginning per cent ranged from 34.9 to 40.5% and 36.6 to 41.0% in the parents and hybrids respectively, these results were supported by those of Ehsan et al. (2008). There were significant differences in all the hybrids in the case of lint index (g) and seed index (g) (Table 3). All the parents and hybrids had wide variation for seed cotton yield per plant. The mean value of seed cotton yield was ranged from 75.2 g to 119.4 g and 73.8 to 139.8 in parents and hybrids. Among hybrids, Narasimha x KC2 recorded lowest value of 8.89 and the highest value of 11.74 was observed in KC2 x TCH1726. Similar findings for seed cotton yield have also been reported (Baloch, 2004; Soomro et al., 2008).

For mean performance of parents, 2.5% span length had a minimum expression of 27.2 mm in TCH 1705 to 30.6 mm in Narasimha. Previous studies indicated that fiber length could vary widely with plant variety and growing conditions. Ehsan et al. (2008), Copur (2006) and Khan et al. (1989) reported similar results for fiber length. The hybrids TCH 1726 x TCH 1705 and MCU 13 x TCH1726 registered minimum (25.8 mm) and maximum (31.9 mm) length. The mean fibre length of hybrids was 29.7 mm. Niagun and Khadi (2001) observed that mean fibre length for Gossypiumbarbadense crosses was 35. 9 mm and this is the greater value compare to our results, and is in conformation with G. barbadanse which is higher in fibre length than G. hirsutum. Fibre fineness or micronaire and fibre strength are very important characteristic of the fiber quality of cotton and are extremely useful for textile industry. In parents, bundle strength was ranged from 20.6 to 22.4 (g/tex). Maximum and minimum valuesrecorded in hybrids were 23.40 g/tex in BW4-1 x MCU 3and 17.40 g/tex in Narasimha x KC 2.These results were supported by earlier studies (Khan, 2002; Karademir et al., 2011). The parentBW 4-1 recorded the greatest micronaire value of 4.7 µg/inch and lowest of 3.9 µg/inch in MCU3. Differences between the cultivars with respect to fiber fineness were also found significant byCopur (2006), Ehsan et al. (2008) and Ashokkumar et al. (2013). Of the 42 hybrids, BW 4-1 xMCU 13 recorded the lowest value of 3.85 µg/inch. The highest value (4.90 µg/inch) was recorded by TCH 1726 × MCU 3. The average value of micronaire value in hybrids was 4.30µg/inch (Table 3). Niagun and Khadi (2001) observed mean micronaire for G. hirsutum and G.barbadense crosses was 2.95 µg/inch, and this showed that the present study significantlyexploited the hybrids than earlier studies.

Estimation of heterosis for yield attributing traits

Estimation of heterotic effects is necessary to identify the new cross combinations that are suitable for direct exploitation. Therefore, the heterotic expression of 42 cross combinations over mid parental and better parental heterosis was examined and results arepresented (Table 3-5). Hybrid Narasimha x KC 2 recorded highest significant positive relative heterosis (28.30%) and heterobeltiosis (26.96%) for plant height. Among fourty two hybrids, 13 crosses recorded significant positive relative heterosis and six crosses showed positivesignificant heterobeltiosis. These results were supported from those of Sayal et al. (1999), Hassan et al. (1999) Rauf et al., (2005) and Ashokkumar and Ravikesavan, (2013) who observed considerable amount of heterosis for plant height. For number of sympodia, the hybrid KC2 xTCH 1726 recorded highest positive significant relative heterosis (45.0%), and heterobeltiosis (45.00%) followed by TCH 1726 x KC 2 with relative heterosis of 35.0%, and heterobeltiosis of 35.0%. This is similar with earlier finding of Koodalingam et al. (1991), Ganapathy and Nadarajan (2008) and Ashokkumar and Ravikesavan, (2013). Among 42 hybrids, 19 hybrids showed positive significant relative heterosis and 16 hybrids showed positive and significant heterobeltiosis for number of bolls per plant. Three hybrids showed positive

significant relative heterosis and two hybrids recorded positive significant heterobeltiosis for boll weight. Of the fourty two hybrids, none of the hybrids recorded positive significant relative heterosis and heterobeltiosis for ginning percentage (Table. 4). For seed index, the cross MCU3 x KC2 recorded a maximum significant positive relative heterosis (29.41%) followed by MCU 13 x KC 2 (26.88%). The cross MCU3 x KC2 exhibited highest and significant heterobeltiosis (26.19 %.). However, seventeen hybrids showed positive significant relative heterosis, 6 hybrids showed positive and significant heterobeltiosis over better parent for seed index (Table 4). Significant positive heterosis as well as heterobeltiosis for seed index also reported in previous studies (Khan, 1986; Rahman et al., 1993; Ashokkumar and Ravikesavan, 2013).

Expression of heterosis for seed cotton yield

Sixteen hybrids exhibited positive and significant relative heterosis, and 11 hybrids showed positive significant heterobetiosis. Ganapathy and Nadarajan (2008) also reported positive heterobeltiotic effect over better parent 20 hybrids for seed cotton yield. Additionally, similar results were already reported (Chaudhari et al.,1992; Ashokkumar and Ravikesavan, 2013) forseed cotton yield. The hybrid KC 2 x TCH 1726 recorded maximum positive significant relative heterosis (81.40%), and heterobeltiosis (77.11%) followed by TCH 1726 x KC 2 with positive significant values of relative heterosis (62.24%), heterobeltiosis (58.40%) for seed cotton yield.

Expression of heterosis for fibre quality traits

For fibre length or 2.5% span length, eighteen hybrids recorded positive and significant relative heterosis over mid parental value (Table 5). The hybrid BW4-1 x MCU 13 recorded maximum positive significant relative heterosis (21.98%) and heterobeltiosis (13.01%). Eight hybrids showed significant and positive heterosis over mid and better parent. The results of heterosis are in conformity with the reports of Tuteja et al. (2005), Iraddi and Kajjidoni (2009), Karademir and Gencer (2010), Karademir et al. (2011) and Ashokkumar et al. (2013). For bundle or fibre strength, one hybrids displayed significant positive relative heterosis over mid parental value and heterobeltiosis for better parental value. Hybrid vigour was also observed by Hassan et al. (1999), Soomro (2000), Rauf et al. (2005) Karademir et al. (2011) and Ashokkumar et al. (2013). The hybrid BW4-1 x TCH 1705 recorded maximum positive and significant relative heterosis (12.77%) and heterobeltiosis (11.961%) for bundle strength. For micronaire, The hybrid TCH 1726 x KC2 recorded maximum positive and significant relative heterosis (16.21%), heterobeltiosis(10.53%) followed by KC 2 x TCH 1726 with significant and positive relative heterosis (13.83%), and heterobeltiosis (8.27%). Two hybrids showed significant and positive heterosis over mid parent, and better parent. Six hybrids showed positive significant relative heterosis, and four hybrids showed positive significant heterobeltiosis for micronaire.

CONCLUSION

Cottonfibre quality traits have a vital influence on the yarn strength. High fibre length and the tensile strength of the fibres becomes the controlling factor of yarn strength. The developing high yields with fibre quality cultivars or hybrids are essential to current modernized spinning mills. Therefore, the present study was carried out for improving yield and fibre quality traits from upland cotton by diallel mating design. The results showed that hybrids are superior to the parents for all the yield and fibre quality traits. A hybrid KC 2 x MCU 3 recorded positive and significant relative heterosis, and heterbeltiosis for number of bolls per plant, and seed cotton yield. Hybrid TCH 1726 X BW4-1 found to be exhibit positive and significant relative heterosisfor most of the yield and firbe quality traits is found to be utilized for direction choice.Further more, increasing the fibre quality traits are a valuable addition to cotton cultivars orhybrids, and it will be useful for textile industries.

REFERENCES

1. Anwar, A.M., M.I. Gill, D. Muhammad, and M.N. Afzal. 2002. Evaluation of cotton varieties at different doses of nitrogen fertilizer. *Pak. Cottons.* 46: 35-41.
2. Ashokkumar, K., and R. Ravikesavan. 2011. Morphological diversity and per se performance in upland cotton (*Gossypiumhirsutum* L.). *J. Agric. Sci.* 3: 107-113.
3. Ashokkumar, K., and R. Ravikesavan. 2008. Genetic studies of combining ability estimates for seed oil, seed protein and fibre quality traits in upland cotton (*G. hirsutum* L).*Research J. Agri. Biological Sci.* 4: 798-802.
4. Ashokkumar, K., and R. Ravikesavan. 2013. Genetic variation and heterotic effects for seed oil, seed protein and yield attributing traits in upland cotton (*G. hirsutum* L.). *Afr. J.Biotechnol.* 12: 5183-5191.
5. Ashokkumar, K., K. Senthil Kumar, and R. Ravikesavan. 2013. Heterosis studies for fibre quality of upland cotton in line x tester design. *Afr. J. Agric. Res.* 8: 6359-6365.
6. Ashokkumar, K., K. Senthil Kumar, and R. Ravikesavan. 2014. An update on conventional and molecular breeding approaches for improving fiber quality traits in cotton - A review. *Afr. J. Biotechnol*. 13:1097-1108.
7. Ashokkumar, K., R. Ravikesavan, and K. SilvasJebakumar Prince. 2010. Combining ability estimates for yield and fibre quality traits in Line X Tester crosses of upland cotton (*G. hirsutum*. L). *Int. J. Biol.* 2: 179-183.
8. Baloch, M.J. 2002. Relative performance of F1 and F2 inter hirsutum hybrids for some quantitative traits in upland cotton. *Pak. J. Sci. Ind. Res.* 45: 407-411.
9. Baloch, M.J. 2004.Genetic variability and heritability estimates of some polygenic traits in upland cotton.*Pak. J. Sci. Ind. Res.* 42: 451-454.
10. Baloch, M.Z., B.A. Ansari, and N. Memon. 2003. Performance and selection of intraspecific hybrids of spring wheat (*Triticumaestivum* L.). *Pak. J. Agric. Agril. Engg. Vet. Sci.*19: 28-31.
11. Basal, H., and I. Turgut. 2003. Heterosis and combining ability for yield components and fiber quality parameters in a half diallel cotton (*G. hirsutum* L.) population. *Turk. J.Agric. For.* 27(2):207-212.
12. Basbag, S., and O. Gencer. 2004. Investigations on the heritability of the cotton seed yield components and technological characters in cotton (*G. hirsutum). Pak. J. Sci.* 7: 1390-1393.
13. Bolek, Y., H. Cokkizgin, and A. Bardak. 2011. Combining ability and heterosis for fiber quality traits in cotton. *Plant Breed.Seed Sci.* 62:3-16.
14. Chandio, M.A., M.S. Kalwar, and G.M. Baloch. 2002. Gene action for some quantitative characters in upland cotton (*G. hirsutum*). Pak. J. Sci. Res. 54(1-2): 31-35.
15. Chaudhari, P.N., D.N. Borole, A.V. Tendulkar, and B.N. Narkhede. 1992. Heterosis for economic traits in intra-specific crosses of desi cotton. *J. Maharashtra Agric. Univ.* 17: 273-276.
16. Copur, O. 2006.Determination of yield and yield components of some cotton cultivars in semi-arid conditions.*Pak. J Biol. Sci.* 9: 2572-2578.
17. Doak, C.C. 1934. A new technique in hybridizing suggested changes in existing methods of emasculation and bagging cotton flowers.*J. Hered.* 25: 201-204.
18. Ehsan, F., A. Nadeem, M.A. Tahir, and A. Majeed. 2008. Comparative yield performance of new cultivars of cotton (*Gossypiumhirsutum* L.). *Pak. J. Life Soc. Sci.* 6:1-3.
19. Ganapathy, S., and N. Nadarajan. 2008. Heterosis studies for oil content, seed cotton yield and other economic traits in cotton (*G. hirsutum* L.). *Madras Agric. J.* 95: 306-310.
20. Gite, V.K., M.B. Misal, and Kalpande. 2006. Correlation and path analysis in cotton (*Gossypiumhirsutum* L.) *J. Cotton Res. Dev.* 20:51-54.
21. Green, C.C., and T.W. Culp. 1990. Simultaneous improvement of yield, fibre quality and yarn strength in upland cotton. *Crop Sci.* 30: 66-67
22. Griffing, B. 1956.Concept of general and specific combining ability in relation to Diallel crossing system.*Aust. J. Biol. Sci.* 9:463-493.
23. Hassan, G., G. Mahmood, N. Khan, A. Razzaq. 1999. Combining ability and heterobeltiosis estimates in diallel cross of cotton (*Gossypiumhirsutum* L.) *Sarhad. J. Agric.* 15: 563–568.

24. Hofs, J.L., B. Hau, and D. Marais. 2006. Boll distribution patterns in Bt and non-Bt cotton cultivars: I. Study on commercial irrigated farming systems in South Africa. *FieldCrops Res.* 98: 203-209.
25. Iraddi, V., and S.T. Kajjidoni. 2009. A Comparative study on heterosis for productivity and fibre quality traits in intra-herbaceum and interspecific (*G. herbaceum* x *G.arboreum*) crosses of diploid cotton. *J. Res. Angrau*. 37:35-43.
26. Islam, Z., H.A. Sadaqat, and F.A. Khan. 2001. Combining ability of some Hirsute cotton types for economic traits. *Int. J. Agri. Biol.* 3:411-412.
27. Karademir, C., E. Karademir, R. Ekinci, and O. Gencer. 2009. Combining ability estimates and heterosis for yield and fiber quality of Cotton in Line x Tester Design. *Not. Bot.Hort. Agrobot. Cluj.*37: 228-233.
28. Karademir, C., E. Karademir, and O. Gencer. 2011. Yield and Fiber Quality of F 1 and F 2 Generations of Cotton (*Gossypiumhirsutum* L.) Under Drought Stress Conditions.*Bulg. J. Agric. Sci.* 17: 795–805.
29. Karademir, E., and O. Gencer. 2010. Combining ability and heterosis for yield and fiber quality properties in cotton (*G. hirsutum* L.) obtained by half diallel mating design.*Not. Bot. Hortic.Agrobot.Cluj.*38: 222-227.
30. Khan, M.A. 1986.Heterotic and heterobeltiotic studies for quality traits in intraspecific crosses of upland cotton.Sarhad J. Agric. 1: 137-144.
31. Khan, N., H. Basal, and G. Hassan 2010. Cottonseed oil and yield assessment via economic heterosis and heritability in intraspecific cotton populations. Afr. J. Biotechnol. 9: 7418–7428
32. Khan, U.Q. 2002.Studies of heterosis in fibre quality traits of cotton. Asian J. Plant Sci. 1: 593-595.
33. Khan, W.S., A.A. Khan, A.S. Naz, and S. Ali. 1989. Performance of six Punjab commercial varieties of Gossypiumhirsutum L. under Faisalabad conditions. Pak. Cottons. 33: 60-65.
34. Kiani, G., G.A. Nematzadeh, S.K. Kazemitabar, O. Alıshah. 2007. Combining ability in cotton cultivars for agronomic traits. Int. J. Agric. Biol. 9: 521-522.
35. Koodalingam, K., S. Rajasekaran, and A. Ramalingam. 1991. Heterosis and inbreeding depression in inter-specific upland cotton. Ann. Agric. Res. 12: 288-291.
36. Marani, A. 1963. Heterosis and combining ability for yield and components of yield in a diallel cross of two species of cotton. Crop Sci. 3: 552-555.
37. Memon, S., B.A. Ansari, and A. Memon. 2005. Estimation of heterosis and interrelationship between important agro-economic traits of bread wheat. Ind. J. Plant Sci. 4: 355-361.
38. Niagun, H.G., and B.M. Khadi. 2001. Progeny analysis of fibre characteristics of DCH 32 an interspecific cotton hybrid. J. Genet. Breed. 55:209-216.
39. Preetha, S., and Raveendran, T.S. 2007.Genetic variability and association analysis in three different morphological groups of cotton (G. hirsutm L.). Asian J. Plant Sci. 6: 122-128.
40. Preetha, S., and T.S. Raveendran. 2008. Combining ability and heterosis for yield and fibre quality traits in line x testercrosses of Upland cotton (G. hirsutum L.). Int. J. Plant Breed. Genet. 2: 64-74.
41. Rahman, S., M.A. Khan, M. Ayub, and M.A. Khan. 1993. Heterosis and heterobeltiosis for lint percentage, seed and lint indices and stable length in different cross combination of five cotton cultivars. Pakistan J. Agric. Res. 14: 115-120.
42. Rauf, S., T.M. Khan, and S. Nazir. 2005. Combining ability and heterosis in (G. hirsutum L.).Int. J. Agri. Biol. 7: 109-113.
43. Sayal, A.U., M.S. Baloch, I. Hussain, and B. Khan. 1999. Exploration of heterosis and heterobeltiosis in American Cotton (Gossypiumhirsutum L.) J. Agric. Res. 37: 201– 208.
44. Senthilkumar, K., K. Ashokkumar, and R. Ravikesavan (2014).Genetic effects of combining ability studies for yield and fibre quality traits in diallel crosses of upland cotton (*G. hirsutum*L.). Afr. J. Biotechnol. 13:119-126.
45. Singh, P. 1982. Note on useful heterosis in upland cotton. Indian J. Agric. Sci. 52: 29-31. Soomro, A.R. 2000. Assessment of useful heterosis in glandless Gossypiumhirsutum cottonstrains through their performance in hybrid combination. Pak. J. Bot. 32: 65–68
46. Soomro, Z.A., A.S. Larik, N.U. Khan, M.J. Baloch, S. Mari, S. Memon, and N.A. Panhwar.2008. Genetic variability studies on quantitative traits in upland cotton. Sarhad J. Agric. 24: 587-592.
47. Tuteja, O.P., S. Kumar, H. Hasan, and M. Singh. 2005. Heterosis and interrelationship between seed cotton

yield and qualitative characters in upland cotton (*Gossypiumhirsutum*). Indian J. Agric. Sci. 75:167-171

48. Williams, W., and N.E. Gilbert. l960. Heterosis and inheritance of yield in the tomato. Heredity, 15: 133-150.
49. Wynne, J.C., Emery, D.A., and Rice, P.M. 1970. Combining anility estimates in Arachis hypogea L. II. Field emergence of F_1 hybrids. Crop sci. 10:719-715.
50. Zhang, Z.S., X.B. Li, D.J. Liu, S.L. Huang, and F.X. Zhang. 2002. Study on heterosis utilization of upland cotton (*G. hirsutum* L.) lines with high fibre quality. Cotton Sci. 14: 264-268.

Table 1.Analysis of variance for means square of yield and fibre quality traits

Source	d.f	Days to first boll bursting	Number of sympodial branches	Plant height (cm)	Number of bolls per plant	Boll weight (g)	Lint index (g)	Seed index (g)	Ginning (%)	Single plant yield (g)	2.5.per cent span length (mm)	Bundle strength (g/tex)	Micronaire
Replications	2	0.659	4.27	14.88	1.55	0.143	0.235	0.157	4.96	493.3	4.62	3.076	0.046
Genotypes	48	6.31**	9.98**	727.6**	39.3**	0.136**	0.758**	1.906**	6.20**	1072.9**	7.367**	3.010**	0.203**
Parents	6	6.09**	5.52*	618.4**	11.93*	0.143**	1.348**	1.719**	14.28**	628.3	16.32**	2.088	0.287**
Hybrids	41	6.24**	10.63**	752.0**	40.1**	0.138**	0.568**	1.538**	5.014**	1141.2**	5.066**	3.054**	0.196**
Parents Vs Hybrids	1	10.44**	10.01**	385.3**	181.4**	0.0006	5.000**	18.13**	6.63**	943.7**	47.92**	6.774*	0.003
F_1's	20	6.73**	10.98**	625.4**	50.86**	0.177**	0.443**	1.319**	5.87**	1207.1**	5.660**	3.552**	0.197**
Reciprocals	20	5.71**	10.77**	906.6**	27.40**	0.102	0.714**	1.436**	4.049**	1124.8**	4.553**	2.703**	0.195**
F_1 Vs reciprocals	1	7.14**	0.79	188.9*	79.36*	0.079	0.147**	7.940**	7.081**	149.4	3.433	0.108	0.186*
Error	96	1.020	1.48	23.35	5.30	0.048	0.221	0.581	0.935	146.3	1.266	1.215	0.039
Total	146	2.757	4.31	254.7	16.50	0.078	0.398	1.011	2.724	455.7	3.317	1.831	0.093

*, Significant at 5% level; **, significant at 1% level

Table 2.Mean performance of parents for yield and fibre quality traits

Genotypes	Days to boll first boll bursting	Number of sympodia per plant	Plant height (cm)	Number of bolls per plant	Boll weight (g)	Lint index (g)	Seed index (g)	Ginning percent (%)	Single plant yield (g)	2.5 % span length (mm)	Bundle strength (g/tex)	Micronaire
Narasimha	102	15	100.0	22	4.10	5.76	8.86	39.2	95.8	30.6	20.7	4.1
KC 2	101	13	102.1	22	4.28	5.89	8.22	38.0	75.2	27.2	22.1	4.4
MCU 3	102	15	127.0	22	4.31	5.59	8.65	39.2	95.7	30.2	22.0	3.9
TCH 1705	102	15	114.7	24	4.50	6.53	9.54	40.5	93.8	27.2	20.9	4.6
BW4-1	105	17	122.3	27	4.70	6.76	10.27	39.7	119.4	28.6	20.6	4.7
MCU 13	103	15	87.0	22	4.58	6.73	10.12	39.9	98.9	27.6	22.3	4.5
TCH 1726	101	13	119.3	21	4.61	4.96	9.23	34.9	78.9	28.7	22.4	4.0
Grand mean	102	15	110.3	23	4.44	6.03	9.27	39.3	93.9	27.8	21.6	4.3

Table 3. Mean performance and expression of heterosis in hybrids (%) for yield attributing traits.

SL. No.	Cross	Days to first boll bursting			Plant height (cm)			Number of Sympodia per plant			Number of Bolls per plant		
		Mean	d_i	^{d}ii	Mean	d_i	^{d}ii	Mean	d_i	^{d}ii	Mean	d_i	^{d}ii
1.	Narasimha x KC 2	107	4.92**	4.58**	129.6	28.30**	26.96**	15	3.53	-2.22	28	23.88**	23.88**
2.	Narasimha x MCU 3	105	2.94**	2.94**	103.4	-8.84**	-18.53**	14	-6.67	-6.67	27	23.31**	22.39**
3.	Narasimha x TCH1705	103	0.98	0.98	116.0	8.10*	1.16	17	15.56**	15.56*	32	38.13**	33.33**
4.	Narasimha x BW4-1	102	-1.93**	-3.48**	131.5	18.34**	7.55*	16	-1.03	-7.69	29	18.92**	8.64
5.	Narasimha x MCU 13	103	0.32	-0.32	94.3	0.86	-5.67	18	18.68**	17.39**	32	44.36**	43.28**
6.	Narasimha x TCH 1726	101	-0.33	-0.65	130.3	18.86**	9.25**	18	29.41**	22.22**	33	50.77**	46.27**
7.	KC2 x Narasimha	103	1.31	0.98	123.6	22.36**	21.08**	16	15.29*	8.89	30	37.31**	37.31**
8.	KC2 x MCU 3	103	1.31	0.98	103.0	-10.10**	-18.90**	17	20.00**	13.33*	33	47.37**	46.27**
9.	KC2 x TCH 1705	104	2.30**	1.96*	98.3	-9.31**	-14.29**	14	20.00**	13.33*	28	22.30**	18.06*
10.	KC2 x BW 4-1	104	0.97	-0.95	92.0	-18.02**	-24.78**	15	-10.87	-21.15**	22	-12.16	-19.75**
11.	KC2 x MCU13	101	-1.63*	-2.58**	96.2	1.70	-5.81	15	4.65	-2.17	26	17.29*	16.42
12.	KC2 x TCH 1726	102	0.66	0.66	103.6	-6.37*	-13.10**	19	45.00**	45.00**	34	58.46**	53.73**
13.	MCU 3 x Narasimha	102	0.00	0.00	88.2	-22.29**	-30.55**	14	-4.44	-4.44	24	6.77	5.97
14.	MCU3 x KC 2	104	2.30**	1.96*	84.8	-25.98**	-33.23**	14	-1.18	-6.67	23	3.76	2.99
15.	MCU3 x TCH 1705	105	2.94**	2.94**	87.2	-27.85**	-31.34**	14	-6.67	-6.67	23	0.00	-4.17
16.	MCU 3 x BW4-1	102	-1.61*	-3.16**	92.6	-25.66**	-27.03**	14	-11.34*	-17.31**	22	-8.84	-17.28*
17.	MCU 3 x MCU 13	103	0.32	-0.32	93.6	-12.48**	-26.25**	14	-5.49	-6.52	24	10.61	10.61
18.	MCU 3 x TCH 1726	102	-0.00	-0.33	91.4	-25.78**	-28.03**	16	10.59	4.44	28	28.68**	25.76**
19.	TCH1705 x Narasimha	105	2.94**	2.94**	109.6	2.14	-4.42	18	22.22**	22.22**	32	38.13**	33.33**
20.	TCH1705 x KC2	103	1.31	0.98	128.2	18.23**	11.74**	14	1.18	-4.44	25	7.91	4.17
21.	TCH1705 x MCU 3	101	-0.65	-0.65	116.0	-4.03	-8.66**	19	26.67**	26.67**	29	27.54**	22.22**
22.	TCH1705 x BW4-1	102	-1.29	-2.85**	111.3	-6.06*	-8.97**	13	-17.53**	-23.08**	22	-15.03*	-19.75**
23.	TCH1705 x MCU 13	103	0.32	-0.32	108.0	7.05*	-5.87	18	18.68**	17.39**	25	8.70	4.17
24.	TCH 1705 x TCH 1726	102	0.33	0.00	122.6	4.83	2.82	15	8.24	2.22	26	14.07	6.94
25.	BW4-1 x Narasimha	105	1.29	-0.32	81.0	-27.13**	-33.77**	15	-5.15	-11.54*	27	8.11	-1.23
26.	BW4-1 x KC2	103	-0.32	-2.22**	92.6	-17.42**	-24.23**	14	-6.52	-17.31**	24	-2.70	-11.11
27.	BW4-1 x MCU 3	105	1.29	-0.32	95.1	-23.68**	-25.09**	15	-7.22	-13.46*	22	-10.20	-18.52**
28.	BW4-1 x TCH 1705	103	-0.64	-2.22**	86.6	-26.87**	-29.14**	15	-7.22	-13.46*	25	-0.65	-6.17
29.	BW4-1 x MCU 13	102	-2.24**	-3.16**	110.6	5.72	-9.51**	13	-20.41**	-25.00**	22	-11.56	-19.75**
30.	BW4-1 x TCH 1726	105	1.61*	-0.32	86.7	-28.20**	-29.08**	18	17.39**	3.85	28	-16.67*	3.70
31.	MCU 13 x Narasimha	103	0.32	-0.32	90.0	-3.77	-10.00*	13	-12.09*	-13.04*	22	-0.75	-1.49
32.	MCU 13 x KC 2	104	1.95**	0.97	95.3	0.78	-6.66	14	-2.33	-8.70	21	-5.26	-5.97
33.	MCU13 x MCU 3	102	-0.65	-1.29	117.3	9.63**	-7.61*	15	-1.10	-2.17	26	18.18*	18.18*
34.	MCU13 x TCH 1705	103	0.32	-0.32	113.8	12.86**	-0.76	13	-14.29*	-15.22*	22	-4.35	-8.33
35.	MCU13 x BW4-1	104	0.00	-0.95	92.0	-12.11**	-24.78**	15	-8.16	-13.46*	25	0.68	-8.64
36.	MCU13 x TCH 1726	102	0.00	-0.97	91.7	-11.09**	-23.11**	14	-2.33	-8.70	22	2.33	0.00
37.	TCH 1726 x Narasimha	102	0.66	0.33	110.1	0.41	-7.71*	16	15.29*	8.89	28	29.23**	25.37**
38.	TCH 1726 x KC 2	103	1.64*	1.64*	105.6	-4.56	-11.43**	18	35.00**	35.00**	26	21.54**	17.91*
39.	TCH 1726 x MCU 3	102	0.33	0.00	134.3	9.08**	5.77	19	31.76**	24.44**	27	25.58**	22.73**
40.	TCH 1726 x TCH 1705	105	3.28**	2.94**	136.0	16.22**	14.00**	14	-3.53	-8.89	23	0.74	-5.56
41.	TCH 1726 x BW4-1	107	3.55**	1.58*	127.6	5.68*	4.39	19	21.74**	7.69	28	15.28*	2.47
42.	TCH 1726 x MCU 13	103	0.33	-0.65	117.8	14.17**	-1.26	15	6.98	0.00	23	6.98	4.55

*significant at 5 per cent level, ** significant at 1 per cent level, di, relative heterosis; dii, heterobeltiosis

Table 4. Mean performance and expression of heterosis in hybrids (%) for boll weight and seed quality traits.

SL. No.	Cross	Boll weight (g)			Lint index (g)			Seed index (g)			Ginning per cent (%)		
		Mean	d_i	d_{ii}	Mean	d_i	d_{ii}	Mean	d_i	d_{ii}	Mean	d_i	d_{ii}
1.	Narasimha x KC 2	4.32	3.10	0.93	6.15	5.52	4.36	8.89	4.02	0.26	40.7	0.70	-2.24
2.	Narasimha x MCU 3	3.96	-5.91	-8.20	6.47	13.95*	12.27	9.59	9.53	8.24	40.1	2.21	2.12
3.	Narasimha x	4.26	-1.01	-5.47	6.36	3.55	-2.55	10.05	9.26	5.38	38.7	-2.96	-4.52
4.	Narasimha x BW4-1	3.91	-11.31**	-17.06**	6.05	-3.30	-10.45	9.43	-1.46	-8.21	39.0	-1.06	-1.60
5.	Narasimha x MCU	4.26	-1.80	-6.98	6.72	7.66	-0.10	9.16	-3.49	-9.49	39.7	6.73	5.84
6.	Narasimha x TCH	4.54	4.24	-1.59	6.43	19.93**	11.63	9.30	2.84	0.79	40.7	9.84	3.74
7.	KC2 x Narasimha	4.09	-2.47	-4.52	6.21	6.55	5.38	10.09	18.10**	13.84	38.0	-6.13	-8.87
8.	KC2 x MCU 3	4.10	-4.62	-4.95	5.68	-1.10	-3.62	9.03	6.97	4.31	38.6	-4.57	-7.43
9.	KC2 x TCH 1705	4.44	1.14	-1.41	6.18	-0.43	-5.31	9.37	5.54	-1.75	39.6	-3.73	-5.04
10.	KC2 x BW 4-1	4.66	3.67	-1.06	6.80	7.46	0.54	10.08	9.01	-1.85	40.2	-1.15	-3.52
11.	KC2 x MCU13	4.50	1.54	-1.82	6.42	1.80	-4.56	10.71	16.74**	5.80	37.4	-8.21	-10.15
12.	KC2 x TCH 1726	4.36	-2.06	-5.63	6.58	21.25**	11.71	9.85	12.91*	6.75	39.9	4.26	-4.24
13.	MCU 3 x Narasimha	4.52	7.57*	4.95	6.68	17.65**	15.91*	11.08	26.55**	25.05**	37.5	-4.42	-4.50
14.	MCU3 x KC 2	4.59	6.95	6.57	6.35	10.57	7.75	10.92	29.41**	26.19**	36.7	-9.19	-11.91
15.	MCU3 x TCH 1705	4.47	1.32	-0.89	6.53	7.76	0.00	11.14	22.50**	16.81*	36.8	-7.73	-9.29
16.	MCU 3 x BW4-1	4.77	5.84	1.34	6.82	10.45	0.89	10.25	8.30	-0.23	39.9	1.14	0.50
17.	MCU 3 x MCU 13	4.37	-1.65	-4.58	6.28	1.95	-6.69	10.35	10.26	2.27	37.7	-4.72	-5.59
18.	MCU 3 x TCH 1726	4.59	2.84	-0.58	6.39	21.16**	14.37*	10.11	13.07*	9.53	38.7	4.63	-1.11
19.	TCH1705 x	4.38	1.86	-2.74	6.75	9.90	3.42	10.50	14.15*	10.10	39.1	-2.05	-3.62
20.	TCH1705 x KC2	4.33	-1.52	-3.99	6.11	-1.61	-6.43	9.50	7.00	-0.38	39.0	-5.19	-6.47
21.	TCH1705 x MCU 3	4.31	-2.23	-4.36	6.31	4.07	-3.42	9.92	9.09	4.02	38.8	-2.72	-4.35
22.	TCH1705 x BW4-1	4.36	-5.39	-7.43	6.87	3.39	1.63	9.99	0.82	-2.76	40.7	1.58	0.49
23.	TCH1705 x MCU 13	4.81	5.76	4.87	6.39	-3.57	-5.00	10.93	11.16*	7.97	36.9	-8.32	-9.04
24.	TCH 1705 x TCH	4.57	0.26	-0.94	6.95	20.94**	6.43	10.61	13.02*	11.18	39.5	4.86	-2.47
25.	BW4-1 x Narasimha	4.38	-0.64	-7.08	7.20	15.07**	6.56	10.71	11.99*	4.32	40.1	1.65	1.09
26.	BW4-1 x KC2	4.40	-2.04	-6.51	6.75	6.72	-0.15	9.94	7.45	-3.25	40.0	-1.56	-3.92
27.	BW4-1 x MCU 3	4.56	1.03	-3.26	7.04	14.01*	4.14	10.98	16.08**	6.95	38.9	-1.23	-1.85
28.	BW4-1 x TCH 1705	4.42	-4.09	-6.16	7.18	8.10	6.26	11.74	18.53**	14.31*	37.9	-5.48	-6.49
29.	BW4-1 x MCU 13	4.60	-1.08	-2.41	6.53	-3.14	-3.35	10.10	-0.93	-1.66	39.2	-1.47	-1.75
30.	BW4-1 x TCH 1726	4.35	-6.72*	-7.64*	7.51	28.12**	11.09	10.65	9.23	3.70	41.0	9.92	3.27
31.	MCU 13 x	4.58	5.57	0.00	7.35	17.64**	9.16	10.91	14.94**	7.81	40.3	1.77	0.92
32.	MCU 13 x KC 2	4.95	11.70**	8.00*	7.26	15.11**	7.92	11.64	26.88**	14.99*	38.3	-6.00	-7.99
33.	MCU13 x MCU 3	4.43	-0.45	-3.42	6.85	11.20*	1.78	11.58	23.40**	14.46*	37.1	-6.15	-7.01
34.	MCU13 x TCH 1705	4.30	-5.46	-6.25	6.06	-8.55	-9.91	10.46	6.41	3.36	36.6	-8.90	-9.61
35.	MCU13 x BW4-1	4.57	-1.65	-2.97	6.57	-2.64	-2.86	11.23	10.15	9.35	36.8	-7.49	-7.76
36.	MCU13 x TCH 1726	4.72	2.54	2.17	6.90	17.96**	2.48	10.92	12.90*	7.94	38.8	3.70	-2.84
37.	TCH 1726 x	4.35	-0.11	-5.70	6.48	20.86**	12.50	9.84	8.81	6.65	39.6	6.97	1.02
38.	TCH 1726 x KC 2	4.77	7.23*	3.32	5.93	9.21	0.62	9.78	12.03	5.92	37.8	-1.22	-9.27
39.	TCH 1726 x MCU 3	4.41	-1.27	-4.55	5.96	13.01*	6.68	9.81	9.71	6.28	37.8	2.11	-3.49
40.	TCH 1726 x TCH	4.43	-2.96	-4.12	5.66	-1.51	-13.32*	9.69	3.25	1.57	36.8	-2.47	-9.29
41.	TCH 1726 x BW4-1	4.68	0.43	-0.57	6.93	18.28**	2.56	10.63	9.03	3.51	39.4	5.72	-0.67
42.	TCH 1726 x MCU	4.52	-1.67	-2.02	6.83	16.76**	1.44	10.08	4.22	-0.36	40.4	8.15	1.34

*significant at 5 per cent level, ** significant at 1 per cent level, di, relative heterosis; dii, heterobeltiosis

Table 5. Mean performance and expression of heterosis in hybrids (%) for yield and fibre quality traits

SL.No	Cross	Seed cotton yield per plant(g)			2.5per cent span length (mm)			Bundle strength (g/tex)			Micronaire		
		Mean	d_i	^{d}ii	Mean	d_i	^{d}ii	Mean	d_i	^{d}ii	Mean	d_i	^{d}ii
1.	Narasimha x KC 2	90.2	5.55	-5.81	27.9	-3.23	-8.61**	17.4	-18.51**	-21.20**	4.15	-3.30	-6.39
2.	Narasimha x MCU 3	86.2	-10.02	-10.05	28.4	-6.36*	-6.97*	21.3	-0.08	-3.17	4.40	9.32**	6.02
3.	Narasimha x TCH1705	120.0	26.57**	25.25*	27.3	-5.65*	-10.78**	19.3	-6.89	-7.34	4.53	3.62	-1.45
4.	Narasimha x BW4-1	102.5	-4.72	-14.13	28.2	4.06	-7.84*	20.5	-0.40	-0.64	4.00	-9.60**	-14.89**
5.	Narasimha x MCU 13	121.9	25.15**	23.17*	29.9	2.63	-2.29	19.9	-7.59*	-11.03**	4.50	4.05	0.00
6.	Narasimha x TCH 1726	130.0	48.77**	35.65**	29.6	-0.17	-3.27	20.5	-4.71	-8.46*	4.50	10.43**	8.43*
7.	KC2 x Narasimha	107.5	25.70*	12.17	28.3	-1.85	-7.30*	21.8	2.02	-1.35	4.50	4.85	1.50
8.	KC2 x MCU 3	118.8	39.05**	24.12*	28.9	0.93	-4.08	21.4	-3.24	-3.46	4.40	5.60	-0.75
9.	KC2 x TCH 1705	115.4	36.62**	23.06*	29.6	8.69**	8.56*	21.5	0.15	-2.71	4.35	-3.69	-5.43
10.	KC2 x BW 4-1	81.3	-16.38	-31.85**	28.7	12.99**	5.51	21.2	-0.86	-4.36	4.15	-9.12**	-11.70**
11.	KC2 x MCU13	95.5	9.74	-3.44	28.7	4.62	3.73	20.6	-7.49*	-7.90	4.65	4.10	3.33
12.	KC2 x TCH 1726	139.8	81.40**	77.11**	28.1	0.54	-2.09	21.6	-3.21	-3.86	4.80	13.83**	8.27*
13.	MCU 3 x Narasimha	84.6	-11.69	-11.72	31.5	3.84	3.16	21.4	0.08	-3.02	4.10	1.86	-1.20
14.	MCU3 x KC 2	83.2	-2.63	-13.09	29.5	2.79	-2.32	21.9	-0.98	-1.20	4.07	4.80	-1.50
15.	MCU3 x TCH 1705	82.5	-12.90	-13.78	31.5	9.63**	4.30	22.3	3.80	1.06	4.35	-5.88	-13.04**
16.	MCU 3 x BW4-1	86.4	-19.69*	-27.64**	30.7	14.37**	1.88	22.2	4.06	0.60	4.10	-4.65	-12.77**
17.	MCU 3 x MCU 13	86.6	-10.99	-12.43	31.1	7.49**	2.98	21.2	-4.28	-4.92	4.07	-3.17	-9.63**
18.	MCU 3 x TCH 1726	105.9	21.31*	10.65	31.0	5.49*	2.87	21.5	-3.14	-4.01	4.35	10.13**	8.75*
19.	TCH1705 x Narasimha	130.2	37.36**	35.93**	28.7	-0.81	-6.21*	21.1	1.44	0.96	4.10	-6.29	-10.87**
20.	TCH1705 x KC2	88.3	4.55	-5.83	28.8	5.75	5.62	21.0	-2.17	-4.96	4.30	-7.01*	-8.70*
21.	TCH1705 x MCU 3	134.8	42.23**	40.79**	28.9	0.81	-4.08	21.9	1.94	-0.76	4.05	1.18	-6.52
22.	TCH1705 x BW4-1	73.8	-30.77**	-38.18**	30.7	20.71**	12.59**	21.7	4.90	4.15	3.90	-12.90**	-13.83**
23.	TCH1705 x MCU 13	123.9	28.60**	25.26*	28.4	3.40	2.65	20.8	-3.85	-7.00	4.20	-14.29**	-15.22**
24.	TCH 1705 x TCH 1726	99.6	15.38	6.22	30.6	9.35**	6.62*	21.4	-1.31	-4.75	4.65	-3.49	-9.78**
25.	BW4-1 x Narasimha	98.6	-8.38	-17.42*	28.8	6.27*	-5.88	19.6	-5.08	-5.31	4.60	5.08	-1.06
26.	BW4-1 x KC2	87.9	-9.59	-26.33**	28.5	12.20**	4.78	20.1	-6.00	-9.32*	4.65	0.73	-2.13
27.	BW4-1 x MCU 3	93.2	-13.31	-21.89**	29.8	10.78**	-1.32	23.4	-3.91	-7.10	4.60	3.49	-5.32
28.	BW4-1 x TCH 1705	94.3	-11.49	-20.97*	29.9	17.82**	9.90**	22.1	12.77**	11.96**	4.35	-6.45*	-7.45*
29.	BW4-1 x MCU 13	74.4	-31.83**	-37.66**	31.2	21.98**	13.01**	21.2	3.18	-0.89	3.85	-16.30**	-18.09**
30.	BW4-1 x TCH 1726	127.1	28.17**	6.45	30.5	16.89**	6.50*	21.2	-1.24	-5.34	4.10	1.15	-6.38
31.	MCU 13 x Narasimha	76.7	-21.25*	-22.50*	28.9	-0.57	-5.34	21.3	-1.08	-4.77	4.40	9.83**	5.56
32.	MCU 13 x KC 2	84.5	-2.89	-14.55	30.8	12.27**	11.33**	21.0	-5.39	-5.81	4.75	6.34*	5.56
33.	MCU13 x MCU 3	94.2	-3.22	-4.78	31.5	9.10	4.53	21.5	-2.93	-3.58	4.75	-1.19	-7.78*
34.	MCU13 x TCH 1705	74.6	-22.54*	-24.55*	30.3	10.32	9.52**	21.7	0.31	-2.98	4.15	-7.69*	-8.70*
35.	MCU13 x BW4-1	95.4	-12.62	-20.10*	29.2	13.91	5.54	20.9	-2.72	-6.56	4.20	-4.35	-6.38
36.	MCU13 x TCH 1726	86.1	-3.13	-12.93	31.9	13.42**	11.38**	21.2	-5.43	-5.64	4.40	4.71	-1.11
37.	TCH 1726 x Narasimha	110.8	26.80**	15.62	29.6	0.06	-3.05	19.5	-9.65**	-13.20**	4.45	3.07	1.20
38.	TCH 1726 x KC 2	125.0	62.24**	58.40**	29.9	6.98*	4.18	20.0	-10.08**	-10.68**	4.20	16.21**	10.53**
39.	TCH 1726 x MCU 3	131.7	50.16**	36.96**	28.7	-2.32	-4.75	19.8	-10.78**	-11.57**	4.90	3.80	2.50
40.	TCH 1726 x TCH 1705	81.6	-5.54	-13.04	25.8	-7.80**	-10.10**	20.6	-4.69	-8.01*	4.10	-3.49	-9.78**
41.	TCH 1726 x BW4-1	129.8	30.92**	8.74	29.8	13.96**	3.83	20.9	-2.63	-6.68	4.15	-6.90*	-13.83**
42.	TCH 1726 x MCU 13	96.0	7.93	-3.00	28.7	2.07	0.23	19.8	-11.38**	-11.57**	4.05	-3.53	-8.89*

*significant at 5 per cent level, ** significant at 1 per cent level, di, relative heterosis; dii, heterobeltiosis

Indian Journal Of Natural Sciences *www.tnsroindia.org.* © *IJONS*

Vol.6 / Issue 33 / December 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

In-Vitro Technology for the Conservation of an Endangered Medicinal Plant, *Celastruspaniculatus*

Pranay Kumar[1]* and Anusree Anand[2]

[1]Dept. of Spices and Plantation crops, Faculty of Horticulture, BCKV, Mohanpur, West Bengal, India.
[2]Dept. of Post-Harvest Technology, College of Horticulture, Bagalkot, Karnataka, India.

Received: 25 Sep 2015 Revised: 29 Oct 2015 Accepted: 25 Nov 2015

***Address for correspondence**
Pranay Kumar
Dept.of Spices and Plantation crops,
Faculty of Horticulture,
BCKV, Mohanpur, West Bengal, India.
Email: pranay.pma@gmail.com

ABSTRACT

An efficient protocol was developed for short-term conservation of an endangered medicinal plant, *Celastruspaniculatus*, using micropropagated nodal segments. Nodal segments maintained in reduced culture condition and low light intensity (2.97 $\mu m^{-2}s^{-1}$)and at 10^0 C. MS medium supplemented with BA(0.886mg/l)+2ip(0.24mg/l) shown highest survival per cent (80%), significantly high shoot length (4.1±0.10), high shoot number/explant (3.1±0.44) and significantly high number of leaves/explant (6.1±1.42) after six months of *in-vitro* conservation.

Keywords: *Celastruspaniculatus*, Nodal segments, *in-vitro*, Conservation

INTRODUCTION

Conservation of biodiversity is considered fundamental because climate change, alien species and use of land for intensive farming and development are causing severethreat to the plant genetic diversity worldwide. *Celastruspaniculatus*is an unarmed woody climbing shrub commonly known as Malkangni, Kangani, Jyotishmati, Sphutabandhani, Svarnalota, Black-Oil tree, Intellect tree, Climbing-staff plant.It grows throughout India up to a height of almost 1,800-2,000 meters. This deciduous vine can grow to a very large size. It belongs to the class Angiospermaeand family Celastraceae.It is a rare and endangered medicinal plant distributed throughout India mostly in tropical forests and subtropical Himalayas.The species is vulnerable in Western Ghats of south India [1].The seeds of *C. paniculatus* contain brownish yellow oil, with a higher proportion of acetic and benzoic acids in addition to other fatty acids, as well as a crystalline substance tetracasanol and sterol[2].Traditional healers have used *Celastrus* oil for centuries to increase mental acuity, improve memory and intellect as well as retention and recalling

power; and to alleviate mental fatigue, stress and minor joint pains. People using the oil were able to learn new information more quickly and were able to accurately and efficiently recall that information over longer periods of time. They used to call it '*magzsudhi*' or brain clearer [3]. The seeds are used to heal indolent ulcers and sores, as well as infectious skin conditions such as scabies in the form of a poultice [4]. The oil being a powerful stimulant is also used as an ointment for relieving rheumatic pains inflicted by malaria [5].Moreover, it has Anti-fertility [6], Wound healing activity [7], Anti-bacterial activity [8] , Anti-fungal activity [9], Analgesic and Anti-inflammatory [10] properties.This highly medicinally significant plant has been subjected to overexploitation by destructive harvesting that affects the survival of this plant in its wild habitat [11].Moreover, the absence of any organized cultivation of this plant calls for immediate conservation measure [12].Hence, this study aimed to optimize the culture condition for short term *in vitro* conservation of the micropropagated nodal segments of *C.paniculatus*.

MATERIALS AND METHODS

The experiment was conducted at Division of Plant Genetic Resources, ICAR-IIHR, Bangalore. Short term conservation of *in-vitro* raised plants of *C.paniculatus*was done for six months to slow down its growth by reducing further sub-culturing and providing limited light intensity for growth. In order to accomplish this, equal number of eight weeks old tissue cultured plants from each treatments (MS medium + Hormonal combinations) were taken and kept under low light intensity(2.97 $\mu m^{-2}s^{-1}$) in a chamber having ambient temperature maintained at 10^0C (Fig. 1). Equal number of replicates from each treatment was kept under standard culture condition in order to compare it with those which are kept under reduced culture condition. Before transferring the replicates from each treatments for *in-vitro* conservation, proper sub-culturing done in every 4 weeks. Readings of growth parameters were recorded at regular intervals (Table 1)

RESULTS AND DISCUSSION

Nodal segments grown in MS medium supplemented with BA(0.886mg/l)+2ip(0.24mg/l) shownhighest survival per cent (80%), significantly high shoot length (4.1±0.10), high shoot number/ explant (3.1±0.44) and significantly high number of leaves/explant (6.1±1.42) (Table 1). A comparison was made between *in-vitro* conserved plantlets and equal number of tissue cultured plantlets regenerated in standard culture conditions for survival per cent, shoot length, number of shoots and number of leaves (Table 2). Plantlets kept in standard culture condition were sub cultured in every 4 weeks. MS medium supplemented with BA (0.886mg/l)+2ip(0.24mg/l) shown highest survival per cent in both *in-vitro* conserved plantlets(80%) and plantlets under normal conditions (100%). The same hormonal combination shown significantly high shoot length in both *in-vitro* conserved plantlets (4.1±0.10) and plantlets kept in normal conditions (5.08±0.29).MS medium supplemented with BA(0.886mg/l)+2ip(0.24mg/l) shown highest number of shoots both *in-vitro* conserved plantlets (3.1±0.44) and plantlets kept in normal conditions (3.4 ±0.54).As far as number of leaves are concerned, MS medium supplemented with BA(0.886mg/l)+2ip(0.24mg/l) shown significantly high number of leaves both in *in-vitro* conserved plantlets (6.1±1.42) and plantlets kept under normal conditions (6.6±0.54).

The objective to go for *in-vitro* conservation by providing limited nutrients, low light intensity (2.97 $\mu m^{-2}s^{-1}$) and maintaining a temperature of 10^0 C was to reduce the growth and other growth related traits in order to conserve it for six months and analysing the survival per cent, shoot length, number of shoots and number of leaves after six months of conservation. Krishnan *et al*. (2011) studied the status of medicinal plants of Western Ghats of India and concluded that not only *in-situ* methods but also *ex-situ* methods through biotechnological tools are required to conserve those important medicinal plant species.MS medium supplemented with BA (0.886 mg/l)+2ip(0.24 mg/l) shown highest survival per cent which indicates that, this particular concentration level of the combination of cytokinins is optimum for the cell division to occur leading to slow shoot growth for six months.The same combination i.e., BA (0.886mg/l)+2ip(0.24mg/l) shown significantly high shoot length, number of shoots/explant and

number of leaves when compared to other treatments indicating the occurrence of constant but slow cell division up to six months. Hence, MS medium supplemented with the hormonal combination of BA (0.886mg /l)+2ip (0.24mg /l) may be used for short term *in-vitro* conservation of *C.paniculatus*.It was found that MS medium supplemented with BA (0.886 mg/l)+2ip(0.24 mg/l) shown highest survival per cent, shoot length, number of shoots and number of leaves in both *in-vitro* conserved plantlets and tissue cultured plantlets regenerated in normal ambient conditions. This shown that, this particular hormonal combination and concentration may be used for short term conservation as well as normal growth of tissue cultured nodal segment.

CONCLUSION

In-vitro conservation was achieved by providing limited nutrients, reduced light intensity (2.97 $\mu m^{-2}s^{-1}$) and maintaining a temperature of 10^0 C to reduce the growth and other growth related traits in order to conserve it for six months. 8 weeks old nodal segment as explant cultured on MS medium supplemented with various combinations of hormones were kept under above mentioned ambient condition. Among various combinations, satisfactory results obtained in terms of survival per cent, shoot length, number of shoots and number of leaves by using a combination of BA (0.886 mg/l) +2ip (0.24 mg/l).

REFERENCES

1. Rajasekharan PE and Ganeshan S., 2002, Conservation of medicinal plant biodiversity,*Journal of Medicinal Aromatic Plant Sciences*, 24: 132-147.
2. Yoganarasimhan SN., 2000, Medicinal plants ofIndia Tamil Nadu. Regional ResearchInstitute Bangalore, India, Vol2., p 441.
3. Nadkarni KM and Nadkharni AK., 1976,The Indian MateriaMedica. Popular Prakashay, Bombay, India, pp: 810.
4. Bhanumathy M, Harish MS, ShivaprasadHN andSushma G., 2010,Nootropic activity of*Celastruspaniculatus* seed. PharmaceuticalBiology, 48(3):324-327.
5. PrakashAnand and Singh KK., 2001, Observationon some threatened plants and theirconservation in Rajaji National Park,Uttaranchal, India. J.Econ.Tax, Bot. 25 (2):363-366.
6. Wangoo D, Bidwai PP., 1988, Anti-spermatogeniceffect of *Celastruspaniculatus* seed extracton the testis of albino rats. Fitoterapia, 59:377-382.
7. Harish BG, Krishna V, Santosh Kumar HS,KhadeerAhamed BM, Sharath R, KumaraSwamy HM., 2008,Wound healing activity anddocking of glycogen-synthase-kinase-3-beta-protein with isolated triterpenoidlupeolin rats. Phytomed., 15(9): 763-767.
8. Pandya KK, Patel RB, Chakravarthy BK., 1990,Antibacterial activity of some Indianmedicinal plants. Indian Drugs., 27: 415-4117.
9. Vonshak A, Barazani O, Sathiyamoorthy P,Shalev R, Vardy D, Golan-Goldhirsh A., 2003,Screening South Indian medicinal plants forantifungal activity against cutaneouspathogens. Phytother Res., 17(9): 1123-1125.
10. Ahmad F, Khan RA, Rasheed S., 1994, Preliminaryscreening of methanolic extracts of*Celastruspaniculatus* and *Tecomellaundulata* for analgesic and anti-inflammatoryactivities. J Ethnopharm., 42(3): 193-198.
11. Krishnan PN, Decruse SW, Radha RK., 2011, Conservation of medicinal plants of Western Ghats, India and its sustainable utilization through in vitro technology. In Vitro Cell Dev.Biol. Plant., 47: 110–122.
12. Jadhav SN, Reddy KN., 2002, *In situ* conservation of medicinal plants in Andhra Pradesh. Proceedings of the National Seminar on Conservation of Eastern Ghats. March 24-26.

Table 1. Estimates of growth after six months of *in-vitro* conservation.

Sl. No.	MS Media + Growth regulators (mg/l)	Survival %	Shoot length(cm) (Mean ± SD)*	No. of shoots/explant (Mean ± SD)*	No. of leaves/explant (Mean ± SD)*
1	BA(0.443)	40	3.04 ± 0.11	1.4 ± 0.54	4.2 ± 0.8
2	BA(0.443)+2ip(0.24)	60	3.0 ± 0.15	2.6 ± 0.54	4 ± 1
3	BA(0.443)+NAA(0.27)	20	3.08 ± 0.08	1.6 ± 0.89	4.8 ± 0.83
4	BA(0.886)	40	3.0 ± 0.12	1.6 ± 0.54	3.8 ± 0.44
5	BA(0.886)+2ip(0.24)	80	4.1±0.10	3.1±0.44	6.1±1.42
6	BA(0.886)+NAA(0.27)	60	3.08 ± 0.08	1.8 ± 0.44	5.2 ± 0.83
7	BA(2.22)	40	2.98 ± 0.08	1.6 ± 0.89	4.2 ± 0.83
8	BA(2.22)+2ip(0.24)	60	3.1 ± 0.07	2.2 ± 0.44	5.2 ± 0.83
9	BA(2.22)+NAA(0.27)	80	3.04 ± 0.11	1.6 ± 0.89	4 ± 0.70
10	BA(5.37)	60	3.0 ± 0.15	1.6 ± 0.89	5 ± 0.70
11	BA(5.37)+2ip(0.24)	60	2.88 ± 0.17	2 ± 0.70	4.4 ± 1.14
12	BA(5.37)+NAA(0.27)	60	2.98 ± 0.13	1.4 ± 0.54	5 ± 1.22
13	BA(10.74)	40	3.0 ± 0.12	2 ± 0.70	3.4 ± 0.54
14	BA(10.74)+2ip(0.24)	60	3.02 ± 0.08	2 ± 1	4 ± 1
15	BA(10.74)+NAA(0.27)	40	2.92 ± 0.23	1.6 ± 0.54	4 ± 1.41

*Mean value of growth parameters of 5 plantlets per treatment.

Fig. 1: Micropropagated *C.paniculatus* plantlets kept for *in-vitro* conservation

Table 2.Comparisons of growth for tissue cultured plantlets kept under standard culture conditions and reduced culture condition after six months.

Sl. No.	MS Media + Growth regulators (mg/l)	Survival %		Shoot length(cm) (Mean ± SD)*		No. of shoots/explant (Mean ± SD)*		No. of leaves/explant (Mean ± SD)*	
		a	b	a	b	a	b	a	b
1	BA(0.443)	40	80	3.04±0.11	3.78 ± 0.19	1.4 ±0.54	2.4 ±0.54	4.2 ± 0.8	4.6 ±0.54
2	BA(0.443)+2ip(0.24)	60	80	3.0 ± 0.15	3.72 ±0.27	2.6 ±0.54	2.6 ±0.54	4 ± 1	4.4 ±0.54
3	BA(0.443)+NAA(0.27)	20	60	3.08±0.08	3.56 ±0.25	1.6 ±0.89	2 ±0.70	4.8 ± 0.83	5.2 ±0.83
4	BA(0.886)	40	80	3.0 ± 0.12	3.74 ±0.24	1.6 ±0.54	2.4 ±1.14	3.8 ± 0.44	5 ±0.70
5	BA(0.886)+2ip(0.24)	80	100	4.1±0.10	5.08 ±0.29	3.1±0.44	3.4 ±0.54	6.1±1.42	6.6 ±0.54
6	BA(0.886)+NAA(0.27)	60	100	3.08±0.08	4.6 ±0.38	1.8 ±0.44	2.2 ±0.83	5.2 ± 0.83	5.2 ±0.44
7	BA(2.22)	40	60	2.98±0.08	3.92 ±0.22	1.6 ±0.89	1.8 ±0.83	4.2 ± 0.83	4.6 ±0.89
8	BA(2.22)+2ip(0.24)	60	80	3.1 ± 0.07	4.78 ±0.19	2.2 ±0.44	3 ±0.70	5.2 ± 0.83	5.4 ±0.54
9	BA(2.22)+NAA(0.27)	80	80	3.04±0.11	4.46 ±0.19	1.6 ±0.89	1.6 ±0.54	4 ± 0.70	4.6 ±0.89
10	BA(5.37)	60	100	3.0 ± 0.15	3.84 ±0.18	1.6 ±0.89	1.6 ±0.89	5 ± 0.70	4.4 ±0.54
11	BA(5.37)+2ip(0.24)	60	100	2.88±0.17	4.06 ±0.15	2 ± 0.70	1.6 ±0.54	4.4 ± 1.14	4.8 ±0.83
12	BA(5.37)+NAA(0.27)	60	80	2.98±0.13	4.04 ±0.15	1.4 ±0.54	2 ±1	5 ± 1.22	5 ±1.4
13	BA(10.74)	40	80	3.0 ± 0.12	3.94 ±0.16	2 ± 0.70	1.8 ±0.83	3.4 ± 0.54	5.4 ±0.54
14	BA(10.74)+2ip(0.24)	60	60	3.02±0.08	4.12 ±0.19	2 ± 1	1.8 ±0.83	4 ± 1	4.8 ±0.83
15	BA(10.74)+NAA(0.27)	40	80	2.92±0.23	3.92 ±0.14	1.6 ±0.54	1.6 ±0.54	4 ± 1.41	4.8 ±1.0

* Mean value of growth parameters of 5 plantlets per treatment.

a: plantlets kept in reduced culture condition

b: tissue cultured plantlets kept under standard culture conditions.

Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.6 / Issue 33 / December 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Invitro Antioxidant and Cytotoxic Properties Cervix Carcinoma Cell Line Hela on *Marine Sponge Spongia tosta*

R.Archana[1*] and G. Rubalakshmi[2]

[1]Department of Biotechnology, SSM College of Arts and Science,Komarapalayam,Tamil Nadu, India.
[2]GRD Bio Clinical Research Services, Rasipuram - 637408, Namakkal District, Tamil Nadu, India.

Received: 21 Sep 2015 Revised: 27 Oct 2015 Accepted: 30 Nov 2015

***Address for correspondence**
R.Archana
Department of Biotechnology,
SSM College of Arts & Science,
Komarapalayam,Namakkal District,
Tamil Nadu, India.

ABSTRACT

The cell damage caused by free radicals is confided to play a prominent role in the aging process and in diseases progression. Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and well being. The need for antioxidants becomes even more critical with increased exposure to free radicals pollution, cigarette smoke, drugs, illness, stress and even exercise can increase free radical exposure because so many factors can contribute to oxidative stress, individual assessment of susceptibility becomes important. The *in vitro* free radical scavenging activity was analyzed from the sponge by nitric oxide, lipid peroxidation and reducing power scavenging assay. The cytotoxicity properties were evaluated. Cervix carcinoma cell line HeLa and compare with the normal cell line L – 6 (Mice, Normal muscle) Using MTT colorimetric assay for 24, 48 and 72 hours. The result shows that the methanolic extract of *Spongia tosta* possess excellent antioxidant and anticancer potential that may be used for therapeutic purpose of free radical scavenging and cancer treatment with proper evaluation processes procedures.

Key words: Antioxidant, Anticancer, marine source and *Spongia tosta*

INTRODUCTION

Cancer is one of the leading causes of human death in the world.Cell division is a physiological process that occurs in tissues. Balance between proliferation and programmed cell death, is being under normal circumstances, usually in the form of apoptosis by tightly regulating both processes. Certain mutations in DNA lead to cancer by disrupting the programmes that regulate the processes.Carcinogenesis is a process by which normal cells are transformed into

cancer cells. It is characterized by a progression of changes at both cellular ad genetic level, that reprogram a cell into undergo uncontrolled division, thus forming a malignant mass(Tumour) that can spread to distant locations (Fearon et al,1990). Biologically active compounds with different modes of action such as antiproliferative, antioxidant, antimicrotubule, have been isolated from marine sources.In recent years, anticancer drugs from natural sources such as plants, marine organisms and microorganisms account approximately 60% of all anticancer drugs. (Cragg et al ,2005). However, many anticancer drugs used in chemotherapeutic treatments developed resistance and side effects (Panchal et al,1998). treatments developed resistance and isolation of new effective non toxic compounds from natural sources is actual problem(Anastyuk et al, 2012).Out of these, reactive oxygen species(ROS) and free radicals attack macromolecules such as DNA, proteins, and lipids leading to many health disorders including cancer(Butter et al, 2006).the harmful effect of the free radicals can however, be blocked by synthetic antioxidants has become crucial.(Choi et al,2007).The aim of the present study is to analyze the anticancer activity of marine sponges as the source of anticancer compounds since with the rich biodiversity and vast marine resources along the Indian coast is a potential useful research in the area of marine drug development and exciting new frontier of scientific discovery and economic opportunity.

MATERIALS AND METHODS

Collection of Sample

The sponge sample was collected as entangled specimens form a bottom trawl fish new operated off Manoli and mare islands of Mandapam group of islands, Gulf of Mannar at Rameshwaram. It was collected by bicatching method. The samples were placed inside sterile ethyl propylene bags under water and transferred to the lab aseptically in ice boxes.

Preparation of sponge extracts

Prior to the extraction, samples were washed with water cleaned air dried Iyophilized and powdered. They were stored for further use for the extraction of crude bioactives, 100g of powdered material was exhaustively extracted with 200ml of methanol using soxhelt apparatus and concentrated in a rotary evaporated at reduced pressure.

Synthesis of nanoparticles

3mM solution of silver nitrate was prepared 20ml of the Marine sponge spongia Tosta extract was mixed with 80ml of 3mM of silver nitrate solution.The colour changed from yellow to reddish brown colour indicating the formation of silver nanoparticles. The AgNPs thus obtained was purified by repeated centrifugation at 7000rpm for 10min. The pellet was collected and dired. The chemical tests were carried out for protein and vitamin C.The pH of the solution was also determined.

In vitro anticancer activity

Chemicals

3 – (4,5 – dimethyl thiazol - 2 -yl) - 5 - diphenyl tetrazoluim bromide (MTT). Fetal bovine serum (FBS), phosphate buffered saline (PBS), Dulbecco's modified eagles from sigma Aldrich co, St. Louis,USA EDTA, Glucose and Antibiotics from Himedia laboratories Ltd., Mumbai. Dimethyl sulfoxide (DMSO) and propane from E. Merck Ltd., Mumbai India all other reagents and chemicals used in the study were of analytical grade.

Cell lines and culture medium

HeLa cell line was procured from national centre from cell sciences (NCCS), Pune, India stock cells are cultured in DMEM supplemented with 10% inactivated Fetal Bovine serum (FBS),penicillin (100μg/ml), streptomycin (100μg/ml) and amphotericin B (5μg/ml) in an humidified atomsophere of 5% Co_2 at 37^{0}c. These cells were dissociated with TPVG solution (0.2% Typsin, 0.02% EDTA, 0.05% Glucose in PBS). The stock cultures were grown in 25cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd. Kolkata, India).

Preparations of test solutions

For cytotoxicity studies, each weighted test drugs was dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of cell viability by MTT assay

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. This assay depends both on the number of cells present and on the mitochondrial activity of the cell. The principle involved in the cleavage of tetra -2-y), -2,5, diphenyl tetrazoluim bromia (MTT) into a blue coloured product. (Formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used.

The monolayer cells culture was trypsinized and the cell count was adjusted and the cell count was adjusted to 1.0 x 10^5 cells / ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hrs, when a partial monolayer was formed. The supernatant was flicked off washed the monolayer once with medium and 100μl of different test concentrations of test drugs were added to the partial monolayer in microtitre plates. The plates were then incubated in 37^{0}C for 3 days in 5% Co_2 atmosphere the supernatant was removed and 100ml propanol was added and the plates were gently shaken to solubilize the formed Formazan. The absorbance was measured using a microplate reader at a wavelength of 540nm .The percentage growth inhibition was calculated using the following formula and concentration of the test drug needed to inhibit cell growth by 50% (CTC 50) values are generated from the dose – response curves for each cell line (Francis et al,1986).

$$\% \text{ Growth inhibition} = 100 - \left[\frac{\text{Mean OD of Individual test group}}{\text{Mean OD of control group}} \right] \times 100$$

Antioxidant Activity

Scavenging of Nitric Oxide Radical

Sodium nitroprusside in aqueous solution at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of modified Griess Ilosvay reaction.In the present investigation, Griess Ilosvay reagent is modified by using Naphthyl ethylene diamine dihydrochloride [0.1%w/v] instead of 1- napthylamine (5%) nitrite ions react with Griess reagent, which forms a purple azo dye. In presence of test components, likely to be scavengers the amount of nitrites will decrease.The degree of decrease in the formation of purple azo dye will reflect the extent of scavenging. The absorbance of the chromophore formed was measured at 540nm (Jayaprakasha et al, 2004).

Reducing power assay

A method developed by Oyaizu, 1986 for reducing power test was used. The above sample including MBO1 together with Ascorbic acid solutions were spiked with 2.5ml of phosphate buffer (0.2M, pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was then kept in 50^0C water – bath for 20min. The resulting solution was then cooled rapidly, spiked with 2.5ml of 10% trichloroacetic acid, and centrifuged at 3000rpm for 10min. The supernatant 5ml was the mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride. The absorbance at 700nm was then detected after reaction for 10min. The higher the absorbance represents the stronger the reducing power. The reducing power assay was expressed in terms of Ascorbic acid equivalent per gram of dry weight basis.

Lipid peroxidation inhibitory activity

The reaction mixture containing egg lecithin 1ml, ferric chloride 0.02ml, ascorbic acid 0.02ml and extract or standard 0.1ml in DMSO at various concentrations was kept for incubation for 1hour at 37^0C. After incubation 2ml of 15% TCA and 2ml of 0.37% TBA were added then the reaction mixture was boiled for 15min, cooled centrifuged and absorbance of the supernatant was measured at 532nm.

RESULTS

The cytotoxic effects of methanol extract of *Marine Sponge Spongia tosta* are shown is table 1. In the presence of investigation *in vitro* anticancer activity of the *Marine Sponge Spongia tosta* was evaluated against the cancer cell lines viz. HeLa (Cerivix, Carcinoma). The anticancer activity displayed by extract of this *Maine Sponge Spongia tosta* was found to be 75. 31± 0.4% at 1000μg/ml concentration against the HeLa (Cervix, Carcinoma). Cell line and CTC_{50} value was recorded as 146. 67±0.6μg/ml.(Figure 1). The free radical scavenging potential of nitric oxide. All of the values are given in mean ± standard error mean (SEM). The methanolic extract of *Marine Sponge Spongia tosta* showed a maximum nitric oxide scavenging activity at a concentration of 1000μg/ml IC_{50} 121.5± μg/ml. table 2 and figure 2. The free radical scavenging potential of lipid peroxidation activity at a concentration of 1000 μg/ml ml IC_{50} 62.5± μg/ml table 2 and Figure 3.The free radical scavenging potential of reducing power acitivty showed only graphical methods Figure 4.

DISCUSSION

On the reactive free radicals formed, in biological systems in hydroxyl radical, known as a highly damaging species.This radical has the capacity to cause numerous cellular disorders such as carcinogenesis and cytotoxicity (Manian et al, 2008). phytochemicals present in sponges can act as antioxidants and prevent disorders due to oxidative damage (Ravikumar et al,2002). Free radicals have long been implicated as mediators of tissue damage in inflammatory mediators of tissue damage in inflammatory diseases.Correspondingly, it has been shown that affected articulations are infiltered by blood derived cells, mainly macrophages, neutrophils and dendritic cells (Bauerova et al, 1999).These cells are responsible for the generation of reactive oxygen species (ROS), which are highly reactive transient chemical species with the potential to initiate cellular damage in tissues (Knight et al 2000).The production of O_2- and oxygen derived metabolites is a highly controlled process. Under adverse circumstances, the oxidizing agents may be released by phagocytes in an uncontrolled manner. These ROS are released in large amounts into the surroundings tissue, which involve in the pathogenesis of arthritis (Morel et al, 1991).When the endogeneous antioxidant defense are meager, the resulting production of free radicals induces impairment and destruction of the joint constituents such as synovial fluid, cartilage and other articular constituents. Nitric oxide is a reactive nitrogen species produced in the mammalian cells, involved in the regulations of various physiological processes the toxicity and damaged caused by NO, O_2- is multiplied as they react to produce reactive peroxiynitrite (DNOO-), which leads to serious toxic substances reactions in the biomolecules (Lekameera et al, 2008). The lipid I by ferrous sulphate takes

place either through ferryl –perferryl complex or through OH radical by Fenton's reaction. The inhibition could be caused either by absence of ferryl perferryl complex, by scavenging the OH by the superoxide radicals, by changing the Fe^{3+}/Fe^{2+}, by chelating the iron itself. Iron catalyses the generation of OH from H_2O_2 and O_2- radicals which is highly reactive and can damage biological molecules(Valentao et al 2002).

CONCLUSION

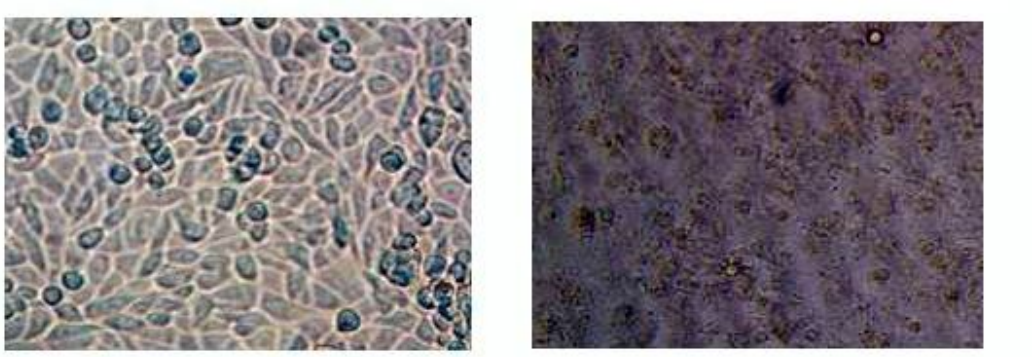
Increasing global warming, and mol nutrition, and various environmental insults continue to increase the incidences of cancer. Natural derivatives play an important role to prevent the cancer incidences a synthetic drug formulation cause various harmful side effects to human beings. Marine floras are potential source of anticancer compounds. The methanolic extract of *Marine Sponge Spongia tosta* appear to contain compounds with antiproliferation and antioxidant properties.

REFERENCES

1. Fearon ER, Vogelstein B.A genetic model for colorectal tumourigenesis. Cell, 1990; 61:759-767.
2. Cragg Gm, Newman DJ. Plants as a source of anticancer agents.J.Ethnopharmacol 2005:100:72-79.
3. Panchal RG.Novel therapeutic strategies to selectively kill cancer cells.Biochem pharmacol 1998;55:247-252.
4. Yang LL, Lee CY, Yen ky. Induction of apoptosis of hydrolysable tannins from Eugenia jambos Lon human Leukemia cell.Cancer Lett 2000;157:65-75.
5. Anastyuk SD, Shevchenko NM,Ermakova SP, vishchuk OS, Nazavenko EL, Dmitrenok PS et al.Anticancer activity in vitro of a fucoidan from the brown algae focus structurally characterized by tandem mass spectrometry.Carbohydrate polymers 2012;87:186-194.
6. Butter jeld DA, Abdul HM, opiiW, Newman sf,Joshi G, Ansari MA et al. Review:Pin/in Alzheimer's disease. Journal of Neuro chemistry 2006;98:1697-1706.
7. Ngo DH, Wijiesekara I, VoTs, Ta Qv, Kim SV.Marine food derived functional ingredients as potential antioxidants in the food industry: an overview.Food research International 2011;44:523-529.
8. Choi Y, Jeong HS, Lee J, Antioxidant activity of methanolic extracts from some grains consume in korea.Food hem.. 2007;103:130-138.
9. Adeolu AA, Florence OJ, SrinivasK, Masika P.Antony JA.Assessment of the medicinal potential of the methanol extracts of the leaves and stem of Buddleja Salign Complem Altern Med 2009;9:21-28.
10. Francis D and Rita L.Rapid Clorometric assay for cell grow and survival modifications to the tetrazolium dye procedure giving improved sensitivity and reliability Journal of Immunological Methods, 198;89:271-277.
11. Jayaprakasha Guddadarangavvanahally k, Lingamallu JR and kunnumpurath ks.Antioxidant activities of Flavidin in different in vitro model systems.Organic medicinal chemistry.2004.12;5141-5146.
12. Manian R.Anusuya N,Siddhuraju P.Manian S.The antioxidant activity and free radicals scavenging potential of two different solvent extracts of Camellia Scnesis (L.)o. Kuntz, Ficusbengalenois L. and Ficusracemosa L.Food hem.. 2008;107:1000-7.
13. Sdal OM and Homazabal V, Antibacterial properties of Extracts from selected cyanobacterium J. Appl. Microbiol., 84 (1998) 1117 – 1124.
14. Prakash Williams, G., Babu, S., Ravikumar, S., Kathiresan, K., Arul Prathap, S., Chinnapparaj, S., marian M.P. and Ziakath Alikhan, S (2007) Antimicrobial activity of tissue and associated bacteria from benthic sea anemone stichodactyla haddoniagainst microbial pathogens. J. Environ, Biol., 28:782-793.
15. Ravikumar S, Anburajan L, Ramanathan G 2 Kaliapperumal N, Screening of seaweed extracts against antibiotic resistant post operative infections pathogen seaweed res. Utilin, 24 (2002) 95 – 99.
16. Bauerova K and Bezek, Role of reactive oxygen and nitrogen species in etiopathogenesis of rheumatoid arthritis, Gen.Physiol.Biophys., 18 (1999)15-20.
17. Knight J.A,Free Radicals, antioxidants, and the immune system, Ann.Clin.Lab.Sci., 30 (2000) 145-158.

18. Morel F, Doussiere J and Vignais P, The superoxide generating oxidase of phagocytic cells.Physiological, molecular and pathological aspects, Eur.J.Biochem., 201[1991}523-546.
19. Lekameera R, Vijayabaskar P and Somasundram ST.Evaluating antioxidant property of brown ALGA *Colpomenia sinuosa[DERB.ETSOL]*African Journal of Food Science,2[2008] 126-130.
20. Valentao P.Fernandes E, Carvalho F, Andrade PB, Seabra R M and Bastos ML, Studies on the antioxidant acyivity of Lippia citriodoru Infusion: Scavenging effect on superoxide radical, hydroxyl radical and hypochlorous acid, Biol.Pharm.Bull., 25[2002] 1324-1327.

Marine Sponge Spongia tosta



A) Control **B) MST 1000µg/ml**

Figure:1.Cell line HeLa

Table 1: Cytotoxic properties of *Marine sponge spongia tosta(MST)* against HeLa cell line.

Sl. No	Name of Test sample	Test concentration (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1	**MST**	1000 500 250 125 62.5	75.31±0.4 67.18±0.6 57.78±0.7 49.22±0.2 42.39±0.8	146.67±0.6

Table 2: *In vitro* antioxidant properties of methanolic extract of MST

Samples	IC_{50} values µg/ml by methods	
	Nitric oxide	Lipid per oxidation assay
MST	121.5	62.5
Standard	Rutin	BHA
	58.63±0.	123.27±4.25

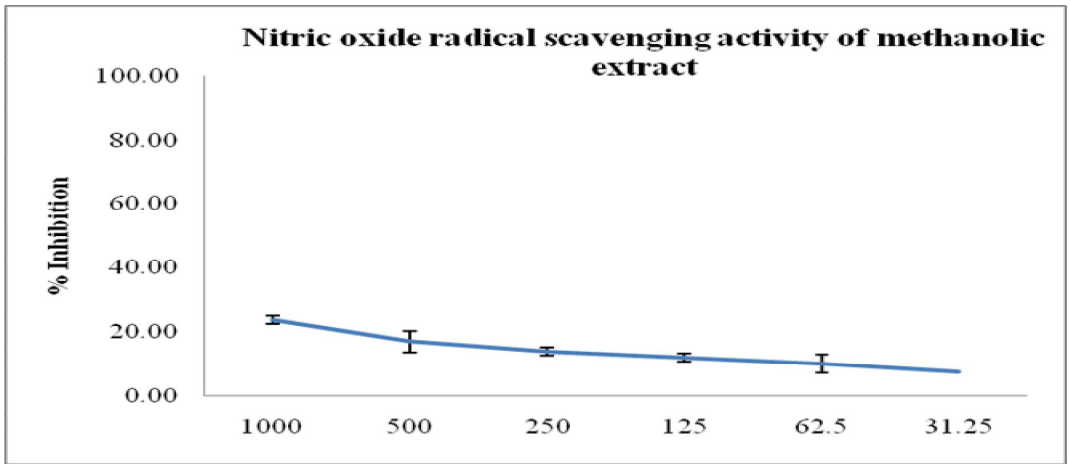


Figure:2.Nitric oxide radical scavenging activity of Methanolic extract of MST

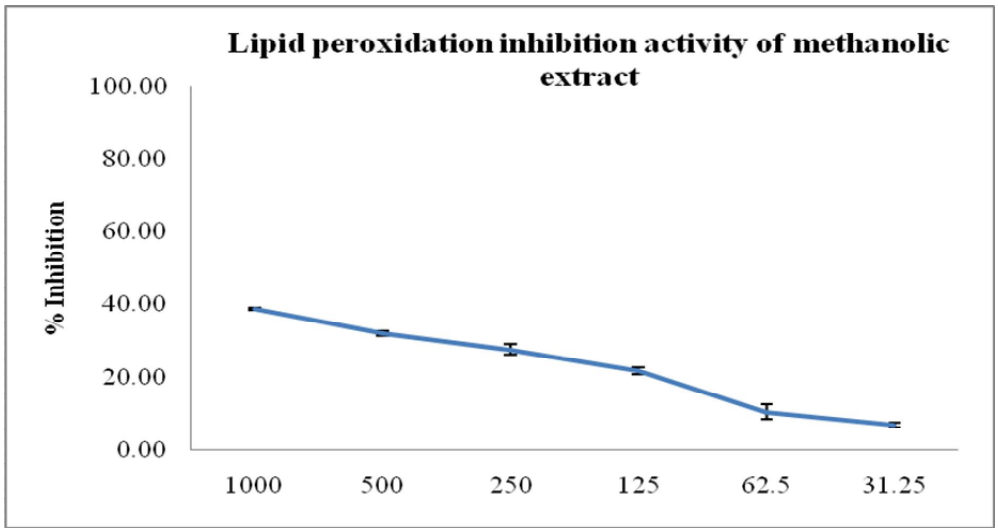


Figure:3.Lipid peroxidation inhibition activity of Methanolic extract of MST

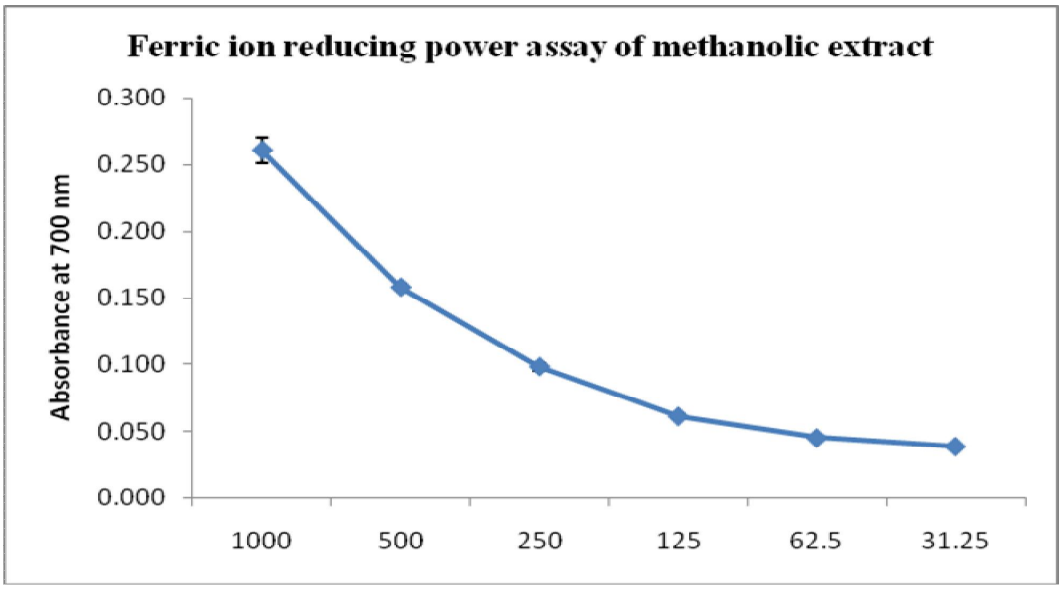


Figure:4.Ferric ion reducing power assay of Methanolic extract of MST

Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.6 / Issue 33 / December 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluation of Pharmacognostic and Antihyperglycemic Activity of *Andrographis echioides* an Indigenous Medicinal Plant

Nirubama K[1], Shabana Begum M[2]* Rubalakshmi G[3] and Kanchana G

[1&2]Department of Biochemistry, Muthayammal College of Arts & Science, Rasipuram- 637408, Namakkal District, Tamil Nadu, India.
[3]GRD Bio Clinical Research, Rasipuram - 637408, Namakkal District, Tamil Nadu, India.

Received: 20 Sep 2015 Revised: 22 Oct 2015 Accepted: 27 Nov 2015

***Address for correspondence**
Shabana Begum M
Department of Biochemistry,
Muthayammal College of Arts & Science,
Rasipuram- 637408, Namakkal District,
Tamil Nadu, India.

ABSTRACT

The present study discusses the need and emphasizes the importance of pharmacognostic and *in vitro* anti diabetic activity of *Andrographis echioides*. All the parameters evaluated in pharmacognostic study such as, powder study, physico chemical analysis (moisture content, loss on drying, ash values, extractive values), fluorescence analysis are enlisted along with their importance. The plant is used in traditional system of medicine for healing various diseases. However, the present study was aimed to evaluate the parameters to determine the quality of the plant. The present study also investigate the *in vitro* antidiabetic glucose up take potential of the hydro alcoholic of extract *Andrographis echioides (HAEAE)*in L6 (rat skeletal muscle cell line) cells. In brief, the 24 hr cell cultures with 70-80% confluency in 40mm petri plates were allowed to differentiate by maintaining in DMEM with 2% FBS for 4-6 days. The extent of differentiation was established by observing multi nucleation of cells. Thus, the study suggests that the hydroalcolic extract of *Andrographis echioides* might be attributed to the observed pharmocogonostic and its anti diabetic properties.

Key words: Pharmacognostic, fluorescence analysis, anti diabetic, *Andrographis echioides*

INTRODUCTION

Diabetes mellitus (DM) is defined as a group of metabolic diseases manifest by hyperglycemia which results from defects in insulin production and/or insulin action. Untreated chronic hyperglycemia can lead to long-term complications including micro-vascular and macro-vascular problems that cause disturbances of carbohydrate, fat

and protein metabolism, and it covers a wide range of heterogeneous diseases. (Sherita and Tamar, 2012). Uncontrolled hepatic glucose output and reduced uptake of glucose by skeletal muscle with reduced glycogen synthesis leads to hyperglycemia. Long term damage and failure of different organs were found along with chronic hyperglycemia. (Rang HP *et al*, 2013).

Plants contain a diverse group of highly valuable and readily available resource of bioactive metabolites *viz.* Alkaloids, tannins, essential oils and flavonoids. According to World Health Organization have estimated that about 80% of the populations in developing countries are unable to afford drugs and rely on traditional medicines especially those that are plant based such as India, Srilanka, Bangladesh, China and Japan. Herbs have provided us some of the very important life savings drugs used in armamentarium of modern medicine. Several herbs have been explored for their anti diabetic potential as a better alternative to blood glucose lowering drugs. Among them, *Andrographis echioides* herbs are constantly being screened for their biological and pharmacological activities such as anti-diabetic, antioxidant, antimicrobial, and anticancer activities. The present study aims to investigate the possible cytoprotective effects of *Andrographis echioides* the ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. Traditionally, many medicinal plants are currently used in India for the treatment of diabetes and its efficacy has been proved scientifically. *Andrographis echioides* or *Indoneesiella echioides* L. Nees (False Water Willow) is an herb widely distributed in the dry districts of tropical India and Srilanka (Chattopadhyay,1992). In traditional medicine, the leaf juice of this plant is used as a remedy for fevers (Gamble, 1956). The plant from genus *Andrographis* is used in goiter, liver diseases (Kirtikarand Basu,1975), fertility problems, bacterial (Nadkarni and Nadkarni,1976), malarial and fungal disorders. Leaf juice boiled with coconut oil is used to control falling and graying of hair (Qadrie et al., 2009).

MATERIALS AND METHODS

Collection and Identification of Plant

*Andrographis echioides or Indoneesiella echioides*used in the study was identified in the ABS Botanical Conservation and Training Centre, India-Southern Circle- Salem, Tamil Nadu, India. The reference material was kept under number [No: AUT/MCAS/035]. Fresh plants were collected randomly from the region of ABS Garden, Salem, Tamil Nadu.

Determination of Moisture Content or Loss on Drying

Take 1gm air dried material in a previously dried and tarred flat bottom dish. Adjust the temperature of hot air oven to 105^{o}C and heat for 1hr. Repeat the procedure for three times. Calculate the loss in weight of the sample with respect to the original weight.

$$\text{Moisture Content} = \frac{W_1}{W_2} \times 100$$

W_1 - weight of raw material after heating, W_2 - Original weight of the raw material

Determination of ASE and WSE

ASE and WSE values are used as a means of evaluating the quality and purity of the drugs, the constituents of which cannot be readily estimated by other means of extraction processes.

Determination of Alcohol Soluble Extractive (ASE)

Macerate 4 g of air dried coarsely powdered drug with 100 ml of 90 % ethanol in a glass-stopper conical flask with frequent shaking for first 6 hours and then allow standing for 18 hours. Thereafter filter rapidly taking care against loss of ethanol. Evaporate about 25ml of the filtrate in a tarred flat-bottomed dish to dryness on water bath and then dry at 105^{0}C for 1 hr, remove the dish, cool in dessicator for 30 minutes and weigh immediately. Repeat the process till the concordant weight is obtained. Calculate the percentage of ethanol soluble extractive with respect to air dried drug.

$$ASE\ \% = \frac{B - A \times 4}{W} \times 100$$

Where, A = sample weight in g B = wt. of dish + contents after drying (g)
W = wt. in g. of empty dish.

Determination of Alcohol Insoluble Extractives

Take the residue from the above step and wash with 5ml ethanol. Dry at 105^{o}c for 1 hr in hot air oven, remove the dish, cool in dessicator and weigh. Repeat the process till the concordant weight is obtained. Calculate the percentage of ethanol insoluble extractive with respect to air dried drug.

$$\%\ Alcohol\ Insoluble\ Extractive = \frac{B - A \times 4}{W} \times 100$$

Where, A = sample weight in g,B = wt. of dish + contents after drying (g),W = wt. in g. of empty dish.

Determination of Water Soluble Extractives

5 g of air dried coarse powder of drugs macerated with 100 ml of 5% chloroform water in a glass-stopper conical flask with frequent shaking for 6 hours and then allowed to stand for 18 hours. Thereafter it was filtered rapidly taking care against loss of solvent. About 25ml of the filtrate was evaporated in a tared flat-bottomed dish to dryness on water bath and then dried at 105^{0}C for 1hr, cool in a dessicator for 30 minutes and weighed immediately. Repeat the process till the concordant weight is obtained. Calculate the percentage of water soluble extractive with respect to air dried drug.

$$WSE\ \% = \frac{B - A \times 4}{W} \times 100$$

Where, A = sample weight in g,B = wt. of dish + contents after drying (g),W = wt. in g. of empty dish.

Determination of Ash Values (Anonymous, 1985)

Ash values such as total ash, acid insoluble ash and water soluble ash were determined. The total ash determination method is designed to measure the total amount of material remaining after ignition. This includes both “physiological ash”, which is derived from the plant tissue itself, and “non physiological ash”, which is the residue of extraneous matter adhering to the plant surface.

Determination of Total Ash

About 3 g each of powdered parts were accurately weighed and taken separately in silica crucible, which was previously ignited and weighed. The powder was spread as a fine layer on the bottom of crucible. The powder was incinerated gradually by increasing temperature to make it dull red hot until free from carbon. The crucible was

cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to the air dried powder.

Acid Insoluble Ash

The ash obtained as described above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a crucible, ignited and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Water Soluble Ash

The ash obtained as described for the total ash, was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred into silica crucible, ignited for 15 min. and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted from the weight of total ash. The difference of weight was considered as water soluble ash. The percentage of water soluble ash was calculated with reference to air dried parts respectively.

Fluorescence analysis

The powdered sample was treated with water, chloroform, 1N sodium hydroxide in methanol, 1N NaOH in water, 10 % HCl and 10% H_2So_4.In most of the cases a definite colour variations was observed under ordinary and ultraviolet rays long (360 nm) and short (254 nm). The effect of both ordinary and ultraviolet lights on fluorescence properties of dried powder is given in table 2(Fig.1).

In vitro anti-diabetic studies

Chemicals

3-(4, 5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Bovine Serum Albumin (BSA), D- glucose, Dulbecco's Modified Eagle's Medium (DMEM), Metformin and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Antibiotics from Hi-Media Laboratories Ltd., Mumbai. Insulin (Torrent Pharmaceuticals, 40IU/ml) was purchased from a drug store. Dimethyl Sulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India**.**

Cell lines and Culture medium

L-6 (Rat, Skeletal muscle) cell culture was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells of L-6 were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 μg/ml) and amphotericin B (5 μg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of Test Solutions

For *in vitro* studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of cell viability by MTT Assay

Principle: The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used (Francis and Rita, 1986).

Procedure: The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37° C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted at 24 h interval. After 24 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37° C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.

$$\% \text{ Growth Inhibition} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

In vitro glucose uptake assay

Glucose uptake activity of test drugs were determined in differentiated L6 cells. In brief, the 24 hr cell cultures with 70-80% confluency in 40mm petri plates were allowed to differentiate by maintaining in DMEM with 2% FBS for 4-6 days. The extent of differentiation was established by observing multinucleation of cells. The differentiated cells were serum starved over night and at the time of experiment cells were washed with HEPES buffered Krebs Ringer Phosphate solution (KRP buffer) once and incubated with KRP buffer with 0.1% BSA for 30min at 37°C. Cells were treated with different non-toxic concentrations of test and standard drugs for 30 min along with negative controls at 37°C. 20µl of D-glucose solution was added simultaneously to each well and incubated at 37°C for 30 min. After incubation, the uptake of the glucose was terminated by aspiration of solutions from wells and washing thrice with ice-cold KRP buffer solution. Cells were lysed with 0.1M NaOH solution and an aliquot of cell lysates were used to measure the cell-associated glucose. The glucose levels in cell lysates were measured using glucose assay kit (ERBA). Three independent experimental values in duplicates were taken to determine the percentage enhancement of glucose uptake over controls

RESULTS AND DISCUSSION

Pharmacognostical Investigation

The detailed and systematic pharmacognostical evaluation would give valuable information for the future studies. This was carried out to support proper identification of physico chemical characterization of *Hydroalcoholic extract of Andrographis echioides* .

DISCUSSION

Diabetes mellitus is a metabolic disorder with increasing incidence throughout the world. Insulin is a key player in the control of glucose homeostasis.Lack of insulin affects carbohydrate, fat and protein metabolism (Rajiv Gandhi and Sasikumar, 2012). Management of diabetes without side effects is still challenge to the medical community. It was proposed that inhibition of the activity of such alpha-amylase and alpha-glucosidase would delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose, as a result the reduction of postprandial blood glucose level elevation (Nirubama et al.,2015). A key finding of the present study is the observation of a significant disturbance in cell viability as well as cell morphology in pancreatic β cells due to their exposure to toxic doses of *HAEAE*. In this present study we evaluated *in vitro* anti diabetic activity of hydroalcolic extract of *Andrographis echioides* demonstrated % of glucose uptake activity in a concentration-dependent manner was found to be 41% and 76%. A positive test result suggests that the extract is a potential for anti diabetic properties. However, the activity was compared with the standard, Rosiglitazone (Table 4).The plant showed significant inhibition activity, so furthers the compound isolation, purification and characterization which is responsible for inhibiting activity, has to be done for the usage of antidiabetic agent.

CONCLUSION

Indian medicinal preparations are often considered being effective due to a mixture of active ingredients rather than a single constituent. To make herbal therapies more effective, it is pertinent to isolate anti-diabetic molecules, define their targets for understanding their modes of action, and establish structure and function relationship for better efficacy and pharmacokinetic profile. Prevention of diabetes is our most powerful intervention and successful implementation of these proven strategies should be the focus of our efforts. In future, these efforts will lead to new chemotypes which will be safer and more cost-effective for the rural Indian population suffering from diabetes, whose numbers are increasing linearly. In the present study, a well known plant for its anti diabetic properties has been investigated.

REFERENCES

1. Angeline Yap, Shin Nishiumi, Ken-ichi Yoshida, and Hitoshi Ashida . Rat L6 myotubes as an in vitro model system to study GLUT4-dependent glucose uptake stimulated by inositol derivatives. Cytotechnology. 2007 December; 55(2-3): 103–108.
2. Anonymous Indian Pharmacopoeia, Vol. II, 3rd Ed., Controller of Publications, Govt. Of India, New Delhi, 1985.
3. Chase, CR. Pratt R. Flourescence of powdered vegetable drugs with particular reference to development of a system of identification. Journal of American Pharmaceutical Association Sciences. 1949 28:324-331.
4. Chattopadhyay , RR., Sarkar , SK, Ganguly,S. Banerjee , RN. Basu, TK. Effect of leaves of *Vincarosea* Linn, on glucose utilization and glycogen deposition by isolated rat hemidiaphragm. Indian J Physiol Pharmacol. 1992 36: 137-38.
5. Evaluation of Free radicals scavenging and glucose uptake by isolated rat hemidiaphragm study of *Andrographis echioides* an indigenous Medicinal Plant *Int.J.Curr.Microbiol.App.Sci* 2015 4(6): 576-583
6. Francis D and Rita L. Rapid colorometric assay for cell growth and survival modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. Journal of Immunological Methods, 1986 89: 271-277.
7. Gamble, J.S. Flora of the presidency ofMadras. Botanical survey of India.Calcutta. 1956 Vol.2.p.1051.
8. Hisako Takigawa-Imamura, Takumi Sekine, Mitsuo Murata, Kiyoshi Takayama, Kiyoshi Nakazawa and Junichi Nakagawa .Stimulation of glucose uptake in muscle cells by prolonged treatment with scriptide, a histone deacetylase inhibitor. Bioscience, Biotechnology, and Biochemistry. 2003; 67:1499–1506.
9. Kokate CK. Practical Pharmacognosy. Vallabh Prakashan. 2004:130.
10. Kokate CK. Purohit AP, Gokhale SB. Text book of Pharmacognosy. Nirali Prakashan. 2008 6:30

11. Nadkarni, A.K ., Nadkarni, K.M. Indian MateriaMedica, Vol.1. Bombay: Popular Prakashan 1976.
12. Qadrie , ZL., BeenaJacob,R. Anandan, et al. Antibacterial activity ethanol extracts of *Indoneesiella echioides* evaluated by the filter paper disc method Pak J Pharm Sci. 2009 22, 123-5.
13. Rajiv Gandhi, G., and Sasikumar, P. Antidiabetic effect of *Merremia emarginata Burm*.F. in Streptozotocin induced diabetic rats. Asian. Paci. J. Tropi. Biomedicine. 2012 2: 281-286.
14. Rang HP, Dale MM, Ritter JM, Flower RJ, Henderson G. Rang and Dale's pharmacology. 7th ed. London: Churchill Livingstone; 2012 p. 377.
15. Rhabaso Lhoret, R., and Chiasson, J.L. - Glucosidase inhibitors. In: Defronzo R.A, Ferrannini E, Keen H, Zimmet P. (Eds.), International Textbook of Diabetes Mellitus, 2004 Vol. 1, third ed. John Wiley and Sons Ltd., UK, pp. 901-914
16. Sherita H. and Tamar S. Methods for Insulin Delivery and Glucose Monitoring in Diabetes: Review *J Manag Care Pharm*. 2012 18(6): 3-17.

Table No: 1- Moisture Content, Extractive Values & Ash Values

S.No.	Sample	***Hydroalcoholic extract of Andrographis echioides (****HAEAE)*
1	Moisture Content %	0.39
2	ASE %	11.24
3	WSE %	38.82
4	AISE %	4.16
5	Total Ash	19.39
6	Acid Insoluble Ash	5.59
7	Water Soluble Ash	2.69

Table No: 2 - Fluorescence Analysis

S.No.	Test sample + Solvent	***Hydroalcoholic extract of Andrographis echioides (****HAEAE)*		
		Ordinary	**UV Short (254nm)**	**UV Long (360nm)**
1	Distilled water	Brown	Dark Green	Dark Green
2	Chloroform	Green	F orange	Light Green
3	1N NaoH in water	Brown	Green	Dark Green
4	1N NaoH in methanol	Orange	Green	Light Green
5	10 % HCL	Orange	Green	Light Green
6	10 % H_2SO_4	Yellow	F Yellow	Light Green

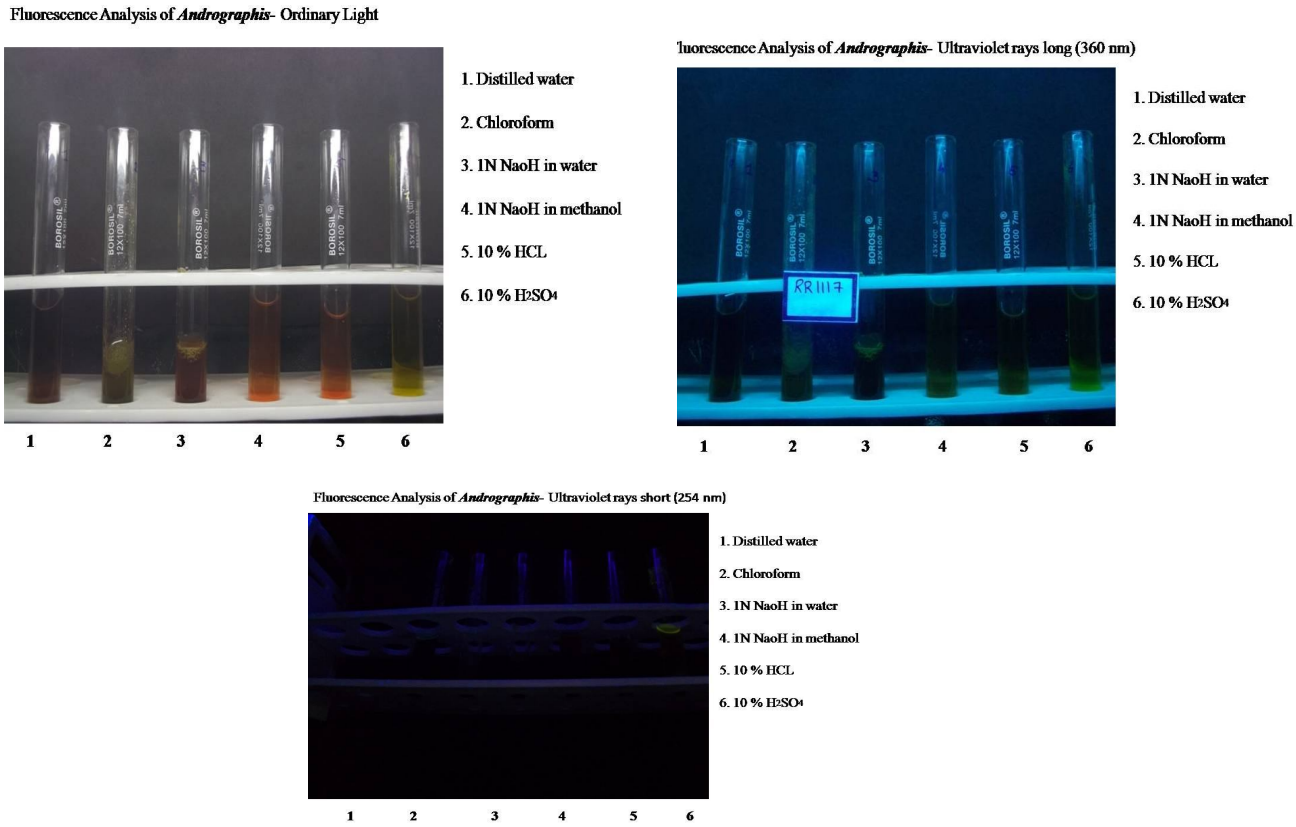


Fig.1.Fluorescence Analysis of *Andrographis echioides*

Table - 3: Cytotoxic Properties of Test Drugs on L6 Cell Line.

Sl. No.	Name of Test sample	Test Concn. (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1	*HAEAE*	1000 500 250 125	38.19±0.9 31.67±1.1 25.64±0.2 24.25±0.7	>1000±0.00

Table 4: *In Vitro* Glucose Uptake Studies In L-6 Cell Line

Sl.No	Name of the extract	Test concentration (µg/ml)	% glucose uptake over control
1	*HAEAE*	1000	76.4±8.64
2	*HAEAE*	500	41.7±1.34
3	Rosiglitazone	50	31.0±6.52

Indian Journal Of Natural Sciences *www.tnsroindia.org.* © *IJONS*

Vol.6 / Issue 33 / December 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Occurrence and Antibiotic Sensitivity of Methicillin-Resistant *Staphylococcus aureus* Isolated from Bovine Mastitis

SunithaR*, Vinod VK, B Sunil, Prejit and Asha K,

Department of Veterinary Public Health College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala- 673 576, India.

Received: 22 Sep 2015 Revised: 26 Oct 2015 Accepted: 29 Nov 2015

***Address for correspondence**
Sunitha R,
M.V.Sc., Scholar,
Department of Veterinary Public Health,
College of Veterinary and Animal Sciences,
Pookode, Wayanad, Kerala- 673 576,India.
Email:dr.sunitha89@gmail.com

ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged worldwide as a significant public health problem. The present study was undertaken to ascertain the prevalence of MRSA in bovine mastitic milk samples. A total of 100 (56 subclinical and 44 clinical) mastitic milk samples were collected from different regions of Wayanad district, Kerala. Out of 100 mastitic milk samples, 62 were found positive for *S. aureus.* Among these 62 isolates of *S. aureus,* MRSA was isolated from seven *S. aureus* isolates. All the MRSA isolates were further subjected to antibiotic sensitivity test. All the MRSA isolates found sensitive to ciprofloxacin (100 per cent), ceftriaxone (100 per cent), tetracycline (85.71 per cent), chloramphenicol (71.42 per cent) and found resistant to gentamicin, co-trimoxoazole, methicillin, ampicillin, amoxyclav, erythromycin, vancomycin, streptomycin.The results indicate the increasing resistance pattern of *S. aureus* organisms towards β lactum antibiotics, paying way for the emergence of MRSA.

Keywords: Methicillin resistant *Staphylococcus aureus,* Bovine mastitic milk, antibiotic sensitivity test

INTRODUCTION

Bovine mastitis has been recognized as a costly disease with losses primarily affecting dairy farms. Among the various causative agents, *S. aureus* is one of the most prevalent and contagious pathogens of intramammary infections in dairy cattle globally. The evolution of antibiotic resistance in *S. aureus* strains is a serious cause of concern in dairy animals (Wang et al. 2008). Methicillin resistance in *S. aureus* is mainly mediated by the expression of the *mec*A gene, which is located on a mobile genetic element, staphylococcal cassette chromosome *mec* (SCCmec) and

encodes an altered penicillin-binding protein (PBP2a) with an extremely low affinity to b-lactam antibiotics, making it possible for *S. aureus* to survive the treatment of b-lactam antibiotics (Hiramatsu, 2001). The emergence of antibiotic-resistant microorganisms in farm animal environments poses a potential public health concern. The use of antimicrobial agents on dairy farms as well as in other food animal production systems is a major concern in the emergence of resistant zoonotic bacterial pathogens (Piddock, 1996). Antibiotics on dairy operations are used to treat highly prevalent infections, such as subclinical mastitis and as a preventive measure during dry cow therapy. Monitoring the emergence of resistant pathogens in animal reservoirs is important particularly for those with zoonotic potential. MRSA isolates are frequently multidrug resistant (MDR), which can result in higher costs, longer treatment times and higher rates of hospitalization and comorbidities. MRSA causes intra-mammary infections in cattle leading to mastitis. MRSA was first reported from bovine sources in 1975 (Devriese and Hommez, 1975). The presence of MRSA in bovine milk and dairy environments poses potential risk to farm workers, veterinarians (Juhász-Kaszanyitzky et al., 2007) and farm animals that are exposed to infected cattle. The present study is envisaged to investigate the occurrence of MRSA among in bovine mastitic milk using routine isolation, PCR characterisation, followed by an antimicrobial susceptibility testing.

MATERIALS AND METHODS

Collection of masitic milk samples

The study was under taken for a period of 10 months from June 2014 to March 2015. On the basis of CMT a total of 100 mastitic milk which included 56 sub-clinical and 44 clinical mastitic milk samples based on consistency of milk and udder changes from three different regions namely, from Kapletta 34, from Thariyode 33 and from Pozhuthana 33 mastitic milk were collected. The milk samples were transported to laboratory in insulated containers and processed in lab within 6 hours of collection.

Isolation of *Staphylococcus aureus* and MRSA

Standard protocol described by Lancette and Bennett (2001) was used for the isolation of *S. aureus* from from clinical as well as subclinical mastitic milk. A total of 10 ml of the sample was transferred to nine ml of Staphylococcal enrichment broth (Himedia, Bombay) and incubated at 37^0C for 24 h. From this enrichment broth, loopful of inoculum was plated on to Baird-Parker (BP) Agar medium (HiMedia) and incubated at 37^0C for 24 to 48 h. After completion of incubation, typical colonies exhibiting circular, smooth, convex, moist, 2-3 mm in diameter, grey-black to jet-black frequently with light coloured margin and surrounded by opaque zone on BP agar were transferred to Luria-Bertani (LB) broth. The pure single colony of these isolates was further subjected to biochemical characterization as described in Table No.1 (Barrow and Feltham, 1993).

Preparation of DNA template

The DNA for the PCR confirmation was isolated by suspending the presumptive colonies in 50µl of deionised water. The suspension was boiled at 95°C for 10 min and centrifuged at 10,000Xg for 10 min. The supernatant was used as DNA template.

Bacterial identification by nuc-PCR and Genotypic identification of MRSA

Identification of MRSA isolates to the species level was verified by PCR using primers specific for *Staphylococcus aureus* thermo stable nuclease, the *nuc* gene and genotypic identification of MRSA using *mec*A gene (Brakstad et al., 1992). The presence of *nuc* and *mec*A gene was verified in *Staphylococcus aureus* isolates using the following primers. For *nuc* gene, *nuc*1 5'-GCGATTGATGGTGATACGGTT-3' and *nuc*2 5'- AGCCAAGCCTTGACGAACTAAAG-3'and for *mec*A gene, *mec*A1 5'-AAAATCGATGGTAAAGGTTGGC-3' and *mec*A2 5'-AGTTCTGCAGTACCGGATTTGC-3'

that are expected to yield a PCR product of 279 bp and 539 bp for *nuc* and *mec*A gene respectively. PCR was performed in a 25µl reaction mixture with a PCR buffer containing 200 µm concentration of each deoxy nucleoside triphosphate (dNTP), 10 mMTris-Hcl (pH 8.3), 1.5 mM MgCl2, 1 unit of Taq polymerase (Promega), 0.25 µM concentration of each primer and 2.5 µl of DNA template. DNA amplification was carried out for 34 cycles in 25 µl of reaction mixture as follows: denaturation at 94°C for 50 seconds, annealing at 57°C for 50 seconds and 58°C for 50 seconds for *nuc* and *mec*A gene respectively, extension at 72°C for 50 seconds with a final extension at 72°C for 5 minutes.

Agarose gel electrophoresis and visualization

Gel electrophoresis was carried out using 1.5 per cent agarose (GeNei™, Mumbai) gel containing ethidium bromide with a submarine gel electrophoresis system (Chromous biotech PVT. LTD) using 1X TAE buffer as running buffer. PCR products mixed with gel loading dye along with 100 bp plus DNA marker ladder were loaded in wells. Products were visualised by gel documentation system (BIO-RAD) after appropriate migration with constant voltage 5V/cm materials contained with ethidium bromide were disposed off as per local guidelines.

Antibiotic Susceptibility Test

The antibiotic susceptibility profile of the isolates of MRSA was studied as per the guidelines provided by Clinical Laboratory Standards Institute (2007). The bacterial isolates obtained in the present study were subjected to disc diffusion assay as per the method described by Bauer et al. (1966). Selection of antibiotics was based on the treatment information obtained from Veterinary and Medical Clinicians. The antibiotic discs used in the study were procured from HiMedia Laboratories Ltd., Mumbai. *E. coli* ATCC 25922 was used as a quality control strain for the *S. aureus*. A loopful of pure culture for each test isolate recovered was transferred into a tube containing 5 ml of nutrient broth medium. The broth culture was incubated in shaker water bath at 37^0C to obtain the turbidity of the 0.5 McFarland standard tube. After incubation, the test isolate culture was spun at 5000 rpm for 5 min, to obtain a pellet which was later dissolved using 1 ml of sterile normal saline solution resulting in an approximately 1 to 2 × 10^8cfu/ml count for each test isolate. This culture was evenly spread on Mueller-Hinton (MH) agar plate for MRSA using a sterile cotton swab. Antibiotic discs were placed on inoculated agar surface at about two to three cm apart. Each disc was pressed down to ensure complete contact with the agar surface. The plates were inverted and incubated at 37°C overnight. The zones of inhibition diameter were measured for each antibiotic, initially for quality control strain and also for all test strains. The obtained data was compared with interpretative chart furnished by the manufacturer to grade the test isolates as sensitive, intermediate and resistant for respective antibiotics.All the isolates were tested against 12 different antibiotics viz. Amoxyclav (30µg), Ampicillin (10µg), Ceftriaxone (30µg), Erythromycin (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Co-Trimoxazole (25µg), Gentamicin (10µg), Methicillin (5µg), Tetracycline (30µg),Vancomycin(30 µg) and streptomycin (10µg).

RESULTS

On the basis of CMT a total of 100 mastitic milk were collected which included 56 sub-clinical and 44 clinical mastitic milk samples based on consistency on consistency of milk and udder changes. From Kalpetta, a total of 21 (61.76 per cent) samples, 23 (69.69 per cent) samples from Thariyode and 18 (54.54 per cent) samples from Pozhuthana were found positive for *S. aureus.* MRSA was detected in seven (11.29 per cent) *S. aureus* isolates which was detected in four (17.39 per cent) from Thariyode, two from (9.52 per cent) Kalpetta and one (5.55 per cent) from Pozhuthana. The antibiotic sensitivity of MRSA isolates revealed that, all the MRSA isolates found 100 per cent sensitive to ciprofloxacin and ceftriaxone, 85.71 per cent to tetracycline, 71.42 per centto chloramphenicol and found resistant to gentamicin, co-trimoxoazole, methicillin, ampicillin, amoxyclav, erythromycin, vancomycin, streptomycin.

DISCUSSION

In the present study MRSA was detected in 11.29 per cent from *S. aureus* isolates. All the MRSA were found sensitive to ciprofloxacin and ceftriaxone, moderately sensitive to tetracycline, and chloramphenicol whereas found resistant to found resistant to gentamicin, co-trimoxoazole, methicillin, ampicillin, amoxyclav, erythromycin, vancomycin, streptomycin. The present study findings are in agreement with 13.1 per cent and 11.25 per cent reported by Kumar et al. (2011) and Joshi et al. (2014) in mastitic milk sanples in Karnal, India and Nepal respectively. The present study findings are higher than 1.5 per cent recorded by Gindonis *et al*. (2013) and 2.99 per cent reported by Chandrashekaran et al. (2014) from mastitic milk samples in Finland and Tamil Nadu, India respectively. Chaalal et al. (2014) found the prevalence of MRSA in 8.69 per cent samples which is lower than the present study observations. The antibiotic sensitivity of MRSA isolates in the present study are in accordance with Kumar et al. (2011) who reported the resistance of the MRSA isolates to gentamicin, ampicillin, streptomycin, penicillin and Haran et al. (2012) reported the resistance of the isolates to gentamicin and erythromycin. Joshi et al. (2014) reported increased resistance of MRSA isolates towards penicillin group of antibiotics and high sensitivity to ciprofloxacin (97.47 per cent), gentamicin (94.95 per cent) and ceftriaxone (91.59 per cent).

The detection of multidrug resistant MRSA over different locations indicates this is a widespread problem. Using various antibiotics can create selection pressure ultimately resulting in the development of antibiotic resistance. High prevalence of MRSA among mastitic cases is another significant finding. The MRSA from all the three regions showed the similar pattern of sensitivity. All the MRSA isolates were found multi drug resistance. In the geographical areas investigated in this study, the penicillin class of antibiotics was the first choice with other types of antibiotic selected subsequently. Higher sensitivity of MRSA isolates to ciprofloxacin and ceftriaxone was observed. This may be because ciprofloxacin and ceftriaxone are less widely used drugs in the study area. Majority of *S. aureus* and MRSA isolates obtained in this study are multi drug resistant this could be due to indiscriminate use of antibiotics. Increased occurrence of MRSA in mastitic milk was one of the significant findings which has got larger zoonotic implications that demands more careful and judicious selection and use of antibiotics. Both veterinarians as well as animal owners should therefore look into importance of ABST during treatment of mastitis to avoid the problem of antibiotic resistance which is a major health hazard for the consumers (Akram *et al.,* 2013).

REFERENCES

1. Wang, Y., Wu, C.M., Lu, L.M., Ren, G.W.N., Cao, X.Y and Shen, J.Z., 2008. Macrolide-lincosamide-resistant phenotypes and genotypes of Staphylococcus aureus isolated from bovine clinical mastitis. *Vet. Microbiol*. 130, 118–125
2. Hiramatsu, K., Cui, L.Z, Kuroda, M, Ito, T., 2001. The emergency and evolution of methicillin-resistant *S. aureus. TRENDS Microbiol,* 9: 486–493.
3. Piddock, L.J.V., 1996. Does the use of antimicrobial agents in veterinary medicine and animal husbandry select antibiotic resistant bacteria that infect man and compromise antimicrobial chemotherapy? *J. Antimicrob. Chemother.* 38, 1–3.
4. Devriese, L.A., Hommez J., 1975. Epidemiology of methicillin-resistant *S. aureus* in dairy herds. *Res. Vet. Sci*. 19, 23–27.
5. Juhász-Kaszanyitzky E., 2007. MRSA transmission between cows and humans. *Emerg. Infect. Dis*. 13, 630–632.
6. Lancette, G.A. and Bennett, R.W., 2001. In: Downes, F.C., Ito, K. (eds.), *Compendium of Methods IUYTR`for the Microbiological Examination of Foods*, (4thEdn). American Public Health Press, pp. 387-403.
7. Barrow, C.J and Feltham, R.K.A., 1993. *Cowan and Steel's manual for the identification of medical bacteria* (3rd Ed.). Cambridge press, London. 238p.
8. Brakstad, O. G., Aasbakk, K and Maeland, J. A., 1992. Detection of *S. aureus* by polymerase chain reaction amplification of the nuc gene. *J. Clin. Microbiol* 30, 1654-1660.

9. Clinical and Laboratory Standards Institute., 2007. Performance standards for antimicrobial susceptibility testing: Twentieth informational supplement M100-S20. CLSI, Wayne, PA, USA.
10. Bauer, A.W., Kirby, W.M.M., Sherris, J.C and Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol* 45, 493–496.
11. Kumar, R., Yadav, B.R and Singh, R.S., 2010. Genetic Determinants of antibiotic resistance in Staphylococcus aureus isolates from milk of mastitic crossbred cattle. Curr. Microbiol 60, 379–386.
12. Joshi, L.R., Tiwari, A., Devkota, S.P., Khatiwada, S., Paudyal, S. and Pande, K.R., 2014. Prevalence of Methicillin-Resistant *S. aureus*(MRSA) in Dairy Farms of Pokhara, Nepal. *Inter J Vet Sci,* 3(2), 87-90.
13. Gindonis, V and Taponen, S., 2013. Occurrence and characterization of methicillin resistant Staphylococci from bovine mastitis milk samples in Finland Anna- *Acta Veterinaria Scandinavica* 55, 61.
14. Chandrashekaran, D., Venkatesan, P., Tirumurugaan, K.G., Nambi, A.P., Thirunavukkarasu, P.S., Kumanan, K., Vairamuthu, S. and Ramesh, S., 2014. Pattern of antibiotic resistant mastitis in dairy cows. *Vet. Wld* 7, 2231-0916.
15. Chaalal, W., Aggad, H., Saidi, N. and Kihal, M., 2014. Prevalence and antimicrobial resistance of methicillin-resistant *S. aureus* isolated from raw meat and bovine milk in Algeria. *Adv. in Environ. Biol* 8(1), 1-5.
16. Haran, K.P., Godden, S.M.,D., Boxrud, D.S., Jawahir, J.B., Bender and Sreevatsana, S., 2011. Prevalence and characterization of *S. aureus*, including methicillin-resistant *S. aureus*, isolated from bulk tank milk from Minnesota Dairy Farms. *J. of Clin. Microbiol*. p. 688–695.
17. Akram, N., Chaudhary, A.H., Ahmed, S., Ghuman, G.M., Nawaz, G. and Hussain, S. 2013. Isolation of bacteria from mastitis affected bovine milk and their antibiogram. *Eur. J. Vet. Med* 2(1), 38-46.

Table.1. Confirmation of *S. aureus* isolates by Biochemical test

TESTS	***Staphylococcus aureus***
a) Primary test reactions	
Grams staining	+
Cell morphology	Cocci, single, pair, cluster or bunch of grapes
Motility	-
Catalase	+
Oxidase	-
b) Secondary test reactions	
VP	+
Urease	+
c) Sugar fermentation reactions	
Lactose	+
Glucose	+
Aerobic	+
Anaerobic	+
Maltose	+
Sucrose	+

Table. 2. Details of primers used in this study

Gene	Target organism	Primer sequence	Amplification size	Reference
nuc	***S. aureus***	**F** 5'-GCGATTGATGGTGATACGGTT-3'	279bp	Brakstad *et al.* (1992)
		R 5'-AGCCAAGCCTTGACGAACTAAAG-3'		
*mec*A	**MRSA**	F 5'-AAAATCGATGGTAAAGGTTGGC-3'	533bp	Frei *et al.* (2013)
		R 5'-AGTTCTGCAGTACCGGATTTGC-3'		

Table. 3 .Occurrence of *S. aureus* and MRSA in bovine mastitic milk

Sl. No.	Areas of collection	No of samples collected	No of samples positive for *S. aureus*		No of samples positive for MRSA
			By culture method	PCR method	
1	Kalpetta	34	22 (64.70%)	21 (61.76%)	Two (9.52%)
2	Thariyode	33	24 (72.72%)	23 (69.69%)	Four (17.39%)
3	Pozhuthana	33	18 (54.54%)	18 (54.54%)	One (5.55%)
TOTAL		100	64 (64%)	62 (62%)	Seven (11.29%)

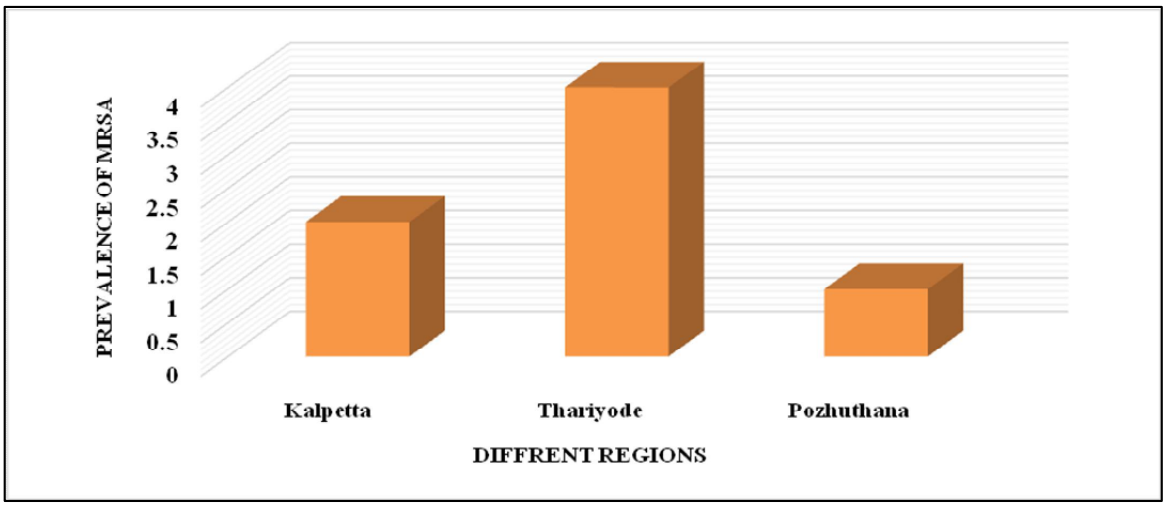


Fig. 1. Prevalence of MRSA in three different regions

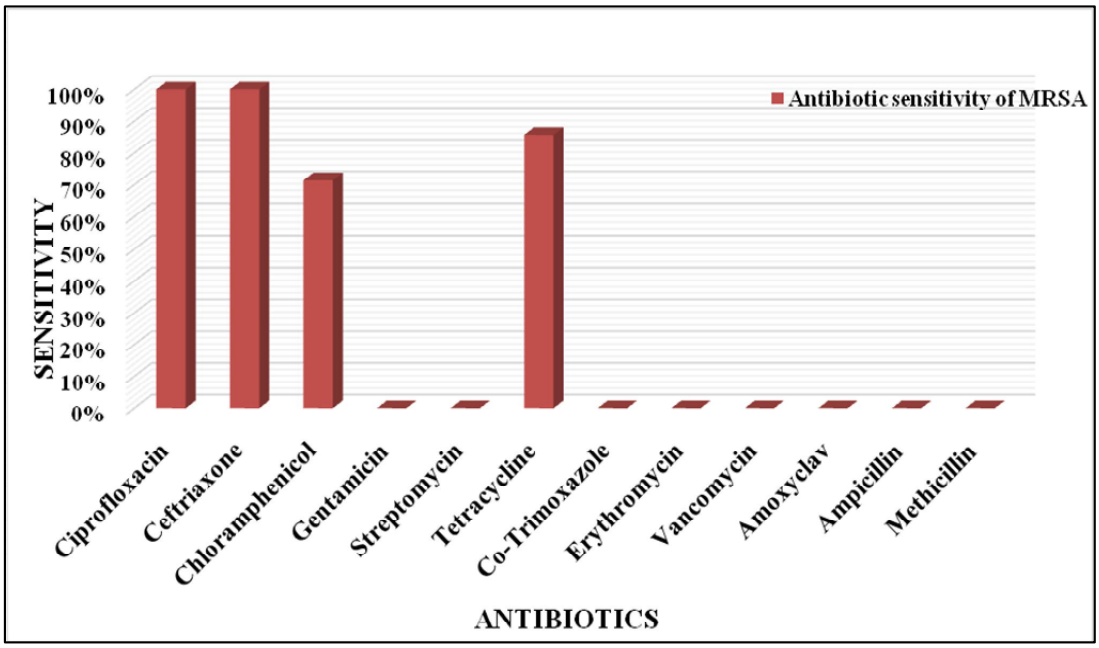


Fig.2.Antibiotic sensitivity of MRSA isolates

Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.6 / Issue 33 / December 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

GSAMSA: Gravitational Search Algorithm for Multiple Sequence Alignment

Rohit Kumar Yadav* and Haider Banka[2]

Department of Computer Science & Engineering, ISM Dhanbad, India-826004

Received: 27 Sep 2015 Revised: 21 Oct 2015 Accepted: 28 Nov 2015

***Address for correspondence**
Rohit Kumar Yadav,
Research Scholar,
Department of Computer Science & Engineering,
ISM DHANBAD, INDIA-826004
Email: rohityadav@cse.ism.ac.in

ABSTRACT

In this paper, a gravitational search based optimization technique is explored for aligning multiple sequences. Gravitational search algorithm (GSA) has been recently emerged as a new randomized heuristic method for both real-valued and discrete optimization problems. This is a nature inspired algorithm based on Newton's laws of gravity and motion which interacts with each other. Here each solution is represented in encoded form as 'position' like 'chromosome' in genetic algorithm (GA). The fitness function (also known as mass in GSA) is designed accordingly to optimize the objective functions i.e., maximizing the matching components of the sequences and reducing the number of mismatched components in the sequences. In GSA, exploration and exploitation can be maintained using Kbest agents in each iteration. Kbest is number of agents (those masses are heavier) which is decreases when number of iteration is increased. Due to this property GSA can solve efficiently Multiple Sequence Alignment (MSA) problem. The performance of the proposed method has been tested on publicly available benchmark datasets (i.e. Bali base) to establish the potential of GSA to solve alignment problem with better and/or competitive performance. The results are compared with some of the well known existing methods such as PRRP, DIALIGN, SB-PIMA, HMMT, SAGA, RBT-GA and GAPAM. The experimental results showed that GSAMSA attained better solutions than the others for most of the cases.

Keywords: Multiple Sequence Alignment. Bioinformatics. Gravitational Search Algorithm. NP-hard Problem. PAM Matrix

INTRODUCTION

More than 3 amino acid sequence or protein sequence alignment at a time is called MSA. MSA is most important tool to solve biological problems. We can solve lots of problem in biology by the use of MSA. MSA help to predict the secondary and tertiary structure of RNA and proteins [11, 8]. We can reconstruct phylogenetic trees by the use of MSA which can predict the function of an unknown amino acid by aligning its sequences with some other known functions. We can also find similarity of the sequences by the use of MSA, which can helps to define similarity in functions and structures [2, 4]. In order for a MSA to be valid entire sequences in the multiple alignments must have a common origin. The goal of MSA is to maximize the matching of protein or amino acid as far as possible. Therefore MSA is an important problem in bioinformatics to study of genetic and phylogenetic relationship. There are several method to solve MSA problem in past. Multiple sequence alignment can be solved and achieve optimal alignment by the use of Dynamic programming (DP). A scoring function used by DP which contains big region. In the article [17] proposed the use of dynamic programming algorithm to solve the problem of two sequence alignment. But problem behind the use of Dynamic Programming (DP) is when the number and length of sequence are increase its complexity also increases in exponential manner. This leads to the MSA problem becomes to NP-hard. Since complexity is supreme embarrassment to solve any problem by the computer. So we have to maximize the matching of protein or amino acid sequence in limited time. This is the reason behind researchers switch to other methods.

MSA problem can be also solved by progressive method. The Progressive approach takes less complexity in terms of time and space for solving MSA problem [24, 9]. According to progressive alignment method initially align more similar sequences after that it inclementally align more divergent sequence or group of sequence in the beginning alignment. CLUSTALW [25] is quality representation of progressive method. In first step, according to this approach we have to assign weight of each pair of sequences in a partial alignment. We assign small-weight of most similar sequences and big-weight for most dissimilar sequences. After this we take exchange matrix which defines the score between two residues of protein sequence based on similarity. In third step two types of gap have been introduced. Residue-specific gap is first kind of gap and second one is locally residue gap penalties. In fourth step, where gap have been introduced in early position receive locally reduced gap penalties to encourage the opening gap. CLUSTALW is a combination of these four steps which is freely available. Progressive alignment method performs better for MSA package in terms of accuracy and time. Even that this method has some restriction. Dependency on initial alignment and choice of scoring scheme is the main problem behind this method. In other words we bound that to align more similar sequence in initial stage. If we have not aligned more similar sequences in initial stage then the solution may be trapped in local optima.

An iterative method does not depend on initial alignment because it starts with initial alignment and improve the solutions per iteration until no more improvement possible. The main objective of the iterative approach for MSA is to globally improve the quality of a sequence alignment. There are some iterative and stochastic approaches for MSA (as example, simulated annealing [14, 16]. HMMT [7] is based on a simulated annealing process. The problem behind these methods solution may be trapped in local optima.Evolutionary algorithms are population based algorithm. According to these algorithms, we generate random initial population in the first step. Next step, we apply some operators to modify initial population for next generation. We repeatedly use these operators until reach the global optimum. When using EAs for MSA, an initial generation is generated by random manner, and then the steps of an EA are applied to improve the similarities among the sequences. There are some evolutionary computations for MSA [3, 5, 12, 13, 18]. There are some other genetic algorithm (GA) based methods for MSA, such as SAGA [18], GA-ACO [15], MSA-EC [20], MSA-GA [10], RBT-GA [21] and GAPAM [27]. We define methodology of some algorithm MSA problem based on Genetic algorithm (GA). In SAGA, the initial generation is generated randomly. According to SAGA, 22 different operators are used to gradually improve the fitness of MSA. But the problem behind SAGA is time complexity due to repeated use of fitness function. RBT-GA is also a GA based method, combined with the rubber band technique (RBT), to find optimal protein sequence alignments [23]. RBT [22] is an iterative algorithm for sequence alignment using a DP table. The authors [27] solved 34 problems from reference sets 2 and 3 of the benchmark Balibse 2.0 dataset. The drawbacks of these evolutionary methods are also local optima due to poor diversity of the solutions.

In GSA, to solve problem of local optima balancing between exploration and exploitation uses Kbest agents. The way of balancing between exploration and exploitation is to minimize the quantity of agents when number of iteration increases. Hence GSA is more powerful technique to solve NP-Hard problem with the proper use of Kbest agents.This paper is organized as follows. After the introduction, Section 2 is a brief discussion about the Gravitational search algorithm. Section 3 represents the steps of the proposed GSAMSA method, Section 4 presents a brief introduction of the test datasets, and the experimental study of the GSAMSA and other methods are discussed in Section 5. In Section 6, our conclusions are provided.

Gravitational Search Algorithm

Gravitational search algorithm (GSA) [19] is a nature inspired technique. It is used to find the approximation solution in the large search space. GSA is based on principle of Newtonian gravity:" Every particle in the universe attracts every other particle with a force that is directly proportional to the multiply of their masses and conversely proportional to the square of the distance between them. Pictorial representation of force of attraction is representing in Fig. 1. In GSA have four steps:

Position

It is corresponding to the solution of the problem. Initially position of the mass are randomly initialized. Now, consider a system with N agents (population size).
Agent ith is shown in Equation 1
$Xi = (Xi^1,\ldots\ldots, Xi^d,\ldots\ldots.., Xi^n)$ *for i=1,2,.....N* (1)
Where Xi^d represents the position of agent *i* in *dth* dimension.

Mass Calculation

We can calculate Gravitational and inertia masses using the fitness function. Massive mass of an agent is a better solution. This means that better agents have higher attractions and roam slowly. Assume equality of the gravitational and inertia mass. We upgrade the gravitational and inertial masses by the following equations 2 and 3

$Mai = Mpi = Mii = Mi \quad i=1,2,\ldots\ldots..,N$

$$m_i(t) = \frac{fit_i(t) - worst(t)}{best(t) - worst(t)} \qquad (2)$$

$$M_i(t) = \frac{m_i(t)}{\sum_{i=1}^{N} m_j(t)} + \in \qquad (3)$$

Where $fit_i(t)$ is ith agent fitness, *worst(t)* is minimum fitness value and *best(t)* is maximum fitness value for maximization problem and *worst(t)* is maximum fitness value and *best(t)* is minimum fitness value for minimization problem.

Force Calculation

At time t, the force acting on mass i from mass j in dth dimension is given in eqn. 4 and 5. Where $Ri,j(t)$ is Euclidean distance between two agents *i* and *j*, *Mpi* is passive gravitational mass, *Maj* is active gravitational mass and € is small constant

$$F^d_{ij}(t) = \frac{G(t) \times M_{pi}(t) \times M_{aj}(t)}{R_{ij}(t) + €} * (x^d_j(t) - x^d_i(t) \qquad (4)$$

$$F^{d}{}_{i}(t) = \sum_{j=1\ j\neq i}^{N} randj \quad F^{d}{}_{ij}(t) \qquad (5)$$

Position update

It is a measure of an resistance on object to changing its state of motion when a gravitational force is applied. Hence acceleration calculation is shown in eqn. 6, where *Mii* is the inertial mass of *ith* agent

$$a^{d}{}_{i}(t) = \frac{F^{d}{}_{i}(t)}{M_{ii}(t)} \qquad (6)$$

After generation of acceleration value, all the agents in population update by velocity and position equations 7 and 8 are shown below.

$$V^{d}{}_{i}(t+1) = randi \times V^{d}{}_{i}(t) + a^{d}{}_{i}(t) \qquad (7)$$

$$X^{d}{}_{i}(t+1) = X^{d}{}_{i}(t) + V^{d}{}_{i}(t+1) \qquad (8)$$

Where *Vid* is the *ith* agent velocity in *dth* dimension at time t, *Xid* is *ith* agent position, $a^{d}{}_{i}$ is acceleration and r is random number between 0 and 1.

PROPOSED METHOD

Agent Representation

In GSA each solution is represented as Agents.

$Xi = (Xi^{1}, \ldots\ldots, Xi^{d}, \ldots\ldots\ldots, Xi^{n})$ (9) *for i=1, 2,.....N*

Where *N* is the number of Solution.

In Initialization State first put the gap in our given MSA is randomly. Suppose our MSA is given in Fig. 2.

Now put Gap in random manner. This is given in Fig 3.

Binary encoding Scheme- In the encoding scheme put 1 in position of Gap and put 0 in the position of protein sequence. Now our MSA has been given in below. After that we are taking decimal value of this binary encoded value from top to bottom of each column. In first column binary value from top to bottom are 0001. Decimal equivalent of this binary is 1. Similarly we can find decimal equivalent of each column. Hence our agent representation of this solution is A_1= (1, 0, 0, 8, 2, 4) and this is given in last row of Fig. 4. Hence the number of columns in the MSA is the number of dimension of agent. Now according to this manner, we can generate 100 number of solution putting Gap in MSA. Hence we can find 100 Agent in initialization.

The complete flow chart of initial generation is given in Fig 5.

Fitness function

The Sum of pair method is used as a fitness calculation for multiple sequence alignments. Here, each column in an alignment is scored by summing the product of the scores of each pair of symbols and their pair weight.

The score of the entire alignment is then summed over all column scores by using (10) and (11)

$$T = \sum_{l=1}^{L} T_l \quad \text{Where} \quad T_l = \sum_{i=1}^{N-1}\sum_{j=i+1}^{N} Cost(A_i, A_j) \qquad (10)$$

Here, T is the cost of multiple alignments. L is the length of columns in the alignment. T_l is the cost of the *lth* column of L length. N is the number of sequences.

Cost (A_i ,A_j) is the alignment score between two aligned sequences A_i and A_j. when $A_i \neq$ "_" and $A_j \neq$"_"Then *Cost (A_i ,A_j)* is defined from the PAM matrix [6]. Also when A_i = "_" and A_j = "_" then *Cost (A_i ,A_j)*= 0.

Finally, the cost function *Cost (Ai ,Aj)* is define from eqn. 11. When A_i = "_" and $A_j \neq$ "_" or *Ai* $\neq$ *"_" and* A_j = "_" using a equation with gap retribution as shown in below

$$Z = Q + nu \qquad (11)$$

Here, Z is the gap retribution, *Q* is the cost of opening a gap, and *u* is the cost of extending gap, and *n* is the total number of the gap.

Illustrations

Suppose in initialization we have taken 5 agents. Initial alignment of one agent out of 5 is given in Fig. 6.Now we take 5 agents after putting random Gap. And initial velocity of each agent of each dimension is 0.

A1 = (4,8,0,2,1) A2 = (2,4,0,1,2) A3 = (4,2,1,0,2) A4 = (8,2,4,0,2) A5 = (4, 1, 0, 2,4)

We can find the fitness value of each agent according to given above fitness function.

Fit (A1) = 11 Fit (A2) = 20 Fit (A3) = 15 Fit (A4) = 10 Fit (A5) = 5

We can find best and worst (since our objective is maximize the score) Hence best=20 and worst=5

Now we can find *Mai = Mpi = Mii = Mi* for i = 1, 2 ,……., N

$m_{A1}(t) = fit(A1)$ *- worst / best – worst*

=11-5/20-5

= .4

Similarly we can find $m_{A2}(t)$, $m_{A3}(t)$, $m_{A4}(t)$ and $m_{A5}(t)$

$m_{A2}(t)$ = 1 $m_{A3}(t)$ = .66 $m_{A4}(t)$ = .33 $m_{A5}(t)$ = 0

After that we can find

Let € =.001

$$M_i(t) = \frac{m_i(t)}{\sum_{i=1}^{N} m_j(t)} + \in$$

M_{A1} (t) = .4 / .4+1+.66+.33+0

= .4/ 2.39

= .673 +.001 = .674

Similarly M_{A2} (t) = .419 M_{A3} (t) = .277 M_{A4} (t) = .139 M_{A5} (t) = .001

Now we can find the force acting on A1 due to all agents in 1st dimension

Let G0 = 0.5, α = .5, *Kbest* =N

G1= G0 $e^{-\alpha t/T}$ = 0.5 / e1/2 = 0.303

$$F^d{}_{ij}(t) = \frac{G(t) \times M_{pi}(t) \times M_{aj}(t)}{R_{ij}(t) + €} * \quad (x^d{}_j(t) - x^d{}_i(t))$$

$$F^1{}_{A_1A_2}(1) = \frac{G(1) \times M_{A_2}(1) \times M_{A_1}(1)}{R_{ij}(1) + €} * \quad (x^1{}_{A_2}(1) - x^1{}_{A_1}(1))$$

$$= \frac{0.303 \times 0.419 \times 0.674}{4.69 + 0.001} \times (2-4)$$

= .303× -.120

= -0.0363

Similarly we can find

$$F^1{}_{A_1A_3}(1) = 0$$

$$F^1{}_{A_1A4}(1) = 0.0241$$

$$F^1{}_{A_1A_3}(1) = 0$$

Hence $F^d{}_i(t) = \sum_{j=1\, j\neq i}^{N} randj \quad F^d{}_{ij}(t)$

$$F^1{}_{A_1}(1) = \sum_{j=1\, j\neq i}^{Kbest} randj \; F^1{}_{A_1Aj}(1)$$

= 0.2 (-0.0363 + 0.0241)

= 0.0024

$$a^d{}_i(t) = \frac{F^d{}_i(t)}{M_{ii}(t)}$$

$$a^1{}_{A_1}(1) = \frac{F^1{}_{A_1}(1)}{M_{A_1}(1)}$$

= -0.0024 / 0.674

= -0.003

$$V^d{}_i(t+1) = randi \times V^d{}_i(t) + a^d{}_i(t)$$

$$V^1{}_{A_1}(2) = randi \times V^1{}_{A_1}(1) + a^1{}_{A_1}(1)$$

$$= 0.01 \times 0 + (-0.003)$$

= -0.003

$$X^d{}_i(t+1) = X^d{}_i(t) + V^d{}_i(t+1)$$

$$X^1{}_{A_1}(2) = X^1{}_{A_1}(1) + V^1{}_{A_1}(2)$$

= 4 – 0.003

= 4

Similarly we can find the value of each dimension of the first agent. Hence our agent A_1 = (4,0,0,0,11). We can put the Gap in each column according to our encoding scheme. This is given in Fig. 7(a). Now we can put protein sequence in vacant position. This is shown in Fig. 7(b) which is Final alignment after 1 generation. The complete algorithm for finding solution of MSA is given in algorithm 1.

(1)Initial MSA Problem;
(2)Predefined Number of Agents N;
(3)Dimension of Agent m;
(4)Kbest=N;
Result: Optimized MSA

Algorithm 1. GSA for solving MSA problem

1. ***Begin***
2. *Initialization*
3. *i=1;j=1;*
4. *Initialize the Agent Ai* $1 \leq i \geq N$
5. ***while*** *(i! = N)*
6. *Calculate Fitness(Ai)*
7. ***end while***
8. ***while*** *(i! = N)*
9. *best = max(Fitness(Ai))*
10. *worst = min(Fitness(Ai))*
11. ***end while***
12. ***while*** *(i! = N)*
13. *Calculate mass(Ai) /*using equation 2 and 3*/*
14. ***end while***
15. ***while*** *(s! = Maxiteration)*
16. ***while*** *(i! = N)*
17. ***while*** *(j! = Kbest)*
18. *Calculate the force of Agent Ai /* using equation 4 and eqn. 5 */*
19. *Calculate the acceleration of Agent Ai /*using equation 6 */*
20. *update the Agent/*using velocity equation 7 and position equations 8 */*
21. *update the Kbest value*
22. ***end while***
23. ***end while***

24. ***end while***

25. ***end***

Termination Condition

We keep the best solution in each generation. If the best solution in next 50 generation consecutively same, then stop our algorithm. This is based on experimental review. We tested our algorithm up to 200 generation after getting best solution we have seen that probability to change best solution is very low.

Test Dataset

We have tested a large number of datasets from Bali base benchmark database to check the quality of our approach. Bali base version 1.0 [26] contains 142 reference alignment which keeps more than 1000 sequences. Bali base version 2.0 [1] is an extended version of Bali base version 1.0. Bali base version 2.0 contains 167 reference alignments which keeps more than 2100 sequences. Bali base version 2.0 contains eight reference sets. Each reference keeps different type of sequences. Small number of equidistance sequence contains in reference set 1. Totally different or unrelated sequence contains in reference set 2. Reference set 3 contains a pair of divergent sub-families. Long terminal extension sequence contains in reference set 4. Reference 5 contains large internal insertions and deletions. Lastly Reference 6-8 contain test case problems where the sequence are repeated and the domains are inverted. Bali score is a score measure the quality of algorithm. Bali score compare between manually alignment sequence (which is available on Bali base version 2.0) and alignment which is come from some existence method. Range of Bali score is 0 to 1. If the manually alignment file and our output file is same then score is 1. If the manually alignment file and our output file is totally different then score is 0. It gives the value between 0 and 1 according to similarity between Bali base manually alignment file and our output file.

EXPERIMENTAL ANALYSES

We have analyzed the performance of our GSA algorithm. After that we compare between our proposed algorithm and some other well-known existence method which is given in paper GAPAM [27]. For fair comparison we considered all the datasets of GAPAM. In this paper, we run our algorithm up to five independent times to each dataset and take the average score.

Effect of GSA

The proposed algorithm GSA uses a law of gravity and *Kbest* (which maintains exploration and exploitation) and modified fitness functions that contribute to it performing better than other algorithms. To analyze the effect of these two components on the algorithms performance, we have designed two set of experiments. In this set, GSA and GAPAM were run. SPM was used as the fitness measure. We have used five Balibse datasets for these experiments (three from reference set 2 and two from the reference set 3). All dataset was run with GSA for five independent runs. The set of experiments proves the superiority of our proposed algorithm. The details of these experiments are reported in Table 1.

Estimation of Performance

There are mainly three components which have been taken major role to define the complexity of any algorithm for MSA problem. In addition, choice of algorithmic parameter also plays an important role to determine the complexity of algorithm. To show the convergence behaviour of our algorithm, we have represented the best and the average

SPM scores against the each generations. As for example, four such plots for four datasets from reference set 2 and 3 are presented in Fig. 8 and Fig. 9. We have seen that from these graphs our algorithm both best and average score improve very fast in initial stage of search process. Although in later stage best score not improved in significant manner but average score up and down in similar manner like in initial stage. This is the kind of solution we want from any good search algorithm.

Assessment of Standard

To determine the quality of our algorithm we have considered all datasets of paper GAPAM [27]. The authors of GAPAM keeps Bali score with respect to best score which is come from out of 10 independent runs. Even that we take Bali score with respect to average score which is come from only 5 independent runs. In GAPAM, the author considered all 23 test datasets of reference 2, and 11 from reference 3. In total, we considered 34 test datasets including 23 from reference 2, 11 from reference 3 out of 12. All these datasets belong to the Bali Base 2.0 benchmark datasets.

Performance of GSAMSA w.r.to Reference 2

There are various types of datasets in Bali-base Reference 2. GSAMSA performed different with different datasets. To determine the performance of GSAMSA with respect to Bali score, we have compared with HMMT, SAGA, RBT-GA, PRRP, DIALIGN, GAPAM, and SB-PIMA. Table 2 show that for the 23 test cases, GSAMSA method has successfully found more accurate solutions than the others in 16 test cases, and GAPAM in 7 test cases. In seven test cases, where GSAMSA method could not obtain the best solutions, they were close to the best solutions. We take average solution of all method w.r.to all datasets of reference 2 and we find that GSAMSA is better than other methods.

Performance of GSAMSA w.r.to Reference 3

There are many datasets in reference 3 which is more divergent. Hence residue identities of these datasets are very low. In this paper, we considered 11 datasets. The experimental results are illustrated in Table 3 show that GSAMSA method found more accurate in 6 test cases, SAGA in 1, GAPAM in 2, and PRRP in 1 and RBT-GA in 1 test case. Although GSAMSA method did not obtain best solution in some test cases but it is close to best solution. We take average solution of all method w.r.to all datasets of reference 3 and we find that GSAMSA is better than other methods. Hence, after experimental analysis we can say that propose method better than all other methods.

CONCLUSIONS

In recent years, various heuristic optimization methods have been developed for MSA. Some of these heuristics are nature inspired swarm intelligence techniques. In this article, a new optimization algorithm that is called Gravitational Search Algorithm (GSA) is used for solving Multiple Sequence Alignment problems. GSA is constructed based on the law of gravity and the notion of mass interactions. The GSA algorithm uses the theory of Newtonian laws of motion and its search agents are the collection of masses. Using the gravitational force, every mass in the system can see the situation of other masses. The gravitational force is acting of transferring information between different masses. In MSA problem initial stage we define population randomly. After that find fitness value of each solution using weighted pair of sum method (SPM) which is described in fitness function section. After finding the fitness value of each solution we can find the Mass of each solution. According to mass value of each solution we can find force between each agent and using this force can find acceleration and finally using acceleration can get updated solution. We used two new approach first one is Gravitational search algorithm and the second one is properly use of *Kbsest* for MSA Problems. To assess the good performance of the algorithm, a number of the experiments tested on GSA and RBT-GA and we find that GSA is better than RBT-GA most of cases. To test our present approach, we considered a good number of benchmark datasets from Bali base 2.0, so as to cover all the test

sets of RBT-GA. GSAMSA method was optimized based on the weighted sum of pair score. Therefore, the corresponding Bali score of this solution was used to compare with other methods, as they used Bali score as their measure of the quality/accuracy of the MSA. The experimental results proved that GSAMSA performed better for most of the test cases. Even the solution of GSAMSA was not always the best for some test cases; it was always close to the best. The GSAMSA method performed better than the others because of its law of gravity and properly use of *Kbest* in each iteration. After the experimental analysis, we can safely conclude that the GSAMSA method can effectively solve multiple sequence alignment problems.

REFERENCES

1. Bahr A, Thompson JD, Thierry JC, Poch O. Bali base (benchmark alignment database): enhancements for repeats, transmembrane sequences and circular permutations. Nucleic Acids Research 2001; 29: 323-326.
2. Bonizzoni P, Della Vedova G. The complexity of multiple sequence alignment with sp-score that is a metric. Theoretical Computer Science 2001; 259: 63-79.
3. Cai L, Juedes D, Liakhovitch E. Evolutionary computation techniques for multiple sequence alignment. In: Evolutionary Computation, Proceedings of the Congress on 2000; 2: 829-835.
4. Carrillo H, Lipman, D. The multiple sequence alignment problem in biology. SIAM Journal on Applied Mathematics 1988; 48: 1073-1082.
5. Chellapilla K, Fogel G.B. Multiple sequence alignment using evolutionary programming. In: Evolutionary Computation, CEC 99. Proceedings of the Congress on, vol. 1 1999.
6. Dayhoff MO, Schwartz RM. A model of evolutionary change in proteins. In: In Atlas of protein sequence and structure. Citeseer 1978.
7. Eddy SR. Multiple alignment using hidden markov models. In: Ismb 1995; 3: 114-120.
8. Feng D, Johnson M, Doolittle R. Aligning amino acid sequences: comparison of commonly used methods. Journal of Molecular Evolution 1985; 21: 112-125.
9. Feng DF, Doolittle RF. Progressive sequence alignment as a prerequisitetto correct phylogenetic trees. Journal of molecular evolution 1987; 25: 351-360.
10. Gondro C, Kinghorn B. A simple genetic algorithm for multiple sequence alignment. Genetics and Molecular Research 2007; 6: 964-982.
11. Gusfield D. Algorithms on strings, trees and sequences: computer science. 1997.
12. Horng JT, Lin CM, Liu BJ, Kao CY. Using genetic algorithms to solve multiple sequence alignments. In: GECCO 2000; 883.
13. Ishikawa M, Toya T, Totoki Y, Konagaya A. Parallel iterative aligner with genetic algorithm. Genome Informatics 1993; 4: 84-93.
14. Kim J, Pramanik S, Chung MJ. Multiple sequence alignment using simulated annealing. Computer applications in the biosciences: CABIOS 1994; 10: 419-426.
15. Lee ZJ, Su SF, Chuang CC, Liu KH. Genetic algorithm with ant colony optimization (ga-aco) for multiple sequence alignment. Applied Soft Computing 2008; 8: 55-78.
16. Lukashin AV, Engelbrecht J, Brunak S. Multiple alignment using simulated annealing: branch point definition in human mrna splicing. Nucleic acids research 1992; 20: 2511-2516.
17. Needleman SB, Wunsch CD. A general method applicable to the search for similarities in the amino acid sequence of two proteins. Journal of molecular biology 1970; 48: 443-453.
18. Notredame C, Higgins DG. Saga: sequence alignment by genetic algorithm. Nucleic acids research. 1996; 24: 1515-1524.
19. Rashedi, E., Nezamabadi-Pour, H., Saryazdi, S. GSA: a gravitational search algorithm. Information sciences 2009; 179: 2232-2248.
20. Shyu C, Sheneman L, Foster JA. Multiple sequence alignment with evolutionary computation. Genetic Programming and Evolvable Machines 2004; 5: 121-144.

21. Taheri J, Zomaya AY. RBT-GA: a novel metaheuristic for solving the multiple sequence alignment problem. Bmc Genomics 2009; 10: (Suppl 1), S10.
22. Taheri J, Zomaya AY. Rbt-l: A location based approach for solving the multiple sequence alignment problem. International journal of bioinformatics research and applications 2010; 6: 37-57.
23. Taheri J, Zomaya AY, Zhou BB. RBT-L: A Location Based Approach for Solving the Multiple Sequence Alignment Problem. School of Information Technologies, University of Sydney 2008.
24. Taylor WR. A flexible method to align large numbers of biological sequences. Journal of Molecular Evolution 1988; 28: 161-169.
25. Thompson JD, Higgins DG, Gibson TJ. Clustal w: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic acids research 1994; 22: 4673-4680.
26. Thompson JD, Plewniak F, Poch O. Balibase: a benchmark alignment database for the evaluation of multiple alignment programs. Bioinformatics 1999; 15: 87-88.
27. Naznin, F., Sarker, R., Essam D. Progressive Alignment Method Using Genetic Algorithm for Multiple Sequence Alignment. IEEE Transaction on Evolutionary Computation 2012; 16: 615-631

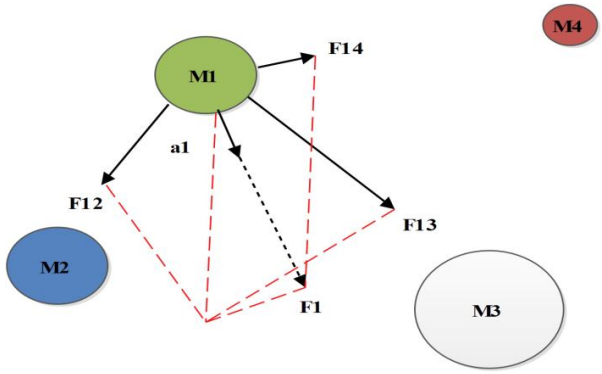


Fig. 1 Every mass accelerate toward the result force that act it from the other masses

A	T	G	C	G
T	A	G	G	G
C	C	A	T	A
A	G	C	A	A

Fig. 2. Initial MSA

A	T	G	-	C	G
T	A	G	G	G	-
C	C	A	T	-	A
-	A	G	C	A	A

Fig. 3. Initial Solution

	0	0	0	1	0	0
	0	0	0	0	0	1
	0	0	0	0	1	0
	1	0	0	0	0	0
Agent →	1	0	0	8	2	4

Fig. 4. Encoding Scheme

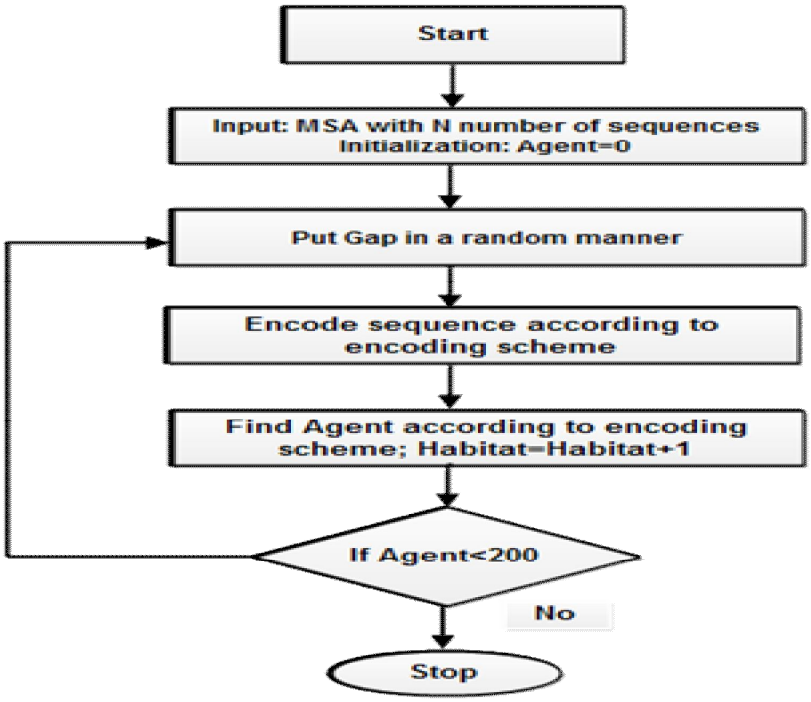


Fig. 5 A complete flow Chart of initial Generation

A	-	C	T	G
C	T	G	A	-
-	T	C	A	G
T	C	C	G	-

Fig. 6. Initial Alignment

				-
-				
				-
				-

(a) Gap insertion

A	C	T	G	-
-	C	T	G	A
T	C	A	G	-
T	C	C	G	-

(b) Final Alignment

Fig. 7. A complete solution after 1 generation

Table 1 Performance test of Gravitational Search Algorithm

DATASETS	GSAMSA	GAPAM
1r69	0.911	**0.965**
1tgxA	**0.932**	0.878
1sbp	**0.778**	0.765
1ajsA	**0.441**	0.311
1ped	**0.814**	0.775
Avg score	**0.775**	0.738

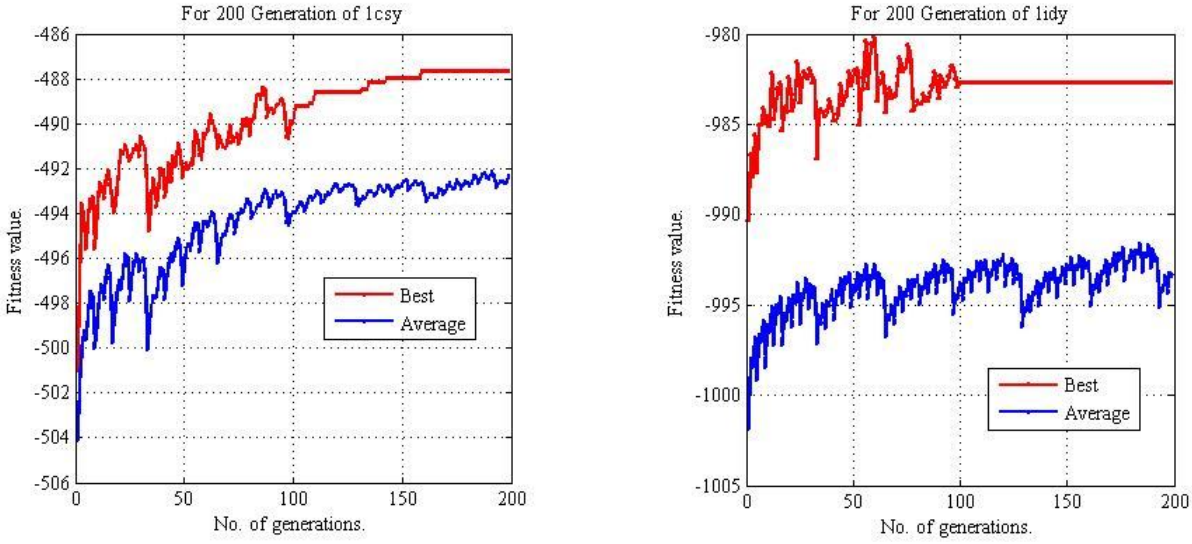


Fig. 8. Graphical presentations of the performance of the GSAMSA method w.r.t. the best and average score per generation.

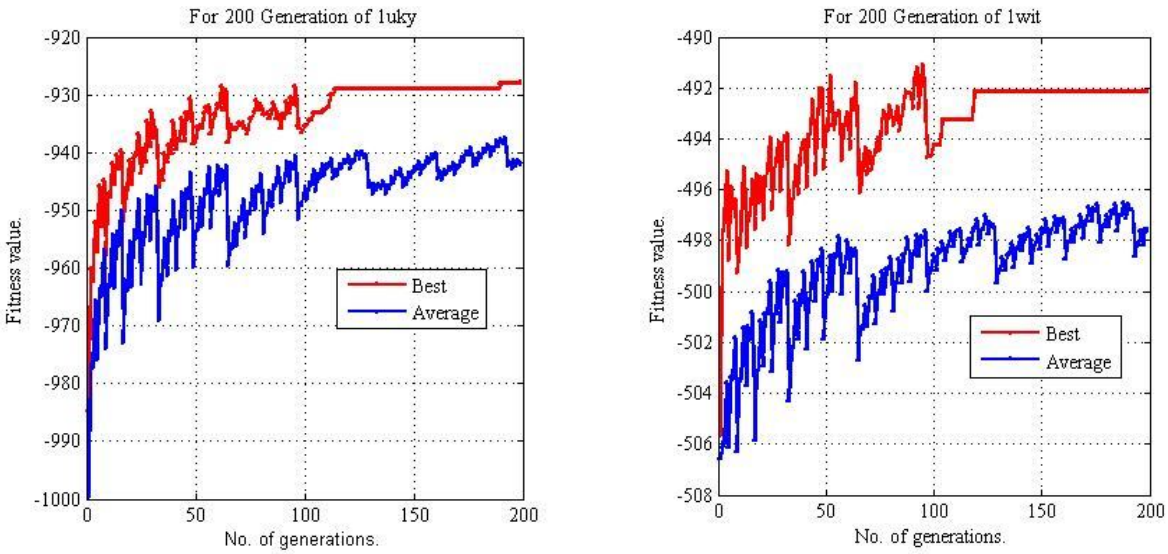


Fig. 9. Graphical presentations of the performance of the GSAMSA method w.r.to the best and average score per generation

Table 2 Experiments on Reference 2 Datasets of Bali base 2.0

Dataset	PRRP	SB-PIMA	HMMT	DIALI	SAGA	RBT-GA	GAPAM	PROPOSED
1aboA	0.256	0.391	0.724	0.384	0.489	0.812	0.796	**0.816**
1idy	0.37	0.000	0.353	0.000	0.548	0.997	**0.989**	0.846
1csy	0.35	0.000	0.000	0.000	0.154	0.735	0.764	**0.841**
1r69	0.675	0.675	0.000	0.675	0.475	0.9	**0.965**	0.911
1tvxA	0.207	0.241	0.276	0.000	0.448	0.891	**0.92**	0.897
1tgxA	0.695	0.678	0.622	0.63	0.773	0.835	0.878	**0.932**
1ubi	0.056	0.129	0.053	0.000	0.492	0.795	0.767	**0.803**
1wit	0.76	0.469	0.641	0.724	0.694	0.825	**0.851**	0.695
2trx	0.87	0.85	0.739	0.734	0.87	0.982	**0.986**	0.984
1sbp	0.231	0.043	0.214	0.043	0.374	0.778	0.765	**0.778**
1havA	0.52	0.259	0.194	0.000	0.448	0.792	0.879	**0.897**
1uky	0.351	0.256	0.395	0.216	0.476	0.625	**0.808**	0.715
2hsdA	0.404	0.39	0.423	0.262	0.498	0.745	0.796	**0.821**
2pia	0.767	0.73	0.647	0.612	0.763	0.730	**0.826**	0.783
3grs	0.363	0.183	0.141	0.350	0.282	0.755	0.746	**0.863**
Kinase	0.896	0.755	0.749	0.692	0.867	0.712	0.799	**0.911**
1ajsA	0.227	0.000	0.242	0.000	0.311	0.892	0.899	**0.945**
1cpt	0.821	0.184	0.388	0.425	0.776	0.584	0.875	**0.932**

1lvl	0.772	0.62	0.539	0.783	0.726	0.567	0.781	**0.891**
1pamA	0.711	0.393	0.53	0.576	0.623	0.66	0.86	**0.888**
1ped	0.881	0.651	0.696	0.773	0.835	0.78	0.912	**0.941**
2myr	0.582	0.727	0.443	0.84	0.825	0.675	0.822	**0.914**
4enl	0.668	0.096	0.213	0.122	0.739	0.812	0.896	**0.932**
Average Score	0.541	0.379	0.401	0.384	0.586	0.777	0.851	**0.866**

Table 3 Experiments on Reference 2 Datasets of Bali base 2.0

Dataset	PRRP	SAGA	DIALI	HMMT	SB-PIMA	RBT-GA	GAPAM	PROPOSED
1idy	0.000	0.364	0.000	0.227	0.000	0.546	0.601	**0.611**
1r69	**0.905**	0.524	0.524	0.000	0.000	0.374	0.709	0.777
1ubi	0.415	**0.585**	0.000	0.366	0.000	0.31	0.386	0.439
1wit	0.742	0.484	0.500	0.323	0.645	**0.78**	0.758	0.756
1uky	0.139	0.269	0.139	0.037	0.083	0.35	0.468	**0.512**
kinase	0.783	0.758	0.650	0.478	0.541	0.697	0.828	**0.869**
1ajsA	0.128	0.186	0.000	0.006	0.000	0.18	0.311	**0.441**
1pamA	0.683	0.579	0.683	0.169	0.546	0.525	**0.835**	0.795
1ped	0.679	0.646	0.641	0.172	0.450	0.425	0.775	**0.814**
2myr	0.646	0.494	0.272	0.101	0.278	0.33	**0.813**	0.467
4enl	0.736	0.672	0.050	0.050	0.393	0.68	0.8	**0.885**
Average Score	0.532	0.506	0.314	0.175	0.267	0.472	0.662	**0.669**

Indian Journal Of Natural Sciences *www.tnsroindia.org.* © *IJONS*

Vol.6 / Issue 33 / December 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Reporting of long tailed Parakeet (*Psittacula longicauda nicobarica*, Gould, 1857) from little Nicobar and great Nicobar island, India.

Dinesh Meena[1], Jai Bahadur Singh Kachhawa[1,2] and Ashish Jangid[3]

[1]Department of Zoology, School of Basic and Applied Sciences, Poornima University, Jaipur – 303905 (Rajasthan) INDIA.
[2]Division of Biological Research, Centre for Advance Research and Development, Jawahar Nagar, Jaipur – 302004 (Rajasthan) INDIA.
[3]Biodiversity Research Laboratory, Maharshi Dayanand Saraswati University, Ajmer – 305009 (Rajasthan) INDIA.

Received: 23 Sep 2015 Revised: 25 Oct 2015 Accepted: 27 Nov 2015

***Address for correspondence**
Dinesh Meena
Department of Zoology,
School of Basic and Applied Sciences,
Poornima University,
Jaipur – 303905 , Rajasthan,India.

ABSTRACT

The study site little Nicobar and great Nicobar island are the politically parts of India's one of the Union Territory Andman and Nicobar islands. The great Nicobar island is geographically situated at 7^{0}01'03.64" N, 93^{0}55'31.05" E. and the little Nicobar island is positioned at 7^{0}22'46.04" N, 93^{0}42'35.54" E. These areas are still with population of traditional tribes. Our observatory sighting record of long tailed Parakeet (*Psittacula longicauda nicobarica,* Gould, 1857) is from little Nicobar and great Nicobar island.

Key-words: Biodiversity, Nicobar island, Parakeet

Andaman and Nicobar islands are one of the last remaining virgin rain forest islands in the world (Figure 1). The **Nicobar Islands** are an archipelagic island chain in the eastern Indian Ocean and one of the most isolated in the world. They are located in Southeast Asia, 150 km north of Aceh on Sumatra, and separated from Thailand to the east by the Andaman Sea. Located 1,300 km southeast of the Indian subcontinent, across the Bay of Bengal, they form part of the Union Territory of Andaman and Nicobar Islands, India.This is fact that parakeets are the smallest members of Parrot family. Parakeets are very attractive birds in concern of their sounds and displaying behaviour. The Parakeets is basically having small body, bright green colour and long tapered tail. India is having 15 species of parakeets, in which 6 species are found in Andman and Nicobar too. Two species out of these 6 species are endemic of Andman and Nicobar islands. Our observatory sighting record of long tailed Parakeet (*Psittacula longicauda nicobarica,* Gould, 1857) is from little Nicobar and great Nicobar island, which is not assessed in the taxon of IUCN Red List, but it is in the catalogue of life. During patrolling at about 1100 Hrs we observed a long tailed Parakeet (*Psittacula longicauda*

nicobarica, Gould, 1857) near a shelter located in the Rajiv Nagar 1 km Campbell Bay, Great Nicobar Island (Figure 2,3,4). Coordinate $7^0$01'03.64"N, $93^0$55'31'.05" E. Elevation 66 ft. It is also observed in the little Nicobar island at Makachua coordinates $7^0$22'46.04"N, $93^0$42'35'.54" E. Elevation 125 feet.

Psittacula longicauda nicobarica is dimorphic species, in which male is of greenish crown and greenish nape. Ear coverts and upper cheeks are of dark red colour. Upper dorsal is of green colour. In vice versa female is of olive green crown and olive green nape too. Even the head is of green colour with dull reddish tone. In female, the super cilliary line is totally absent. Both sexes showed green rump and greyish eyes. Average body length of *Psittacula longicauda nicobarica* could be found upto 45 cm with an average weight 100-140gms. The total life span is 15-20 years.

ACKNOWLEDGEMENTS

I would like to express my deep gratitude and thanks to Sub Inspector Benedict, Sub Inspecor Samuel, PC 2889 Sameer Kumar Minj of Andaman & Nicobar Police. Very special thanks to Prof. K.K. Sharma, Former Vice Chancellor, Maharshi Dayanand Saraswati University, Ajmer (Rajasthan) for his motivational spirit. I extend my sincere thanks to Dr Mahesh Bundele, Coordinator, Research, Poornima University, Jaipur. I am highly indebted to Dr Vivek Sharma, Dr S.P. Mehra for their time to time guidance and support. I am also thankful to Forest Department Andman & Nicobar Islands.

REFERENCES

1. A Field Guide to the Birds of Indian Subcontinent: Krys Kazmierczak, Ben Van Perlo, 2000.
2. Birds of Indian Subcontinent: Richard Grimmett, Carol Inskipp, Tim Inskipp; Christopher Helm, London, 2010.
3. Management plan for Campbell Bay National Park (For the Period of 2008-2013): Dr. P. Vishwakannan, IFS, Divisional Forest Officer, Nicobar Division; Andman and Nicobar Administration & Department of Environment and Forests, 2008.
4. The Book of Indian Birds: Salimali, 2003.
5. www.iucnredlist.org/search

Figure 1: Geographical position of little Nicobar and great Nicobar islands.

Figure 2: ***Psittacula longicauda nicobarica***

Figure 3: ***Psittacula longicauda nicobarica***

Figure 4: ***Psittacula longicauda nicobarica***

Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.6 / Issue 33 / December 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

In vitro Antioxidant Activity of Ethanol Extract of Leaves of *Phyllanthus virgatus*F.

Uma V[1], Ruba P[1], Subramaniam P[1] and Shabana Begum M[2]*

[1]Dept.of Botany, A.A.Govt.Arts College, Namakkal District - 637 002, TamilNadu, India.
[2]Dept.of Biochemistry,Muthayammal College of Arts and Science, Rasipuram, Namakkal District – 637 408, TamilNadu, India.

Received: 20 Sep 2015 Revised: 28 Oct 2015 Accepted: 29 Nov 2015

***Address for correspondence**
Shabana Begum M
Dept.of Biochemistry,
Muthayammal College of Arts and Science,
Rasipuram, Namakkal District – 637 408,
TamilNadu, India.

ABSTRACT

*Phyllanthus virgatus*F (Eurphobacea) is has been used in Ayurvedic medicine for over 2,000 years and has a wide number of traditional uses. This family includes several plant species among all the species; *P. amarus, P. urinaria, P. maderaspatensis* and *P. fraternus* are the most popular ones due to their antioxidant properties as well as their extensive use in the treatment of disease related to kidney, liver, urinary bladder, intestinal infection, cancer, and diabetes.This study was designed to examine the invitro antioxidant activities of ethanol extract of leaves of *Phyllanthus virgatus*. Ethanol extract of leaves of*Phyllanthus virgatus* showed its scavenging effect in concentration dependent manner on 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2'-azino-bis (3- ethylbenzthiazoline-6-sulfonic acid) (ABTS), reducing power, hydroxyl radical, nitric oxide, superoxide, lipid peroxidation assay by thiobarbituric acid and hydrogen peroxide (H 2 O 2)and they were compared with standard antioxidant ascorbic acid. From the study the results revealed that the ethanol extract of *Phyllanthus virgatus*were found to exhibit maximum free radical scavenging and these compounds may be used as a good natural antioxidant and it may be an alternative source to existing synthetic antioxidant and could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases.

Key words: Antioxidant, *Phyllanthus virgatus*,DPPH, ABTS, Radical scavenging, Lipid peroxidation

INTRODUCTION

*Phyllanthus virgatus*F (Eurphobacea) is has been used in Ayurvedic medicine for over 2,000 years and has a wide number of traditional uses. This family includes several plant species among all the species; *P. amarus, P. urinaria, P. maderaspatensis* and *P. fraternus* are the most popular ones due to their antioxidant properties as well as their extensive use in the treatment of disease related to kidney, liver, urinary bladder, intestinal infection, cancer, and diabetes (Kirtikar and Basu, 1933; Girach*et al*., 2007). *P. virgatus* is also known traditionally for its remedial properties and extensively known for antioxidant property and used in the treatment of intestinal, liver, kidney and bladder problem (Hashim*et al*., 2013; Shabeer*et al*., 2009; Kirtikar and Basu, 1933; Calixto*et al*., 1998; Kumaran and Joel Karunakaran, 2007). *P. virgatus* is also rich in tannins, flavonalsulfonates, norlignan compounds (Huang *et al*., 1998), lignans (Huang *et al*., 1996), hypophyllanthin, isointetralin, nirathin, nirtetralin, phyltetraline, virgatusui, lactone and acids like indole-3-carboxylic acid (Calixto*et al*., 1998), 2,4,5- trimethoxypropenylbenzene, 11-octadecenoic acid, 9,12-octadecadienoic acid, hexadecanoic acid, benzenedicarboxylic acid, tridecyl ester and 6-octadecynoic acid (Hashim*et al*., 2013).

MATERIALS AND METHODS

Chemicals

ABTS (2,2'-azinobis[3-ethylbenzothiazoline-6-sulphonate]), DPPH(2,2- diphenyl-1 picrylhydrazyl) were purchased from Sigma Aldrich Co, St Louis, USA. All reagents used, including solvents used in this experiment were of analytical grade and obtained from Himedia, India.

Collection of Plants

Fresh leaves of *Phyllanthus virgatus* were collected in around Namakkal district. The fresh fully-grown plant having sufficient leaves is selected. Collected plant materials were cleaned to remove mud and other adhering weed plants. Fresh plants materials were dried at the room temperature first and then shade dried for 2-3 days. The shade dried coarsely powdered leaves of *Phyllanthus virgatus* (50g) was extracted with 500 ml of ethanol by using Soxhlet extractor. After extraction, the extract was filtered, concentrated to dryness in rotavapour under reduced pressure and controlled temperature (40-50ºC).

Radical scavenging activity

The efficacy of the ethanol extract of leaves of *Phyllanthus virgatus* was studied under in vitro conditions. DPPH radical scavenging activity was carried out by the method of Molyneux (2004).ABTS radical scavenging activity was performed as described by Re *et al.,* (1999) with a slight modification.Hydrogen peroxide radical scavenging activity of the test sample was estimated by the method of Ruch*et al.,* (1989) with slight modification. Superoxide radical scavenging activity of the test sample was studied by the slightly modified method of Liu *et al.,* (1997) with slight modifications. Nitric oxide radical scavenging activity was measured spectrophotometrically by the method of Govindharajan*et al*., (2003). Different concentration of ascorbic acid was used as reference compound.

Statistical Analysis

The biochemical parameters studied were subjected to statistical analysis using Sigma Stat statistical package (Version3.1). The experimental results were expressed as mean ± SD.

RESULTS AND DISCUSSION

The present study was undertaken to analyze the antioxidant activity of ethanol extract of leaves of ***Phyllanthus virgatus***.Different concentrations ranging from 75-500 µg/ml of the ethanol extract of leaves of *Phyllanthus virgatus*were tested for their antioxidant activity in different *in vitro* models. The percentage of inhibition was observed and found that the free radicals were scavenged by the test compounds in a concentration dependent up to the given concentration in all the models.

Radical Scavenging Activity

Different concentrations ranging from 75-500 µg/ml of the ethanol extract of leaves of *Phyllanthus virgatus*were tested for their antioxidant activity in different *in vitro* models. The percentage of inhibition was observed and found that the free radicals were scavenged by the test compounds in a concentration dependent up to the given concentration in all the models.

DPPH Radical Scavenging Activity

The activity of DPPH radical scavenging of the ethanolic extract of leaves of *Phyllanthus virgatus*was presented in Table 1 and Figure 1. The percentage of inhibition in DPPH in different concentration like 75, 125, 250, 500 µg/ml were observed in 34.75±3.42, 56.95±1.59, 77.15±2.92 and 87.89±5.75 respectively whereas the percentage inhibition of ascorbic acid in concentration like 75, 125, 250, 500 µg/ml were found to be 58.55±3.56, 77.15±2.42, 95.82±3.82 and 99.95±1.65 respectively. The IC_{50} values for DPPH scavenging activity forethanol extract of leaves of *Phyllanthus virgatus* and ascorbic acid were 103.5 µg/ml and 77.6 µg/ml respectively. Values are the average of triplicate experiments and represented as mean ± standard deviation. The DPPH method has been recommended as an easy and accurate technique to measure the antioxidant activity of fruit and vegetable juices or extracts (Oskoueian*et al.*, 2011). The result of DPPH scavenging activity assay in thisstudy indicated that the leaves was potently active. This suggested that the peel extract contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity.

ABTS Radical Scavenging Activity

Table 2 and Figure 2 shows the percentage of inhibition in ABTS in different concentration like 75, 125, 250, 500 µg/ml were observed in 39.68±2.43, 58.95±4.55, 83.19±1.34 and 99.78±0.97 respectively whereas the percentage inhibition of ascorbic acid in concentration like 75, 125, 250, 500 µg/ml were found to be 54.43±2.39, 76.27±1.77, 92.63±0.98 and 99.89±0.78 respectively. The IC_{50} values for ABTS scavenging activity for ethanol extract of leaves of *Phyllanthus virgatus* and ascorbic acid were 97.0µg/ml and 80.0 µg/ml respectively. Values are the average of triplicate experiments and represented as mean ± standard deviation. ABTS radical cation is another common organic radical that has been used to determine the antioxidant activity of single compounds and other complex mixtures (Zhou *et al*., 2004). *Phyllanthus virgatus* showed very potent ABTS radical scavenging activity. The scavenging activity of $ABTS^{+}$ radical by the plant extract was found to be appreciable; this implies that the plant extract may be useful for treating radical related pathological damage especially at higher concentration.

Hydrogen PeroxideRadical Scavenging Activity

Table 3 and Figure 3 shows the percentage of inhibition of H_2O_2 in different concentration like 75, 125, 250, 500 µg/ml were observed in 66.85±2.24, 78.97±3.51, 86.72±3.46 and 96.81±2.51 respectively whereas the percentage inhibition of ascorbic acid in concentration like 75, 125, 250, 500 µg/ml were found to be 74.70±2.18, 86.59±2.23, 94.73±1.75 and 97.81±2.63 respectively. The IC_{50} values for hydrogen peroxide scavenging activity for ethanol extract of leaves of *Phyllanthus virgatus* and ascorbic acid were 67.3 µg/ml and 50.6 µg/ml respectively. Values are the average of

triplicate experiments and represented as mean ± standard deviation. Hydrogen peroxide is an important reactive oxygen species because of its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell (Gulcin*et al.,* 2003). The extract was capable of scavenging hydrogen peroxide in a concentration dependent manner.

Superoxide Radical Scavenging Activity

Superoxide free radicals scavenged in different concentration like 75, 150, 250, 500 µg/ml were observed in 67.91±3.11, 79.92±2.44, 84.37±0.54 and 90.17±1.80 and 86.47 ± 1.80 respectively whereas the percentage inhibition of ascorbic acid in concentration like 75, 150, 250, 500 µg/ml were found to be 76.63±3.66, 87.12±2.09, 95.56±0.99 and 99.56±0.43 respectively. The IC_{50} values for superoxide scavenging activity for ethanol extract of leaves of *Phyllanthus virgatus* and ascorbic acid were 64.6 µg/ml and 46.6 µg/ml respectively which was presented in Table 4 and Figure 4. Values are the average of triplicate experiments and represented as mean ± standard deviation. Superoxide dismutase catalyzes the dismutation of the highly reactive superoxide anion to oxygen and hydrogen peroxide (Kamalakkannan, 2003). Superoxide anion is the first reduction product of oxygen (Ray, 2002). According to the results, it can be said that ethanol extracts of leaves of *Phyllanthus virgatus*have significant activities on the superoxide and the activities could be occurred due to phenolic compounds of these plants.

Nitric Oxide Radical Scavenging Activity

Table 5 and Figure 5 depicts the percentage of inhibition of nitric oxide in different concentration like 75, 125, 250, 500 µg/ml were observed in 65.61±2.32, 75.89±3.12, 83.85±0.55 and 99.92±1.70 respectively whereas the percentage inhibition of ascorbic acid in concentration like 75, 125,250,500 µg/ml were found to be 74.52±1.35, 85.87±2.28, 94.92±1.62 and 99.88±0.51 respectively. The IC_{50} values fornitric oxide radical scavenging activity for ethanol extract of leaves of *Phyllanthus virgatus* and ascorbic acid were 77.6µg/ml and 51.3 µg/ml respectively. Values are the average of triplicate experiments and represented as mean ± standard deviation. Nitric oxide (NO) is a reactive free radical produced by phagocytes and endothelial cells, to yield more reactive species such as peroxynitrite which can be decomposed to form OH radical. The result indicated that the extract might contain compounds able to inhibit nitric oxide and offers scientific evidence for the use of the leaves in the indigenous system in inflammatory condition.

CONCLUSION

The present study reveals that the ethanol extracts of leaves of *Phyllanthus virgatus* possess good antioxidant activity. This plant is promising sources of potential antioxidant and may be efficient as preventive agents in some diseases and can be considered as a natural herbal source in pharmaceutical industry.

REFERENCES

1. Calixto JB, Santos ARS, Filbo V, Yunes RA. Review of the plants of the genus Phyllanthus. Med Res Rev. 1998;18:225–258.
2. Girach RD, Aminuddin AM, Brahman M. Euphobiaceae in native health practices glucose levels over time. Diabetologia. 2007;50:2239–2244.
3. Hashim A, Khan MS, Khan MS, Baig MH, Ahmad S. Antioxidant and alpha-amylase inhibitory property of Phyllanthus virgatus: an in vitro and molecular interaction study. Biomed Res Int. 2013;2013:729393.
4. Huang YL, Chen CC, Hsu FL, Chen CF. A new lignan from Phyllanthus virgatus. J Nat Prod. 1996;59:520–1.
5. Huang Y-L, Chen C-C, Hsu F-L, Chen C-F. Tannins, flavonol sulphates and a norlignan from Phyllanthus virgatus. J Nat Prod. 1998;61(1):194–197.
6. Kirtikar KP, Basu BD. Indian medicinal plants.Vol. 3. Allahabad: Lalit Mohan Basu; 1933. pp. 2217–2227.

7. Kumaran A, Joel Karunakaran R. In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT - Food Sci Technol. 2007;40:344–52.
8. Shabeer J, Srivastava RS, Singh SK. Antidiabetic and antioxidant effect of various fractions of Phyllanthus simplex in alloxan diabetic rats. J Ethnopharmacol. 2009;12:34–8.
9. Molyneux, P . (2004) The use of stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin Journal of Science and Technology 26 , 211 – 219
10. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cationdecolorization assay. Free RadicBiol Med. 1999;26:1231–1237. doi: 10.1016/S0891-5849(98)00315-3.
11. Ruch RT, Cheng S J, Klaunig J E. Spin trapping of superoxide and hydroxyl radicals, methods in enzymology; 1984, 105: 198 – 209.
12. Liu, F., Ooi,V.E.C., & Chang, S.T. (1997). Free radical scavenging activity of mushroom polysaccharide extracts. Life science, 60, 763-771.
13. Govindarajan R., Rastogi S., awat.A K.S., Vijayakumar M., Shirwaikar A., Mehrotra S. and Palpu P. Studies on the Antioxidant Activities of *Desmodiumgangeticum*. Biolog. Pharmaceut. Bulletin. 2003; 26: 1424- 1427.
14. Oskoueian E, Abdullah N, Ahmad S, Saad W Z, Omar AR, Ho YW (2011a). Bioactive compounds and biological activities of *Jatrophacurcas* L. kernel meal extract. Int. J. Mol. Sci. 12:5955- 5970.
15. Zhou, K., J.J. Laux, and L. Yu. 2004. Comparison of Swiss red wheat grain and fractions for their antioxidant properties. J. Agric. and Food Chem ., 52: 1118-1123.
16. Gulcin I, Oktay M, Kirecci E, Kufrevioglu OI:Screening of antioxidant and antimicrobial activities of anise (*Pimpinellaanisum* L.) seed extracts. Food Chem 2003;83:371-382.
17. Kamalakannan, A., Mohan, L., Kavith, K., Harish, S., Radjacommare, R., Nakkeeran, S., Parthiban, V. K., Karuppiah, R. and Angayarkanni, T. (2003). Enhancing resistance to stem and stolon rot of peppermint (*Menthapiperita* Linn.) using biocontrol agents. ActaPhytopathologicaEntomologicaHungarica. 38: 293-305.
18. Ray Gibanananada and Husain Syed Akhtar (2002). Oxidents, antioxidants and carcinogenesis. Indian J. Exp. Biol.,40 : 1214.

Table: 1 DPPH radical scavenging activity of ethanol extract of leaves of *Phyllanthus virgatus*

Group	Concentration (µg/ml)	% of Inhibition	IC_{50} Value
Ethanol extract of leaves of *Phyllanthus virgatus*	75	34.75±3.42	103.5 µg/ml
	125	56.95±1.59	
	250	77.15±2.92	
	500	87.89±5.75	
Ascorbic Acid	75	58.55±3.56	77.6 µg/ml
	150	77.15±2.42	
	250	95.82±3.82	
	500	99.95±1.65	

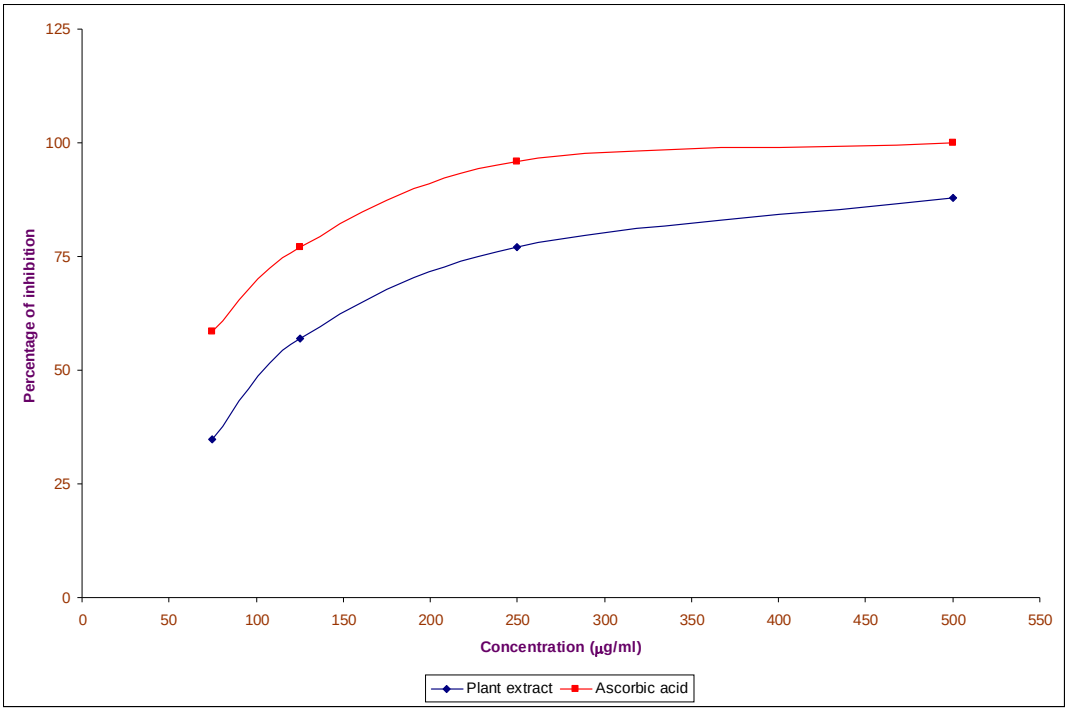


Figure: 1.DPPH radical scavenging activity of ethanol extract of leaves of *Phyllanthus virgatus*

Table: 2. ABTS radical scavenging activity of ethanol extract of leaves of *Phyllanthus virgatus*

Group	Concentration (µg/ml)	% of Inhibition	IC_{50} Value
Ethanol extract of leaves of *Phyllanthus virgatus*	75	39.68±2.43	97.0 µg/ml
	125	58.95±4.55	
	250	83.19±1.34	
	500	99.78±0.97	
Ascorbic Acid	75	54.43±2.39	80.0 µg/ml
	150	76.27±1.77	
	250	92.63±0.98	
	500	99.89±0.78	

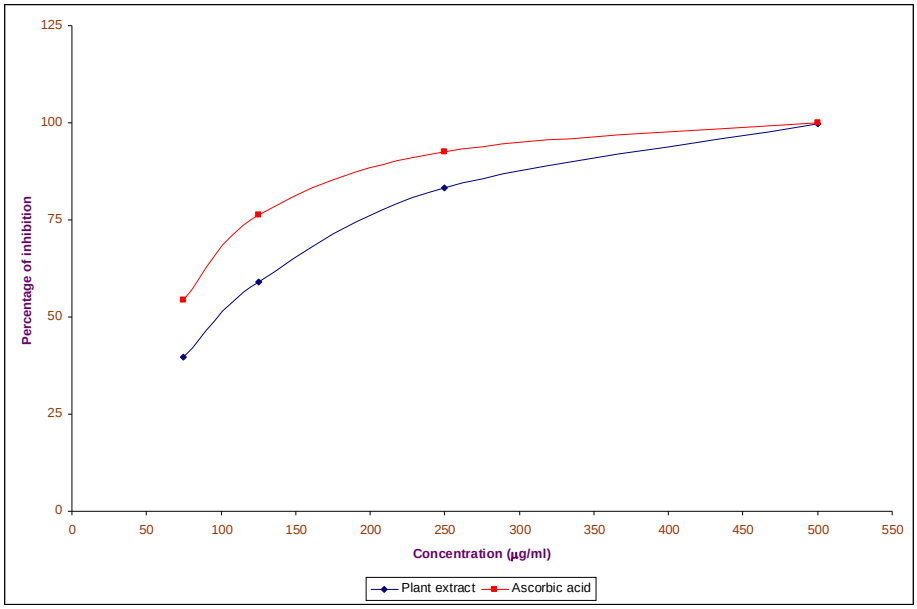


Figure: 2. ABTS radical scavenging activity of ethanol extract of leaves of *Phyllanthus virgatus*

Table: 3. H_2O_2 radical scavenging activity of ethanol extract of leaves of *Phyllanthus virgatus*

Group	Concentration (µg/ml)	% of Inhibition	IC_{50} Value
Ethanol extract of leaves of *Phyllanthus virgatus*	75	66.85±2.24	67.3 µg/ml
	125	78.97±3.51	
	250	86.72±3.46	
	500	96.81±2.51	
Ascorbic Acid	75	74.70±2.18	50.6 µg/ml
	150	86.59±2.23	
	250	94.73±1.75	
	500	97.81±2.63	

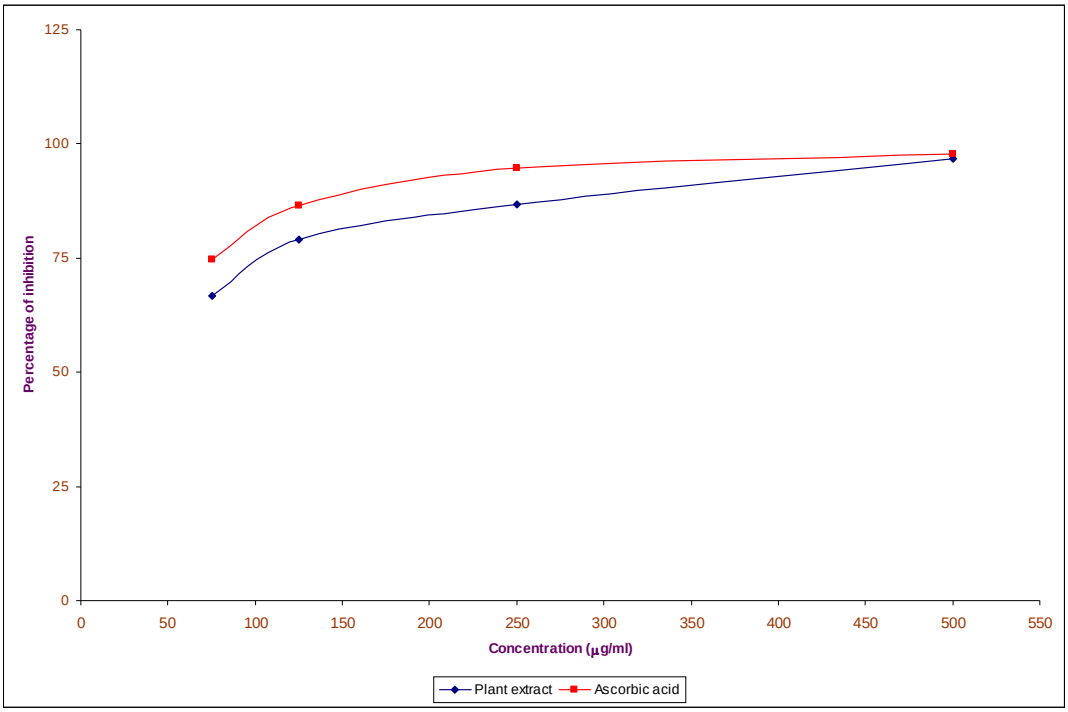


Figure: 3. H_2O_2 radical scavenging activity of ethanol extract of leaves of *Phyllanthus virgatus*

Table: 4. Superoxide radical scavenging activity of ethanol extract of leaves of *Phyllanthus virgatus*

Group	Concentration (µg/ml)	% of Inhibition	IC_{50} Value
Ethanol extract of leaves of *Phyllanthus virgatus*	75	67.91±3.11	64.6 µg/ml
	125	79.92±2.44	
	250	84.37±0.54	
	500	90.17±1.80	
Ascorbic Acid	75	76.63±3.66	46.6 µg/ml
	150	87.12±2.09	
	250	95.56±0.99	
	500	99.56±0.43	

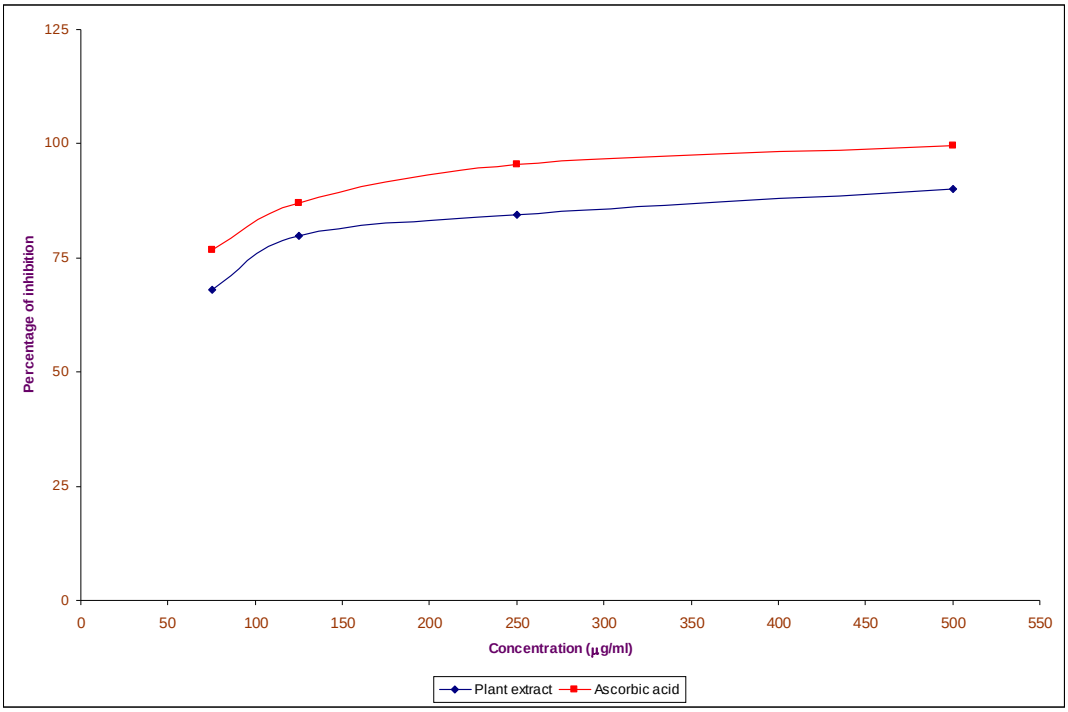


Figure: 4. Superoxide radical scavenging activity of ethanol extract of leaves of *Phyllanthus virgatus*

Table: 5. NO radical scavenging activity of ethanol extract of leaves of *Phyllanthus virgatus*

Group	Concentration (µg/ml)	% of Inhibition	IC_{50} Value
Ethanol extract of leaves of *Phyllanthus virgatus*	75	65.61±2.32	77.6 µg/ml
	125	75.89±3.12	
	250	83.85±0.55	
	500	99.92±1.70	
Ascorbic Acid	75	74.52±1.35	51.3 µg/ml
	150	85.87±2.28	
	250	94.92±1.62	
	500	99.88±0.51	

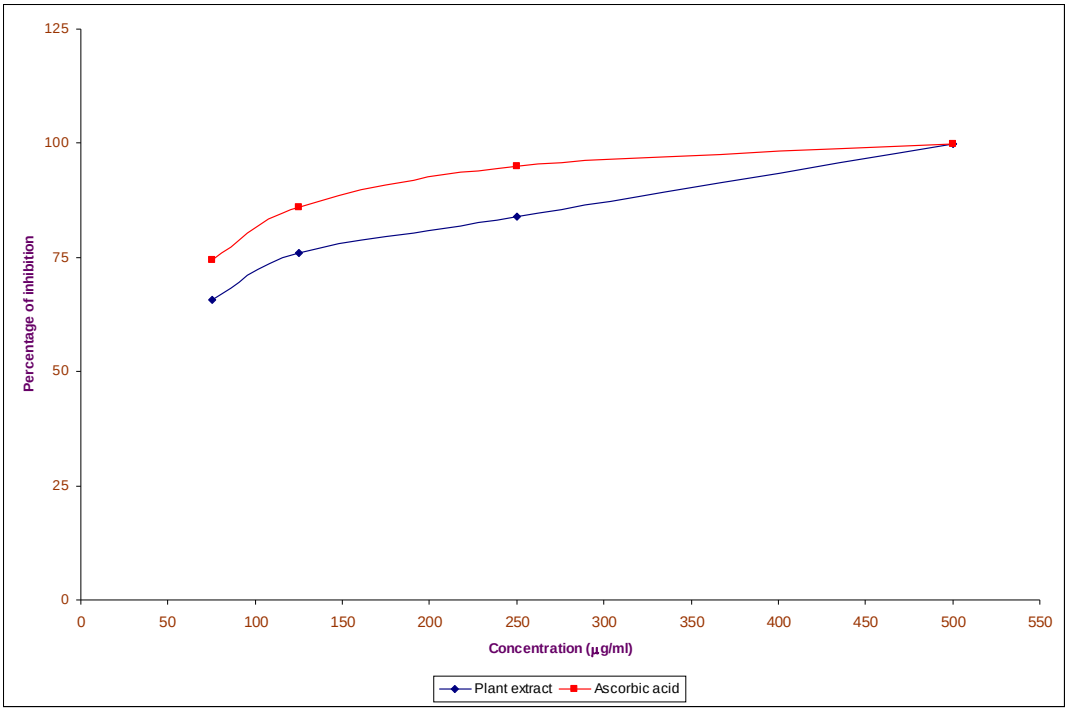


Figure: 5. NO radical scavenging activity of ethanol extract of leaves of *Phyllanthus virgatus*

Indian Journal Of Natural Sciences www.tnsroindia.org. © IJONS

Vol.6 / Issue 33 / December 2015 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Screening and Identification of Bacteriuria Diversity (*Asymptomatic Bacteriuria*) in Diabetic Patients

Shalini Gnanam T[1]* and M.Vivekanandan[2]

[1]Department of Zoology, Holy cross college, Trichirappalli- 620 002, TamilNadu, India
[2]Center for Distance Education, Bharathidasan University, Thiruchirappalli-620 023, TamilNadu, India.

Received: 25 Sep 2015 Revised: 22 Oct 2015 Accepted: 30 Nov 2015

***Address for correspondence**
T.Shalini Gnanam
Assistant Professor,
Department of Zoology,
Holy cross college,
Thiruchirappalli - 620 002, TamilNadu, India.
Email:shalurosh@gmail.com

ABSTRACT

Diabetes mellitus is a common occurrence among the patients. If not treated properly or due attention is not paid by the patients, the urinary tract infection will become more acute. Because of the wide-spread prevalence of diabetes among the Indian population the present study has been undertaken to find out the occurrence of pathogenic bacteria (bateriuria) among the diabetic men and women patients and also the results are compared with the normal individuals with no diabetes.Pus cells were found to be present or absent among the diabetic patients as well as in normal individuals, it is generally believed that presence of pus cells more than 5 in number is considered as pyuria case and the bacterial population exceeding 10^5 is considered as asymptomatic bacteriuria. In the present study no correlation has been observed between the pyuria level and asymptomatic bacteriuria. Even in non pus cell diabetic patients, bacteriuria was recorded and vice versa. It is evidently clear from the critical analysis of diabetic and non-diabetic patients; the occurrence of bacteriuria in diabetic patients is much higher because the diabetic sugar level serves as a carbon source for the growth of bacteriuria.The following bacteriuria were recorded in the present study:

1. Escherichia coli
2. Pseudomonas aeuroginosa
3. Klebsiella pneumonia

A strange feature observed in the study is that the different isolates of E.coli for instance revealed susceptibility or resistance differently to different antibiotics and this incidentally points out that doctors should exercise utmost caution in prescribing antibiotics to control these bacteriuria, otherwise the present day acute problem of multiple drug resistance will shoot up still further and search must be made to discover new antibiotics and chemicals from natural sources to control the ever-growing antibiotic resistance by the pathogenic bacteria.

Key words : Diabetes mellitus, antibiotics, pathogenic bacteria, pyuria, bacteriuria.

INTRODUCTION

Urinary tract infections (UTI) are common health problem affecting millions of people each year. They are second in seriousness only to respiratory infection. They are among the most common bacterial infection in elderly and the most frequent source of bacteremia that lead patients to seek medical care. It has been estimated that more than 6 million outpatients visit and 300,000 hospital stays every year are due to urinary tract infections. Approximately 10% of humans will have an UTI at some time during their lives.(betty Forbes et al.,1998).Urinary tract is a complex drainage system consisting of distinct anatomical and physiological areas. Majority of bacterial infections with or without symptoms occur in bladder after the ascending migration of bacteria from urethra or perineum. Infection of kidney may follow the hematogenous spread of bacteria. Urinary tract infection means the presence of bacteria undergoing multiplication in urine within the urinary drainage system. Based on the general anatomy of urinary tract, infection can be categorized into lower tract infection (Urethritis, Cystitis, Prostatitis) and upper tract infection (acute pyelonephritis, intrarenal and perinephric abscesses). Infecton at these various sites occur together or independently and may be either aymptomatic or symptomatic. (Geoffrey Smith et al., 1990)

Bacteriuria refers to the the presence of bacteria in urine. It may be symptomatic or asymptomatic. Significant bacteriuria is a term that has been used to describe the number of bacteria in voided urine that exceed the number usually due to contamination from the anterior urethra .i.e greater or equal to 100,000 bacteria /mL. The implication is that in the presence of atleast 100,000 bacteria/mL of urine infection must be seriously considered. Asymptomatic bacteriuria refers to significant bacteriuria in a patient without symptoms.(Gerald Mandell et al.,2000)Urinary tract infections are important complication of diabetes, renal disease, renal transplantation and structural and neurological abnormalities that interfere with urine flow. (Betty Forbes et al., 1998)Diabetes mellitus is a life-long disease for which there is not yet complete cure. It is a chronic metabolic disorder affecting the body' ability to make or use insulin. Insulin is the hormone that transports glucose from digested nutrients into the body's cell for energy and growth. Diabetes is of two types:

1. Type-1
2. Type-2.

In Type-1, Insulin is not produced by the body and so referred as insulin dependent Diabetes mellitus.
In Type-2 body produces insulin but is unable to process it and/or use it correctly. It is also known as Non Insulin dependent diabetes mellitus.It can be controlled by diet and exercise in most cases.Due to the severity and frequency of urinary tract infection in diabetic patients prompt diagnosis and early therapy is essential. In this background present study is carried out to determine the prevalence of asymptomatic bacteriuria in diabetic patients and its relationship with pyuria employing both diabetic men and women as well as non-diabetic men and women patients.

Aim of the Work

1. To Find out the frequency of asymptomatic bacteriuria in diabetic patients.

2. To find out the common causative organisms and treatment response with antibiotics.
3. To know if asymptomatic bacteriuria is common in males or females
4. To know the association between pus cell and bacteriuria.

Screening the samples for asymptomatic bacteria was carried out by culture test.(Geoffrey smith et al.,1990) Bacterial count or 100,000 organisms /mL in two clean catch midstream urine specimen greater than 24 hours apart obtained from a asymptomatic patient is considered as a criteria to identify asymptomatic bacteriuria (Gerald Mandell et al.,2000)urine containing greater than 5 pus cells/High power field considered as pyuria (Barnabas Rozsal et al., 2003). In screening of Asymptomatic bacteriuria detection of Mycobacterium tuberculosis is also included because most of the TB patients who were found to be diabetic were asymptomatic and TB is a frequent complication in patients with established Diabetes mellitus.Pyuria with a sterile routine culture may be found with renal Tuberculosis.(Monica Cheesbrough et al.,1983).RT PCR is carried out to detect Tuberculosis.

MATERIALS AND METHODS

1. Urine samples for the present study was obtained from Doctors Diagnostic center, Trichy.
2. 20 men and 20 women with type 2 diabetes and 20 individuals without diabetes (control) were studied. Clean catch midstream urine specimen was obtained for study.
3. Examination of urine include microscopy and quantitative culture. It was carried out within 1 hour of collection. Test was repeated the next day also with a new urine sample collected from the same patient.
4. Purpose of microscopy is to determine the number of white cells. Pyuria without bacteriuria may be an indication of Tuberculosis. (Geoffrey Smith et al., 1990)
5. Culture of urine sample carried out in Blood and MacConkey Agar. Colony forming units (CFU) were counted. The test was repeated in consecutive day also. Asymptomatic bacteriuria was defined as the presence of 100,000 or more colony forming unit/mL in two consecutive culture greater than 24 hours apart.(Gerald Mandell et al., 2000; Godfrey Harding, 2003) Diabetic patients were examined for blood sugar level.

Media used in the present study

Blood Agar:

Peptone	15gm
Liver digest	2.5gm
Yeast extract	5gm
Sodium chloride	5gm
Agar	15gm
pH	7.4

MacConkey Agar

Peptic digest of animal tissue	20gm
Lactose	10gm
Sodium taurocholate	5gm
Neutral red	0.04gm
Agar	20gm
Distilled water	1000mL
pH	7

Mueller Hinton Agar

Beef infusion	300 gm
Casein acid hydrolysate	7.50gm
Starch	1.50gm
Agar	1000mL
pH	7

Criteria for patient selection

1. 40 diabetic patients randomly selected for study.
2. No signs of symptom of Urinary tract infection.
3. Urine containing greater than 5 pus cells/High power field are considered as pyuria.
4. Asymptomatic bacteriuria(ASB)was defined as the presence of $>10^5$ colony-forming units/ml of one and the same bacterial species in both samples without symptoms of urinary tract infection (UTI).(Barnabas Rozsai et al.,2013;Gerald Mandell et al.,2000)
5. 20 non-diabetic patients without signs of symptoms of UTI were randomly selected for study and considered as control.

Collection of sample

Morning clean catch midstream urine specimen were obtained for analysis (plate 1) (Geoffrey Smith et al.,1990) Following instructions were given to patients in written form for getting optimal results.

Instructions given

Wash the genital organ with soap water and thoroughly rinse it with clean water. Once cleaning is complete, retract the labial fold or penis and collect urine directly on the given sterile container and close it immediately (Betty Forbes et al., 1998)

Microscopic Analysis

This was done to identify pus cells and bacteria on fresh uncentifuged urine

Wet preparation of fresh uncentrifuged urine

1. Three loopfulls of well mixed fresh urine was placed on a slide and covered with a cover glass.
2. The preparation was observed under 10x and 40x objective.
3. Pus cells are usually reported as the number per high power field.(Monica cheesbrough et al.,1983)

Direct gram smear preparation of fresh uncentrifuged urine:

1. Three loopfulls of well mixed fresh urine were placed on a slide.
2. It was air-dried and heat fixed and then gram staining procedure was done.
3. The preparation was observed under 10 x and 100 x oil immersion objectives.

Gram staining

1. It was done to differentiate the organism as gram positive or gram negative.

2. Those organisms are called Gram positive which after being stained dark purple with crystal violet are not decolorized by acetone or alcohol. Those organisms are called Gram negative which after being stained with crystal violet lose their color when treated with acetone or ethanol and stain pink with safranin. The iodine solution used in this technique acts as a mordant for crystal violet.
3. A thin smear of bacterial suspension was prepared on the slide, air dried and heated to fix.
4. 1%Crystal violet was applied and stained for a minute. (primary stain)
5. The stain was washed off with water.
6. Gram's iodine was applied and allowed to act for a minute.(mordant)
7. The iodine was washed off with water.
8. The slide was kept inclined and decolourised with acetone for 5 seconds and washed immediately with water.
9. It was counter stained with saffranin immediately for 1 minute.
10. It was washed with water and the back of the slide was wiped clean and air dried.
11. The smear was observed under oil immersion objective of light microscope.

Isolation of organism: (on Mac'Conkey and Blood Agar)

1. Urine was mixed thoroughly and then the top of the container was removed.
2. Calibrated loop 0.001mL was flamed, allowed to cool and inserted vertically into the urine.
3. Loopful of urine was spread over the surface of the agar plate by quadrant streaking. The plates were incubated at 37^0 c for 24 hours.
4. After incubation the number of bacteria in the urine sample was estimated by counting the number of colonies that appear on the surface of the media. To determine the number of microorganisms per milliliter in the original specimen, the number of colonies counted were multiplied by 1000(0.001 ml loop is used)
5. If no growth was observed incubation was prolonged for another 24 hours.(Betty Forbes at al., 1998)
6. Plates with significant colony was subjected to biochemical identification.
7. Culture test was repeated with a fresh urine sample from the same patient the next day.

Guidelines for interpretation

Categories	Interpretation
Fewer than 10^5 bacteria/mL in both the tests	No Asymptomatic Bacteriuria
Greater than 10^5 bacteria/mL in one test and fewer then 10^5 bacteria/mL in another test	No Asymptomatic Bacteriuria
Greater than 10^5 bacteria/mL in both the tests	Asymptomatic Bacteriuria
Greater than 5 pus cells/ High power field	Pyuria
Lesser than 5 pus cells/ High power field	No Pyuria
No growth	Culture sterile

Interpretation	Workup
Asymptomatic Bacteriuria with or without pyuria	Antibiotic sensitivity test
No Asymptomatic Bacteriuria	Discard sample
Culture sterile with pyuria	Test for Tuberculosis by RT-PCR

Catalase Test, Oxidase Test, Indole Test, Methyl Red Test, Voges Proskauer Test, Citrate utilization Test, Triple Sugar Iron Agar Test, Urease Test, Antibiotic sensitivity Test,

RESULTS AND DISCUSSION

Diabetes mellitus is a most prevalent metabolic disorder among the population of developing countries. Diabetes is classified in recent times into Type 1 and Type 11 category. Although this is not a serious disease but if left unchecked, it may lead is a common health problem in the developing countries due to unhygienic conditions and unhygienic practices followed. Therefore because of widespread prevalence of diabetic metabolic disorder(disease), the present study has investigated the problem of bacteriuria, asymptomatic bacteria, presence or absence of pus cell, sugar levels in diabetic and control patients, and the relationship among all these yardsticks. The present work has also investigated the above parameters in both diabetic men and women patients, from whom the urinary samples were collected for analysis of the parameters listed.In a patient population of 20 women, 10 patients did not produce any pus cell whereas in all other patients pus cells were observed in varying numbers as indicated in Table 1. The patients showing more than 5 pus cells are considered as pyuria patients. (Bamabas Rozsal et al.,2003).The urine samples of the patients were cultured on blood and MacConkey medium.(Monica cheesbrough et al.,1983)The following bacterial species were recorded(Table 1).

1. Escherichia coli
2. Pseudomonas aeuroginosa
3. Klebsiella pneumonia
4. Mycobacterium tuberculosis.

In the bacterial population wherever the number increased over 10^5, the respective bacterium was named as Asymptomatic bacterium.(Gerald Mandell et al.,2000). The presence and absence of bacteria is notified in Table 1 as +or-. There was no correlation between pus cell formation and the occurrence of bacteriuira. This is because patients with nil pus cells also exhibited bacteriuria population. Wherever the pus cells are not present in DM patients, the bacterial population was always found to be in a range of 1.2×10^5 to 1.5×10^5 indicating the bacterial population was lower(Table 2).Similarly the presence and absence of pus cells in a total population of 20 men and 20 women diabetic numbering 40 was worked out and the data are presentation in Fig.1. In women patients out of 20, in 11 cases, pus cells were absent and in 9 patients, pus cells were found to be present. But an opposite version was present in male population. Out of 20 patients, only in one patient, pus cells were found. It is evident from Fig.1 and Table 6 that the presence or absence of pus cells cannot be taken as a criterion for the presence or absence of bacteriuira. This conclusion is arrived at because in normal non-diabetic individuals consisting of males and females, inspite of pus cells being present (2 to 4), no growth of bacteria was observed.(Table 3) This is also evident from Table 6 that in a male population of 20 diabetic patients, in 19 cases no pus cells were observed with no bacterial growth in the culture medium.

In all the cases analysed only one particular kind of bacterium alone was present. No cases were observed where more than 1 bacterium was present. The pathogenic bacteria were identified following various biochemical tests such as indole test, Mannitol motility test, Citrate test, Urease test, Methyl red and Oxidase test.(Table 8) In a population of 20 patients,12 patients were considered as asymptomatic bacterial carriers, where the bacterial colony number exceeded more then 10^5. In one of the cases where higher number of the pus cells was observed to an extent of 20 but no bacterial growth (E.coli,P.aeuroginosa,K.pneumoniae) was observed in Blood and MacConkey agar. But later on this patient was found to be a TB patient carrying M.tuberculosis which was identified by Real-Time PCR. It is reported in literature that if pus cells (pyuria) are present with no pathogenic bacteria (Bacteriuria), patient is prone to Tuberculosis (Geoffrey smith et al.,1990). In one of the non-diabetic Individual(Table 3:S.No:16 Age:62F) although 15 pus cells were observed, culture result was negative and even Real-Time PCR technique could not identify any pathogenic bacteria. Therefore, it is evident from Table 1 that, the presence of pus cells to such a large number as 20,

could be correlated to the occurrence of more severe pathogenic bacterium such as M.tuberculosis. Generally in non-diabetic individuals, inspite of pus cells being present to such a large number as 15, they are devoid of any kind of pathogenic microbes in the urine sample. Tuperculosis is a frequent complication in patients with established diabetes mellitus. Diabetes has 3 to 4 times higher prevalence of Tuberculosis than non-diabetic controls. The common occurrence of bacteriuria in diabetic patients may be attributed to Glucose providing a source of energy for the growth of these urinary pathogenic bacteria.(osullivan et al.,1961)In a total population of 40 diabetic patients, pyuria was observed only in 5 patients, whereas bacteriuria was observed in 13 patients and this clearly reveals that there is no correlation between the occurrence of pyuria and occurrence of asymptomatic bacteriuria.(Fig 2)

In Figure 3, asymptomatic bacteriuria number in diabetic male and female patients is presented. Out of total number of 40 cases (men and women), 13 patients were found to have bacteriuria (bacterial population sxceeding $10^{5)}$ In a female diabetic population of twenty, twelve were found to have bacteriuria , which is a quite higher percentage of occurrence. In male diabetic population of 20, only one bacteriuria case has been observed which is a quite negligible number.Most patients with asymptomatic bacteriuria are women, and are generally in the older age group. Individuals with DM (diabetes mellitus) exhibit a greater frequency and severity of infection. The reason for this increase may be abnormalities in cell-mediated immunity and phagocytic function associated with hyperglycaemia. Hyperglycaemia generally leads in the colonisation and growth of a variety of organisms including Candida and other Fungai species. Urinary tract infections are the result of common bacterial agents such as E.coli and Candida. (Eugene Braunwald et al,) 2001:Stapleton 2002) Infection in diabetes is 2 to 3 times more common in adult diabetic patients than in non-diabetic patients. (Vejlsgaard,1966). Bacterial counts tend to be high in diabetic urine because of increased glucose content (O'Sullivan et al.,1961) From the sample survey made as indicated in Table 1 and Figure 3, distribution of asymptomatic bacteriuria in diabetic patients, a pie-diagram has been prepared as shown in Fig-4, where E.coli occuples the major pathogenic bacterium to an extent of 53.8% and then P.aeuroginosa occupying a percentage of 23.1% and the remaining forms such as K.pneumoniae, M.tuberculosis and Candida species occupying 7.7% each. (Table 4: Plate 2,3a and 3b). The incidence of asymptomatic bacteriuria in diabetic patients is 32.5% from a total population of 40(Table:5).The occurrence was more among women than men as stated earlier. (Fig 3)

A similar attempt was made on men diabetic patients as well as done on women patients. The data are presented in Table 6. Out of 20 DM male patients only one was found to contain pus cells(6) In the urine sample (S.No:3, Age:50) with only Candida species identified in the urine culture. All the other patients did not reveal any pus cell and pathogenic bacteria in the culture.(Table 6 and Plate 4). From the observation made so far, it is quite evident that the number of urinary tract infections in women is 30 times greater than in men. (Lois Bergquist et al.,1998).From the above results, a correlation has been drawn between age group and the occurrence of asymptomatic becteriuria as indicated in Figure 5. It is interesting to note that bacteriuria consists of only of E.coli between the age grounp of 31 and 40 years. In the age group between 40 and 50, the E.coli colony size was reduced and the fungus, Candida albicans made its appearance and in the last age group of 51-60 years. P.aeuroginosa dominance was observed with a rare occurrence of K.pneumoniae in this age group.

In Table-1, one of the patients (S.No:18,Age:36, Sugar level uncontrolled)as briefly mentioned earlier exhibited value of 20 pus cells and the routine culture experiments, indicated no organism and the patient's urine was checked for M.tuberculosis.,(Monica cheesbrough et al., 1983) using Real-Time PCR (Fig:6 and 7) The patient's name was Mrs.Helan from whom the DNA was extracted and purified for amplification. Positive and negative controls were also run along with the isolated and purified DNA from the urine sample. In Real-Time PCR, if the amplification reaches the exponential phase and the curve raised above the threshold level, it signals positive for Mycobacterium and also the exponential phase indicated the amplification of the PCR product.(Mycobacterial DNA).Exponential curve in FAM channel is specific for M.tuberculosis(Fig.6) In Figure 7 the Real- Time PCR is repeated with same DNA of Mrs.Helan. But CYS channel revealed a curve below the threshold level indicating that CYS fluorophore is specific for Mycobacterium other than tuberculosis. Therefore, CYS channel was negative for Mycobacterium tuberculosis. Therefore using the Real-Time PCR, the FAM channel confirms the presence of Mycobacterium

tuberculosis.The Real-Time PCR was repeated on a non-diabetic patient by name Mrs.Noorjahan who had 15 pus cells but no growth was observed in culture media. Her case was resorted through the Real-Time PCR. But no amplification of the microbial DNA was observed employing both FAM and CYS channel, which evidently proved the patient did not harbour any species of Mycobacterium. (Fig 8 and 9)The present observation using Real-Time PCR clearly indicated that diabetic patients are prone to tuberculosis and this is evidently proved by no results in non-diabetic although carrying as many as 15 pus cells but with no evidence for tuberculosis.(Table 3)As another phase of the study, antibiogram of bacteriuria was investigated in E.coli, P.aeuroginosa and K.pneumoniae. The following antibiotics were employed. Among the seven isolated prepared, in E.coli, each group of isolates responded differently to antibiotic sensitivity. The 4 isolates of E.coli listed in Table 7 exhibited susceptibility to Amikacin, Cephotaxime, Chloramphenicol and Nitrofurantoin, whereas to Gentamicin, Ciprofloxacin and Co-Trimoxazole the 4 E.coli isolates revealed resistance by way of noinhibition (Plate 5). A similar result was observed for the single isolate of E.coli in the same Table. As regards P.acuroginosa only the antibiotics Amikacin and Piperacillin susceptibility was observed and to all other antibiotics resistance was exhibited. Regarding the 2 isolates of P.acuroginosa quite surprisingly susceptibility was evident to all the antibiotics (Table 7 and Plate 6 and 7). Lastly regarding K.pneumoniae, to the only isolate prepared susceptibility was observed with Ciprofloxacin and Co-Trimoxazole and to all other antibiotics resistance was observed (Table 7). From the for-going results, it is evident that E.coli isolates, P.acuroginosa isolates prepared from different individuals responded differently to different kinds of antibiotics and even among the same species the different isolated exhibited, different patterns of susceptibility and resistance, as E.coli isolates prepared from different individuals responded differently to different kinds of antibiotics and therefore, doctors will have to exercise caution in prescribing antibiotics after critical evaluation of the culture test against antibiotics in a well established clinical laboratories; otherwise indiscriminate administration of antibiotics will lead to further multiple drug resistance among the bacterial population.

ACKNOWLEDGEMENTS

I am glad to take this opportunity to express my deep gratitude to my guide Dr.Vivekanandan. M.Sc, Ph.D. prof & Head, Dept. of Bio-technology, Bharathidasan University for his inspiring guidance, valuable suggestions, discussions besides his busy schedule and constant encouragement throughout the course of the experiments and completion of this dissertation.I extend my heartfelt thanks to Dr.Rath, Managing Director, Doctors Diagnostic centre, Trichy for giving permission to use the lab facilities and enabled me to complete the work.I sincerely thank Dr. Dhanpaul, Head, Department of Microbiology, KAPV Medical college. Trichy. For the valuable suggestions and giving permission to use the lab facilities and library facilities which was of great use in completion of the project work.

REFERENCES

1. Abu- Bakare A and Oyaide SM.1986. Asymptomatic bacteriuria in Nigerian diabetics.J Trop Med Hyg. Feb;89 (1):29-32
2. Alsbiosu CO, Osinupebi O A and Olajubu F A 2003. Significant asymptomatic bacteriuria among Nigerian type 2 diabetics. J Nati Med Assoc 5:344-349
3. Balasoiu D, Van Kessel KC, Kats-Renaud HJ, collet TJ and Hoepelman AI. 2000 Diabetes care.23 (12) :1737-1741
4. Barnabas Rozsai, Eva Lanyi, and Gyula Soltesz 2003. Asymptomatic Bacteriuria and Leukocyturia in Type 1 Diabetic Children and young Adults, Diabetes care 26:2209-2210
5. Betty Forbes A. Daniel Sahm F and Alice Weisefeld S. 1998. Diagnostic Microbiology 10th ed. Mosby publishers: 351-357.
6. Bonadio Meini M, Gigli C, Longo B and Vigna A. 1999. Urinary tract infection in diabetic patients. Urologia Internationalis. 63(4):215-219

7. Caksen H, Arslan S, Abuhandan M, Celik A, Bozkurt H and Odabas D. 2001Asymotomatic bacteriuria in infants in Eastern Turkey.42(6):338-339
8. Eknoyan G, Qunibi WY 1982 Medicine 61:55
9. Elmer Koneman W, Stephen Allen D, William Janda M and Paul Schreckenberger C.1997. color Atlas and Textbook of Diagnostic Microbiology 5th ed. Lippincott-Raven pulishers:172-185
10. Elokle MA, Hamza SA and Abdel Aziz AA. 2004. Prevalence of asymptomatic bacteruiuria among Egyptian elder persions living in the rural areas. J Egypt Soc Parasitol.34(2):705-712
11. Eugene Braunwald, Anthony Fauci S, Dennis Kasper L and Stephen Hauser L. 2001 Principle of Internal medicine. 15th edition. McGraw-Hill Medical publishing division :1620-1626
12. Geerlings SE Stolk RP, Camps MJ, Netten PM, collet TJ and Hoepelman AI.2000. Risk factors for asymptomatic urinary tract infection in women with diabetes. Diabetes Care 23:1737-41
13. Geerlings SE, Meiland R and Hoepelman IM. 2001. Urinary tract infections in women with diabetes mellitus. Ned Tidschr Geneeskd. 145(38):1832-1836
14. Geerlings SE, stolk RP, camps MJ and Netten M. 2001. Consequences of Asymptomatic becteriuria in women with Diabetes mellitus. Arch Intern Med. 161 :1421-1427
15. Geoffrey Smith R and Charles Easmon S F 1990. Principles of Bacteriology, Virology, and immunity 8th ed. Hodder and Stoughton. London:198-210
16. Gerald Mandell L, John Bennett and Raphael Dolin.2000 Principles and practices of infectious diseases 5th ed. Churchill Livingstone publishers 2 :2301-2323
17. Godfrey Harding KM George Zhanel G, Lindsay Nicolle E and Mary Cheang 2003. Antimicrobial treatment in diabetic women with Asymptomatic bacteriuria Diabetes Care. Jul ;26(7):2209-2210
18. Hermidia Perez JA and Lorro Ferrer JF 2004. Asymptomatic bacteriuria in women. Epidemiological, pathologic and therapeutic study. Arh Esp Urol. Oct;57(8):784-804
19. Higuchi R, Dollinger G, Walsh PS and Griffith R. 1992. Simullaneous amplification and detection of specific DNA sequences. Biotechnology April:10(4):413-417
20. Kass EH. 1955. Asymptomatic infections of the urinary tract. Trans Assoc Am Physicians. 69:56-64.

TABLE: 1 Culture Result of Female Diabetic Patients

No	Age	Blood Sugar Level(mg/dl)	Pus Cell No	Bacterial Population In Urine		ASB$
1	35	C 143	6*	E.coli	2.5×10^5	+
2	39	U 211	3	E.coli	3×10^5	+
3	44	U 231	3	E.coli	3×10^5	+
4	49	U 351	NIL	E.coli	1.5×10^5	+
5	60	U 175	5	Klebsiella pneumoniae	1.3×10^5	+
6	54	U 211	4	P.aeuroginosa	2.6×10^5	+
7	54	C 145	NIL	E.coli	20×10^5	-
8	40	C 140	NIL	E.coli	50×10^5	-
9	39	C 158	6*	E.coli	3×10^5	+
10	48	C 151	NIL	NIL	NIL	-
11	36	C 145	6*	E.coli	2.3×10^5	+
12	59	C 155	NIL	NIL	NIL	-
13	55	C 160	NIL	NIL	NIL	-
14	50	C 150	NIL	NIL	NIL	-
15	60	C 159	NIL	NIL	NIL	-
16	52	U 352	4	P.aeuroginosa	2.5×10^5	+
17	49	U 351	NIL	E.coli	1.2×10^5	+

18	36	U 351	20*	Mycobacterium tuberculosis	RT PCR +	+
19	58	U 292	NIL	P.aeuroginosa	1.5×10^5	+
20	52	U 300	NIL	NIL	NIL	-

$ASB: Asymptomatic bacteriuria:whose number$\times10^5$ greater.
The data are average of two different experiments.
C: Control diabetic: (140-160mg/dl) U: Uncontrolled diabetic: (160mg/dl>)
*Pyuria cases :where the pus cells exceeded a number of 5 (5>)

TABLE: 2 Relationship Between Presence Of Pus Cells And Bacteria In Urine Among The Diabetic Patients

No	No of Pus Cells	Blood Sugar Level	Bacteria	Colony Count(CFU)
1	6	C	E.coli	2.5×10^5
2	3	U	E.coli	3×10^5
3	3	U	E.coli	3×10^5
4		U	E.coli	1.5×10^5
5	6	C	E.coli	3×10^5
6	6	C	E.coli	2.3×10^5
7		U	E.coli	1.2×10^5
8	4	U	P.aeuroginosa	2.6×10^5
9	4	U	P.aeuroginosa	2.5×10^5
10		U	P.aeuroginosa	1.5×10^5
11	5	U	K.pneumoniae	1.3×10^5
12	20	U	No growth in culture	M.tuberculosis Real time PCR positive
13	6	U	Candida albicans	3×10^5

C-Control diabetic (140-160mg/dl) U-Uncontrolled diabetic (160mg/dl)

TABLE: 3 Culture Results Of Normal Individuals

Sl No	Age	Sex	Pus Cell	Culture Result
1	61	F	3	NO GROWTH
2	63	M	2	
3	34	M	3	
4	36	F	3	
5	38	F	4	
6	54	M	3	
7	45	M	2	
8	56	M	3	
9	62	M	2	
10	45	M	2	
11	49	M	2	
12	39	M	3	

13	60	M	3	
14	47	M	2	
15	50	F	2	
16	62	F	15	
17	58	F	3	
18	38	F	2	
19	36	F	2	
20	45	F	2	

M-Male F-Female

Table 4: Isolated Asymptomatic Bacteriuria from Diabetic Patients

Total Number of Patients Analysed (Urine Samples)	Numebr of Patietns With ASB*	Number of Patients Carryung the Bacteria		Percentage
40	13	E.coli	7	53.8%
		P.aeuroginosa	3	23.1%
		K.pneumoniae	1	7.7%
		Candida albicans	1	7.7%
		Mycobacterium tuberculosis	1	7.7%

*ASB: Asymptomatic bacteriuria

TABLE 5: Incidence of Asymptomatic Bacteriuria in Diabetic Patients

Total Number of Patients Analysed	Number of Positive Patients	Patients Harbouring Bacteriuria		Incidence of Asymptomatic Bacteriuria Among Patients(%)
40	12 (women) 1 (Men)	E.coli	7	32.5%
		P.aeuroginosa	3	
		K.pneumoniae	1	
		Candida albicans	1	
		Mycobacterium tuberculosis	1	

TABLE 6: Culture Results of male Diabetic Patients

Sl. No	Age	Blood Sugar Level(Mg/Dl)	Pus Cell	Culture Result
1	30	C 150	NIL	NIL
2	35	U 300	NIL	NIL
3	50	U 351	**6**	Candida albicans(3×10^5)
4	52	U 292	NIL	NIL
5	60	C 150	NIL	NIL
6	58	U 391	NIL	NIL
7	54	U 211	NIL	NIL
8	52	U 214	NIL	NIL
9	40	C 145	NIL	NIL
10	43	C 150	NIL	NIL
11	50	C 155	NIL	NIL
12	55	C 140	NIL	NIL
13	60	U 220	NIL	NIL

14	50	U 300	NIL	NIL
15	48	U 380	NIL	NIL
16	47	C 150	NIL	NIL
17	32	C 155	NIL	NIL
18	39	C 149	NIL	NIL
19	60	U 383	NIL	NIL
20	40	C 156	NIL	NIL

C: Control diabetic: (140-160 mg/dl) U: Uncontrolled diabetic : (160 mg/dl>)

Table 7: Antibiogram of Bacteriuria

A: Escherichia coli

Organism E.coli	Antibiotics Diameter of inhibition (mm)						
	Ak	Ce	C	Nf	G	Cf	Co
4 Isolates	19 S	29 S	22 S	18 S	NIL R	NIL R	NIL R
2 Isolates	16 S	6 R	NIL R	NIL R	3 R	NIL R	NIL R
1 Isolates	16 S	NIL R	13 R	NIL R	NIL R	NIL R	NIL R

B.Pseudomonas acuroginosa

Organism P.aeuroginosa	Antibiotics Diameter of inhibition (mm)						
	G	Tb	Ca	Cb	Ak	Ce	Pc
1 Isolate	NIL (R)	12 (R)	NIL (R)	13 (R)	20 (S)	10 (R)	18 (S)
2 Isolates	15 (S)	15 (S)	18 (S)	17 (S)	17 (S)	23 (S)	18 (S)

C:Klebsiella pneumoniae

Organism K.pneumoniae	Antibiotics Diameter of inhibition (mm)						
	A	Ak	G	Nx	Cf	Co	C
1 Isolate	13 R	14 R	12 R	10 R	21 S	16 S	10 R

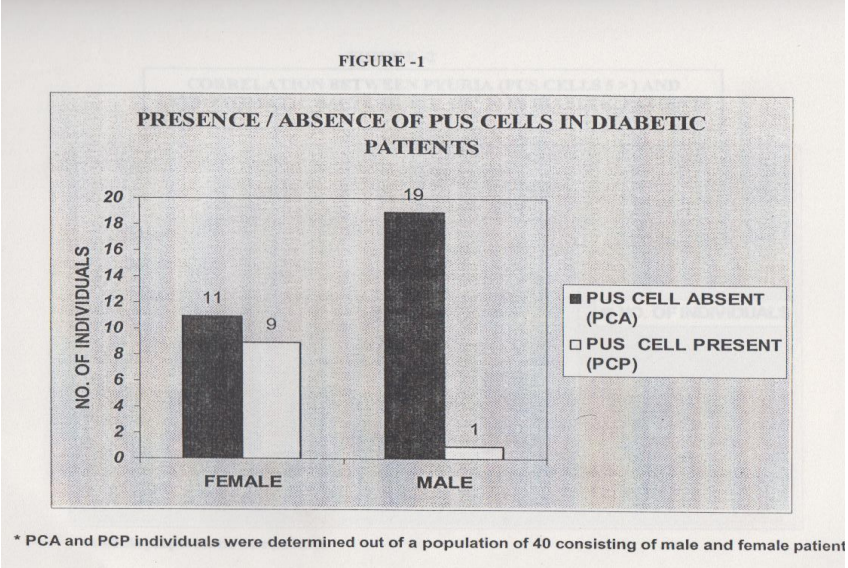
Antibiotics: Ak-Amikacin Ce-Cephotaxime C-Chloramphenicol NF-Nitofurantoin G-Gentamicin Cf-Ciprofloxacin Co-co-Trimoxazole Tb-Tobramicin Ca-Ceftazidime Cb-Carbencillin Pc-Piperacillin A-Ampicillin Nx-Norfloxacin S-Sensitivity (inhibition positive)
R-Resistance (No inhibition) Number indicate diameter of inhibition in mm

Table 8: Biochemical Test

Organisms	Indole	Mannitol Motility	Citrate	Urease	TSI	Methyl Red	Oxidase
E.coli	+	+	-	-	A/A Gas+,H_2S-	+	-
P.aeuroginosa	-	-	+	-	K/K Gas,H_2S-	-	+
Klebsilla .pneumoniae	-	+	+	-	K/A Gas+,H_2S-	--	-

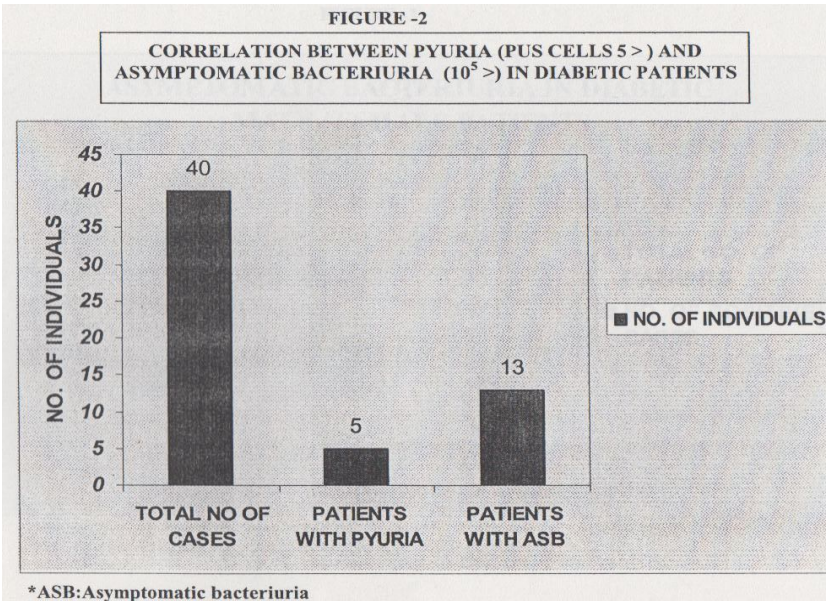
+ Positive, - Negative A/A- Acid slant/ Acid deep K/K-Alkaline slant/ Alkaline deep K/A-Alkaline slant/ Acid deep

FIGURE -1



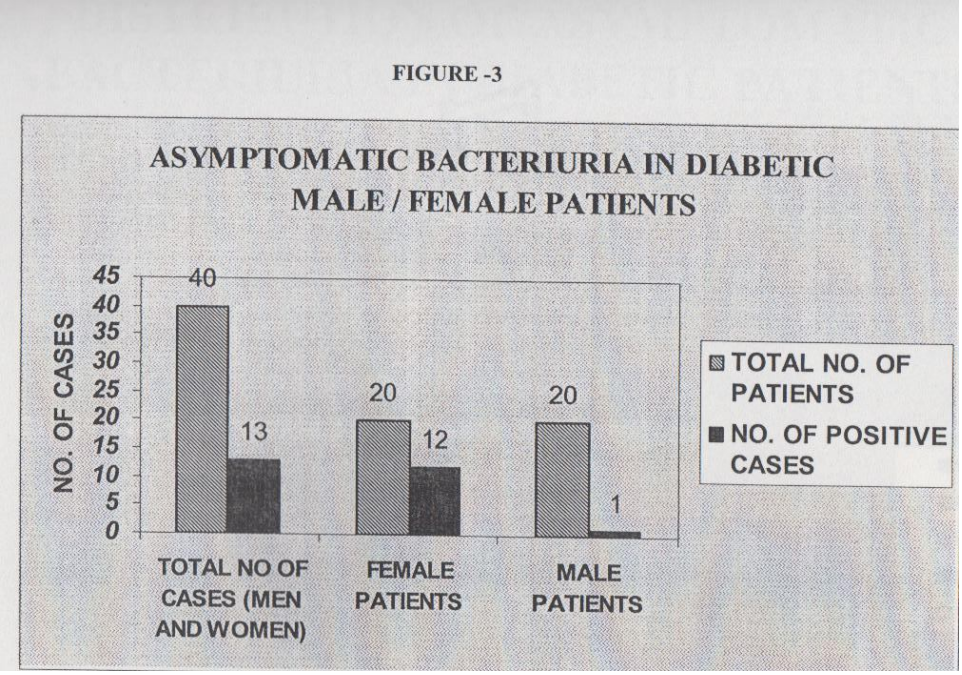
* PCA and PCP individuals were determined out of a population of 40 consisting of male and female patient

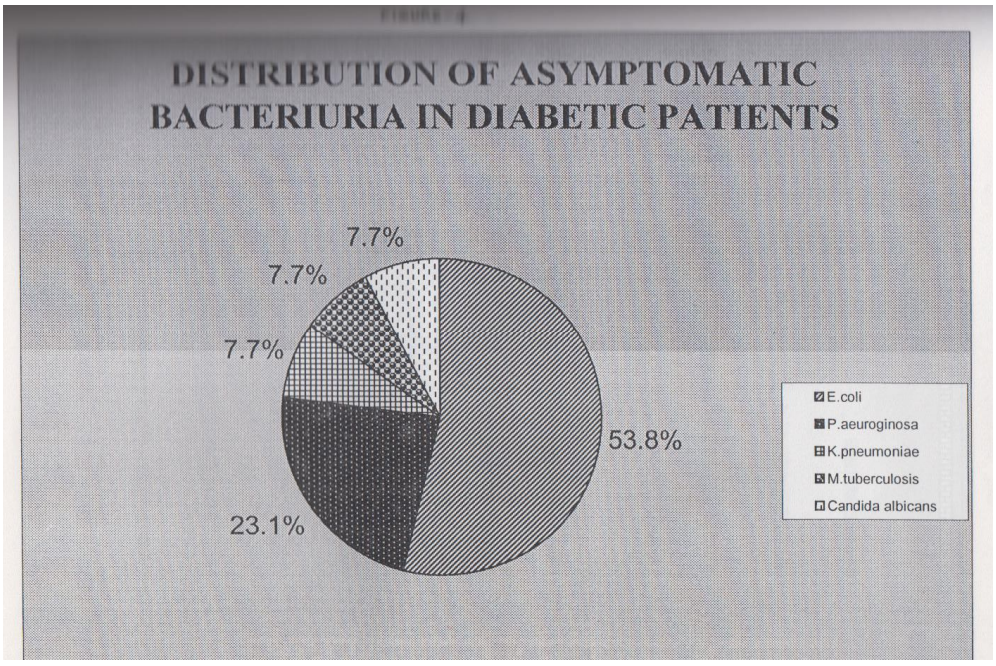
FIGURE -2

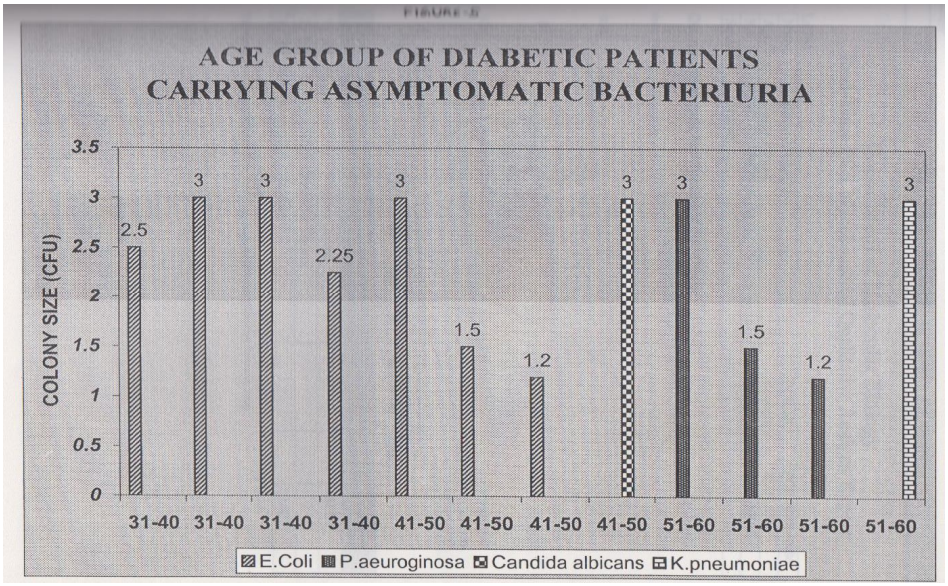


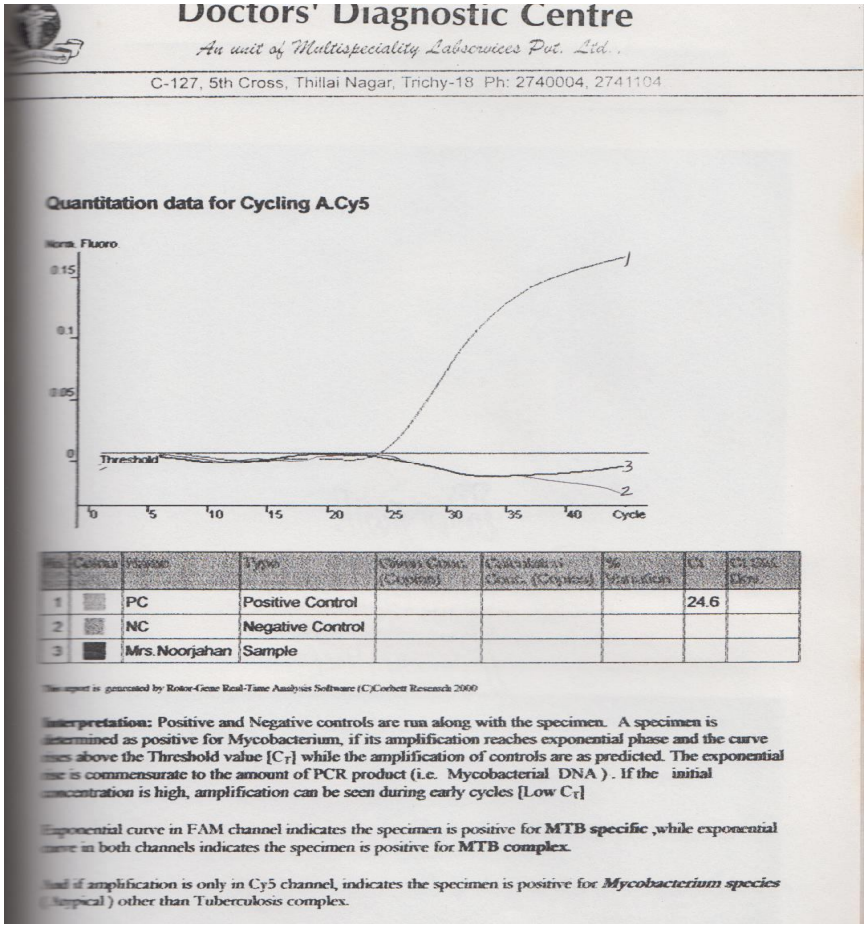
*ASB:Asymptomatic bacteriuria

FIGURE -3


ASYMPTOMATIC BACTERIURIA IN DIABETIC MALE / FEMALE PATIENTS
NO. OF CASES
45
40
35
30
25
20
15
10
5
0
40
13
20
12
20
1
TOTAL NO OF CASES (MEN AND WOMEN)
FEMALE PATIENTS
MALE PATIENTS
TOTAL NO. OF PATIENTS
NO. OF POSITIVE CASES


DISTRIBUTION OF ASYMPTOMATIC BACTERIURIA IN DIABETIC PATIENTS
7.7%
7.7%
7.7%
53.8%
23.1%
E.coli
P.aeuroginosa
K.pneumoniae
M.tuberculosis
Candida albicans





Doctors' Diagnostic Centre

An unit of Multispeciality Labservices Pvt. Ltd.

C-127, 5th Cross, Thillai Nagar, Trichy-18 Ph: 2740004, 2741104

Quantitation data for Cycling A.Cy5

Norm. Fluoro

0.15
0.1
0.05
0
Threshold
3
2
0 5 10 15 20 25 30 35 40 Cycle

No.	Colour	Name	Type	Given Conc. (Copies)	Calculated Conc. (Copies)	% Variation	Ct	Ct Mean
1		PC	Positive Control				24.6	
2		NC	Negative Control					
3		Mrs.Noorjahan	Sample					

The report is generated by Rotor-Gene Real-Time Analysis Software (C)Corbett Research 2000

Interpretation: Positive and Negative controls are run along with the specimen. A specimen is determined as positive for Mycobacterium, if its amplification reaches exponential phase and the curve rises above the Threshold value [C_T] while the amplification of controls are as predicted. The exponential rise is commensurate to the amount of PCR product (i.e. Mycobacterial DNA). If the initial concentration is high, amplification can be seen during early cycles [Low C_T]

Exponential curve in FAM channel indicates the specimen is positive for **MTB specific** ,while exponential curve in both channels indicates the specimen is positive for **MTB complex**.

Lack of amplification is only in Cy5 channel, indicates the specimen is positive for ***Mycobacterium species*** (Atypical) other than Tuberculosis complex.

Plate 1
Mode of collection of urine sample

Plate 2
Photograph showing one of the isolates of *E.coli*

Plate 3a
Photograph showing one of the isolates of *Pseudomonas aeuroginosa* on blood agar

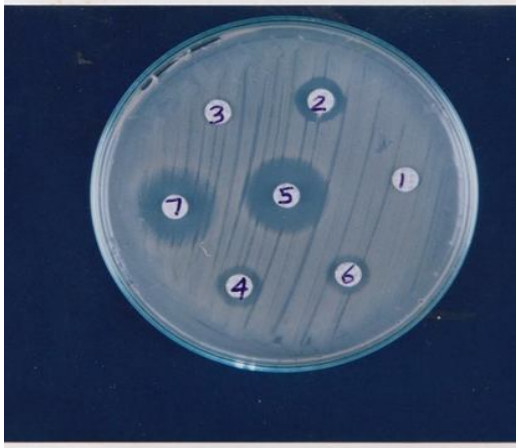
Plate 3b
Photograph showing one of the isolates of *Pseudomonas aeuroginosa* on MacConkey agar

Plate 4
Photograph showing *Candida albicans* in Sabouraud agar medium

Plate 5
Antibiogram assay in different isolates of E.coli

1 – Amikacin
2 – Cephotaxime
3 – Chloramphenicol
4 – Nitrofurantoin
5 – Gentamicin
6 – Ciprofloxacin
7 – Co-trimoxazole

Plate 6
Antibiogram assay in the isolate of *Pseudomonas aeur*



1 – G...
2 – Tobramicin
3 – Ceftazidime
4- Carbenicillin
5 – Amikacin
6 – Cephotaxime
7 – Piperacillin

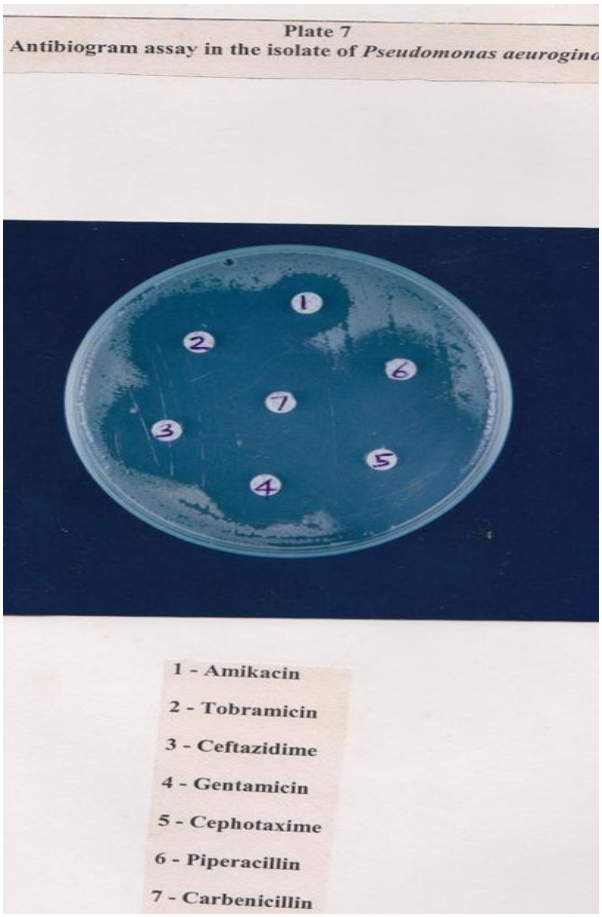


Plate 7
Antibiogram assay in the isolate of Pseudomonas aeurogino
1
2
3
4
5
6
7
1 - Amikacin
2 - Tobramicin
3 - Ceftazidime
4 - Gentamicin
5 - Cephotaxime
6 - Piperacillin
7 - Carbenicillin