MOHAMED SATHAK COLLEGE OF ARTS AND SCIENCE

(Affiliated to the University of Madras, Accredited with NAAC)

PG AND RESEARCH DEPARTMENT OF BIOTECHNOLOGY

SHOLINGANALLUR, CHENNAI- 600 119

PROCEEDINGS

NATIONAL CONFERENCE ON EMERGING TRENDS IN MANAGEMENT OF INFECTIOUS DISEASES AND PUBLIC HEALTH (ETMIDPH 2016)

ISBN: 978-81-931973-6-3

Organised by

PG AND RESEARCH DEPARTMENT OF BIOTECHNOLOGY

DATE: 4 - 5 FEBRUARY 2016

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OF
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Janab Alhaj S.M. Mohamed Yousuf
Chairman
Mohamed Sathak Trust

January 28, 2016

Foreword

My hearty congratulations for this souvenir published about "Emerging Trends in Management of Infectious Diseases and Public Health" – ETMIDPH 2016 on the occasion of Silver Jubilee celebrations of Mohamed Sathak College of Arts and Science . This will certainly kindle and illuminate the young sparkling minds. Research abstracts on many areas of Biotechnology have been scrutinized and reviewed with utmost care.

This souvenir covers the current concepts of Biotechnology like nanomedicine, which is the medical application of nanotechnology, Medical Bioinformatics, Diagnosis and various other fields to improve the quality of research in Biotechnology. The information contained in this souvenir will provide a medium for dissemination of knowledge among scientists.

I wish the conference all success.

Chairman

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S.M.H. Sharmila
Secretary
Mohamed Sathak Trust

January 28, 2016

Foreword

I am very happy to release this souvenir on the Silver Jubilee celebrations of Mohamed Sathak College of Arts and Science. The National Conference "Emerging Trends in Management of Infectious Diseases and Public Health" – ETMIDPH 2016, on 4th and 5th February 2016, is a dedicated compilation of many scientists, research scholars and faculty members.

This conference integrates many disciplines of Biotechnology to a central theme of Infectious diseases, which is now a major area of concern in today's world. Modern and recent techniques like Medical Bioinformatics, Diagnosis, Herbal Biotechnology and Vector control have been included. The Souvenir will definitely have an impact on young scientists for future research.

I wish Department of Biotechnology for their great and successful work.

Sharmila

Secretary

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Janab S.M.A.J. Abdul Haleem
Director

January 28, 2016

Foreword

I take great pleasure to wish the Department of Biotechnology for the excellent souvenir published on the occasion of National conference on "Emerging Trends in Management of Infectious Diseases and Public Health" – ETMIDPH 2016 in this Silver Jubilee year. This souvenir is packed with a lot of research abstracts which are very useful to scientists, research scholars, faculty members and students. The topics covered in this souvenir reflect the current scenario about management of infectious diseases which are helpful to the society at large.

Professionals from different areas of biological sciences have come together to discuss valuable, useful and informative ideas in the single platform, to arrive and solve many issues related to Biotechnology.

I wish everyone who have accomplished this task a great future.

Director

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Date : January 28, 2016

Foreword

The souvenir published about "Emerging Trends in Management of Infectious Diseases and Public Health" – ETMIDPH 2016 on the occasion of Silver Jubilee celebrations of Mohamed Sathak College of Arts and Science is a tremendous work of the Department of Biotechnology. It contains a lot of research articles which are very useful to the scientific community.

The content deserves great appreciation as it contains articles on Herbal Biotechnology, a thrust area for research, Marine Biotechnology for the development of Marine natural products, and various novel areas with many contributions. This conference will provide an opportunity of intensive learning on many areas of Biotechnology to develop useful products and applications.

I wish everyone who have contributed to this souvenir a grand success.

Dr.MAJOR M.JAILANI,
DEAN

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28-01-2016

MESSAGE

In commemoration of silver jubilee celebrations of our college, I am delighted to associate with ETIMPDH -2016 conference which brings together academics and Industrial experts, researchers and students from around the country.

The conference features 8 sessions of special invited lectures on emerging trends in management of infectious disease and public health.

The conference arrangements were handled with diligence and creativity by the Post Graduate and Research Department of Biotechnology, deserves sincere appreciation for organising this conference in fantabulous manner.

I hope that you will find the conference informative and enjoyable, that you will take the opportunity to share and enrich your knowledge and that will have a great day & stay in our college.

Dr. M. Abul Hasan Sathali

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28-01-2016

MESSAGE

It is my sincere pleasure to write foreword for this souvenir published on the occasion of Silver Jubilee celebrations of Mohamed Sathak College of Arts and Science which coincides with the Department of Biotechnology conducting National Conference on "Emerging Trends in Management of Infectious Diseases and Public Health" – ETMIDPH 2016.

It contains a concordant view about the infectious diseases and their management, written by scientists, research scholars and faculty members. The deliberations of this conference will help the young scientists to understand the various areas of Biotechnology like Environmental Biotechnology, Herbal Biotechnology, Marine Biotechnology and Medical Bioinformatics, which will help the young scientist to understand and make a more focused effort in undertaking research which will result in alleviating many problems plaguing the world.

My wishes to the staff and students of Biotechnology for their exemplary work.

Dr. R. Meganathan

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Tamil Nadu Scientist Awardee
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23.1.2016

Message

I am happy to know that PG & Research Department of Biotechnology is going to organize a national conference on "Emerging trends in management of Infectious diseases and public health ETMIDPH 2016" to be held during February 4 & 5, 2016 in Mohamed Sathak College of Arts and Science at Chennai.

Infectious diseases are disorders caused by bacteria, viruses, fungi or parasites. Many organisms live in and on our bodies. They are normally harmless or even helpful, but under certain conditions, some organisms may cause disease. Some infectious diseases can be passed from person to person.

Infectious diseases are ever growing threat to the well-being of organisms including human. The infectious diseases are likely to increase many-fold by climate change impacts. Due to global warming, human disease, such as malaria, dengue, influenza, diahorrae, cancer, cholera, bacterial and viral diseases besides malnutrition are increasing. In this regard, the biotechnological and microbiological tools are increasingly helpful in identification and diagnosis of the diseases and in discovery of novel drugs from natural sources to treat the diseases efficiently. It is of importance to manage the infectious diseases most efficiently and to tackle the problem of antibiotic resistance among pathogens through advanced research efforts.

My hearty congratulations are due to the sincere effort of Dr. M. Syed Ali, Head of the Department and his team, in organizing the national conference of current importance to India.

I wish the conference all success

Prof. Dr. K. Kathiresan
Dean & Director

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Dr.S.Ravikumar
Professor

MESSAGE

In the past sixty years, antibiotics have been critical to fight against infectious disease caused by bacteria and other microbes. Antimicrobial chemotherapy has been a leading cause for the dramatic rise of average life expectancy in the 21th century. However, disease causing microbes that have become resistant to antibiotic drug therapy are an increasing public health problem. The main problem is that bacteria and other microbes have developed several ways to resist antibiotics and other antimicrobial drugs. Now-a-days, about 70% of the bacteria that cause infections in hospitals are resistant to at least one of the drugs most commonly used for treatments and some organisms are resistant to all approved antibiotics. An alarming increase in resistance of bacteria forced as to find out new antibacterial agents. I understood from the conference title that, the Department of Biotechnology, Mohamed Sathak College of Arts and Science, Sholinganallur , Chennai has a long vision to explore novel and drug wonders from natural resources. Before step in to the new venture it is very good effort to organize the National conference by inviting several experts in this research to have a better arena to discuss about the future prosperity of human being by taking effort to explore novel drugs. I congradulate the Organising secretary for choosing this title to hold a conference for saving the excellent creature of human being. I wish the conference for very fruitfull deliberations and bringout with novel recommendations and success.

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PG AND RESEARCH DEPARTMENT OF BIOTECHNOLOGY
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ABOUT THE INSTITUTION

Mohamed Sathak College of Arts and Science (MSCAS) is a Self-financing college affiliated to the University of Madras. It was started in the year 1991 by Mohamed Sathak Trust, a public, charitable and educational body established in 1973 by the Philanthropic Mohamed Sathak family of Kilakarai, Ramanathapuram district. The College has been recognized by UGC and accredited by NAAC with B^+ Grade in 2004. At present the college offers 17 Under Graduate programmes, 16 Post graduate programmes, 3 Post Graduate Diploma programmes and 3 Ph.D., programmes. This year MSCAS is celebrating Silver Jubilee.

The College is situated in pleasant surroundings in the midst of inspiring green fields of Rajiv Gandhi Salai (OMR). The enchanting campus has large playgrounds with international standards, separate hostels for Men and women, a branch of IOB, centralized library with all modern facilities and a collection more than 20,710 volumes of books, 71 journals, 181 online journals and periodicals.

ABOUT THE BIOTECHNOLOGY DEPARTMENT

The Department of Biotechnology, established in the year 2001 with M.Sc programme and B.Sc from the year 2005 and upgraded to Research Department (M.Phil) in the year of 2009 and Ph.D from the academic year 2011. An innovative PG Diploma in Gene manipulation technology is also offered by the Department. The Department is actively engaged in research on Bioremediation, Bioprospecting, Nanotechnology, Micropropagation, Enzyme technology, Secondary metabolite production from plant and microbial sources etc., Faculty members have been regularly presenting papers in International conferences and publishing papers in International and National journals with high impact factor. The students of the department are securing University ranks every year.

The Department has signed an Memorandum of Understanding (MoU) with the prestigious premier research and development organisation of our country Indian Institute of Crop Processing Technology (IICPT), Thanjavur under the ministry of Food Processing Industries, Government of India to facilitate the research activities of the Department.

ABOUT THE CONFERENCE

The main objective of the National Seminar on Emerging Trends in Management of Infectious Diseases and Public Health (ETMIDPH 2016) is to explore advances in Biotechnological and Microbiological research in disease management across the Globe. There is an alarm over emerging and re-emerging diseases which has resulted in a number of national and international initiatives to restore and improve surveillance and control of communicable diseases. In response to this, WHO urged all its Member States to strengthen surveillance on infectious diseases in order to promptly identify emerging diseases and also to discover new molecules from marine sources. There is a need of bringing scientists, experts and academicians together on a common platform to share their knowledge, innovative ideas and approach towards tackling the challenges that creep every now and then in a biotechnology-based society. Keeping this in view, the proposed seminar is highlighted with various plenary lectures and poster presentations sessions on thematic areas and it will cover a wide array of topics from the field of current status, prospects and challenges in management of infectious diseases

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PREFACE

Infectious diseases are disorders caused by microorganisms- such as bacteria, viruses, fungi or parasites. Many organisms live in and on our bodies. They are normally harmless or even helpful, but under certain conditions, some organisms may cause disease. Some infectious diseases can be passed from person to person. Infectious diseases, including HIV/AIDS, tuberculosis, malaria, polio, and several neglected tropical diseases (NTDs) are easily spread through personal contact, water, and air, (many NTDs are vector borne transmitted by mosquitoes, flies, etc) and are a particularly significant problem in developing countries. In the past, infectious diseases have been widespread in developing countries and chronic diseases were found primarily in high income countries. However, the global pattern of disease burden is shifting.

Viral Hepatitis, Influenza, and Tuberculosis (TB) remain among the leading causes of illness and death in the United States and account for substantial spending on the related consequences of infection. The infectious disease public health infrastructure, which carries out disease surveillance at the Federal, State, and local levels, is an essential tool in the fight against newly emerging and re-emerging infectious diseases. Other important defenses against infectious diseases include: Proper use of vaccines, Antibiotics, Screening and testing guidelines, scientific improvements in the diagnosis of infectious disease-related health concerns.

Today's infectious disease challenges are broader and more complex than they were in 1998, when CDC last issued a comprehensive plan to guide national efforts to prevent and control emerging infectious threats. Since then, new microbes or new forms of old ones have been discovered nearly every year, and infectious disease outbreaks triggering international responses have been reported on nearly every continent. While our changing, globalized world has provided increased opportunities for emergence and spread of infectious diseases, it has also brought significant advances toward their control. The ID Framework takes into account many of the scientific, demographic, technological, and economic developments currently modifying efforts to protect public health, challenging us to rethink our processes and strategies and take advantage of new ways to prevent disease and improve health. Emerging infectious diseases may be considered of public health importance based on a variety of criteria, including their designation as a emerging disease. It may be considered of public health importance based on a variety of criteria, including their designation as an emerging disease by international, federal, and/or provincial health authorities; their potential for preventability or public health action; and the seriousness of their impact on the health of the population and potential spread.

With this background, the present conference will definitely help to come to know the advances and various strategies used to tackle the challenge of infectious diseases.

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BIO-PROSPECTING OF MARINE MICROORGANISMS FOR FOOD AND MEDICINE FOR FUTURE PROSPERITY

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Diverse group of marine microorganisms are yet to be explored due to lack of isolation and identification efforts by the marine biologists. The photosynthetic and non photosynthetic microorganism particularly, the associated microorganism from seaweeds, seagrass and sponges have recently been identified as a potential source of organisms for the exploitation of bioactive principles. Technologies available for the extraction of bioactive compounds from marine microorganisms are not the same for marine microorganism as they are all the pure marine forms. Standardisation of methodologies for the isolation and identification of microorganism from marine resources, extraction of bioactive compounds, hurdles for the mass production of bioactive compounds by continuous and batch fermentation process will be discussed. Standardisation of drugs from marine microorganisms for drug development will be discussed. Besides that, a modified method of extraction of herbal salt from the marine salt intruders (mangrove plants) over traditional Indian methods will also be highlighted and their bioactive potential for the drug development will also be discussed. Moreover the bio potential of extremophilic microorganisms such as Halobacteria and solar saltern Cyanobacteria for the treatment of wastes from coconut retting waste water and water discharge from the industries with cyanide and metal contamination and the methods standardized for the mathematical kinetic modeling will be discussed. Moreover recent effort on the "Impact of ocean acidification on the marine Drug Loss", the current research on the identification of potential drugs for the treatment of Malaria and further the enhancement of some beneficial microorganisms in the open ocean through bio inoculation with the mangrove root associated microorganism will also be discussed in detail at that time of conference.

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VECTOR BORNE DISEASES: DEVELOPMENT OF RISK PREDICTION MODELS, USING RS - GIS TECHNOLOGIES

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While on the one side our country is proud of major achievements in science and technology including space and our march towards a knowledge society, it is true on the other side that a large proportion of our population has no access to even safe drinking water, to cite one of the major problems facing the country. The continued practice of open drainage system, indiscriminate disposal of water and industrial effluents into water bodies, and added to this, the increased migration of people from rural areas have resulted in large slums in our urban centres creating an environment unsuitable for healthy living, thus aggravating the spread of water borne diseases. Yet another area of concern to the country is the spread of vector-borne diseases (VBDs) such as malaria, filariasis, Japanese encephalitis and dengue to newer areas with mosquitoes, the vectors carrying these diseases breeding in water bodies. The World Health Organization (WHO) and other international bodies highlight the threat posed by these VBDs to the world's population in general and to India in particular. It is said that in India alone, over two million cases of VBDs are reported every year. VBDs are spreading to newer areas due to increased risk of transmission fuelled by changing climatic conditions, developmental activities, more specifically the urbanization and industrialization along with demographic changes to name a few possible causes. With advanced knowledge on the principles underlying the disease transmission dynamics, the prediction of areas of health risk is possible based on geo-environmental factors. Modern tools like Remote Sensing (RS) and Geographical Information Systems (GIS) have now come in handy to address the issues on health risk, and predicting the trend in disease prevalence for undertaking intervention measures. Mosquito vectors thrive on water, vegetation and dwellings (with the availability of vertebrate host). Study of mosquito population dynamics with the change of environmental variables helps in understanding the criticality of those variables. Satellite remote sensing technique along with GIS enable surveillance of environmental conditions for vector development and disease transmission providing information on epidemiology of a region, viz. favourable ecological conditions, habitat types providing breeding sites and their characterization, prevailing disease, past history of epidemics and environment and social and economic factors associated with the epidemics. Major factors such as climate, landscape and developmental activities responsible for risky conditions are being studied with the help of remotely sensed data analysis. The satellite imagery is being used to explain these variables on a desired spatial and temporal scale, and GIS facilitates acceptance of satellite information, fit it to a vector mosquito model and produce imagery, indicating the areas of risk of transmission of VBDs. Our responsibility in the immediate future should be to provide technical information on these, facilitate formulation of policy statement, preparation of strategic plan, ease advocacy steps at different stages and foster effective linkages with all partners.

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INSECTS AND MICROBES: FRIENDS OR FOES?

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Insects (Phylum Arthropoda; Class Insecta) are one of the most diverse groups of living creatures on Earth. First appeared in the late Carboniferous period about 300 million years ago, they have subsequently radiated into a group that now includes about 850,000 to several million species. Some 80–95% of insect species have yet to be collected, named and described, most of them living in the tropics. Even for the 850,000-plus species that have been named, we know little about how they are distributed or what they feed on. Equally diverse are the feeding habits and behaviors exhibited by the group as a whole, and there is virtually no terrestrial food source that escapes exploitation by one or more species. However one property shared by all insects is their common association with microorganisms. The associations range from loose and nonspecific ones, in which the insect merely serves as an inadvertent carrier and distributor of microbes, to much tighter, highly interdependent and remarkably regulated symbiotic interactions, and they include literally all gradations in between these extremes. Microbes on the other hand still more diversified than insects and the only group on the earth which can surpass the insects in diversity. Microorganisms include prokaryotes (a cell characterized by the lack of a distinct membrane-bound nucleus) such as bacteria and archea and eukaryotes (cells whose chromosomes are contained within a membrane-bound nucleus) such as fungi and protozoa. For over 3.8 billion years, these organisms have formed the foundation of the biosphere, surviving in extremes of heat, cold, radiation, pressure, salt, acidity, and darkness. For 2 billion years microbes were the only forms of life on Earth. During this long history, all of the basic biochemistries of life evolved, and all life forms have developed from these microbial ancestors. With their ability to harvest energy in almost any form, and thrive with or without oxygen, microbes have spent over a billion years making nitrogen available to plants while transforming the atmosphere with oxygen. Microbes are found throughout the entire planetary ecosystem including niches where higher animal species are rare or absent (e.g. the ocean depths, the planet's subsurface, thermal and polar environments, and oxygen-free environments). This wide ecological range reflects their vast metabolic capabilities that allow different microbial species to inhabit different environments.

Current evidence suggests that perhaps 1.5 million species of fungi exist yet only 5 % are described. For bacteria there may be 300,000 to 1 million species on earth yet only 5000 bacteria are described. A gram of typical soil contains about 1 billion bacteria, but only 1 % of those can be cultured. Hence, most microbes remain to be discovered. Estimates suggest that up to 99% of microbes could not be cultured in a laboratory using conventional methods. Detection and characterization could be achieved only by Culture-independent techniques, including sequencing of the 16S rRNA gene, a

relatively recent methodology adopted by microbial taxonomists. Microbes provide the fundamental underpinning of all ecosystems. Without microorganisms, alllife on earth would cease to exist. Insects have a delicate and intricate set of relationships with a microbial world of astonishing diversity. All insect species are known to harbour a rich and complex community of microorganisms in their guts and other body regions. This microbiota participates in many types of interactions ranging from prey & predator and pathogenesis to obligate mutualism. One reason for the microbial diversity is that different groups of insects have different feeding habits; this results in different gut structures and functions and promotes the establishment of different phylotypes. In recent years there has been renewed interest in the understanding of insect gut microorganisms for two reasons. First, this diverse microbiota is a potential source of novel bioactive compounds such as antimalarial, antiviral and antitumour peptides, enzymes and novel metabolites. Second, manipulating these microbial symbionts is thought to be an effective strategy for controlling the spread of pathogens that use insects as hosts.

Early studies revealed the often striking anatomical and behavioral adaptations of insects to harbor the microbial partners in, on, or around them and to ensure transmission of microbial symbionts to their offspring. The distinct microbial symbionts or communities of symbionts were common and often essential in insects that feed on restricted and or relatively refractory food resources. Such diets are often deficient in nutrients such as amino acids (eg. Plant sap) or vitamins (eg. Animal blood), and lignocellulosic plant material is not only poor in nitrogenous compounds, vitamins, and sterols, but it is also difficult to digest. Symbiotic microbes are providing such missing nutrients or digestive enzymes to the insect host. However, microbial symbionts are also involved in numerous other aspects of insect biology eg. Detoxification of plant defensive secretions; production of insect behavior modifying compounds; protection against microbial pathogens and pest; and alteration of host reproductive patterns. Moreover, the microbial biomass itself is consumed by a large group of insects as well as its larval forms as their main source of food. On the other hand, natural population of insects is kept under check by the activities of parasites and predators. Several species of viruses, bacteria, fungi, protozoa and nematodes are known to cause infection in insects. The positive and negative association of insect and microbes will be discussed in detail.

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LEPROSY - A NEGLECTED TROPICAL DISEASE

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Leprosy or Hansen's disease is a Neglected Tropical Disease (NTD) caused by *Mycobacterium leprae* and is the leading infectious cause of permanent physical disability. A chronic disease, Leprosy continues to be a major challenge to public health in several countries of the world including India. WHO recommended multi drug therapy is an effective intervention strategy against leprosy and its implementation in India through the National Leprosy Eradication Programme (NLEP) has immensely contributed to significant decline in trend of leprosy prevalence and annual new case detection rates (ANCDR). The presentation will encompass important aspects of historical and current research on leprosy in India.

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Phytopharmacological profile and Brine Shrimp lethality assay of the Methanolic and Ethanolic extracts of the leaf and bark of *Symplocos cochinchinensis.*

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Abstract
Cancer is one of the deadliest diseases in the world. Researchers all over the world are in constant search for new drugs to cure cancer. Traditional medicinal practices have utilized various plant sources for the treatment of cancer. Medical scientists all over the world have started exploring the traditional medicinal practices to prepare new formulations to treat cancer. The present study is one such approach. The cytotoxicity of the methanolic and ethanolic extracts of the leaf and stem powder of *Symplocos cochinchinensis* against brine shrimp larvae is done for the first time. It was found that the ethanolic extracts of the leaf showed 100% mortality at 2 mg/ml concentration in the 24th hour. The methanolic and ethanolic extracts of the leaf and bark showed 100% mortality at 3 mg/ml concentration in the 24th hour. The methanolic and ethanolic extracts of *Symplocos cochinchinensis* showed the presence of various phytoconstituents like phenols, flavonoids, tannins, saponins and glycosides. The methanolic extract of the leaf has the maximum phytoconstituents viz. Total phenolic content of 13.67$\pm$0.29 mgTAE/g, Total flavonoid content 6.34$\pm$0.4 µgQE/g. The phytopharmacological profile of the two extracts of the leaf and bark of *S. cochinchinensis* are also analysed. This study proves that the methanolic and *ethanolic* extracts of the leaf and bark of *S. cochinchinensis* are potential candidates for further research.

Introduction
Cancer is one of the deadliest diseases worldwide. Every year millions of people are diagnosed with various types of cancer, millions die and millions are under treatment. Cancer still remains to be an aggressive killer.

The International agency for Research on Cancer estimates of the incidence of mortality and prevalence from major types of cancer, at national level, for 184 countries of the world revealed that there were 14.1 million new cancer cases, 8.2 million cancer deaths and 32.6 million people living with cancer worldwide. (1). By 2030, it is projected that there will be 26 million new cancer cases and 17 million cancer deaths per year (2).

The development of novel synthetic drugs or novel drug delivery systems has not been successful in the curing of cancer. So, much focus is on the development of new, effective and affordable anticancer drugs (3). Natural products have been

receiving increasing attention over the past 30 years for their potential as novel cancer preventive and therapeutic agents (4,5)

The favorite source is the compounds from the medicinal plants. There are millions of medicinal plants worldwide which are unexplored till date. Many of these plants may contain a number of active compounds which can be potential drugs against cancer. Tannins are naturally occurring water soluble phenolic compounds (6) A review of tannins and human health had been carried out by Chung et al. (7). Condensed tannins of higher molecular weight are commonly described as phlobatannins (8). They are formed due to the aging of tissues (9) or due to enzymatic action on dead cells (10) Flavonoids and phenolics are the most important group of secondary metabolites and bioactive compounds in plants (11).They possess diverse biological activities such as anti ulcer and anti inflammatory (12) and as an anti oxidant (13). Antidiabetic activity and anti cancer activity of flavonoids have been reviewed (14,15)

Bioactive compounds are often toxic to shrimp larvae. Hence, invitro lethality to shrimp larvae can be used as a rapid and simple preliminary monitor for bioactive compounds during the isolation of natural products. Cytotoxicity to brine shrimp, *Artemia salina*, larvae is a rapid, inexpensive, in house, general bioassay which has been developed for screening, fractionation and monitoring of physiologically active natural products. (16)

Plant species belonging to many genera have medicinal properties.(17) One such genus is *Symplocos.* This genus is widespread all over the world but only a very few species from this genus have been extensively studied for their medicinal properties. A species little explored in this genus is *Symplocos cochinchinensis* which is distributed in tropical and sub tropical Asia. It is a small evergreen tree reaching a height upto 7 m with thin, smooth, light grey bark and white wood.(18)

S. cochinchinensis has many uses in the indigenous system of medicine. The bark is astringent, acrid, ophthalmic, expectorant, anti inflammatory, depurative, febrifuge, haemostatic and stomachic. According to the Ayurveda system of medicine, it is useful in vitiated conditions of *pitta and kapha,* asthma, bronchitis, dropsy, arthritis, ulcers, leprosy, skin diseases, ulmeorrhagia, dyspepsia and gonorrhea (19). Its bark is described as bitter and pungent which is used as an aphrodisiac and in menorrhagia, the diseases of "raktpitta" and against the disease of the eyes. (20). Bark is used in ophthalmia and in threatened abortion (21)

The "Sarabendra vaidya muraigal" (a text generated by many ayurvedic, siddha and unani physicians at the period of King Sarfoji II) reports the use of *Symplocos cochichinensis* (Lour.)S.Moore. to treat Diabetes mellitus (22,23). Ved (24) reported the use of three species of *Symplocos* viz *Symplocos racemosa, Symplocos paniculata* and *Symplocos cochinchinensis* as *"lodhra"* for treating diabetes mellitus. The decoction of the leaves is valued in Indian medicines to treat diabetes. Paste of the leaves, boiled in oil is used for application in the scalp diseases (21). The leaves impart a yellow dye which is used as a mordant. The fruits and seeds are strung into rosaries (21). The wood is white, soft and even grained. It is

used for making temporary rafts (21) and as fuel (26) and is used for match splints (21).

Materials and methods

Plant source and preparation of the plant extracts:

The leaves and barks of *Symplocos cochinchinensis* (Figures 1,2) were collected from the Western Ghats, Nilgiris, India in the month of June 2015. The plant parts were authenticated by Dr.Chelladurai, Research officer, Central Council for Research in Ayurveda and Siddha. The leaves and bark were shade dried and powdered. The methanolic and ethanolic extracts of the dried powders were prepared. 10 g of the dried powder was dissolved in 100 ml of methanol and 1:2 ratio of ethanol – water. The contents were stirred well and left for 48 hours at room temperature. The filtrate collected after cold percolation was used for further analysis.

Phytochemical analysis

The methanolic and ethanolic extracts obtained from the dried leaves and bark powder of *Symplocos cochinchinensis* were tested for the presence of phytochemicals – Tannins, Phlobatannins, Flavonoids, Terpenoids, Cardiac glycosides and Steroids following the methodology described by Evans.(8)

Tests for Tannins

To 5 ml of the extracts, a few drops of 0.1% of Ferric chloride were added. The presence of brownish green or blue black colour indicated that the plant material possessed Tannins.

Tests for Phlobatannins

10 ml of the plant extracts were boiled with 1% HCl in a test tube. The presence of Phlobatannins was confirmed by the deposition of red precipitate in the test tube.

Tests for Saponins

To 10 ml of the extracts, 3 ml of distilled water was added and shaken well, so as to obtain froth. To the froth formed, a few drops of olive oil were added. The formation of emulsion indicates the presence of saponins.

Test for Flavonoids

A few drops of 1% liquid ammonia were taken in test tubes, to which the methanolic and ethanolic extracts were added. Yellow colouration of the solution confirmed the presence of Flavonoids

Test for Terpenoids

Around 2 ml of chloroform and 3 ml of concentrated sulphuric acid were added consecutively to 5 ml of the plant extracts. A reddish brown interface in the solution denoted the presence of Terpenoids.

Test for Cardiac Glycosides:

To 5 ml of the plant extracts, 2 ml of glacial acetic acid containing a drop of Ferric chloride was added. This was followed by the addition of 1 ml of concentrated Sulphuric acid. The brown ring thus obtained yield positive result for the test.

Test for Steroids

A couple of grams of plant powder were mixed with 10 ml of chloroform followed by boiling and filtration. To the above 2 ml of the filtrate 2 ml of acetic anhydride and a few drops of concentrated sulphuric acid were added. Stable presence of blue green ring in the solution confirms the presence of steroids.

Determination of Total phenolic content

Folin- ciocalteau method was followed for the determination of the total phenolic content. Distilled water (500µl) and Folin –ciocalteau reagent (500µl) were added to 100 µl of the plant extracts and incubated at room temperature for 6 minutes. The final volume was made up to 3 ml with 7% sodium carbonate solution. The absorbance was measured at 760 nm using UV-visible spectrophotometer after an incubation period of 90 min. The total phenolic content was expressed as milligrams of tannic acid equivalents per gram of dry weight (mg TAE/gDW) of the plant using a standard plot of tannic acid.(9)

Determination of Total flavonoid content

The total flavonoid content of the plant was determined by the method adopted by Moussa *et al*. (2011). The plant extracts were taken in test tubes and the solvent were allowed to evaporate. To the residue 5 ml of 0.1 M Aluminum chloride was added and shaken well. This was followed by incubation for 40 minutes at room temperature and the absorbance value was measured at 415 nm using UV -Vis spectrophotometer. A standard plot of quercetin at varying concentration was used to evaluate the total flavonoid content, expressed as milligrams of Quercetin equivalent per gram dry weight (mg QE/gDW) of the plant material.(10)

Determination of the Total antioxidant activity

The total antioxidant activity was estimated by phosphomolybdenum method. To the plant extract (0.5 ml), 4.5 ml of the reagent solution (0.6 M sulphuric acid, 28 mm sodium phosphate and 4 mM of ammonium molybdate) was added. The solution was maintained in a boiling water bath at 95 C for 90 min. The solution was cooled to room temperature and the absorbance was measured at 695 nm using UV- visible spectrophotometer. The total antioxidants in the plant were expressed as mg TAE/g DW of the plant material (11).

2, 2 – Diphenyl-1- picryl hydrazyl (DPPH) free radical scavenging assay

The two plant extracts were taken at various concentrations (10,20,30,40,50 µg/ml) in small test tubes and made up to 1 ml using methanol. 1 ml of 0.01 mM DPPH dissolved in methanol was added to all the test tubes and maintained in dark for 30 minutes, at room temperature. The absorbance of the solutions was read at 517 nm. The percentage inhibition and IC 50 values were calculated with DPPH as the control and butylated hydroxyl anisole (BHA) as the reference. The concentration in µg of dry material per ml of solvent (µg/ml) that inhibits the formation of DPPH radicals by 50% is defined as the IC 50 value.(12)

$$\% \text{ inhibition} = \frac{(\text{absorbance of control (Ac)} - \text{Absorbance of the sample (As)}) \times 100}{\text{Absorbance of the control (Ac)}}$$

Ferric thiocyanate (FTC) assay

The assay involves the addition of 120 µl of 98% ethanol, 100 µl of 2.5 % linoleic acid and 9 ml of 40 mM phosphate buffer (pH 7) to 100 µl of the plant extract. To 100 µl of the mixture, 9.7 ml of 75% ethanol, 100 µl of 30% ammonium thiocyanate and 100 µl of 20 mM $FeCl_3$ in 3.5% HCl were added after maintaining the solution in the dark, at 40 ° C. The absorbance of the solution was measured at 500 nm using UV-visible spectrophotometer after 3 min. The percentage of inhibition was calculated with Tannic acid as the standard (Deepa *et al*., 2013). (13)

$$\% \text{ inhibition} = \frac{\text{(absorbance of control (Ac) - Absorbance of the sample (As))} \times 100}{\text{Absorbance of the control (Ac)}}$$

Thiobarbituric acid (TBA) assay

Equal volume (200 µl) of 20% trichloroacetic acid and 0.67% thiobarbituric acid were mixed with 1 ml of 2.51% linoleic acid and 1 ml of plant extract. The solution was maintained in boiling water bath for 10 min; cooled to room temperature and centrifuged at 3000 rpm. The supernatant was subjected to UV–visible spectrophotometric analysis at 532 nm. The percentage inhibition of the plant against the secondary products of lipid peroxidation was evaluated with reference to the standard solution of butylated hydroxyl toluene (BHT) (Deepa *et al*., 2013) (13)

$$\% \text{ inhibition} = \frac{\text{(absorbance of control (Ac) - Absorbance of the sample (As))} \times 100}{\text{Absorbance of the control (Ac)}}$$

Ferric reducing antioxidant power (FRAP) assay

1 ml of plant extract, 2.5 ml phosphate buffer (of 0.2 M, pH 7) and 1% potassium ferricyanide (2.5 ml) were mixed and incubated at 50 ° C for 30 min. To the solution, 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 6500 rpm for 10 min. Distilled water (2.5 ml) and 0.5 ml of 0.1% $FeCl_3$ were added to 2.5 ml of the supernatant. The absorbance of the solution was measured at 700 nm using UV–visible spectrophotometer. The reducing ability of the plant was evaluated in terms of percentage by relating to the standard, $FeSO_4$ (Kalita *et al.,* 2013) (14).

$$\% \text{ inhibition} = \frac{\text{(absorbance of control (Ac) - Absorbance of the sample (As))} \times 100}{\text{Absorbance of the control (Ac)}}$$

[2,2⁻ -Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] ABTS assay

A solution of 7 mM ABTS [2,2 -azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] and 2.45 mM potassium persulphate was incubated in the dark for 12–16 h, after which the solution was diluted with ethanol till the absorbance reached 0.7 ± 0.02 at 734 nm. 1 ml of the diluted solution was mixed with 100 µl of plant extract and the absorbance was evaluated at 734 nm after 6 min. The percentage reduction against ABTS was calculated with reference to the standard, Tannic acid (Deepa et al., 2013) (13).

Larvicidal activity

Culture of larvae

The *Artemia salina* seeds were obtained from Marina labs, India. The seeds were incubated in marine water for 48 hours for hatching in a small water tank. Aeration was provided with an aerator pump. Required light was provided with Philips 40 Watts lamp for 12 hours cycle . After 48 hours, the larvae were removed and used for the experiments. The hatched larvae were used in the nauplii stage (26).

Bioassay

Larvae of *Artemia salina* were taken in different test tubes containing the extracts of *Symplocos cochinchinensis,* leaf and bark powder at different concentrations. Then five concentrations (1,2,3,4.5 ml) of each extract were added to 10 ml of sea water and 20 larvae were added to each of the test tubes. After 24 and 48 hours the viability of the larvae was recorded. The test tubes were maintained in triplicates. At the end of the experimental period, the numbers of mobile and dead larvae in each test tube were checked using a hand lens. Nauplii were considered dead when they were immobile and stayed at the bottom of the test tube. (26)

RESULT

The present study was done to find out the phytochemicals, anti oxidant activity, free radical scavenging activity and the toxicity assay of the methanolic and ethanolic extracts of the bark and leaf powder of *Symplocos cochinchinensis.* It was found that the ethanolic extracts of the leaf and bark showed the presence of phytochemicals like flavonoids, tannins and saponins. (Table 1) Both the extracts had significant larvicidal activity. The % mortality of the ethanolic extract of the leaf powder at the concentration of 2 mg/ml was 100 in the 24^{th} hour whereas the methanolic leaf extract showed 100% mortality at 24^{th} hour at the concentration of 3 mg/ml.

Similarly methanolic and ethanolic extracts of the bark showed 100% mortality at 3 mg/ml in the 24^{th} hour.(Table 2, Figure 3) At the 48^{th} hour ethanolic extracts of both the leaf and bark showed 100% mortality at 2 mg/ml whereas the methanolic extracts showed the same at 3 mg/ml.(Table 3 , Figure 4) The ethanol water extracts of the leaf and the bark showed maximum larvicidal activity at 24^{th} and 48^{th} hour.

The total phenolic, total flavonoid, total antioxidant content were estimated. The total phenolic content was expressed as milligrams of tannic acid equivalents per gram of dry weight(mg TAE/gDW)of the plant using a standard plot of tannic acid. The ethanol water leaf extract had the maximum phenolic content of 13.81 ± 0.21. (Table 4, Figure 5,6,7,8)

The total flavonoid content is expressed as milligrams of Quercetin equivalent per gram dry weight (mg QE/gDW) of the plant material. The methanol extract of leaf had the maximum flavonoid content with 6.34 ± 0.21.(Table 5, Figure 5,6,7,8)

The total antioxidants in the plant were expressed as mg TAE/g DW of the plant material. The methanol extract of leaf had the maximum antioxidant content with 16.34 ±0.21.(Table 6, Figure5,6,7,8) The methanol extracts of leaf and bark showed maximum antioxidant activity. (Table 7,8,9,10,11, Figure 9,10)

Table 1 Phytochemical analysis

	Leaf		**Bark**	
Solvent / Phytochemical	**Methanol**	**Ethanol-Water**	**Methanol**	**Ethanol-Water**
Flavonoids	-	+	-	+
Tannins	+	+	+	+
Saponins	-	+	-	+
Terpenoids	-	-	-	-
Cardiac glycosides	-	-	-	-
Steroids	-	-	-	-
Phlobatannins	-	-	-	-

+ indicates presence - Indicates absence

Table 2 Larvicidal activity - At 24th hour

Plant part	**Solvent**	**% mortality**					**LD_{95} (mg/ml)**
		Concentration (mg/ml)					
		1	**2**	**3**	**4**	**5**	
Leaf	Methanol	60	65	100	100	100	2.001
	Ethanol-Water	70	100	100	100	100	1.001
Bark	Methanol	75	90	100	100	100	2.02
	Ethanol-Water	70	85	100	100	100	2.007

Values are representatives of mean ± SD (n=3)

Table 3 Larvicidal activity

At 48th hour

Plant part	**Solvent**	**% mortality**					**LD_{95} (mg/ml)**
		Concentration (mg/ml)					
		1	**2**	**3**	**4**	**5**	
Leaf	Methanol	70	80	100	100	100	2.003
	Ethanol-Water	80	100	100	100	100	1.003
Bark	Methanol	80	95	100	100	100	2
	Ethanol-Water	90	100	100	100	100	1.02

Values are representatives of mean ±SD (n=3)

Table 4 Total phenolic content

Plant part	**Solvent**	**TPC (mg TAE/g)**
Leaf	Methanol	13.67±0.29
Leaf	Ethanol-Water	13.81±0.21
Bark	Methanol	11.67±0.25
Bark	Ethanol-Water	13.43±0.24

Table 5 Total flavonoid content

Plant part	Solvent	TFC (µg QE/g)
Leaf	Methanol	6.34±0.21
Leaf	Ethanol-Water	1.23±0.25
Bark	Methanol	3.63±0.15
Bark	Ethanol-Water	1.93±0.2

Table 6 Total antioxidant content

Plant part	Solvent	TAC (mg TAE/g)
Leaf	Methanol	6.34±0.21
Leaf	Ethanol-Water	1.23±0.25
Bark	Methanol	3.63±0.15
Bark	Ethanol-Water	1.93±0.2

Table 7: DPPH free radical scavenging assay

Plant part	Solvent	10 mg/ml	20 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml	EC_{50}
Leaf	Ethanol-water	34.34	56.57	62.63	83.84	97.98	29.96267
Bark	Methanol	7.07	33.33	49.5	63.64	83.84	31.41443
Bark	Ethanol-water	7.07	27.27	33.33	56.57	84.85	30.02581

Plant part	Solvent	1 mg/ml	2 mg/ml	3 mg/ml	4 mg/ml	5 mg/ml	EC_{50}
Leaf	Methanol	20.05	29.87	37.33	47.78	59.67	4.037885

Values are representatives of mean ±SD (n=3)

Table 8: Ferric thiocyanate assay

Plant part	Solvent	% Inhibition
Leaf	Methanol	84.54
Leaf	Ethanol-Water	18.56
Bark	Methanol	22.68
Bark	Ethanol-Water	46.39

Values are representatives of mean ±SD (n=3)

Table 9 Thiobarbituric acid assay

Plant part	Solvent	% Inhibition
Leaf	Methanol	88.89
Leaf	Ethanol-Water	62.63
Bark	Methanol	82.83

Bark	Ethanol-Water	64.65

Values are representatives of mean ± SD (n=3)

Table 10 Ferric reducing antioxidant power

Plant part	Solvent	% Inhibition
Leaf	Methanol	17.17
Leaf	Ethanol-Water	7.07
Bark	Methanol	73.74
Bark	Ethanol water	64.65

Values are representatives of mean ± SD (n=3)

Table 11 ABTS free radical scavenging assay

Plant part	Solvent	% Inhibition
Leaf	Methanol	42.86
Leaf	Ethanol-Water	55.71
Bark	Methanol	57.14
Bark	Ethanol-Water	38.57

Values are representatives of mean ± SD (n=3)

Fig 1: *Symplocos cochinchinensis* leaf

Fig 2: Plant *S. cochinchinensis*

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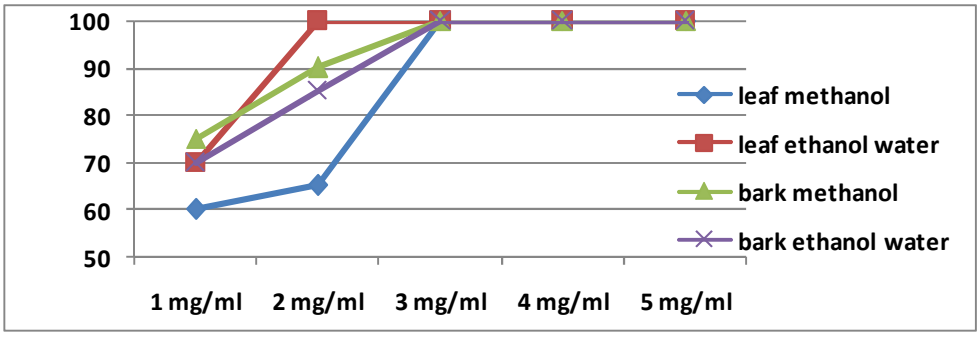


Fig 3: Larvicidal activity (24th hour) at the different concentrations by the ethanol and methanol extracts of the leaf and bark powder of *Symplocos cochinchinensis*

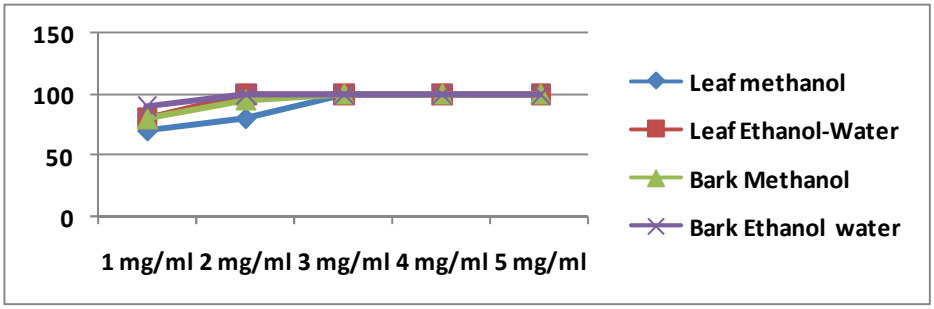
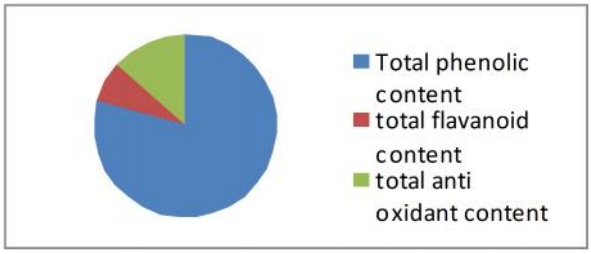


Fig 4: Larvicidal activity (48th hour) at the different concentrations by the ethanol and methanol extracts of the leaf and bark powder of *Symplocos cochinchinensis*



FFig5: Total Phenolic, Flavonoid and Antioxidant content in the Ethanol extracts of the leaf powder Methanol

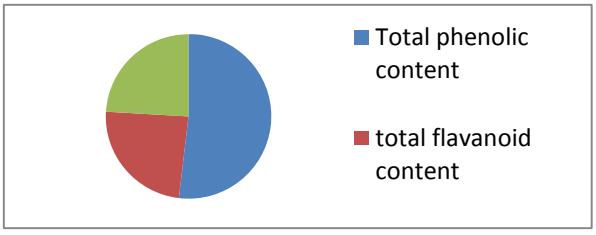
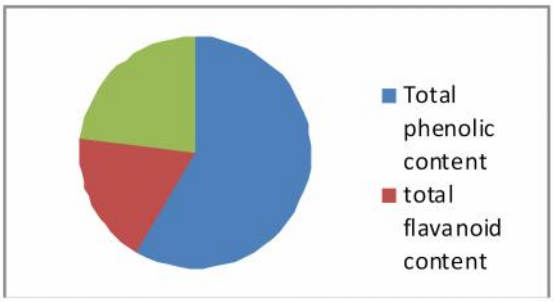


Fig 6: Total Phenolic, Flavonoid and Antioxidant content in the extracts of the leaf powder



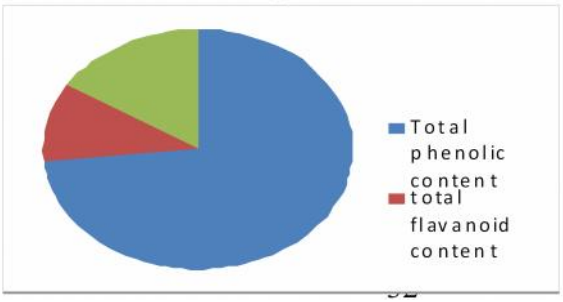


Fig 7: Total Phenolic, Flavonoid and Antioxidant content in the Ethanol extracts of the bark powder

Fig 8: Total Phenolic, Flavonoid and Antioxidant content in the Methanol extracts of the Bark powder

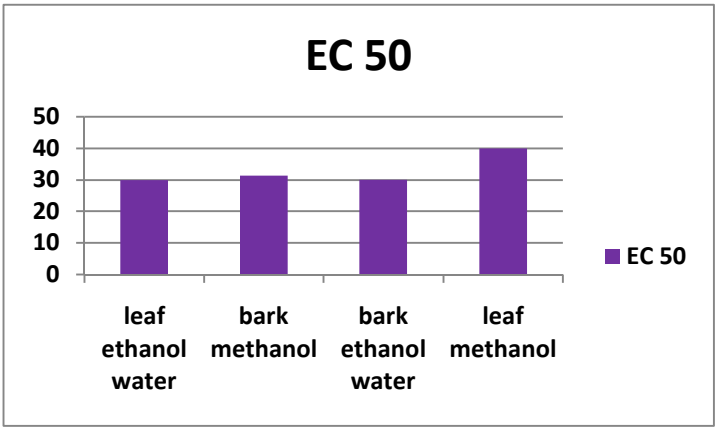


Fig 9: DPPH free radical scavenging activity of the ethanol and methanol extracts of the leaf and bark of *Symplocos cochinchinensis***.** Values are representatives of mean ± SD (n=3)

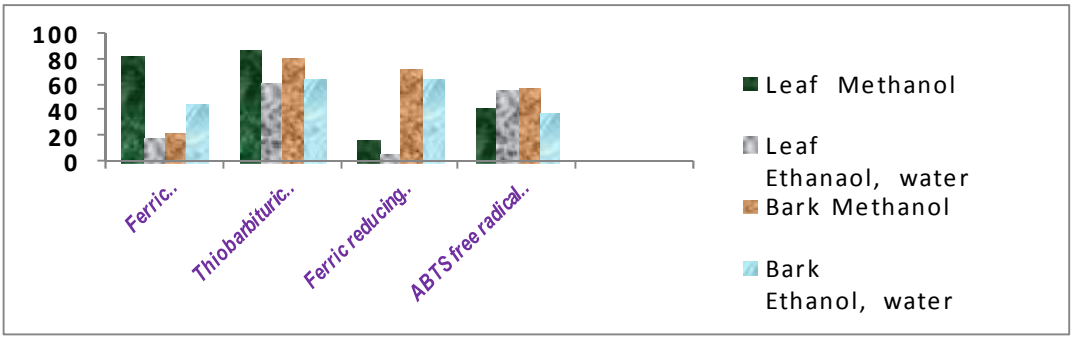


Fig 10: Antioxidant assays of the Methanolic and Ethanolic Extracts of the leaf and Bark powder. The results are the average of the triplicates.

Discussion:

Medicinal plants have been traditionally used in the treatment of various diseases. Asian traditional medicinal systems such as Chinese medicine (TCM), Korean-Chinese medicine, Japanese-Chinese medicine (kampo), Ayurveda from India and Jamu from Indonesia are well known systems to cure various ailments (1).In India, various plant extracts are used to cure tumors. Recently, different plant extracts are tested for their anti inflammatory, anti cytotoxic and anti tumor activities. Many

plant species are still unexplored, the reason being the lack of access to these plants (4).One such genus is *Symplocos*. The present study was aimed to study the anti inflammatory and anticytotoxic activity of *S. cochinchinensis*-an evergreen tree found at the higher altitudes of Western Ghats.

Various studies prove that methanol helps in the extraction of different phytoconstituents and WHO has recommended extraction with ethanol: Water in the ratio 1:2 to be safe for human consumption. So, in the present study, the methanol extract ETOH water extracts of the bark and leaves of *S. cochinchineasis* have been used.

Flavonoids seem to have an important role in human food since they have medicinal properties. They are found only in plants and they have antioxidant, anticancerous, cardiotonic, and capillary fragility and antithrombic properties. They are also reported to lower cholesterol, protect the liver and the stomach. Flavonoids are reported to be anti-inflammatory, antimicrobial and analgesic (27).

The phytoconstituents in the extracts were assayed using the standard protocol and the results were found to be consistent with the work done by Sofia Banu *et al* (2013).

The Brine shrimp lethality assay shows the cytotoxic activity of the plant extracts and this is supposed to be the pioneering attempt with this plant species. The cytotoxic activity was found to be effective and thus promise this plant species to be an effective anti cancer agent.

The bioefficacy of a plant depends on its phytoconstituents. In the investigation of the biological activity of the plant extracts and the natural products, the assay on *Artemia salina,* brine shrimp larvae is a valuable tool for establishing the cytotoxicity and toxicity parameters. (26) Thus the lethality towards brine shrimp larvae is recommended as an effective prescreen for the existing invitro cytotoxicity and antitumor assays.(27)

The standard antioxidant assays were done with the leaf and bark extracts. The methanol extracts of the leaf and bark showed maximum inhibition. This is so supportive in accordance to the report given by Sofia Banu *et al (2013).* Similarly the different extracts of the leaf and bark of *Symplocos cochinchinensis* were found to have free radical scavenging activity which is the major indicator for the presence of antioxidant compounds in the extracts.

Conclusion

Many new plant species are being tested for their anticancer properties. Many factors are vital to prove their potential anti cancer activity. Any plant part which has shown the presence of certain bioactive compounds is subjected to further investigations. The ethanolic and methanolic extracts show significant lethality to brine shrimp larvae, at a very low concentration, making them potential candidates for further cytotoxic assays. In the present study the preliminary analysis for the antioxidant activity and the phytochemical constituents of the methanolic and ethanolic extracts of the leaf and bark of *S. cochinchinensis* was done. Based on the results, it can be concluded that these plant parts can be subjected to further investigations to find out their potential as effective drugs.

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References:

1. GLOBOCAN,2012, http://globocan.iarc.fr/Default.aspx.
2. M.J. Thun, J.O. DeLancey, M.M.Center, A.Jemal and E.M.Ward, "the global burden of cancer : priorities for prevention", Carcinogenesis, Vol 3, no.1, 100-110, 2009.
3. S.Coseri, " Natural products and their analogues as efficient anticancer drugs," Mini reviews in Medicinal Chemistry, vol 9, no.5, 560-571, 2009.
4. D.J. Newman, "Natural products as leads to potential drugs: an old process or the new hope to drug discovery?", Journal of Medicinal Chemistry, Vol 51, no.9, 2589-2599,2008.
5. D.J.Newman, G.M. Cragg and K.M.Snader, "Natural products as sources of new drugs over the period 1981-2002," Journal of Natural products , vol 66, no.7, 1022-1037,2003.
6. J.S. Martin, M.M.Martin, "Tannin assays in ecological studies: lack of correlation between phenolics, proanthocyanidins and protein precipitating constituents in mature foliage of six oak species," Oecologia, vol 54, 205-211,1982.
7. K.T.Chung, T.Y.Wong, C.I.Wei, Y.W. Huang, Y.Lin,'Tannins and human health: A review', Critical review on food science and nutrition, vol 38, 421-464, 1998.
8. L.Y.Foo, J.J. Karchesy "The chemistry and significance of condensed Tannins" Plenum press, 1989.
9. L.J.Porter "Structure and chemical properties of condensed Tannins" Plenum press, 1992.
10. A.Scalbert, "Tannins in woods and their contribution to microbial decay prevention" Plenum press, 1992.
11. D.O. Kim,S.W. Jeond, C.Lee, "Antioxidant capacity of phenolic phytochemicals from various cultivars of plums", Journal of food chemistry, vol 8, 321-326, 2003.
12. A.Villar, M.A.Gasco, M.J. Alcaraz, "Anti inflammatory and anti ulcer properties of hypolaetin –8- glucoside, a novel plant flavonoid", Journal of pharmacy and pharmacology, vol 36, 820-823, 1984.
13. A.Ghasemzadeh, H.Z.E.Jaafar, "Anticancer and antioxidant activities of Malaysianyoung ginger (Zingiber officinale R) varieities grown under different CO2 concentration", Journal of medicinal plant research, vol 5, 3247-3255, 2011.
14. G.Brahmachari , " Bio-Flavonoids with promising anti diabetic potentials: A critical Survey.", Opportunity, challenge, scope Natural products Medicinal Chemistry, 187-212, 2011.
15. M.K.Chahar, N.Sharma, M.P. Dobhal, Y.C. Joshi, "Flavonoids- a versatile source of anticancer drugs" Pharmacognosy review, vol 5, 1-2, 2011.
16. B.N.Meyer, N.R. Ferrigni, J.E.Putnam, "Brine Shrimp: A convenient general bioassay for active plant constituents", Journal of Medicinal plant research, vol 45, 3134, 1982.

17. N.K.Udhayaprakash, S.Bhuvaneshwari, B.Jahnavi, "A study on the antimicrobial activity of common weeds in Northern Districts of Tamilnadu, India," Research Journal of medicinal Plant, vol 6, 341-345,2012.
18. Sofia Banu , Karishma Kashyap, " Symplocos laurina : an unexplored medicinal plants of Shola Ecosystem", International Journal of Medicinal and Aromatic plants, vol 3, no 3, 366-374, 2013.
19. Anonymous, Sushrutha Samhita, Chaukhambha Orientalia, Varanasi, 120-125, 1980.
20. P.K. Warrier, V.P.K. Nambiar, C.Ramakutty, " Indian medicinal plants -A compendium of 500 species", Orient longman, vol 5, 1996.
21. Anonymous, " The wealth of India ; Raw materials", CSIR publications, 424-426, 1986.
22. N.Gangadharan, " The state of Ayurveda in the eighteenth and nineteenth centuries", Indian Journal of history of science, Vol 17, 154-163, 1982.
23. S.P.Nair, " Native collection of natural knowledge(1798-1832): Raja Serfoji II of Tanjore as 'Centre of calculation', Journal of the Royal Asiatic Society of Great Britain and Ireland, vol 15, 279-302, 2005.
24. D.K.Ved, G.S. Goraya, " Demand and supply of medicinal plants in India", NMPB, 2007.
25. D.K.Hore, " Symplocaceae: Fascicles of flora in India", vol 20, 1990.
26. Udaya et al, " Studies on the Antimicrobial, Antioxidant, larvicidal, pesticidal Activity and Phytochemistry of the leaves of *Alangium Salvifolium (L.f .) Wang*, International journal of Pharmacy and Pharmaceutical Sciences, Vol 5, Issue 2, 86-89.
27. http://www.botanical-online.com/flavonoids.
28. Quignard ELJ,Pohlit AM, Nunomura SM, Pinto ACS etal, " Screening plants found in Amazonas State for lethality towards brine shrimp" Acta Amazonica 2003;33:93-104

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ANALYSIS OF SOIL PHYSICO- CHEMICAL CHARACTERISTICS OF ORGANIC FARMING AND CONVENTIONAL FARMING PRACTICES

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Abstract

With an increasing awareness about the harmful effects of agrochemicals, the demand for technologies and product based on biological processes such as organic farming has been increasing steadily worldwide. The objective of our research was to evaluate the impact of organic and conventional management strategies on grower fields in Chennai on soil physico-chemical characteristics. Physico-chemical properties such as temperature, pH, moisture content, organic matter, macronutrients (Nitrogen, Phosphorous and Potassium), micronutrients (Zinc, Manganese, Iron and Copper), respiration and heavy metals were determined. There wasn't much difference in the temperature measurement. Soil pH was slightly higher in organic field than control and conventional field. Moisture content was higher in organic field than conventional and control field. T*he results showed that organic farming practices showed higher organic matter, respiration, micronutrients, macronutrients except potassium which was followed by conventional field and control field.* Heavy metals content was higher in conventional fields than the organic fields. Hence organically managed soils establish ecological systems that are able to sustain biological productivity as well as agricultural productivity in the long-term.

Key words: Organic farming, conventional farming, soil, physic-chemical characteristics.

Introduction

In the past decades and up to the present, high crop yields have been achieved through conventional farming, i.e., the use of high-yielding varieties (HYVS), agrochemical inputs (inorganic fertilizers and pesticides), farm machineries that facilitate land preparation, and irrigation that relives the crop from any yield-depressing effect of water deficit. The long-term ecological consequences of this production approach have since been recognized, i.e., soil and water pollution, loss of biodiversity, genetic erosion, receding underground water etc. The intensive uses of agrochemicals and monocropping have resulted in the degradation of the environment.

High yields continue to be the most important indicator of assessing the success or failure of crop farming. But if high yields are obtained with negative consequences, then it is imperative to re-think about continuing to practice much more supporting or subsiding conventional farming. As early as the 1980's, various organizations started the campaign for organic and sustainable agriculture. So there is a need to promote chemical-free agriculture or environment-friendly pest management techniques and/or organic farming. Now there is a growing realization that the adoption of ecological and sustainable farming practices can only reverse the declining trend in the global productivity and environment protection.

Organic agriculture is the oldest form of agriculture on earth. The system avoids applications of synthetic fertilizers and pesticides, use of organic inputs and recycling for nutrient supply and emphasizes cropping system design and biological processes for pest management. In a long-term field trial, microbial biomass was higher in soils from organic plots (Gelsomino *et al.*, 2004; Tu *et al.*, 2005; Liu *et al.*, 2007). Other researchers have shown that incorporation of organic amendments increased soil microbial activity, microbial diversity (Girvan *et al.*, 2004; Grayston *et al.*, 2004), densities of bacteria (Bruggen and Semenov, 2000) and nematodes (Abawi and Widmer, 2000).

So organic agriculture is gaining worldwide acceptance. Here was a surprising lack of research regarding a variety of aspects of organic agriculture. The number of studies comparing organic and conventional systems is also limited to India. Most of the research related to these studies was carried out on foreign countries like North Carolina (Liu *et al.*, 2007), New Zealand (Condron *et al.*, 2000), Brazil (Araujo *et al.*, 2009), England (Jenkins *et al.*, 2010), China (Hu and Zhiping Cao, 2007). The objectives of our research were to evaluate the impact of organic and conventional management strategies on grower fields in Chennai on soil physical and chemical characteristics. The results will help to provide a platform for future research into the impacts of organic farming.

Materials and Methods

Selection of field for experimental study

Six soil samples were collected with a history of organic and conventional crop production farms in and around Chennai. Three farms were certified organic and did not use synthetic fertilizers or pesticides. They were located in Wallajabadh, Redhills and Chengelpet. Three of the farms were classified as conventional farms where chemical fertilizers and pesticides were used. They were located in Porur, Kundrathur and Kovur. Control field also set up without the addition of any fertilizers (biofertilizers and chemical fertilizers).

COLLECTION OF SOIL SAMPLE

Well mixed composite soil samples of about 20g were taken at a depth of 20cm from four corners and from the middle of the farm field. All the soil samples were mixed thoroughly to get a homogenous mixture. Stones and crop residues are discarded. The soil samples were stored at 4°C for microbial analysis and the remaining were air dried and sieved for the determination of physico-chemical properties of soil.

PHYSICO-CHEMICAL ANALYSIS OF SOIL SAMPLES

TEMPERATURE

Soil temperature was noted by using soil thermometer at the time of sample collection.

MEASUREMENT OF SOIL pH

To 40ml of distilled water, 20g of soil was added and stirred thoroughly with a glass rod. It was kept undisturbed for 15min. The electrode of pH meter was dipped into the suspension and the pH was noted. For standardization, buffer solutions of pH 4.0, 7.0 and 9.2 were used.

MOISTURE CONTENT

The moisture content of the soil samples were determined gravimetrically by weighing, drying in a hot air oven at 105°C for 24 hours and then reweighing.

ORGANIC MATTER

Soil organic matter was found by rapid titration method of Walkely and Black (1934).

MACRO AND MICRONUTRIENTS

Macronutrients such as Nitrogen (Sankaran, 1966), Phosphorus (Olsen *et al*., 1954) and Potassium (Sankaran, 1966) and Micronutrients (Lindsay and Norwell, 1978) like Zinc, Manganese, Copper and Iron were also analyzed.

RESPIRATION

100g of soil sample was dissolved with distilled water and its Water Holding Capacity (WHC) was adjusted to 33%. The soil sample was kept in a 1000ml flask in which a test tube containing 10 ml of freshly prepared N/10 NaOH was hung using a thread. Another flask devoid of soil sample was used as control. The flask was incubated at 30°C for 2-3 weeks. At weekly intervals NaOH was taken out, to which 2-3 drops of phenolphthalein was added and titrated against N/10 HCl solution (Haynes And Gower, 1995).

HEAVY METALS

The heavy metals which were present in all the three soil samples were determined by using X-ray fluorescence spectroscopy (XRF).

STATISTICAL ANALYSIS

All the experiments were conducted in triplicates and data shown in results are means and standard deviation.

Results and Discussion

The physico-chemical analysis of soil sample was carried out by studying the temperature, pH, moisture content, organic matter, macronutrients (Nitrogen, Phosphorous and Potassium), micronutrients (Zinc, Copper, Iron and Manganese), respiration and heavy metal content of all the three fields (Organic, Conventional and Control field). The results were tabulated (Table.1).

There wasn't much difference in the temperature measurement. Soil pH was slightly higher in organic field (7.05±0.01) than control (7.0±0.03) and conventional field (6.8±0.02). The results were correlated with the findings of Padmavathy and Poyyamoli, (2011), who reported that pH of the organic field (7.6±0.1), was slightly higher than conventional field (7.2±0.2). There were some significant differences in the soil pH which was agreed by Poudel *et al.,* (2002) studies of soils from California. However other researchers have shown that pH was not significantly different between organically and conventionally managed soils (Mader *et al.*, 2002; Diepeningen *et al.,* 2006). The change of pH in soil sample generally occurred as a consequence of mineralization of organic compound.

Moisture content was higher in organic field (0.920±0.1%) than conventional (0.883±0.05%) and control field (0.865±0.2%). This is in accordance to the study done by Chauhan *et al.,* (2011) who reported that moisture content was higher in organic field (45.460±1.44%) than conventional field (44.360±1.31%). The result was also correlated with the findings of Liu *et al.,* (2007).

The highest organic matter was observed in organically treated plots (3.58±0.3%) and the least in control (2.62±0.1%) and conventional fields (0.96±0.1%).This result is correlated with the study done by Chauhan *et al.,* (2011) which showed higher organic content in organic fields (2.320±0.04%) than conventional field (2.290±0.04%). Soil from

the organic field showed an increase in organic matter, compared to other fields; this might be due to the addition of organic contents as they are the sources of nitrogen and carbon to soils. A positive effect of organic fertilizers on the microbial biomass nitrogen and the organic matter content in the soil was also observed by Cerny *et al.,* (2008). The greater amounts of organic matter contribute to the better soil structure observed in organic field.

The total Nitrogen content was higher in organic field (1.79±0.04%) than other two fields (control-1.31±0.01%, conventional-0.48±0.02%). This is in accordance with the study done by Padmavathy and Poyyamoli, (2011) who reported that the total Nitrogen content in organic fields were higher than the conventional fields, due to the external chemical inputs effects microbial population in conventional fields. This result is also correlated with the findings of Condron *et al.,* (2000), who reported that the higher level of microbiological activity in the organic soil leads to the increase in nitrogen content of soil. The present result was contrast to the results showed by Chauhan *et al.,* (2011), where his study showed higher Nitrogen content in conventional field (0.570±0.05%) than organic (0.550±0.03%) and control field (0.440±0.31%).

The phosphorous content was significantly higher in organic field (16.369±0.59 µg g^{-1}dry soil) than conventional field (14.960±0.75 µg g^{-1}dry soil). This was agreed to the study done by Padmavathy and Poyyamoli, (2011) who reported higher phosphorous content in organic field (17±2 kg ha^{-1}) than conventional field (8±1 kg ha^{-1}). But there was a contrast in results showed in the study made by Condron *et al.,* (2000). His study showed higher phosphorous content in conventional field (66.2 mg kg^{-1}) than organic field (45.7 mg kg^{-1}). This is also correlated with the results of Chauhan *et al.,* (2011) because conventional fields amended with the mixture of urea showed highest content in phosphorous.

The potassium content was lower in organic field (0.031±0.003 µg g^{-1}dry soil) than conventional field (0.036±0.004 µg g^{-1}dry soil). The result is correlated with the findings of Condron *et al.,* (2000) who reported that the potassium content was higher in conventional field (1.00 cmol kg^{-1}) than organic field (0.97 cmol kg^{-1}). Mader *et al.,* (2002) reported that the lower nutrient inputs in organic fields cause lower amount of potassium. But the potassium level was more variable among farms from year to year (Liu *et al.,* 2007).

Soil micronutrients were higher in organic field (Zn-21±1 ppm, Cu-10±2 ppm, Fe-5.2±0.2 ppm, Mn-0.3±0.01 ppm) than conventional (Zn-20±1 ppm, Cu-6±2 ppm, Fe-2.8±5 ppm, Mn-0.09±0.01 ppm) and control field (Zn-10.1±1 ppm, Cu-4.8±2 ppm, Fe-1.2±2 ppm, Mn-0.05±0.01 ppm). This was in accordance with to Liu *et al.,* (2007) who reported that the micronutrient content was higher in organic field than conventional field. The present study was also supported by Bending *et al.,* (2000). He reported that the increase in microbial numbers leads to the higher content of micronutrients in soil. The experimental study carried out by Padmavathy and Poyyamoli (2011) also showed higher micronutrient content in organically treated plots (Zn-26±1 ppm, Cu-9±2 ppm, Fe-4.2±0.2 ppm, Mn-0.3±0.01 ppm) than convetional plots (Zn-15±1 ppm, Cu-7±2 ppm, Fe-3.2±0.2 ppm, Mn-0.1±0.01 ppm).

Soil microbial respiration rates were higher in soils from organic fields (55.4±1.9%) than conventional field (35.8±2.8). This was in accordance with Liu *et al.,* (2007) who showed higher respiration rates in organic field (96.7 mg/kg) than conventional field (11.0 mg/kg). The results suggested that largest activity of soil microbes existed in soil organic farms leads to higher respiration rates (Mader *et al.,* 2002; Diepeningen *et al.,* 2006).

Heavy metals content was higher in conventional fields (Cr-101±3 ppm, Ni-28±4 ppm, Ar-14±2 ppm, Pb-13±2 ppm) than the organic fields (Cr-45±5 ppm, Ni-11±2 ppm, Ar-5±3 ppm, Pb-3±1 ppm). This was supported by the study done by Padmavathy and

Poyyamoli (2011) where they stated that the usage of chemical fertilizers leads to the higher rate of heavy metal content in conventional field (Cr-89±2 ppm, Ni-20±4 ppm, Ar-6±2 ppm, Pb-6±2 ppm).

On comparing the Physico-chemical analysis of three soil samples (Organic, Conventional and Control field), the results showed that organic farming practices showed higher organic matter, respiration, micronutrients, macronutrients except potassium which was followed by conventional field and control field. Hence organically managed soils establish ecological systems that are able to sustain biological productivity as well as agricultural productivity in the long-term.

References

Araújo S.F.A., Luiz F.C. Leite., Valdinar B. Santos and Romero F.V. Carneiro. 2009. Soil Microbial Activity in Conventional and Organic Agricultural Systems. *Sustainability.* 1:268-276

Abawi G.S. and T.L. Widmer. 2000. Impact of soil health management practices on soil borne pathoge-,nematodes and root diseases of vegetable crops. *Appl. Soil Ecol.* **15**: 37-47.

Bending G.D., Putland C., Rayns F. 2000. Changes in microbial community metabolism and labile organic matter fractions as early indicators of the impact of management on soil biological quality. *Biol.Fertil.Soils*. **31**:78–84.

Bruggen V.A.H.C. and A.M. Semenov. 2000. In search of biological indicators for plant health and disease suppression. *Appl. Soil Ecol.* **15**: 13-24.

Cerny J., J. Balik., M. Kulhanek and V. Neded. 2008. *Plant Soil Environ.* **54**: 212-218.

Chauhan P.K., V. Singh., Vinod Kumar Dhatwalia and Abhishek. 2011. Physico-chemical and Microbial activity of soil under Conventional and Organic Agricultural Systems. *J. Chem. Pharm. Res.* **3(3)**:799-804.

Condron L. M., K. C. Cameron., H. J. Di., T. J. Clough., E. A.Forbes., R. G. McLaren & R. G. Silva. 2000. A comparison of soil and environmental quality under organic and conventional farming systems in New Zealand. *New Zealand Journal of Agricultural Research.* **43(4):** 443-466.

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S.No	Physico-chemical properties	Control field	Organic field	Conventional field
1	Temperature ºC	28	29	28
2	pH	7.0±0.03	7.05±0.01	6.8±0.02
3	Moisture content%	0.865±0.2	0.920±0.1	0.883±0.05
4	Organic matter%	2.62±0.1	3.58±0.3	0.96±0.1
5	Available Nitrogen%	1.31±0.01	1.79±0.04	0.48±0.02
6	Available Phosphorous µg g^{-1} dry soil	12.246±0.53	16.369±0.59	14.960±0.75
7	Available Potassium µg g^{-1} dry soil	0.025±0.003	0.031±0.003	0.036±0.004
8	Micro nutrients			
	Zinc ppm	10.1±1	21±1	20±2
	Copper ppm	4.8±2	10±2	6±2
	Iron ppm	1.2±2	5.2±0.2	2.8±5
	Manganese ppm	0.05±0.01	0.3±0.01	0.09±0.01
9	Respiration %	30.3±1.8	55.4±1.9	35.8±2.8
10	Heavy metals			
	Chromium ppm	20±2	45±5	101±3
	Nickel ppm	5±1	11±2	28±4
	Arsenic ppm	2±1	5±3	14±2
	Lead ppm	1±1	3±1	13±2

Haynes BE and Gower ST. 1995. Belowground carbon allocation in unfertilized and fertilized red pine plantations in northern Wisconsin. Tree Physiol., 15(5): 317-25.

Jenkins L.J.H.A, R. J. GodwinA, B. PearceB, R SakrabaniA and A. WhitmoreC. 2010. A comparison between conventional and organic farming practices 1: Soil physical properties. *19th World Congress of Soil Science.*

Lindsay, W.L. and W.A. Norvell. 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci. Soc. Amer. J. 42:421-428.

Liu Bo., Cong Tu., Shuijin Hu., Marcia Gumpertz., Jean Beagle Ristaino. 2007. Effect of organic, sustainable, and conventional management strategies in grower fields on soil physical, chemical, and biological factors and the incidence of Southern blight. *Appl.Soil Ecol*. **1082**: 1-13.

Mader P., Andreas Fliebbach., David Dubois., Lucie Gunst., Padruot Fried., Urs Niggli. 2002. Soil Fertility and Biodiversity in Organic Farming. *Science.* **296**:1694.

Olsen, S.R., C.V. Cole, F.S. Watanabe, and L.A. Dean. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Dep. of Agric. Circ. 939.

Padmavathy A and G Poyyamoli. 2011. Effects of conventional and organic management strategies on soil quality and biodiversity in agriculture fields of Bahour, Puducherry, India. *American-Eurasian J. Agric. And Environ. Sci.* **10(4)**:644-652.

Poudel D.D., Horwarth W.R., Lanini W.T., Temple S.R., vanBruggen A.H.C. 2002. Comparison of soil N availability and leaching potential, crop yields and weeds in organic, low-input and conventional farming systems in northern California. *Agric. Ecosyst. Environ.* **90**:125–137.

Sankaran, A. 1966. Laboratory manual for Agricultural Chemistry. Madras: Asia Publishing House.

Tu C., Frank J. Louws., Nancy G. Creamer., J. Paul Mueller., Cavell Brownie., Ken Fager., Melissa Bell., Shuijin Hu. 2005. Responses of soil microbial biomass and N availability to transition strategies from conventional to organic farming systems. *Agriculture, Ecosystems and Environment.* **113(1-4)**:206-215.

Walkley, A.; Black, I.A. 1934. An examination of Degtjareff method for determining soil organic matter, and proposed modification of the chromic acid tritation method. Soil Science 37:29-38.

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STUDIES ON NUTRITIONAL GROUPING OF STREPTOMYCETES FROM FISHES OF THREE ENVIRONMENTAL BIOTOPES, THEIR ANTIBIOGRAM AGAINST *VIBRIO CHOLERAE*

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Abstract

For thousands of years, natural products have played an important role throughout the world in treating and preventing human diseases. Marine, estuarine and fresh-water organisms produce many of the pharmaceutically active natural compounds (drugs). Natural products are the carbon compounds isolated from diverse living things. These compounds may be derived from primary or rather secondary metabolism of living organisms. The primary metabolites (polysaccharides, nucleic and fatty acids) are common in all biological systems. The secondary metabolites are, however, of low molecular weight (MW<3000), chemically and taxonomically extremely diverse compounds with obscure function, characteristic mainly to some specific and distinct types of organisms. The practical importance of antibiotics and other secondary metabolites is tremendous. They are widely used in the human therapy, veterinary, agriculture, scientific research and in countless other areas. Microbial sources are one among them synthesizing antibiotics and other secondary metabolites. Considering the above facts, we attempted to isolate antibiotics from *Streptomyces* that were isolated from the gut of fishes from three environmental biotopes – marine (*Epinephelus diacanthus* [Grouper]), estuarine (*Oreochromis mossambicus* [Tilapia]) and fresh-water (*Cyprinus carpio* [Common carp]). From the initial screening, around 121 cultures of *Streptomyces* were obtained and from these, 23 cultures were selected for the study on the basis of their anti-microbial activity. Four cultures showed positive antibiogram against *Vibrio cholerae*. TLC and U.V spectral analysis results also supported this observation, by revealing an R_f value of the compounds to be in the range of 0.40 to 0.78 and Maximum absorbance peaks ranged from 215 to 300 nm, which corresponds high bioactivity and polyene nature. These strains produced either a broad spectrum antibacterial compound or several compounds with different activities against *Vibrio cholerae*.

Key words: Nutritional grouping, microbes, *Streptomyces*, *Vibrio cholera*

INTRODUCTION

Natural product medicines have come from various source materials including terrestrial plants, terrestrial microorganisms, marine organisms and terrestrial vertebrates and invertebrates (Newman DJ et al, 2000). Marine, estuarine and fresh-water organisms produce many of the pharmaceutically active natural compounds

(drugs). Drug discovery research from Marine, estuarine and fresh-water organisms has been accelerating and now involves interdisciplinary research including biochemistry, biology, ecology, organic chemistry, and pharmacology. An important part of the natural products, the group of small molecular secondary metabolites of microorganisms, usually exhibits some kinds of biological activities, and their compounds, the bioactive secondary microbial metabolites. Most characteristic feature of secondary metabolites is their incredible array of unique chemical structures and their very frequent occurrence and versatile bioactivities. The secondary metabolites isolated from microbe's exhibits either anti microbial (antibacterial, antifungal, anti protozoan) anti tumor and antiviral activities) used to be called as "antibiotics". Various antibiotics were mainly isolated from bacteria of different species, the search for new bioactive substances has been remarkably successful and approximately two third of naturally occurring antibiotics including many medical importance have been isolated from actinomycetes (Okami and Okazaki, T. 1976) and majority from the genus *Streptomyces*. Compared to terrestrial forms, aquatic *Streptomyces* are important sources for the discovery of novel antibiotics. The light of our accumulated knowledge and in the statistical data, however, the potency of *Streptomyces* spp should not be under estimated. Their capacity to produce promising new compounds will certainly be unsurpassed and for a long time and they still have been producing the majority of the chemotherapeutically applied antibiotics. Most *Streptomyces* and other actinomycetes produce a diverse array of antibiotics including amino-glycosides, anthracyclins, glycopeptides, β-lactams, macrolides, nucleosides, peptides, polyenes, polyethers and tetracycline's (Good Fellow *et.al*., 1988). Considering the above facts which leads to studies on (1) isolation and nutritional grouping of *Streptomyces* spp from gut of fishes of three environmental biotopes – marine (*Epinephelus diacanthus* [Grouper]), estuarine (*Oreochromis mossambicus* [Tilapia]) and fresh-water (*Cyprinus carpio* [Common carp]) and (2) their antibiogram against *V.cholerae* and its antibiotic spectrum.

MATERIALS AND METHODS

In the present study we aimed to isolate and enumerate the nutritional grouping of *Streptomyces spp* from the gut of fishes includes of three environmental biotopes – marine (*Epinephelus diacanthus*[grouper]) Vizhinjam, estuarine (*Oreochromis mossambicus* [Tilapia]) Veli lake and fresh-water (*Cyprinus carpio* [Common carp]) CARE (Centre for Aquatic and Research Extension). Then the samples were transported to the laboratory within the minimum possible time to avoid the external microbial contamination. After transportation to the laboratory fish gut were removed. Normally for nutritional grouping of microorganisms they use basal media for isolation but instead of that used selective media like Glycerol asparagine agar and some additional growth factors like amino acids, vitamins, yeast extract, sediment extract, for enrichment as well as isolation of more number of *Streptomyces* colonies. The selective medium used is Glycerol asparagine agar, the best medium

allowing good development of actinomycetes micelles while suppressing bacterial growth which were containing starch or glucose as the carbon sources with casein & asparagines or nitrate as the nitrogen source, which is enriched with amino acids, vitamins, and yeast, sediment extracts. Totally 7 different media were prepared. **1. GA + C, 2. GA + AA, 3. GA + V, 4. GA + AA + V, 5.GA + Y, 6. GA + S, 7.GA + Y + S** .[**GA**- Glycerol asparagine agar,**C**- Control, **AA**-Amino acid (Alanine) - 0.100g in 100ml, **V**-Vitamin (Ascorbic acid) – 0.005g in 100ml, **Y**-Yeast extract – 0.025g in 100ml, **S**-Sediment extract – 25ml in 75 ml of media].

Enumeration and Maintenance of Cultures

In each selective media, numbers of colonies of *Streptomyces spp* were found. There selective colonies of *Streptomyces* species are sub cultured in slants by using Glycerol asparagine agar medium enriched with nutrients. Later they were kept in refrigerator (4^0C) till further analysis was to be carried out.

Characterization of selected *Streptomyces spp*

The characterization of the selected *Streptomyces spp* were carried out according to the methods employed by collaborator in International *Streptomyces* Project (ISP) (Shirling and Gottlieb, 1966).

Colour Determination

For colour determination cultures were streaked on different media such as Glycerol asparagine agar and they were observed after 7 days. Aerial and substrate mycelial colours were recorded in a simple way (Grey, white, red, yellow, orange, green, cream etc).

Antibacterial Activity of Selected *Streptomyces spp*

A loop full of *Streptomyces* strains was inoculated into Glycerol asparagine broth and incubated for 28^0C at 120h done in shaker. Then it was centrifuged at 5000 rpm for 15 minutes and the cells are separated. 2ml of the cells was transferred to fermentation broth (100ml) and incubated at 28^0C for 120h in shaker at 105 rpm. After growth it was centrifuged at 10,000 rpm for 20 minutes to separate the mycelial biomass. Then the supernatant was mixed with ethyl acetate, hexane, and toluene in 1:1 proportion. Then it was shaken for 2hrs in shaker and transferred to separating funnel, solvent was separated. Again it was centrifuged at 5000 rpm for 15 minutes to remove the traces from fermentation broth. Then the solvent was dried in water bath of 80-90^0C and residue was weighed. The residue is mixed and concentrated with little ethanol. Then it was impregnated with filter paper disk (6mm diameter) and dried. Then it was placed in microbes cultivated plates and kept in 37^0C for 48h. Inhibition zone was noted and measured.

TLC and UV Vis Spectral Analysis

Quantitative analysis of antibacterial compounds in the experimental sample was carried out by using Thin Layer Chromatography (TLC). Then, applying slurry made by silica gel G for TLC grade and applied over the glass slides, TLC slides were made. This was dried at 60°C for an hour of period. The dried slides were pre-activation base was drawn on the TLC slides 1.0 cm away from the base line on the portion of the TLC slides.

After that sample were spotted on the baseline of the TLC slides at 1.0 cm and then allowed to dry at room temperature. Then sample applied TLC slides were placed in pre-saturated TLC chamber contains mobile phase of two combination solvents like ethyl acetate (3.5 ml) : chloroform (1.5 ml) for marine fish, ethyl acetate(1.5 ml) : benzene(3.5 ml) for estuarine fish, ethyl acetate (2 ml) : benzene (3 ml) for fresh-water fish. Then the chromatogram was developed up to a mark. Then the slides were taken out dried for few minutes. Using iodine vapour the slides were stained and spots were marked. The distances traveled by each spot in base line and relative R_f values were calculated. The residue mixed and diluted with ethanol was taken for spectral analysis at 200-400nm using UV spectrophotometer.

RESULTS AND DISCUSSION

The present investigation was an attempt to understand the distribution pattern of *Streptomyces spp* in the micro-environment of gut regions of fishes of three environmental biotopes – marine (*Epinephelus diacanthus* [grouper]), estuarine (*Oreochromis mossambicus* [Tilapia]) and fresh-water (*Cyprinus carpio* [common carp]). The primary isolation of *Streptomyces* was carried out under selective media like Glycerol asparagine agar (Dhevendaran and Annie, 1999a). This is the first time by choosing selective media instead of basal media and other six nutritional enriched media for isolation of *Streptomyces* was attempted successively. Although the nutritional classification of terrestrial bacteria has been established (Lochhead, 1952; Rouaft, 1968) a systematic approach to understand. The original nutritional grouping of microorganisms in the normal gut of three environmental biotopes fishes has not so far been made. It is interesting to note that in some fishes nutritional enriched media are essential to activate the inactive genes and to harbour more number of diversified *Streptomyces* spp.

Tables 1, 2 and 3 showed the number of microbial colonies in selective media from gut of fishes of three environmental biotopes – marine (*Epinephelus diacanthus*[grouper]), estuarine (*Oreochromis mossambicus* [Tilapia]) and fresh-water (*Cyprinus carpio* [common carp]). In that bacterial and fungal colonies were minimum in numbers because of these selective media (Glycerol asparagine agar) have glycerol in the medium which inhibit the growth of bacterial and fungal population (Zobel, 1946; 1963). But maximal growths of *Streptomyces spp* were

found particularly in the selective media enriched with amino acids and vitamins. This maximal occurrence of *Streptomyces* colonies inhibited the growth of bacteria because it has already been proved that marine *Streptomyces* synthesized antibiotics, anticancer agents, L-asparaginase enzyme as reported earlier (Nishino *et al*., 1999).

Tables-4 showed mycelial colour characteristics of selected strains of *Streptomyces* in different selective media like Glycerol Asparagine agar. The colouration pattern of aerial and substrate mycelia were totally different in each media with enrichment this may be due to that the enriched media provides certain nutrients for triggering of genes for the conversion as well as expression of other metabolic products. Which in turn lead to different aerial and substrate mycelial colourations (Figure 1). This colouration difference may be due to other secondary metabolites production provided by the enriched media (Dhevendaran and Annie, 1999; Dhevendaran *et al.*, 2004; Dharmaraj 2011). These secondary metabolites of different colouration are the rich source of certain compounds like amino acids, sugars, fatty acids, terpenes,etc.

Table 5 exhibited the antibacterial activity. The antibacterial activities of some *Streptomyces* strains were found to be active against test organism like *Vibrio cholerae*. The maximum inhibition zone (5-8mm) occurred in strains obtained from gut of fishes (Figure 2). Antagonistic *Streptomyces spp* are famous for their antibiotic production against bacteria, fungi and virus (Annie Mathew, 1995 and Dhevendaran and Annie, 1999b). Suja Devan (2000) screened *Streptomyces* from Veli Lake for antagonistic property but in the present study the components were isolated antibacterial from fermentation media and were tested for antagonism against *Vibrio cholerae*. The inhibition zone proves that these strains showed effective antagonism against particular pathogen. Further purification of components can be done and be studied for individual compound's activity against any other pathogens (Sahin and Ugur, 2003; Augustine *et al*., 2005). Our findings are corroborated the above observations.

Then TLC was carried out for the ethyl acetate, hexane, and petroleum ether extract samples of the selected strains. The R_f values ranged from 0.40 to 0.78 (Table- 6; Figure 3). The similar results were observed earlier (Illic *et al* .,2005; Dharmaraj and Sumantha 2009) and their bioactive regions were detected on TLC plates and the R_f values were ranges from 0.70 to0.88.

The U.V spectral data for the ethanol extract of selected active strains from fermented broth are shown in Figure 4. Maximum absorbance peaks ranged from 215 to 300nm. The range of peaks was observed from 200 to 400nm and the characteristics of absorption peaks indicate a highly polyene nature. These strains produced either a broad spectrum antibacterial compound or several compounds with different activities. The spectral data are consistent with those reported earlier (Swaadoun *et al*., 1999; Sheeja *et al*., 2011; Sanjana *et al*., 2014). It is quite obvious

that the *Streptomyces spp* are inherent in marine ornamental fish, synthesizing commercially valuable bio active compounds.

CONCLUSION

As far as fishes of three environmental biotopes – marine (*Epinephelus diacanthus*[grouper]), estuarine (*Oreochromis mossambicus* [Tilapia]) and fresh-water (*Cyprinus carpio* [common carp]) are concerned , which have wider association with muddy soils. The nutrient rich diversity provided the fish with all required habitats. Here we studied the interaction or association of microbes particularly *Streptomyces spp* in the gut of fishes of three environmental biotopes and their ability of producing antibacterial components against human pathogen *Vibrio cholerae*. The result of active extracts clearly proved from antibiogram, TLC, and spectral analysis that these strains are very much effective producers of antibacterial components against pathogens. It has already been shown that our *Streptomyces spp* fermented active extracts are effective against other bacteria also *viz, Bacillus subtilis, Staphylococcos aureus, Mycobacterium smegmates, and E. coli*. The similar result was obtained against *Vibrio cholerae* also. The inhibition zone measurement ranging from 5 to 8mm was also observed. This proves that these extracts have the effective antibacterial components production against pathogen *Vibrio cholerae.* The TLC 'R_f' values also exhibited the same. It ranges from 0.40 to 0.78. The bioactive components revealed the maximal U.V absorption peaks ranging from 215 to 300nm. They confirmed the active extracts may contain compounds of polyene nature. These strains produced either a broad spectrum antimicrobial compounds with different media or nature spectrum for specific target pathogen like, *Vibrio cholerae.*

TABLE-1 -Number of *Streptomyces* colonies obtained after Nutritional grouping of from gut of marine fish (*Epinephelus diacanthus*[Dot Grouper]).

Strains	Number of Colonies	Selected strains
GA + G	4	1
GA + V	7	1
GA + S	5	1
GA + Y	4	1
GA + Y + S	8	2
GA + AA + V	9	1

GA- Glycerol asparagine agar, G- Control, AA- Amino acid, V- Vitamins, S-Sediment extract, Y - Yeast extract.

TABLE-2 -Number of *Streptomyces* colonies obtained after Nutritional grouping of from gut of estuarine fish (*Oreochromis mossambicus* [Tilapia]).

Strains	Number of Colonies	Selected strains
GA + T	9	1
GA + V	7	1
GA + AA + V	6	2
GA + Y	9	1
GA + Y + S	7	1
GA + S	6	1
GA +AA	5	1

GA- Glycerol asparagine agar, T- Control, AA- Amino acid, V- Vitamins, S- Sediment extract, Y - Yeast extract.

TABLE-3 - Number of *Streptomyces* colonies obtained after Nutritional grouping of from gut of fresh-water (*Cyprinus carpio* [Common carp]).

Strains	Number of Colonies	Selected strains
GA + Y	5	1
GA + AA	4	1
GA + S	7	1
GA + AA + V	5	1
GA + Y	4	1
GA + Y + S	7	2
GA + Y_1	3	1

GA- Glycerol asparagine agar, Y- Control, AA- Amino acid, V- Vitamins, S- Sediment extract, Y - Yeast extract.

Table 4 – Mycelial colour characteristics of selected strains of *Streptomyces* in selective media, isolated from gut of 1) Marine (*Epinephelus diacanthus*[grouper]), 2)Estuarine (*Oreochromis mossambicus* [Tilapia]) and 3)Fresh-water (*Cyprinus carpio* [common carp]) fishes

Strains	Glycerol asparagine agar medium	
	Aerial mycelium	Substrate mycelium
GA + T	White	Orange
GA + Y + S	White	Yellow
GA + S	Dark green	Pale yellow
GA + AA + V	Pure white	Yellow

GA- Glycerol asparagine agar, T- Control, AA- Amino acid, V- Vitamins, S- Sediment extract, Y - Yeast extract.

Table 5 –Antibiogram of *Streptomyces* spp associated in gut of 1) Marine (*Epinephelus diacanthus*[grouper]), 2)Estuarine (*Oreochromis mossambicus* [Tilapia]) and 3)Fresh-water (*Cyprinus carpio* [common carp]) fishes.

Strains of fishes	Inhibition zone(mm)
GA + T	6.7
GA + Y + S	8.3
GA + S	2.0
GA + AA + V	7.0

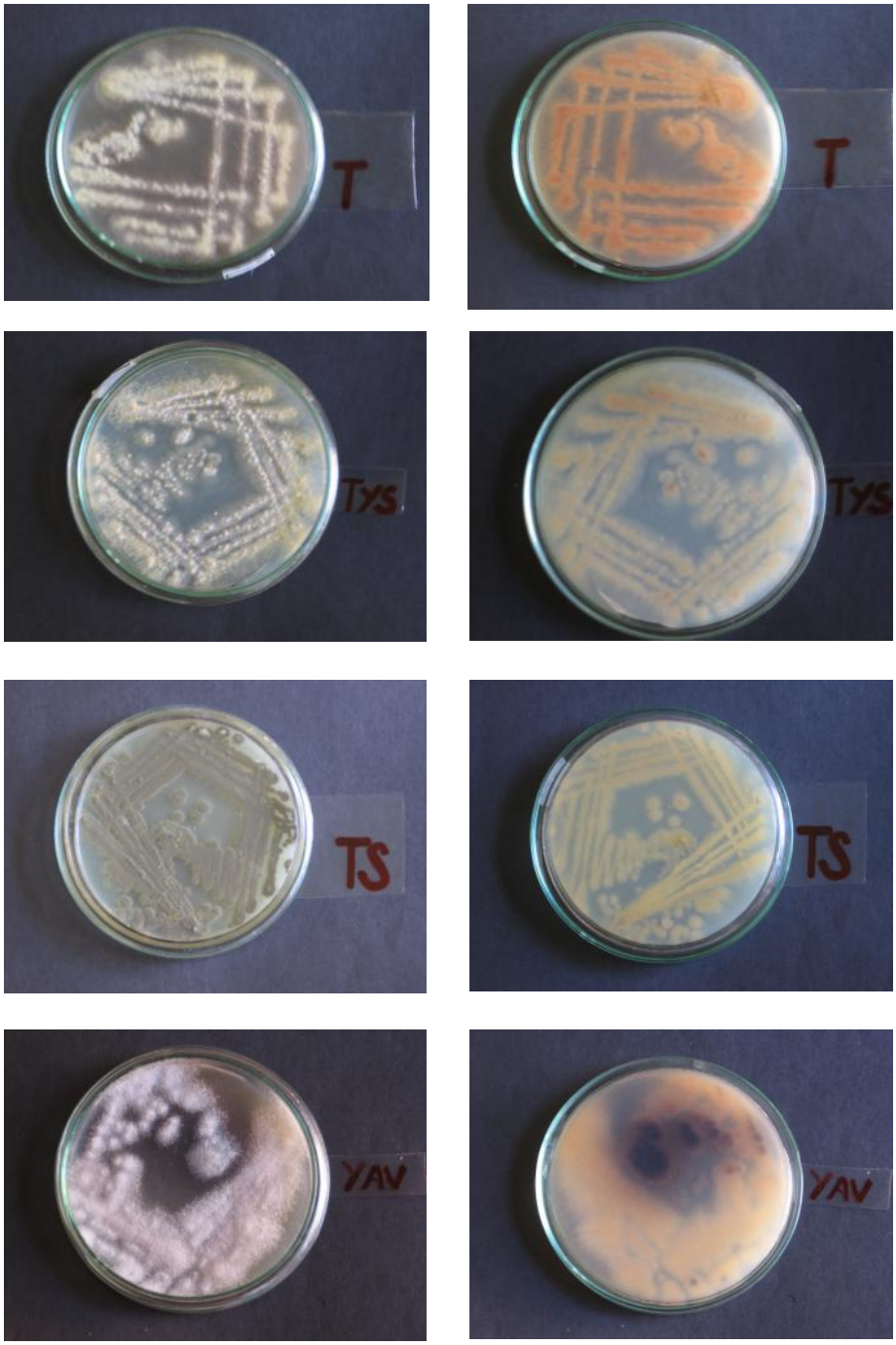
GA- Glycerol asparagine agar, T,Y- Control, AA- Amino acid, V- Vitamins, S- Sediment extract, Y - Yeast extract

Table 6 -Qualitative analysis of antibacterial components isolated from selected *Streptomyces spp* associated in gut of 1)marine (*Epinephelus diacanthus*[grouper]), 2)estuarine (*Oreochromis mossambicus* [Tilapia]) and 3)fresh-water (*Cyprinus carpio* [common carp]) fishes by using TLC

Strains of fishes	R_f values
GA + T	0.63 0.69
GA + Y + S	0.67
GA + S	0.78
GA + AA + V	0.51 0.46

GA- Glycerol asparagine agar, T,Y- Control, AA- Amino acid, V- Vitamins, S- Sediment extract, Y - Yeast extract

Figure 1- Colour dertermination of selected *streptomyces spp* associated in gut of 1) Marine (*Epinephelus diacanthus*[grouper]), 2)Estuarine (*Oreochromis mossambicus* [Tilapia]) and 3)Fresh-water (*Cyprinus carpio* [common carp]) fishes.



GA- Glycerol asparagine agar, T,Y- Control, AA- Amino acid, V- Vitamins, S- Sediment extract, Y - Yeast extract.

Figure 2: Thin Layer Chromatographic separations of Antibacterial components from 1) Marine (*Epinephelus diacanthus*[grouper]), 2)Estuarine

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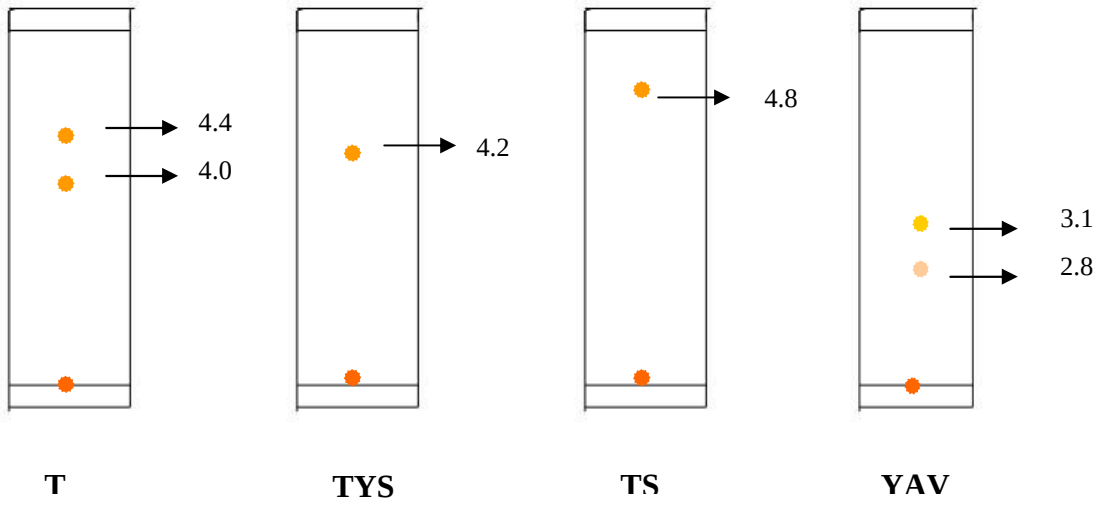
(*Oreochromis mossambicus* [Tilapia]) and 3)Fresh-water (*Cyprinus carpio* [common carp]) fishes.



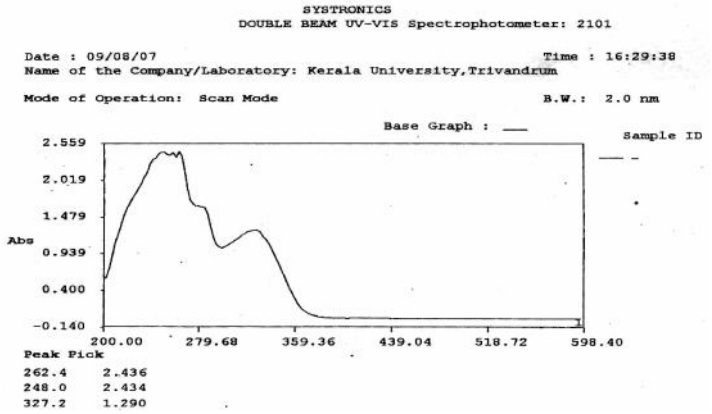
Starting point

Above the R_f values 3.0-3.9

Above the R_f values 4.0 -5.0

Above the R_f values 2.0-2.9

Figure 3- U.V Spectral analysis of antibacterial components isolated from *Streptomyces* spp isolated from gut of 1)marine (*Epinephelus diacanthus*[grouper]), 2)estuarine (*Oreochromis mossambicus* [Tilapia]) and 3)fresh-water (*Cyprinus carpio* [common carp]) fishes



1. GA + T

2. GA + Y + S

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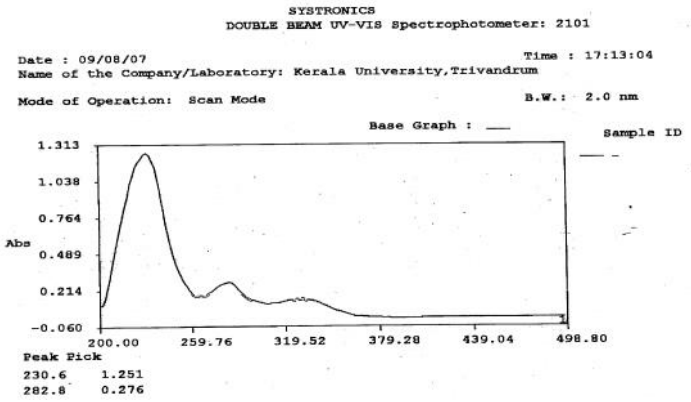
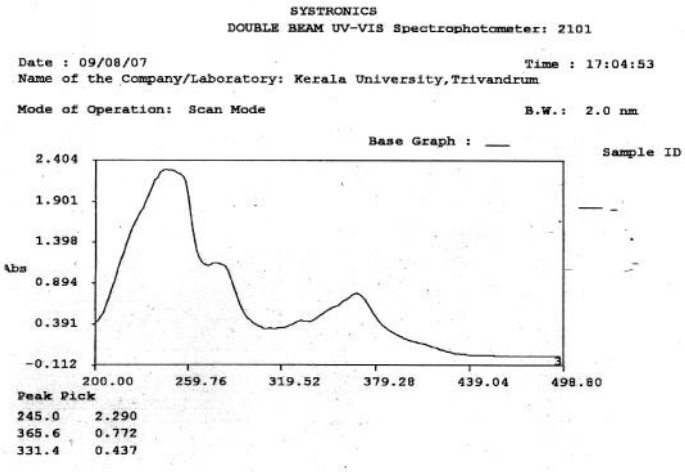
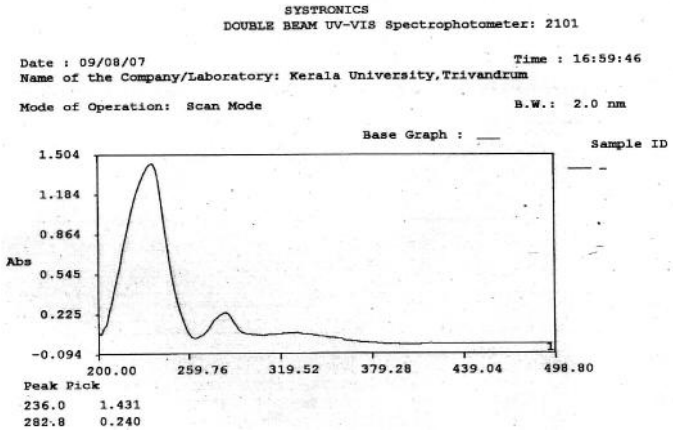


3. GA + S



4. GA + AA + V



REFERENCES

1. Annie Mathew A.K. (1995) Studies on antagonistic *Streptomyces* associated with fish and shell fish of Veli Lake, Kerala. Ph. D Thesis, University of Kerala.
2. Augustine, S.K. (2004) Bioactive compounds from actinomycetes with potential to inhibit pathogenic fungi, Ph.D Thesis, University of Pune, Pune.
3. Dharmaraj, S. and Sumantha, A.,(2009)"Bioactive potential of *Streptomyces* isolated from marine sponges, *World. J. Microbiol. Biotechnol*., 25, pp. 1971-1979.
4. Dharmaraj, S., (2011) Antagonistic potential of marine actinobacteria against fish and shellfish pathogens *Turkish. J. Biol*., 35, pp. 303-311.
5. Dhevendaran, K and Annie Mathew. K. (1999 a) Systematics of *Streptomyces* associated with the gut of alimentary canal of estuarine fish and shell fish of India. *Fish. Technol*, 36: 90-95.

6. Dhevendaran, K and Annie Mathew. K. (1999b) Antibiotic and L. asparaginase activity of Streptomyces isolated from fish, shell fish and sediment of *Veli* estuarine Lake along the Kerala coast. *Indian. J.Mar. Sci;* 28: 335-337.
7. Dhevendaran, K; Sukumaran, M and Georgekutty, (M.I. 2004) Growth hormone (IAA) - producing bacteria and Streptomycetes and their bio-fertilizer effect on mangrove seedling, *Avicennia officinalis*.L. *Mar.Biotechnol*.6. S209-213.
8. Good-fellow, M; Williams, S.T and Mordarski, M. (1988) Actinomycetes in biotechnology. London. Academic press.
9. Ilic, S.B., Konstantinovic, S.S., and Todorovic, Z.B., (2005) UV/Vis analysis and antimicrobial activity of *Streptomyces* isolates *Medi. Biol*., 12, pp. 44-46.
10. Lochead A.G. (1952) The nutritional classification of soil bacteria. *Proc .Soc .Appl.Bact*, 15:15-20
11. Newman DJ, Cragg GM , Snader KM (2000) The influence of natural products upon drugs discovery. Nat Prod Rep. 17: 215-234
12. Nishino, H., *et al.* (1999) Cancer prevention by carotenoids, *Journal of Pure and Applied Chemistry*, 71:2273-2278
13. Okami, Y and Okazaki, T. (1976) Studies on Actinomycetes isolated from shallow sea and their antibiotic substances. In: T. Arai (ed). *Actinomycetes the boundary microorganisms*. Toppen Co. Ltd. Tokyo. Japan, 123-161
14. Rouaft, J. W. (1968) Nutritional classification of soil bacteria and their value in ecological studies. *In the Ecology of Soil Bacteria*, T.R.G. Gray and Parkinson,D (eds) ,360-370
15. Sahin, N and A. Uğur, (2003). Investigation of the antimicrobial activity of some *Streptomyces* isolates. Turk. J. Biol., 27: 79-84.
16. Sajana, A.S., Dharmaraj, S., Rajan, R., and Vijaya, P.P., (2014) Screening of polyene compounds from *Streptomyces* associated with retting areas of Murukkumpuzha along Kerala coast, India, *Proceedings of National Seminar on Recent Adv. Biotechnol. Appl. Microbiol.*, pp. 284-291.
17. Sheeja, M.S., Dharmaraj, S., and Dhevendaran, K., (2011) Bioactive potential of *Streptomyces* associated with gut of marine ornamental fishes, *Middle-East. J. Sci. Res*.,7, pp. 327-334.
18. Shirling, E.B and Gottieb, D. (1966) Co-operative descriptive of type cultures of *Streptomyces. The International Streptomyces Project. Intr. J.System, Bacteriol*, 17: 315-322.
19. Suja Devan, V. (1999) Environmental impact assessment. Studies on microbial population of Parvathy Puthnar, Thiruvananthapuram, M.Phil Dissertation, University of Kerala.
20. Swaadoun, I; Hameed, K.M and Moussadui, A. (1999) Characterization and analysis of antibiotic activity of some aquatic actinomycetes. *Microbios*, 99: 173-179
21. Zobell, C.E. (1946) *Marine microbiology*. Chronica Botanica, Watham, Mass, 240pp.
22. Zobell, C.E.(1963) *Domain of marine microbiologist*. In: Symposium on marine microbiology (Oppeinheirmer, Charles C. Thomas, ed.), USA.,3-24.

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ANTIPLASMODIAL PHYTOTHERAPY AND DRUG DISCOVERY- AN OVERVIEW

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ABSTRACT

Malaria is the most destructive and dangerous parasitic infection in many tropical and subtropical countries. The burden of this disease is getting worse, mainly due to the increasing resistance of *Plasmodium falciparum* against the widely available antimalarial drugs. There is an urgent need for new, more affordable and accessible antimalarial agents possessing original modes of action. Natural products have played a dominant role in the drug discovery to treat human diseases and this fact anticipates that new antimalarial drugs may certainly emerge from tropical plant sources. This present review covers a list of antimalarial plants, natural compounds from plants with antiplasmodial and antimalarial properties, besides the majority of papers describing antiplasmodial crude extracts published in the last five years and prospecting of natural compounds. In addition, some perspectives and remarks on the development of new drugs and phytomedicines for malaria.

INTRODUCTION

Malaria is a foremost health problem in tropical, subtropical world and to the world which are socioeconomically backward. In India, 95% population resides in malaria endemic regions and it is major public health problem in most part of the country. Malaria is a parasitic protozoan disease, caused by parasites of *Plasmodium* genus. About 92% malaria cases and 97% of deaths due to malaria is reported from North-eastern states ,Chhattisgarh, Jharkhand, Madhya Pradesh, Orrissa, Andhra Pradesh, Maharashtra, Gujarat, Rajasthan, West Bengal and Karnataka (1). Around 2.5 million malaria cases are reported annually from South East Asian Nations (2) of which India alone contributes 76% of in the global malaria incidences (WHO, 2012). Its general symptoms are high fever come with chills, intermittent fever, and very often headache as well as trembling as major symptom. Over millennia, its victims have included Neolithic dwellers, early Chinese and Greeks, princes and paupers. In the 20th century alone, malaria claimed between 150 million and 300 million lives, accounting for 2 to 5 percent of all deaths (3). Although its chief sufferers today are the poor of sub Saharan Africa, Asia, the Amazon basin, and other tropical regions, 40 percent of the world's population still lives in areas where malaria is transmitted. Malaria is caused by single-celled protozoan parasites called *Plasmodium* and transmitted to man through the *Anopheles* mosquito. It is one of the major fatal diseases in the world, especially in the tropics, and is endemic in some 102 countries, with more than half of the world population at risk with fatality rates being extremely high among young children below 5 years of age.(4) The World Health Organization estimates that there are between 300 and

500 million new cases of malaria worldwide, every year, mostly in Africa, Asia, South Pacific Islands and South America, which causes at least 1 million deaths annually. In spite of control programs in many countries, there has been very little improvement in the control of malaria, and infections can reduce the effectiveness of labor and can lead to both economic and human losses. Control of malaria is complex because of the appearance of drug resistant strains of *Plasmodium* and with the discovery that man becomes infested with species of simian (monkey) malaria (4) At the same time, the *Anopheles* mosquitoes have developed resistance to many insecticides(5).

Malaria is an infectious disease endemic throughout tropical countries. Malaria is also prevalent in subtropical areas, where the disease is contagious affecting both indigenous population and travelers (6). Malaria is caused by *Plasmodium* parasites that are transmitted through the bite of *Anopheles* mosquitoes and have a life cycle in mosquito and human hosts (6). Of all parasite types, *Plasmodium falciparum* (*P. falciparum*) is the most dangerous *Plasmodium*, causing human malaria with a mortality of 1–2 million people annually. According to surveys conducted between 1900 and 2008 in 2,366 locations in Indonesia, four species of *Plasmodium* may infect humans, *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. *P. falciparum* is the most common parasite that is contagious in Indonesia, with prevalence rates of 33% in Papua, 29% in Lesser Sundas and 21% in Sumatra (7). Findings of studies performed in other parts of Indonesia, including the Thousand Island district (8), Nias Island (9), Sumba Island (10) and Aceh (11), have shown that *P. falciparum* was the most frequent parasite that caused malaria.

APPLICATION OF PLANTS IN THE TREATMENT OF MALARIA

Apart from the pharmacological treatment, various options are being used since ancient timesfor many health ailments. Nearly 80% of the global population still depends upon the herbal drugs for their health care. In India, the use of several medicinal plants to cure specific ailments has been practiced since ancient times. Various cultural traditions are associated with use of wild plants as medicinal herbs. This medico-lore is passed over generations traditionally all over the world. Reliance on plants is primarily due to their safety, effectiveness, cultural preferences, Inexpensiveness and abundant availability all the time. The medicinal virtues of plants are identified by instinct/intuition or by trial and errors. Globally, traditional healers are using various medicinal plants for the treatment of malaria; however, this practice is not really completely recognized by modern medical Science. Knowledge about traditional medicinal practices and plants is currently transmitted from generation to generation principally by word of mouth. Large number of plant species has been identified as anti-malarial medicinal plants.

Medicinal plants have been used as traditional medicines for hundreds of years. The first antimalarial agents, including quinine as well as the next generation of antimalarial agents, such as lapachol and artemisinin, have been isolated from plants (12,13). Traditional medicines have a high potential as novel drug candidates, can provide valuable clues to find novel drugs and may shift the drug discovery paradigm from 'finding newentity drugs' to 'combining existing agents' (14,15). Certain approaches in finding novel drugs use plants that are consumed by particular groups (16). Primates have a close similarity with human physiology and also have similar characteristics of disease as humans. Humans use drugs to

cure these diseases, whereas primates can only rely on the foods they eat to protect themselves against these diseases.

Sr.No	Botanical Name	Common name/ Vernacular name	Family	Parts used
1	*Clerodendrum viscosum* Vent[17]	Viti, Bhat Pata, Bhati, Vaita,Foksha, Baadbagora.	Lamiaceae	Whole plant
2	*Duranta repens* L[17]	Kata-mehandi, Kata-mehendhe	Verbenaceae	Whole plant
3	*Lantana camara* L[17]	Chaturaangi, Jangoli-janglog	Verbenaceae	Leaf, root, flower
4	*Nyctanthes arbor tristis* L[17]	Shefali, Sheuli, Sheuly-phang	Oleaceae	Leaf
5	*Dracaena reflexa* Lamk[18].	Hasina Pleomele, Song of India	Asparagaceae	Leaf and bark decoction
6	*Cinnamosma fragrans* H[18]	Sakarivohazo,	*Canellaceae*	A decoction of the leaf and bark
7	*Andropogon schoenanthus/nardis* [18]	veromanitra	*Gramineae*	The leaf decoction
8	*Desmodium mauritianum D.C[18].*	Bean of the hare oganana	*Leguminosae*	A decoction of the leaf and bark
9	*Desmodium hirtum Grill* and Perr[18].	Tsilavindrivotro	*Leguminosae*	A decoction of the leaf and bark
10	*Tristellateia madagascariensis* Poir[18].	Menahelika	*Malpighiaceae*	The leaf decoction
11	*Ficus megapoda* Bak[18].	Mandresy	*Moraceae*	A decoction of the leaf and bark
12	*Nymphaea lotus* L[18].	Voahirana or retsimilana	*Nymphaeaceae*	A decoction of the leaf and bark
13	*Vepris ampody* H. Perr[18]	Ampody	*Rutaceae*	A decoction of the leaf and bark
14	*Zanthoxylum tsihanimposa* Bak[18].	Tsihanihimposa	*Rutaceae*	A decoction of the leaf and bark
15	*Peddiea involucrata* Bak[18]	Montana	*Thymelaeaceae*	A decoction of the leaf and bark
16	*E. angolense[19]*	Mukus, Edinam	*Meliaceae*	Plant extract
17	*P. nitida[19]*	waboom, blousuikerbos	*Apocynaceae*	Plant extract
18	*T. hensii[19]*		*Thomandersiaceae*	Plant extract
19	*Shumanniophyton magnificum[19]*	Sierra Leone Mend	*Rubiaceae*	Plant extract
20	*Rauvolfia vomitoria Afzel.[20]*	Omuatabusinde/Kinyabusinde	*Apocynaceae*	The leaves decoction
21	*Canarium schweinfurtii Engl[20]*	Muubani	*Burseraceae*	The bark scent

22	*Zehneria scabra Sond[20]*	Akabindizi	*Cucurbitaceae*	The leaves decoction
23	*Bridelia micrantha (Hochst.) Baill[20]*	Mshamako	*Euphorbiaceae*	Root decoction
24	*Tragia furialis Bojer[20]*	Omugonampili	*Euphorbiaceae*	The leaves decoction
25	*Abrus precatorius L[20]*	Kaligaligo	*Fabaceae*	The leaves decoction
26	*Dolichos kilimandscharicus Taub[20]*	Khat	*Fabaceae*	The leaves
27	*Sesbania microphylla E.Phillips & Hutch[20]*	Msenga, Mbondo	*Fabaceae*	The leaves
28	*Senna occidentalis (L.) Link[20]*	Omwetanjoka	*Fabaceae*	The leaves decoction
29	*Tetradenia urticifolia (Baker) Phillipson[20]*	Lwamo	*Lamiaceae*	The leaves decoction
30	*Solanum aculeastrum Dunal[20]*	Omulembezi, Entobatobe	*Solanaceae*	Fruits
31	*Caesalpinia nuga (L.)W. T. Aiton[21]*	Krung-khai	*Fabaceae*	Seed
32	*Adansonia digitata L[21]*	Kattio-daghor	*Bombacaceae*	Leaf, root, Flower
33	*Jatropha gossypifolia[21]*	Titto-long	*Euphorbiaceae*	Seed
34	*Rauwolfia serpentina Benth[21]*	Sharpagandha	*Apocynaceae*	Root
35	*Hodgsonia macrocarpa Cogn[21]*	Keha-pang	*Cucurbitaceae*	Fruit
36	*Erythrina variegata L[21]*	Mada-kamiama-fang	*Fabaceae*	Bark
37	*Streblus asper Lour[21]*	Sarwa	*Moraceae*	Bark
38	*Clerodendrum viscosum Vent[21]*	Kung-sroi-ma	*Verbenaceae*	Leaf
39	*Amaranthus spinosus[21]*	Kang-chuo	*Amaranthaceae*	Root
40	*Mussaenda corymbosa A.L. de Jussieu[21]*	Mok-ae	*Rubiaceae*	Leaf
41	*Scoparia dulcis[21]*	Tapra-amkanlu	*Scrophulariaceae*	Leaf
42	*Ocimum sanctum Linn.[22]*	Tulsi	*Lamiaceae*	Plant extract
43	*Cryptolepis Sanguinolenta[23,24]*	Nibima	*Apocynaceae*	aqueous extract
44	*Artemisia annua[23,24,25]*	sweet wormwood	*Asteraceae*	aqueous extract
45	*Dichroa Febrifuga[23]*	Gigil,Tataruman	*Hydrangeaceae*	aqueous extract
46	*Kalanchoe pinnata[24]*	Air Plant, Life Plant	*Crassulaceae*	Leaf
47	*Esenbeckia febrifuga (A.St.-Hil.) A.Juss. ex Mart[25]*	Três folhas	*Rutaceae*	Hexane/ethanolic extracts
48	*Boerhavia hirsuta[25]*	Pega Pinto	*Nyctaginaceae*	Hexane/ethanolic extracts
49	*A. austral[25]*	Carrapicho	*Cerambycidae*	Hexane/ethanolic extracts

50	*Tachia guianensis[25]*	Caferana	*Gentianaceae*	Hexane/ethanolic extracts
51	*Cecropia glaziouvii[25]*	Umbauba	*Urticaceae*	Plant
52	*Bidens pilosa[25]*	Cobbler's Pegs or Spanish Needle	*Asteraceae*	Extracts and fractions of plants
53	*A.amazonicus Ducke[25]*	Indian beer	*Rhamnaceae*	Dried Ground roots
54	*Alstonia scholaris (L.) R.Br[26]*	Yaknalae's Ita	*Apocynaceae*	Leaves and bark
55	*Aristolochia indica L. [26]*	Yaki'Itchale	*Meliaceae*	Leaves and bark decoction
56	*Maclura sp. [26]*	Iveriate	*Moraceae*	Leaves
57	*Polygala paniculata L. [26]*	Nuva gihi	*Polygalaceae*	Leaves and fruits
58	*Polyscias filicifolia[26]*	Iriduki'Imetchale	*Araliaceae*	Leaves
59	*Setaria sp. [26]*	Nomu suva	*Poaceae*	whole plant
60	*Tasmannia piperita (Hook. f.) Miers.[26]*	Iridukichale	*Winteraceae*	Leaves
61	*Tristiropsis sp. [26]*	Longola	*Sapindaceae*	Soft Leaves
62	*T. herba-barona[27]*	Caraway thyme	*Lamiaceae*	Aerial parts
63	*S. thymbra[27]*	Thyme-leaved savory	*Lamiaceae*	Aerial parts
64	*M. communis[27]*	Myrtle	*Myrtaceae*	Aerial parts
65	*Cymbogon citratus[27]*	Lemon grass, oil grass	*Poaceae*	Vapours of a decoction
66	*Ocimum gratissimum[27]*	Clove Basil, African Basil	*Lamiaceae*	Vapours of a decoction
67	*Cuviera longiflora[28]*	-	*Rubiaceae*	Leaves
68	*Dacryodes edulis[28]*	Zo'o	*Burseraceae*	Leaves
69	*Eucalyptus globules[28]*	Klatusse	*Myrtaceae*	Leaves
70	*Kotschya speciosa[28]*	Hepper	*Leguminoceae*	Whole plant
71	*Coula edulis[28]*	Walnut	*Olacaceae*	Stem bark
72	*Vernonia amygdalina[28]*	Bitter leaf	*Asteraceae*	Leaves, root bark
73	*Vismia guinensis[28]*	-	*Asteraceae*	Stem bark
74	*Nauclea latifolia[29]*	Pin cushion tree	*Rubiaceae*	stems and roots
75	*Pseudocedrela kotschyi[29]*	Dry zonecedar; Hard cedar-mahogany	*Meliaceae*	Leaves
76	*Prosopis africana[29]*	African mesquite	*Fabaceae*	Plant
77	*Trichilia emetica[29]*	Natal Mahogany	*Meliaceae*	Plant

78	*Diospyros quaesita Thw. [30]*	Calamander	*Ebenaceae*	Leaf and stem
79	*Gongronema napalense (Wall.) Decne.[30]*	-	*Asclepiadaceae*	Whole flower(wine)
80	*Nauclea orientalis (L.) [30]*	yellow cheesewood	*Rubiaceae*	Dried plant/stem
81	*Rourea minor (Gaertn.) Aubl. [30]*	A woody vine	*Connaraceae*	Dried stem
82	*Amaranthus hybridus[31]*	Slim amaranth	*Amaranthaceae*	Leaves
83	*Uvaria scheffleri Diels[31]*	Mukukuma	*Annonaceae*	Leaves
84	*Carissa edulis (Forssk.) [31]*	Simple-spined num-num	*Apocynaceae*	Root bark
85	*Landolphia buchananii (Hallier f.)Stapf [31]*	Apricot vine	*Apocynaceae Juss*	Leaves
86	*Rauwolfia Cothen. [31]*	-	*Apocynaceae Juss.*	Root bark
87	*Vernonia amygdalina A. Chev. [31]*	Ewuro	*Asteraceae*	Leaves
88	*Tridax procumbens L. [31]*	lilac tassel flower	*Asteraceae*	Whole plant
89	*Commiphora schimperi (O.Berg)Engl. [31]*	Glossy-leaved corkwood	*Burseraceae Kunth*	Roots, stem bark
90	*Combretummolle Engl. & Diels[31]*	Velvet bush willow	*Combretaceae*	Leaves
91	*Gerrardanthus lobatus C. Jeffrey[31]*	Cogniaux	*Cucurbitaceae*	Roots
92	*Momordica foetida Schumach. [31]*	Concombre sauvage	*Cucurbitaceae Juss*	Leaves
93	*Ricinus communis L. [31]*	Castor Bean	*Euphorbiaceae*	Roots, leaves
94	*Suregada zanzibariensis Baill. [31]*	Prota	*Euphorbiaceae Juss*	Root bark
95	*Albizia anthelmintica Brongn. [31]*	Kyoa	*Fabaceae*	Stem bark
96	*Acaciaseyal Delile[31]*	Shittimwood	*Fabaceae*	Roots
97	*Dichrostachys cinerea (L.) Wight&Arn. [31]*	Sickle bush	*Fabaceae*	Roots
98	*Tamarindus indica L. [31]*	Tamarind	*Fabaceae Lindl*	Roots, leaves
99	*Harungana madagascariensis Lam. Ex Poir.[31]*	Praying hands	*Hypericaceae Juss.*	Root bark, stem bark
100	*Hoslundia opposita Vahl[31]*	Orange bird berry	*Lamiaceae*	Roots
101	*Ocimum gratissimum L.[31]*	African basil	*Lamiaceae*	Leaves
102	*Clerodendrum myricoides R. Br. [31]*	Bandamuchenene	*Lamiaceae*	Root bark
103	*Adansonia digitata L. [31]*	Baobab, Dead-Rat Tree	*Malvaceae*	Leaves
104	*Grewia hainesiana Hole[31]*	Phalsa	*Malvaceae Juss.*	Leaves
105	*Grewia trichocarpa Hochst.exA.Rich. [31]*	Ecol. Status	*Malvaceae Juss.*	Roots

106	*Azadirachtaindica A. Juss. [31]*	Neem	*Meliaceae*	Roots, stem bark, leaves
107	*Cissampelos mucronata A. Rich. [31]*	Heart-leaved vine	*Menispermaceae Juss*	Root bark
108	*Ficus bussei Warb.ex Mildbr.& Burret[31]*	Rudraksha	*Moraceae*	Roots
109	*Securidaca longifolia Poepp. [31]*	-	*Polygalaceae*	Roots
110	*Canthium glaucum Hiern[31]*	Bluish-Green, Glaucous	*Rubiaceae*	Fruits
111	*Pentas longiflora Oliv. [31]*	Golden Shrimp Plant	*Rubiaceae*	Root bark
112	*Clausena anisata (Willd.) Hook. f. ex Benth[31]*	Horsewood	*Rutaceae*	Leaves
113	*Zanthoxylum chalybeum Engl. [31]*	Bemba	*Rutaceae*	Root bark
114	*Toddalia asiatica (L.) Lam. [31]*	Orange climbe	*Rutaceae*	Root bark
115	*Fagaropsis angolensis (Engl.) Dale[31]*	Caper-bush	*Rutaceae Juss.*	Leaves
116	*Teclea simplicifolia (Engl.) I. Verd. [31]*	Teclea nobilis	*Rutaceae Juss.*	Roots
117	*Flacourtia indica (Burm.f.)Merr. [31]*	Governor plum	*Salicaceae*	Roots
118	*Lantana camara L. [31]*	Shrub verbenas	*Verbenaceae*	Leaves
119	*Aloe vera L. ex Webb[31]*	English musambra aloe	*Xanthorrhoeaceae*	Leaves
120	*Hypericum lanceolatum[32]*	Curry bush	*Hypericaceae*	Stem bark
121	*Caesalpinia pluviosa[33]*	Sibipiruna	*Fabaceae*	Crude extract
122	*Icacina senegalensis A. Juss.[34]*	Bankanas, False yam	*Icacinaceae*	Leaf extracts
123	*Holarrhena antidysenterica[35]*	Bitter Oleander	*Apocynaceae*	Plant extracts
124	*Viola canescens[35]*	Viola serpens var.	*Violaceae*	Plant
125	*Aframomum sp[36]*	Grains of paradise	*Zingiberaceae*	Plant
126	*Vernonia guineensis[36]*	Ginseng	*Asteraceae*	Plant
127	*Spilanthes oleracea[36]*	Toothache Plant	*Asteraceae*	Plant
128	*Alstonia boonei[36]*	Alstonia, Cheesewood	*Apocynaceae*	Plant
129	*Ambrosia Maritima[37]*	Damsisa	*Asteraceae*	Whole plant
130	*Aristolochia Bracteolata[37]*	Um Galagel	*Aristolochiaceae*	Leaves
131	*Citrullus colocynthis[37]*	El-Handal	*Cucurbitaceae*	Seed
132	*Croton zambesicus[37]*	Um-Geleigla	*Euphorbiaceae*	Fruit
133	*Nigella sativa[37]*	Kamun-Aswad	*Ranuneulaceae*	Seed

134	*Solenostema argel[37]*	El-Hargel	*Ascepiadaceae*	Leaves
135	*Allium sativum L.[38]*	Sunkurtae	*Alliaceae*	Fresh or dry fruits
136	*Artemisia afra Jack. ex Wild[38]*	Chugughee	*Asteraceae*	Fresh/dry leaves
137	*Lepidium sativum L. [38]*	Feaxxo	*Brassicaceae*	Seeds
138	*Croton macrostachyus Del. [38]*	Bissano	*Euphorbiaceae*	Fresh/dry leaves
139	*Clerodendrum viscosum* Vent *[39]*	Viti, Bhat Pata	Lamiacieae	Leaf, Root, Flowers.
140	*Duranta repens[39]*	Kata- Mehandi	Verbenaceae	Leaf
141	*Laltana Camara* L*[39]*	Shefali	Verbenaceae	Leaf and Bark Decoction
142	*Zanthoxylum tsihanimposa* Bak*[39]*	Tsihanihimposa	Rutaceae	A decoction of the leaf and bark
143	*Nymphaea lotus[39]*	Voahirana or retsimilana	Rutaceae	A decoction of the leaf and bark
144	*Bridelia micrantha* (Hochst.) Baill*[39]*	Mshamako	Euphorbiacea	Root decoction
145	*Senna occidentalis [39]*	Omwetanjoka	Fabaceae	Root
146	*Rauwolfia serpentina* Benth*[39]*	Sharpgandha	Apocynaceae	Root
147	*Ocimum sanctum* Linn *[39]*	Tulsi	Apocynaceae	Plant Extract
148	*Cryptolepis Sanguinolenta [39]*	Nimbima	Asteraceae	Plant Extract
149	*Artemisia annua[39]*	sweet wormwood	Hydrangeaceae	aqueous extract
150	*A.amazonicus* Ducke*[39]*	Indian Beer	Apocynaceae	Ground Root and Leaves
151	*Cymbogon citratus[39]*	Lemon Grass	Lamiaceae	Vapours of a decoction
152	*Coula edulis[39]*	Walnut	Asteraceae	Leaves and root bark
153	*Vernonia amygdalina[39]*	Bitter leaf	Asteraceae	Stem Bark
154	*Tamarindus indica[39]*	Tamarind	Fabaceae Lindl	Root
155	*Azadirachta indica* A. Juss*[39]*	Neem	Meliaceae	Root, Stems, Bark, Leaves
156	*Ficus bussei* Warb.ex Mildbr.& [39] Burret	Rudraksha	Moraceae	Fruit

LIST OF PLANTS IN APPLIED AS ANTIMALARIAL AGENTS
PLANT SOURCE FOR ANTIMALARIAL DRUG

One can make an argument that biologically derived secondary metabolites and synthetic compounds derived from them perform better as drugs than do randomly synthesized compounds. Primary and secondary metabolites, receptors, enzymes, transporters and regulatory proteins originated from a limited number of parent molecules. These originating molecules were present in primitive life forms and therefore coevolved to interact with one another, thus granting direct ecological benefit to the producing organism, whether in competition for resources, avoiding predation or combating pathogens. Although a divergence in function and structure has subsequently occurred, some structural relationship still remains that make natural products, on average, better ligands for biological targets than randomly synthesized compounds [39, 40]. Biosynthesis uses a very parsimonious set of building blocks. It diversifies by taking its limited battery of building blocks and distributes them into many different pathways. Nature has a tendency to form oxides by hydrolysis or abstraction of oxygen from organic compounds by enzymes that delicately achieve site selective C–H activation to introduce oxygen and discriminate between numerous functional groups at different oxidation levels.

In contrast, medicinal chemistry focuses on nitrogen and often includes additional atoms such as sulfur and halogens that are relatively rare in nature [41]. Generally, natural metabolites have a high 'sterical complexity'. This is an evident outcome of the spatial dimensionality and the chirality of the enzymes involved in biosynthesis, their molecular targets and the metabolites themselves [42]. Bioactive compounds share pharmacophoric features, and natural products provide a rich source of potentially attractive scaffolds and molecular building blocks for synthesis. Since natural compounds have not evolved as therapeutic agents, sometimes their chemical structure must be further improved, whether in terms of efficacy and selectivity for the target or achieving optimal pharmacokinetic and pharmacodynamic properties. The numerous ring system scaffolds have no orthologs in the drug synthesis database. Hence, natural products could provide new starting points in drug discovery. The vastly unexplored flora and fauna (90 % of total species) could provide other new leads and drugs for chemotherapy, for the isolation of certain natural products in large amounts, total synthesis by chemical approaches, or limited scope for chemical modification.

PROSPECTING FOR NATURAL COMPOUNDS

The validation of traditional remedies can be problematic because of the lack of sufficient information, documentation and of standardization of extracts to be evaluated, but these remedies deserve deep and thorough consideration [43]. In spite of these problems, such materials serve as a valuable source for novel compounds. They also conceal an abundant combination of secondary metabolites which might act in concert to enhance the therapeutic effect [44, 45]. Because the quantitative and qualitative composition of secondary metabolites in a plant is notoriously varied, standardization is obligatory. Modern agronomic and plant genetic methods could be harnessed to domesticate some of the most promising medicinal plants, so as to ensure a reproducible qualitative and quantitative production of active ingredients, in spite of changes in biotic and abiotic constraints. Achieving such goals may provide indigenous populations in malarious areas with new commercial crops. In addition, the road to antimalarial development could be shortened considerably, since it is

much easier to approve the use of extracts than that of their active ingredients. This does not mean that the use of such extracts does not have to pass through rigorous clinical test to assess that toxicity, teratogenicity, mutagenicity etc are not a problem.

CONCLUSION

In this review, the rationale for using natural compounds for drug development has been presented with specific consideration of antimalarial drugs. The underlying principles for using drug combination and the benefits of using multicomponent plant extracts have been argued. It seems that the scientific and pharmacological justification of these principles could provide the basis for further development of ethnic/traditional medicine as a valid, cheap and locally available means to treat malaria.

REFERENCE

1. Malaria situation in India. Delhi: National Vector Borne Disease Control Programme, Ministry of Health and Family Welfare, Govt. of India. Available from:http://www.nvbdcp.gov.in/Doc/malaria1.pdf
2. World Health Organization, World Malaria Report 2012, Geneva http://www.who.int/malaria/publications/world_malaria_report_2012/en/
3. Carter R, Mendis KN. 2002. Evolutionary and historical aspects of the burden of malaria. Clinical Microbiology Reviews 15(4):564594.

4. Symth JD. Animal Parasitology. Cambridge, England: Cambridge University Press; 1994.
5. Srisilam K, Veersham C. Antimalarials of plant origin. In: Khanum A, Khan IA, editors. Role of Biotechnology in Medicinal and aromatic plants. Vol. 7. Hyderabad India: Ukaaz Publishers; 2003. pp. 17–47.
6. Hyde JE. Drugresistant malaria an insight. FEBS J. 2007;274:4688–4698.
7. Elyazar IR, Hay SI, Baird JK. Malaria distr ibution, prevalence, drug resistance and control in Indonesia. Adv Parasitol. 2011;74:41–175.
8. Maguire JD, Tuti S, Sismadi P, et al. Endemic coastal malaria in the Thousand Islands District, near Jakarta, Indonesia. Trop Med Int Health. 2005;10:489–496.
9. Syafruddin D, Asih PB, Wahid I, et al. Malaria prevalence in Nias District, North Sumatra Province, Indonesia. Malar J. 2007;6:116.
10. Asih PB, Rogers WO, Susanti AI, et al. Seasonal distribution of antimalarial drug resistance alleles on the island of Sumba, Indonesia. Malar J. 2009;8:222.
11. Asih PB, Rozi IE, Herdiana, et al. The baseline distribution of malaria in the initial phase of elimination in Sabang Municipality, Aceh Province, Indonesia. Malar J. 2012;11:291.
12. Wells TN. Natural products as starting points for future antimalarial therapies: going back to our roots? Malar J. 2011;10(Suppl 1):S3.
13. Ginsburg H, Deharo E. A call for using natural compounds in the development of new antimalarial treatments an introduction. Malar J. 2011;10(Suppl 1):S1.
14. Kong DX, Li XJ, Zhang HY. Where is the hope for drug discovery? Let history tell the future. Drug Discov Today. 2009;14:115–119.
15. Wagner H, Ulrich Merzenich G. Synergy research: approaching a new generation of phytopharmaceuticals. Phytomedicine. 2009;16:97–110.

16. Koshimizu K, Murakami A, Hayashi H, Ohigashi H, Subarnas A, Gurmaya KJ, Ali A. Biological activities of edible and medicinal plants from Indonesia and Malaysia. Proceedings of The Tokyo International Forum on Conservation and Sustainable Use of Tropical Bioresources; Tokyo: Japan Bioindustry Association; 1998. pp.203–208.
17. WHO (2010). Guidelines for the treatment of Malaria- Second edition. Available from: http://whqlibdoc.who.int/publications/2010/9 789241547925_eng.pdf. Last accessed on 18th Jan 2013
18. Rahmatullah et al. Folk medicinal uses of verbenaceae family plants in Bangladesh . Afr J Tradit Complement Altern Med 2011; 8(S):53- 65.
19. Milijaona Randrianarivelojosia et al. Plants traditionally prescribed to treat *tazo* (malaria) in the eastern region of Madagascar. *Malaria Journal* 2003, 2:25.
20. Jean Bickii, Guy Raymond Feuya Tchouya, Jean Claude Tchouankeu and Etienne Tsamo. Antimalarial activity in crude extracts of some Cameroonian medicinal plants. *Afr. J. Trad. CAM*. 2007; 4(1):107-11
21. Moshi et al. Ethnomedicine of the Kagera Region, north western Tanzania. Part 3: plants used in traditional medicine in Kikuku village, Muleba District. *Journal of Ethnobiology and Ethnomedicine* 2012, 8:14.
22. Mohammed Rahmatullah et. al. Medicinal Plants Used by Various Tribes of Bangladesh for Treatment of Malaria. Malaria Research and Treatment. Volume 2012, Article ID 371798: 1- 5.
23. Priyabrata Pattanayak et.al. *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An Overview. Pharmacogn Rev. 2010 Jan-Jun; 4(7): 95–105.
24. Alaíde B. Oliveira et al. Plant-derived antimalarial agents: new leads and efficient phythomedicines. *An Acad Bras Cienc* 2009;81(4):715-40.
25. Merlin L Willcox, Gerard Bodeker. Traditional herbal medicines for malaria. BMJ 2004; 329:1156-59.
26. Antoniana U Krettli et al. The Search for New Antimalarial Drugs from Plants Used to Treat Fever and Malaria or Plants Ramdomly Selected: a Review. *Mem Inst Oswaldo Cruz, Rio de Janeiro* .November 2001; 96(8):1033-42.
27. Louis R Barrows.et.al. An ethnobotanical survey of medicinal plants used in the eastern highlands of Papua New guinea. *Journal of Ethnobiology and Ethnomedicine* 2012, 8:47.
28. Dell'Agli et al. Anti-plasmodial and insecticidal activities of the essential oils of aromatic plants growing in the Mediterranean area *Malaria Journal* 2012, 11:219.
29. Denis Zofou et al. In Vitro Antiplasmodial Activity and Cytotoxicity of Extracts of SelectedMedicinal Plants Used by Traditional Healers of Western Cameroon. *Malaria Research and Treatment*; Volume 2011.
30. Tchacondo et al., Herbal remedies and their adverse effects in tem tribe traditional medicine in Togo. *Afr J Tradit Complement Altern Med.* 2011.8(1):45-60.
31. Soejarto et al. An ethnobotanical survey of medicinal plants of Laos toward the discovery of bioactive compounds as potential candidates for pharmaceutical development. *Pharm Biol*. 2012 ; 50(1): 42-60.
32. Ngutaetal.et.al.Ethnodiagnostic skills of the Digo community for malaria: a lead to traditional bioprospecting. FrontiersinPharmacology. June 2011;2: Article 30.
33. Zofou et al. Hypericum lanceolatum (Hypericaceae) as a potential source of new anti-malarial agents: a bioassay-guided fractionation of the stem bark. *Malaria Journal* 2011;10:167.

34. Kayano et al. In vitro and in vivo assessment of the antimalarial activity of Caesalpinia pluviosa. *Malaria Journal* 2011;10:112.
35. Sarr et al. Icacina senegalensis (Icacinaceae), traditionally used for the treatment of malaria, inhibits in vitro Plasmodium falciparum growth without host cell toxicity. *Malaria Journal* 2011;10:85.
36. Verma et al. Anti-malarial activity of Holarrhena antidysenterica and Viola canescens, plants traditionally used against malaria in the Garhwal region of north-west Himalaya .Malaria Journal 2011;10:20.
37. Fokunang et al. Traditional medicine: past, present and future research and development prospects and integration in the national health system Of cameroon. *Afr J Tradit Complement Altern Med* 2011; 8(3):284-95.
38. Ahmed et al. Antiplasmodial Activity of Some Medicinal Plants Used in Sudanese Folk- medicine. *Environmental Health Insights* 2010; 4:1-6.
39. Ganesan A: The impact of natural products upon modern drug discovery. *Curr Opin Chem Biol* 2008, 12:306317.
40. Clardy J, Walsh C: Lessons from natural molecules. *Nature* 2004, 432:829837.
41. Feher M, Schmidt JM: Property distributions: differences between drugs, natural products, and molecules from combinatorial chemistry. *J Chem Inf Comput Sci* 2003, 43:218227.
42. Grabowski K, Baringhaus KH, Schneider G: Scaffold diversity of natural products: inspiration for combinatorial library design. *Nat Prod Rep* 2008, 25:892904.
43. Corson TW, Crews CM: Molecular understanding and modern application of traditional medicines: triumphs and trials. *Cell* 2007, 130:769774.
44. Keith CT, Borisy AA, Stockwell BR: Multicompo nent therapeutics for networked systems. *Nat Rev Drug Discov* 2005, 4:7178.
45. Li XJ, Zhang HY: Synergy in natural medicines: implications for drug discovery. *Trends Pharmacol Sci* 2008, 29:331332.
46. Bahekar, Satish and Kale, Ranjana, " Herbal Plants Used For the Treatment of Malaria" Journal of Pharmacognosy and Phytochemistry 1, (6), 123-149.

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Green Synthesis of Silver Nanoparticles using *Citrus Medica(Rutaceae)* leaf extract and its Antibacterial Efficacy

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Abstract
Nanoparticles behave differently than other similarly sized particles. It is therefore necessary to develop specialized approaches to testing and monitoring their effects. It was observed that to use natural processes such as use of plants based biological systems makes a fast and convenient method for the synthesis of silver nanoparticles(AgNPs) and can reduce silver ions into AgNPs within minutes of reaction time without using any severe conditions.The present study describes a rapid and green eco-friendly method for the synthesis of AgNPs from aqueous solution of silver nitrate using *Citrus Medica* (Rutaceae) leaf extract. UV–visible spectrum of the aqueous medium containing silver ion showed a peak at 420 nm corresponding to the plasmon absorbance of AgNPs. Scanning electron microscopy (SEM) micrograph showed formation of well-dispersed crystalline AgNPs in the range of 50-63nm. Electron dispersive spectroscopy (EDX) reveals the elemental composition of produced AgNPs. X-ray diffraction (XRD) spectrum of the AgNPs exhibited 2θ values corresponding to the AgNPs of crystalline nature. The process of reduction being extracellular and very fast may lead to the development of an easy bioprocess for synthesis of silver nanoparticles. Antimicrobial activity of the produced silver bionanoparticles was performed by well diffusion method against *Pseudomonas aeruginosa* and *Escherichia coli*. Further, the produced silver nanoparticles showed an effective antibacterial activity towards the pathogenic bacteria.

Key words: Green synthesis, *Citrus Medica*, SEM, XRD, EDX, AgNPs, Antibacterial efficacy.

Introduction
Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic molecular structures. The uses for nano sized particles are even more remarkable. Different types of nanomaterials like copper, zinc, titanium [1], magnesium, gold [2], alginate [3] and silver have come up but AgNPs have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic microorganisms [4]. As the AgNPs are playing a major role in the field of nanotechnology and nanomedicine. The use of nanoparticles derived from noble metals has spread too many areas including, medical fields, and electronics. The application of nanoparticles as delivery vehicles for bactericidal agents represents a new paradigm in the design of antibacterial therapeutics. Numerous microorganisms and plant extracts have been applied to synthesize inorganic nanostructures either intracellularly or extracellularly [5-7].The use of microbial cells for the synthesis of nanosized materials has recently emerged as a novel approach for the synthesis of metal nanoparticles. More and more research inputs are rendered for this and there has been upsurge of interest in the biological synthesis of nanomaterials by using, microorganisms, several plants in the past few years, microbes like bacteria [8-9], fungi [10-11] and plant extract. In recent

times, AgNPs have been synthesized using various plant extracts such as *Hibiscus rosa sinensis* [12], *Svensonia hyderabadensis* [13], *Trianthema decandra* [14], *Dioscorea batatas* [15], *Catharanthus roseus* [16], *Bacopa monniera* [17], *Citrus limon* [18], *Crossandra infundibuliformis* [19], *Ficus benghalensis* [20], *Mentha piperita* [21], *Piper betle* [22] etc. The metal nanoparticles are extensively used in many biomedical and bioelectrochemical applications due to their extraordinary electrocatalytic activity. Although metal is a poor catalyst in bulk form, nanometer-sized particles can exhibit excellent catalytic activity due to their relative high surface area to volume ratio and their interface dominated properties [23 & 21], which drastically differ from those of the bulk material.

Here, we report an inexpensive, versatile, and very reproducible method for the large-scale synthesis of AgNPs by reduction process using leaf extract of *Citrus Medica*. This leaf extract acts both as reducing and stabilizing agent. *Citrus Medica* is an important medicinal plant, belongs to the family Rutaceae. It is a common constituent of diet worldwide in various forms with considerable effects as potent antibiotic agent, etc.

Materials and methods

Collection and preparation of leaf extract

The collected *Citrus Medica* leaves were washed 2–3 times with de-ionized water. Aqueous extract of citrus medica leaf was prepared using freshly collected leaves of 20g were boiled with 100ml of de-ionizeds water at 100°C for 30 minutes.

Biosynthesis of silver nanoparticles(AgNPs)

In Erlenmeyer flask 2.5 ml of ammonium solution was added with 5 ml of 1 mM AgNO3 solution, followed by addition of leaf extract 1–10 ml and the final volume was adjusted to 50 ml by adding the appropriate amount of de-ionized water. A control set up was also maintained without leaf extract. The Erlenmeyer flasks were incubated in dark conditions at 37 °C under agitation (200 rpm) for 24 h. For the synthesis of AgNPs, the solution containing the *Citrus Medica* leaf extract turned from yellow to dark brown.

Characterization of silver nanoparticles

To determine the time point of maximum production of AgNPs, the absorption spectra of the samples were taken at 300–540 nm, using UV–vis spectrophotometer (HITACHI, Model U-2800 spectrophotometer). The sample of AgNPs produced from *Citrus Medica* leaves were air-dried and allowed to be characterized by SEM and XRD patterns. In addition, presence of metals in the sample was analysed by EDX.

Antimicrobial activity by well diffusion method

The AgNPs produced from aqueous leaf extract of *Citrus Medica* were subjected to their antimicrobial activity by well diffusion method against pathogenic organisms like *P. aeruginosa* and *E. coli*. The pure cultures of organism were sub cultured on Muller–Hinton broth at 35 °C on rotary shaker at 200 rpm. Each strain was swabbed uniformly on the individual plates using sterile cotton swab. Wells of size 6 mm have been made on Muller–Hinton agar plates using gel puncture. Using micropipette the samples of nanoparticles, plant extract and antibiotics were poured into wells on all plates. After incubation at 37°C for 24 h, the different levels of zone of inhibition were measured.

Results and discussion

The detailed study on biosynthesis of AgNPs by natural plant extract of *Citrus Medica* has carried out in this work. The aqueous Ag+ ions were reduced to AgNPs when added to the natural leaf extract of *Citrus Medica*. It was observed that the color of the solution turned from yellow to bright yellow and then to dark brown after 1- 24 h of the reaction indicated the formation of silver nanoparticles.

UV-Vis Spectra analysis

The formation and stability of the reduced AgNPs in the colloidal solution was monitored by UV–Vis spectrophotometer analysis by taking readings at regular time intervals. The strong surface plasmon resonance band positioned at 420 nm was observed for AgNPs Fig.1. which increased in intensity as a function of time of reaction and attained its maximum. The observation indicates the reduction of the Ag+ ions took place extracellularly. It is reported earlier that absorbance at around 430 nm for silver is acharacteristic of these nobel metal nanoparticles [24].

SEM analysis

Fig .2. Shows the scanning electron micrograph of the *Citrus Medica* leaf extract treated with 1 mM silver nitrate solution. SEM exhibited the cubic structure of well dispersed AgNPs as measured to be in the range of 10-63nm in size.

EDAX

Citrus Medica leaf extract reduced silver solutions were dried, drop coated on to carbon film. The EDAX pattern thus clearly shows that the AgNPs are crystalline in nature by the reduction of silver ions by using leaf broth. It exposes strong signal in the silver region and confirms the formation of AgNPs. Metallic silver nanocrystals generally show typical optical absorption peak approximately at 3 keV due to surface plasmon resonance [25]. Other elemental (O, Cl) signals are recorded possibly due to elements from enzymes or proteins present within the flower extract Fig.3.

XRD pattern

The crystalline nature of AgNPs produced from the aqueous leaf extract of citrus medica was confirmed by XRD analysis. Fig.4. shows six different clear peaks in the range of 10-100 with 2θ values. The mean particle diameter of AgNPs was calculated from the XRD pattern according to the line width of the plane, refraction peak using the Scherrer's equation [26].

Antimicrobial activity

As shown in the fig.5. The AgNPs synthesized by citrus medica leaf extract was found to have highest antimicrobial activity against *P. aeruginosa* (30 mm) and *E. coli* (25 mm), compare to the activity of leaf extract without the silver ion and the antibiotics respectively.

The AgNPs showed efficient antimicrobial property compared to other compounds due to their extremely large surface area, which provides better contact with microorganisms. As the mechanism involved is nanoparticles get attached to the cell membrane and also penetrated inside the bacteria. The bacterial membrane contains sulfur containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. When AgNPs enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death.The

nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity [27 & 28].

The results of present study indicates that the plant extract is found to be more efficient in the production of AgNPs, this may be due to mass of young cells with different ploidy in the plants which can be metabolically active to produce various types of chemicals responsible for the reduction of silver ions. Many researchers have reported the biosynthesis of nanoparticles with plants extracts. Synthesis of quasi spherical AgNPs using purified apiin compound, extracted from henna leaf at ambient conditions [29]. Using green tea, *C. sinensis* extract as reducing and stabilizing agents gold nanoparticles and silver nanostructures could be produced in aqueous solution at ambient conditions [24]. Plant extracts from live alfalfa, the broths of lemongrass, geranium leaves and others have served as green reactants in AgNPs synthesis [30 - 32]. The reaction of aqueous AgNO3 with an aqueous extract of leaves of a common ornamental geranium plant, *P. graveolens* gave AgNPs after 24 h of incubation. Biosynthesis of AgNPs was also conducted using *Cycas* leaf extract [33]. Plants extract from *Ocimum tenuiflorum, Solanum tricobatum, Syzygium cumini, Centella asiatica* and *Citrus sinensis* was used for the synthesis of AgNPs from silver nitrate solution [34]. Evaluated study on antimicrobial activity of synthesised AgNPs using *Millingtonia hortensis* shows the best possibility of using plant materials [35].

Conclusion

The silver nanoparticles have been produced by *Citrus Medica* leaf extract is a green eco-friendly, economical and efficient process. UV–vis spectrophotometer, XRD and SEM techniques have confirmed the reduction of silver nitrate to silver nanoparticles of crystalline nature. The zones of inhibition were formed in the antimicrobial screening test indicated that the AgNPs synthesized from the aqueous leaf extract of *Citrus Medica* has the efficient antimicrobial activity against pathogenic bacteria. The biologically synthesized silver nanoparticles could be of immense use in medical field for their efficient antimicrobial function.

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References

[1]. Retchkiman-Schabes, P.S., Canizal, G., Becerra-Herrera, R., Zorrilla, C., Liu, H.B., Ascencio, J.A., (2006). Biosynthesis and characterization of Ti/Ni bimetallic nanoparticles.
Opt. Mater. 29, 95–99.

[2]. Gu, H., Ho, P.L., Tong, E., Wang, L., Xu, B., (2003). Presenting vancomycin on nanoparticles to enhance antimicrobial activities. Nano Lett. 3 (9), 1261–1263.

[3]. Ahmad, Z., Pandey, R., Sharma, S., Khuller, G.K. (2005). Alginate nanoparticles as antituberculosis drug carriers: formulation development, pharmacokinetics and therapeutic
potential. Ind. J. Chest Dis. Allied Sci. 48, 171–176.

[4]**.** Gong, P., Li, H., He, X., Wang, K., Hu, J., Tan, W. (2007). Preparation and antibacterial
activity of Fe3O4@Ag nanoparticles. Nanotechnology 18, 604–611.
[5]**.** A.B Bazylinski and B.R. Frankel. (2004). Magnetosome formation in Procayotes,Nature
reviews-Microbiology. 2, 213-230.
[6]. F.M. Lengke E.M Fleet., G. Southam. (2007). Biosynthesis of silver nanoparticles by
filamentous cyanobacteria a from a silver (I) nitrate complex, Langmuir. 23, 2694-2699.
[7]. V.Parashar., R. Prashar., B. Sharma., & A.C. Pandey. (2009). Parthenium leaf extract
Mediated synthesis of silver nanoparticles: a novel approach towards weed utilization,
Digest journal of nanomaterials and biostructures. 4(1), 45-50.
[8]**.** M. Gericke and A.Pinches. (2006). Microbial Production of Gold Nanoparticles, Gold
Bulletin.1-39.
[9]. M. Saravanan., A.K. Vemu., S.K.Barik. (2011). Rapid biosynthesis of silver nanoparticles
from Bacillus megaterium (NCIM 2326) and their antibacterial activity on multi drug
resistant clinical pathogens, Colloids and Surfaces B: Biointerfaces. 88, 325– 331.
[10]. M. Sastry., A. Ahmad., M.I. Khan and R. Kumar. (2003). Biosynthesis of metal
nanoparticles using fungi and actinomycete, Current science. 85, 2- 25.
[11]. K.T Dhermendra., J. Behari1., and P. Sen. (2008). Time and dose-dependent antimicrobial
potential of Ag nanoparticles synthesized by top-down Approach, current science. 95, 5-10.
[12]. D. Philip. (2010).Green synthesis of gold and silver nanoparticles using Hibiscus rosa
sinensis, Physica E. 42, 1417–1424.
[13]. M. L. Rao and N. Savithramma. (2011). Biological Synthesis of Silver Nanoparticles using
Svensonia Hyderabadensis Leaf Extract and Evaluation of their Antimicrobial Efficacy, J.
Pharm. Sci. & Res.3, 1117-1121.
[14]. R.Geethalakshmi and D.V.L. Sarada. (2010). Synthesis of plant-mediated silver
nanoparticles using *Trianthema decandra* extract and evaluation of their anti microbial
activities, International Journal of Engineering Science and Technology. 2, 970-975.
[15]. P.C.Nagajyothi and K. D. Lee. (2011). Synthesis of Plant-Mediated Silver Nanoparticles

Using Dioscorea batatas Rhizome Extract and Evaluation of Their Antimicrobial Activities,
Journal of Nanomaterials.10, 1155-2011.
[16]. K.S. Mukunthan., E.K. Elumalai., T. N. Patel., V. R. Murty. (2011). Catharanthus roseus: a
natural source for the synthesis of silver nanoparticles, Asian Pacific Journal of Tropical
Biomedicine.270-274.
[17].B. Mahitha., B. Deva., P. raju., G.R. Dillip., C.M. reddy., K. Mallikaruna., I. manoj.,
S.Priyanka., K.J. rao., N.J. sushma. (2011). Biosynthesis, Charecterization and
antimicrobial studies of AgNPs extract from Bacopa monniera whole plant, Digest Journal
of Nanomaterials and Biostructures . 6, 135-142.
[18]. T.C. Prathna., N. Chandrasekaran., A. M. Raichur., A. Mukherjee. (2011). Biomimetic
synthesis of silver nanoparticles by Citrus limon (lemon) aqueous extract and theoretical
prediction of particle size, Colloids and Surfaces B: Biointerfaces. 82, 152–159.
[19]. S. Kaviya., J. Santhanalakshmi., B. Viswanathan.(2011). Biosynthesis of silver nano-flakes
by Crossandra infundibuliformis leaf extract, Materials Letters. 3, 12709.
[20]. A. Saxena., R.M. Tripathi., F. Zafar., P.Singh. (2011). Green synthesis of silver
nanoparticles using aqueous solution of Ficus benghalensis leaf extract and characterization
of their antibacterial activity. Materials Letters. 4, 12724.
[21]. D. M. Ali, N. Thajuddin., K. Jeganathan., M. Gunasekaran. (2011). Plant extract mediated
synthesis of silver and gold nanoparticles and its antibacterial activity against clinically
isolated pathogens, Colloids and Surfaces B: Biointerfaces. 85, 360–365.
[22]. P. U. Rani and P. Rajasekharreddy. (2011). Green synthesis of silver-protein (core–shell)
nanoparticles using Piper betel L. leaf extract and its ecotoxicological studies on Daphnia
magna, Colloids and Surfaces A: Physicochemical and Engineering Aspects.7, 17211.
[23]. S. Thangavel., R. Ramaraj. (2008). The need for monitoring the actual nitric oxide
concentration in tumors, J. Phys. Chem. 112, 19825.
[24]**.** Nestor, A.R.V., Mendieta, V.S., Lopez, M.A.C., Espinosa, R.M.G., Lopez, M.A.C.,
Alatorre, J.A.A. (2008). Solventless synthesis and optical properties of Au and Ag
nanoparticles using Camielliasinensis extract. Mater. Lett. 62, 3103–3105.

[25]**.** P. Magudapatty., P. Gangopadhyayrans., B.K. Panigrahi., K.G.M.Nair., S. Dhara. (2001).
Electrical transport **s**tudies of Ag nanoparticles embedded in glass matrix, Physica B. 299,
142–146.
[26]. Balaji, D.S., Basavaraja, S., Deshpande, R., Bedre Mahesh, D., Prabhakar, B.K.,
Venkataraman, A. (2009). Extracellular biosynthesis of functionalized silver nanoparticles
by strains of Cladosporium cladosporioides fungus. Colloids Surf. B Biointerf. 68, 88–92.
[27]. Sondi, I., Salopek-Sondi, B. (2004). Silver nanoparticles as antimicrobial agent: a case
study on E. coli as a model for Gram-negative bacteria. J. Colloids Interface Sci. 275, 177–
182.
[28]. Morones, J.R., Elechiguerra, J.L., Camacho, A., Holt, K., Kouri, J.B., Ramfrez, J.T.,
Yacaman, M.J. (2005). The bactericidal effect of silver nanoparticles. Nanotechnology 16,
2346–2353.
[29]**.** Kasthuri, J., Veerapandian, S., Rajendiran, N. (2009). Biological synthesis of silver and
gold nanoparticles using apiin as reducing agent. Colloids Surf. B: Biointerf. 68, 55–60.
[30]. Torresdey, J.L.G., Gomez, E., Videa, J.R.P., Parsons, J.G., Troiani, H., Yacaman, M.J.,
(2003). Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles. Langmuir
19, 1357–1361.
[31]. Shankar, S.S., Ahmad, A., Sastry, M. (2003). Geranium leaf assisted biosynthesis of silver nanoparticles. Biotechnol. Prog. 19, 1627–1631.
[32]. Shankar, S.S., Rai, A., Ahmad, A., Sastry, M. (2005). Controlling the optical properties of lemongrass extract synthesized gold nanotriangles and potential application in infrared- absorbing optical coatings. Chem. Mater. 17, 566–572.
[33]. Jha, K.A., Prasad, K. (2010). Green synthesis of silver nanoparticles using Cycas leaf. Int. J. Green Nanotechnol. Phy. Chem. 1 (2), 110–117.
[34]. Peter Logeswari., Sivagnanam Silambarasan., Jayanthi Abraham. (2012). Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property. Journal of Saudi Chemical Society. 10.1016.
[35].Gnanadhas Gnanajobitha., Mahendran Vanaja., Kanniah Paulkumar., Shanmugam Rajeshkumar., Chelladurai Malarkodi., Gurusamy Annadurai., Cellapandian Kannan. (2013). Green Synthesis of Silver Nanoparticles using *Millingtonia hortensis* and Evaluation of their Antimicrobial Efficacy. International Journal of Nanomaterials and Biostructures. 2277-3851.

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Inhibition of the Biosynthesis of Epicatechin in tea, *Camellia sinensis* (L. Kuntze) by insecticides deployed for the control of the tea mosquito bug, *Helopeltis theivora* Waterhouse

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Abstract
Herbal medicines have vital role in prevention and treatment of cancer. Most drugs for cancer care have originated from natural products. Epicatechin, a known anti-cancerous compound derived from tea (*Camellia sinensis* (L. Kuntze)) is produced due to the action of the enzyme anthocyanidin reductase (1.3.1.77) on its substrate cyanidin. *Helopeltis theivora* Waterhouse (Heteroptera: Miridae) or the tea mosquito bug (TMB) is a major sucking pest of tea (*Camellia sinensis* L.) in most tea-producing countries. Pesticides are the routine approaches to control this pest. Application of pesticides may be successful in controlling this pest, but could also cause an indirect loss to the production of vital secondary metabolites. Here, we have studied the effect of pesticides that are commonly used in the tea fields of India such as clothianidin, bifenthrin, cypermethrin, deltamethrin, endosulfan, imidacloprid, phosalone, profenofos, quinolphos, thiacloprid, thiamethoxam, dimethoate on the production of Epicatechin using molecular modeling approaches. This study reveals that the pesticides studied share the same binding site as the substrate, indicating competitive means of inhibition of Epicatechin biosynthesis by pesticides.

Introduction:

Herbal medicine is being practiced since ancient cultures and recent popularity can be tied to the belief that herbs can provide benefit over and above allopathic medicines. Recent surveys suggest one in three Americans use dietary supplements daily and the rate of usage is much higher in cancer patients (in some cases, up to 50% of patients treated in cancer centers) (1). Tea (*Camellia sinensis*) is one of the most consumed beverages in the world. Green tea is derived from *Camellia sinensis*, an evergreen shrub of the Theaceae family; has been reported to have antioxidant, anti-inflammatory, and anticancer properties (2, 3 & 4). Green tea and its extracts are a rich source of flavonoids which contain flavon-3-ols, commonly known as catechins, which include (–)-epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin- 3-gallate (ECG) and (–)-epicatechin (EC) (5 & 6). Anthocyanidin reductase (ANR) is an enzyme that participates in flavonoid biosynthesis. In the flavonoid biosynthesis pathway the substrates cyanidin and delphidin get converted to epicatechin and epigallocatechin respectively via ANR by using reduced nicotinamide adenine dinucleotide phosphate (NADPH) as a coenzyme (7). *Helopeltis theivora* is a major pest of tea in India and Asia. It causes severe crop loss to the tune of up to 25% (8). The nymphs and adults of this pest suck the sap of the young leaves and tender stem and can produce 100 spots within 24 hr (9). Pesticides are the routine approaches to control this pest.

Most often used pesticides to control this pest include clothianidin, bifenthrin, cypermethrin, deltamethrin, endosulfan, imidacloprid, phosalone, profenofos, quinolphos, thiacloprid, and thiamethoxam (10). Integrated Pest Management (IPM) talks about sustainable agriculture, meaning no loss to non target flora and fauna. However, application of pesticides might be successful in controlling this pest, but could also cause an indirect loss to the production of vital secondary metabolites. Studies have indicated this irreparable situation quite often. One of the concerns includes the effect of these pesticides on the production of vital anti-cancerous secondary metabolites. Therefore, we studied the effect of these commonly used pesticides in the tea fields of India on the production of flavonoids using molecular modeling approaches (11).

Materials and Methods

The Kegg pathway with the accession number map00941 (flavonoid biosynthesis) provided the basis for the understanding of biosynthetic pathway of catechins. Anthocyanidin reductase (ANR; EC: 1.3.1.77) are involved in the synthesis of five vital secondary metabolites proven to have anti-cancerous properties quercitin, (+)-gllocatechin and (+)-catechin, and (-)-epigallocatechin and (-)-epicatechin respectively.

Sequence Retrieval and Structure Modeling:

The enzyme sequences were retrieved from the Uniprot database and the 3D structures were predicted using the swissmodel server. The predicted models were tested for their accuracy by the Ramachandran plot analysis and procheck analysis. The binding site of these enzymes was predicted by 3D Ligand site server.

In order to understand the most commonly used pesticides in tea fields, the Indian tea research institutes UPASI, Coonoor, Tamilnadu and Tea Research Foundation, Tocklai, Jorhat, Assam in India were visited. The pesticide list was compiled based on the interactions had with the experts at these places. These compounds were downloaded from NCBI's Pubchem database and were further pruned to the required format using OpenBabel application.

Molecular Docking:

Docking was performed using Autodock 4.0 version. Grid was generated by taking the binding site residues into due consideration. We employed the Lamarckian genetic algorithm (LGA) for ligand conformational searching, which is a hybrid of a genetic algorithm and a local search algorithm. For every docking experiment ten different poses were generated and best one was chosen based on the binding energy. Binding energies that are reported represent the sum of the total intermolecular energy, total internal energy and torsional free energy minus the energy of the unbound system.

Results and Discussion

Molecular Docking Studies:

Substrate when bound to an enzyme yields product. This enzyme may also bind to other compounds in a competitive or non-competitive manner and will regulate the end product. Anthocyanidin Reductase when bound to delphinidin and cyanidin leads to the synthesis of -(-)epigallocatechin and epicatechin respectively.

Molecular docking studies performed with selected pesticides and their respective substrates gave greater interesting insights in to the binding affinity of various pesticides and these results are tabulated in table 1.
The mode of interaction between the substrates (cyanidin, delphinidin) and anthocyanidin reductase was mainly due to a maximum of two hydrogen bonds which was relatively lesser in comparison to some of the pesticides where a maximum of four hydrogen bonds were witnessed.

Binding energy is an important attribute to understand the affinity between the compounds. Deltamethrin, an organophosphate was found to have best binding energy of -9.59 kcal/J**.** This was a result of higher contribution by the electrostatic interaction and the van der waals interaction. All the compounds, but azadirachtin reported to have binding affinity with ANR with favorable binding energy and atleast one hydrogen bond. Clothianidin and Imidacloprid reported four hydrogen bonds. However, the interacting residues for these two were different. It was competitive inhibition in case of clothianidin and non competitive for Imidiacloprid. Tyr17 eventually turns out to be an important residue for an interaction for almost all the residues.

Table **1 Docking results of pesticide with ANR**

Parameters	**BE**	**LE**	**IC**	**IME**	**TE**	**refRMS**	**IR**
AZADIRACHTIN	95.74	1.88	nil	74.41	16.48	14.8	T17, K47, F92, Y19
BIFENTHRIN	-4.19	-0.14	855.35	-7.29	1.04	17.85	T17, F92
CLOTHIANIDIN	-5.46	-0.36	99.56	-6.06	-0.52	20.36	V86, F92, T17, K47
CYPERMETHRIN	-6.64	-0.24	13.63	-7.88	-1.33	17.85	V86
DELTAMETHRIN	-9.59	-0.34	93.07	-11.16	-0.09	19.72	T17,F92
DIMETHOATE	-4.01	-0.33	1.14	-5.15	-0.36	21.15	K47, G15
ENDOSULFAN	-6.34	-0.33	22.59	-6.34	0	18.81	T17, K47
IMIDACLOPRID	-6.08	-0.36	35.1	-6.34	0.48	21.69	V20, N91, V90, N91
PHOSALONE	-5.25	-0.25	141.99	-7.09	0.48	19.09	V86, K47
PROFENOFOS	-5.24	-0.29	144.99	-6.61	-0.97	18.64	F92
QUINLOFOS	-3.93	-	1.31	-5.59	-0.44	21.61	R40

		0.21					
THIACLOPRID	-5.16	-0.32	166.15	-5.54	-0.24	19.93	D98
THIAMETHOXAM	-5.6	-0.31	77.98	-6.34	1.41	20.41	V86, F92, K47
CYANIDIN	-6.38	-0.3	20.98	-6.38	0	35.01	T17
DELPHINIDIN	-6.76	-0.31	11.02	-6.76	0	35.17	T17, F92

BE: Binding energy; LE: Lowest energy; IC: Inhibition constant; IME: Intermol energy; TE: Torsional energy;
IR: Interacting residue; ANR: Anthocyanidin reductase.

Conclusion

In this study, we attempted to understand the potential implications of the pesticides used by the tea cultivators in India on the physiological changes in C. sinensis. Molecular docking approaches gave insights in to the fact, that the pesticides indeed have an affinity towards the enzyme ANR. In most cases these pesticides indicated their binding affinity to be the same as that of the substrate, an indication of competitive inhibition between these two compounds. Hence, a possible change in the physiology and metabolism of C. sinensis. So, the possibility of the vital anti-cancerous substances epicatechin and epicatechin gallate production getting inhibited. Therefore, this study has proven the complications of using pesticides on the nutritional quality of tea. These findings could further be validated in the laboratory for better quatitative understanding.

References

1. Richardson, M. A., Sanders, T., Palmer, J. L., Greisinger, A. & Singletary, S. E. (2000) Complementary/alternative medicine use in a comprehensive cancer center and the implications for oncology. *J. Clin. Oncol.* **18**:2505-2514

2. Yang F, de Villiers WJ, McClain CJ, and Varilek GW (1998) Green tea polyphenols block endotoxin-induced tumor necrosis factor-production and lethality in a murine model. J Nutr 128:2334–2340

3. Yang CS, Chung JY, Yang G-y, Chhabra SK and Lee M-J (2000) Tea and tea polyphenols in cancer prevention. J Nutr 130:472S–478S
4. Zhong Z, Froh M, Connor HD, Li XL, Conzelmann LO, Mason RP, Lemasters JJ, and Thurman RG (2002) Prevention of hepatic ischemia-reperfusion injury by green tea extract. Am J Physiol 283:G957–G964.
5. Yang F, Oz HS, Barve S, de Villiers WJ, McClain CJ, Varilek GW. The green tea polyphenol (-)-epigallocatechin-3-gallate blocks nuclear factor-kappa B activation by inhibiting I kappa B kinase activity in the intestinal epithelial cell line IEC-6. Mol Pharmacol. 2001; 60 (3): 528-33. PMID: 11502884

6. Yang GY, Liao J, Kim K, Yurkow EJ, Yang CS. Inhibition of growth and induction of apoptosis in human cancer cell lines by tea polyphenols. Carcinogenesis. 1998; 19 (4): 611-6. PMID: 9600345
7. Characterisation of anthocyanidin reductase from Shuchazao green tea.
Zhang X[1], Liu Y, Gao K, Zhao L, Liu L, Wang Y, Sun M, Gao L, Xia T.

8**.** Prasad, S.: Infestation of Helopeltis. Tea Times Sandoz (India) Ltd. Bombay. 1-6 (1992).
9. Banerjee, B.: Annual Scientific Report of Tocklai Exptt Stn for 1977-78. p. 50 (1978).
10. A K Barooah. Present status of use of agrochemicals in tea industry of eastern India and future directions.
11. R. J. Rabindra. Sustainable pest management in tea: Prospects and challenges**.** Two and a Bud 59:1-10, 2012

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A PILOT STUDY ON PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTIINFLAMMATORY ACTIVITIES OF *PUNICA GRANATUM* SEED EXTRACTS

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ABSTRACT

Punica granatum L. belongs to the family Puniaceae which includes only one genus and two species, the other one, little known, being *P. protopunica Balf*. peculiar to the island of Socotra. The pomegranate is considered to have originated from Iran to northern India and has been cultivated since ancient times throughout the Mediterranean region. Many research activities on P. garanatum leaves, flowers, fruits have been carried out. The number of seeds in pomegranate vary from 200 to about 1400 in number. Natural products sometimes have pharmacological or biological activity that can be of therapeutic benefit in treating diseases. As such, natural products are the active components not only of most traditional medicines but also many modern medicines. Furthermore, because the structural diversity of natural products exceeds that readily achievable bychemical synthesis, and synthetic analogs can be prepared with improved potency and safety, natural products are often used as starting points for drug discovery. In fact, natural products are the inspiration for approximately one half of U.S. Food and Drug Administration-approved drugs. Though the nutritional content and nutritional value of pomegranate seeds have been studied extensively, their medicinal and therapeutic properties are yet to be well documented. A preliminary phytochemical screening can be done to reveal the phytoconstituents present within the seeds and antioxidant and anti-inflammatory studies can also be performed to gain an insight into the therapeutic properties of the pomegranate seeds extracts.

Keywords:- *Punica granatum*, seed extracts, phytochemical, antioxidant, anti-inflammatory

Introduction

Natural products have contributed significantly towards the development of modern medical science. Recently traditional medicine worldwide is being re-evaulated by extensive research on different plant species and their active therapeutic principles. The rich wealth of plant kingdom can represent a novel source of newer compounds with significant anti-inflammatory activities. The major merits of herbal medicine seem to be their perceived efficiency, low incidence of serious adverse effects and low cost.

Antioxidant activity is one of the most important medical advances of the last decades of the twentieth century. It is a new understanding of the way in which a wide range of disease and other agencies cause damage to the human body. The essence of all research conducted in the past is that the damage to our cells and tissues at the root of most diseases is caused by

highly active and dangerous chemical groups called "free radicals". Free radicals are constantly being formed in the body as a result of basic disease processes. They are formed following exposure to cigarette smoke, car exhaust, industrial fumes, and almost all forms of radiation. Antioxidants are now recognized as being of considerable importance to health. Several reports concern the antioxidant activities of natural compounds in fruits, vegetables, dietary and rhizomatous plants with medicinal properties (Stajneret al., 1998; Moreno et al., 1999; Mukherjee, 2002; Liu et al., 2003, Hu and Kitts, 2000).

Inflammation is a pathophysiological response to injury, infection or destruction characterized by heat, redness, pain, swelling and diet distributed functions. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactive or destroy the invading organisms, to remove the irritant and set the stage of tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells (Anonymous; 2005). The most commonly used drug for management of inflammatory condition are non-steroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects especially gastric irritation leading to formation of gastric ulcers.(Tripathi K.D. 2008, Bennett P.N., Brown M.J. 2005).

Inflammation involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair (Vane *et al.,* 1995).It is a complex process, which is frequently associated with pain and involves occurrences such as: the increase in vascular permeability, increase of protein denaturation and membrane alterations (Umapathy *et al.,* 2010). Harmful stimuli including pathogens, irritants or damaged cells initiate response of vascular tissue as inflammation. Inflammation is a protective attempt by the organism to remove injurious stimuli as well as initiate the healing process for the tissue (Denko.1992). However, if inflammation is not treated it leads to onset of diseases like vasomotor rhinorrhoea, rheumatoid arthritis and atherosclerosis (Henson *et al.,* 1989).

The pomegranate (*Punica granatum* L.) is one of the oldest edible fruits and is widely grown in many tropical and subtropical countries [Salaheddin, 1984]. It is an important commercial fruit in Iran with a total production of 665,000 tons in 2003. Pomegranate juice and peel contain substantial amounts of polyphenols such as ellagic tannins, ellagic acid and gallic acid [Loren et al., 2005]. An earlier study has reported the use of leaf and bark extracts of *Punica granatum* as a good antibacterial agent [Egharevba et al., 2010]. It has been used in the preparation of tinctures, cosmetic, therapeutic formula and food recipes [Finkel et al., 2000] and in this regard pomegranate peel is a good source of antioxidants [Singh et al., 2001]. The synergistic action of the pomegranate constituents appears to be superior to that of single constituents. In the past decade, numerous studies on the antioxidant, anticarcinogenic, and anti-inflammatory properties of pomegranate constituents have been published, focusing on treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, bacterial infections and antibiotic resistance, and ultraviolet radiation-induced skin damage (Jurenka, 2008).

The present study is aimed at revealing the phytoconstituents present within the *Punica granatum* seed extracts by a preliminary phytochemical screening technique and also to study their antioxidant and anti-inflammatory properties.

MATERIALS AND METHODS

I.Fruit collection

Healthy fruits of *Punica granatum* were collected 2013 December from Chennai, Tamil Nadu, India and were maintained and shaded conditions for drying process at Poonga Biotech Research centre.

II.Preparation of the seed extract

Preparation of the extracts was done according to a combination of the methods used by Janarthanam et al., 2010. About 1g of fleshy dried powder of *Punica granatum* plant seed materials were extracted with 20 mL ethanol 75%, acetone, chloroform, aqueous and petroleum ether (Merck, extra pure) for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-vator at 40 °C to a constant weight and then dissolved in methanol ethanol and water. The dissolving rate of the crude extracts was approximately 100 %. The solution was stored at 18 °C until use.

III.Phytochemical Screening from seed extracts of *Punica granatum*

The phytochemical screening of seed extracts were assessed by standard method as described by Brinda *et al.,* 1981; Siddiqui and Ali, 1997 and Savithramma *et al.,* 2011. Phytochemical screening was carried out on the leaf extracts using different solvents to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides**,** coumarins and steroids. General reactions in these analysis will reveal the presence or absence of these compounds in the leaf extracts tested.

IV.Qualitative Antioxidant Assay

Antioxidant assay Ozkan *et al.,* (2004) and George *et al.,* (1996) on plant seed dried powder extracts of ***Punica granatum*** plant were estimated for their free radical scavenging activity by using DPPH (1,1-Diphenyl-2,Picryl-Hydrazyl) free radicals.

The radical of sample is calculated by the following formula,

Principle
DPPH (1,1-Diphenyl-2-picrylhydrazyl) is a stable free radial with purple colour (absorbed at 517nm). If free radials have been scavenged, DPPH will degenerate to yellow colour. This assay uses this character to show free radical scavenging activity.

$$\text{Inhibition} = [(\text{Absorbance of control (Ac 517)} - \text{Absorbance of sample (As517)}) / (\text{Absorbance control (Ac517)}] \times 100$$

Screening method

50µL of seed dried powder extracts of *Punica granatum* medicinal plant were taken in the microtiter plate. 100µL of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

V.Quantitative assay of antioxidant activity

Selected samples of 100µl from qualitative assay were mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Subsequently, at every 5 min interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of (0.16%) of Butylated Hydroxy Toluene (BHT).

VI.Estimation of Total phenol content in *Punica granatum*

Total phenolic content in the ethanolic seed extracts was determined by the Folin Ciocalteau colorimetric method (Slinkard and Singleton, 1984). For the analysis, 0.5 ml of dry powdered ethanolic seed extracts were added to 0.1 ml of Folin- Ciocalteau reagent (0.5N) and the contents of the flask were mixed thoroughly. Later 2.5 ml of Sodium carbonate (Na2CO3) was added and the mixture was allowed to stand for 30 min after mixing. The absorbance was measured at 760 nm in a UV-Visible Spectrophotometer. The total phenolics contents were expressed as mg gallic acid equivalents (GAE)/g extract.

VII. ASSESSMENT OF *INVITRO* ANTI-INFLAMMATORY ACTIVITY
VII a. INHIBITION OF ALBUMIN DENATURATION

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of the test extract (***Punica granatum*** seed extract) so that final concentrations become 1.56, 3.125, 6.25, 12.5, 25.0, 50.0 mg/ml. Similar volume of double-distilled water served as control. Then the mixtures were incubated at 37±2ºC in a incubator for 15 minutes and then heated at 50ºC for 5 minutes. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using vehicle as blank. Diclofenac sodium at the final concentration of (78.125, 156.25, 312.5, 625, 1250 µg/ml) was used as reference drug and treated similarly for determination of absorbance (Dey *et al.,* 2011; Chandra *et al.,* 2012). The percentage inhibition of protein denaturation was calculated by using the following formula:

% Inhibition of Protein Denaturation = 100 x [Vc – Vt / Vc]
Where, Vt = absorbance of test sample, Vc = absorbance of control.
The extract/drug concentration for 50% inhibition (IC50) was determined form the dose response curve by plotting percentage inhibition with respect to control against
treatment concentration.

VII b. MEMBRANE (HUMAN RED BLOOD CELLS) STABILIZATION TEST

Drug used as Standard: Acetylsalicylic acid available in the commercial name of Ecosprin -75 marketed by USV Limited, Mumbai, Maharashtra was used as a source of Acetylsalicylic acid.

Human Blood: The blood was collected from a healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and collected in heparinzed vacutainer. The blood was washed three times with 0.9% saline and centrifuged simultaneously for 10 minutes at 3000 rpm. The packed cells were washed with 0.9% saline and a 40% v/v suspension made using isotonic phosphate buffer which was composed of 154mM NaCl in 10mM Sodium Phosphate Buffer at pH 7.4 used as Stock erythrocyte or RBC suspension.

Hypotonic solution –induced haemolysis or membrane stabilizing activity: This test was done according to the method described (Shinde et al., 1999) with slight modifications. The test sample consisted of stock erythrocyte (RBC) suspension 0.030ml mixed with 5ml of hypotonic solution (154mM NaCl in 10mM Sodium Phosphate Buffer at pH 7.4) containing ***Punica granatum*** seed extract ranging from concentration 1.56 - 50 mg/ml. The control sample consisted of 0.030ml RBC suspension mixed with hypotonic buffered solution alone. The standard drug acetylsalicylic was treated similar to test at 78.125, 156.25, 312.5, 625, 1250 µg/ml concentrations. The experiment was carried out in triplicate. The mixtures were incubated at 10 minutes at room temperature, centrifuged for 10 minutes at 3000rpm and absorbance of the supernatant was measured spectrophotometrically at 540 nm.

The percentage inhibition of haemolysis or membrane stabilization was calculated by following equation.

% Inhibition of haemolysis = 100 x [A 1 –A 2 /A 1]

Where:

A 1 = Absorbance of hypotonic buffered solution alone

A 2 = Absorbance of test /standard sample in hypotonic solution.

Results

I.Fruit Collection

Healthy seeds of *Punica granatum* were collected from healthy fruits. Around 100g was taken and taken for further processing.

Fig 1:-

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II. Preparation of seed extract

The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-vator at 40 °C to a constant weight and then dissolved in methanol ethanol and water. The dissolving rate of the crude extracts was approximately 100 %. The solution was stored at 18 °C until use.

III. Phytochemical screening

The seed extracts were tested strong positive for alkaloids and terpenoids, positive for steroids, flavonoids and quinones. The rest of the phytoconstituents were present in an average range whereas the glycosides were almost nil.

Table 1:-

Phytocontituents	**Seed extract**				
	Acetone	**Ethanol**	**Aqueous**	**Chloroform**	**Petroleum ether**
Alkaloids	+ +	+ +	+ +	+ +	-
Glycosides	-	-	-	-	-
Cardiac glycosides	+	+	-	-	-
Terpenoids	+ +	+ +	+	-	+
Steriods	+	+	+	+	+
Flavonoids	+	+	+	+	+
Phenol	+	+	-	-	-
Tannins	+ +	-	-	-	-
Saponins	+	-	-	-	-
Quinones	+	+	+	+	+
Coumarins	+	+	+	-	-

++ = strong positive
+ = positive
¯ = negative

IV. Determination of antioxidant activity in dried seed extracts of *Punica granatum*
Quantitative Assay
Punica granatum dried seed extracts samples were used for antioxidant studies. Analysis on different extraction of 75% ethanol, acetone, chloroform, petroleum ether and aqueous extract showed the presence of antioxidants.
Five solvent extractions were tried for DPPH assay, using 75% ethanol, aqueous, acetone, chloroform and petroleum ether extract.
The absorption maxima of the sample was noted and compared with known synthetic standard of (0.16%) Butylated hydroxyl toluene.

Antioxidant activity in dried seed extracts of Punica granatum
Table 2:-

Samples	**Time duration**						
	0	**5**	**10**	**15**	**20**	**25**	**30**
Acetone	0.33	0.12	0.08	0.06	0.06	0.05	0.05
Chloroform	0.55	0.45	0.44	0.43	0.43	0.42	0.41
Petroleum ether	0.43	0.38	0.37	0.37	0.36	0.36	0.36
Ethanol	0.38	0.25	0.23	0.21	0.20	0.20	0.19
Aqueous	0.83	0.80	0.80	0.79	0.78	0.78	0.76

The percent DPPH scavenging activities of *Punica granatum* seed dried powdered extract were summarised in (Fig. 2). At all five solvent extractions tested, *Punica granatum* exhibited a dose-dependent DPPH radical-scavenging activity. The order of the scavenging activity was found to be acetone (95.7 %), aqueous (87.5 %), ethanol (86.3%), chloroform (56.9%) and petroleum ether (69.0%). The scavenging effect was comparable to that of the standard BHT with percentage value of 98.3 %.

V. Total phenol estimation
Table 3:-

particulars	B	S1	S2	S3	S4	S5	T1	T2
working standard(gallic acid)	-	0.2	0.4	0.6	0.8	1	-	-
Sample	-	-	-	-	-	-	100ml	100ml

Distilled water	1	0.8	0.6	0.4	0.2	-	0.9	0.9
Na2co3 2%		1.5ml						
Folin and ciocelate		0.5ml						
	Incubation in 15 mins							

The total phenolic content was measured using spectrophotometrically according to the Folin- Ciocalteau colorimetric method. The highest total phenolic content was observed in the acetone extract of the pomegranate seed sample.

VII a.INHIBITION OF ALBUMIN DENATURATION

Table 4:-
Influence of *Punica granatum* against Protein Denaturation

S.No	Concentration (mg / mL)	% Inhibition of Protein Denaturation
1	**1.56**	**25**
2	**3.125**	**62.5**
3	**6.25**	**80**
4	**12.5**	**88.75**
5	**25**	**96.25**
6	**50**	**97.5**

VII b. Assessment Of *Invitro* Anti-Inflammatory Activity
The extract/drug concentration for 50% inhibition (Ic_{50}) was determined from the dose response curve by plotting percentage inhibition with respect to control against treatment concentration.

Table 5:- Influence of Diclofenac Sodium against Protein Denaturation

S.No	Concentration (mg / mL)	% Inhibition of Protein Denaturation
1	**0.078**	**84.37**
2	**0.156**	**92.5**
3	**0.312**	**96.25**
4	**0.625**	**97.5**
5	**1.250**	**98.75**

Table: 6:- IC_{50} values of *Punica granatum* and Diclofenac Sodium against Protein Denaturation

S.No	Treatments	IC_{50} values (mg / mL)
1	***Punica granatum***	**2.5**

2	**Diclofenac Sodium**	**0.046**

The results showed that seed extract of ***Punica granatum*** at concentration of 6.25mg/ml protect significantly the erythrocyte membrane against lysis induced by heat (Table7). Aspirin 1.25mg/ml offered a highest percentage of stabilization against damaging effect of heat solution.

Membrane (Human Red Blood Cells) stabilization test

Table: 7. Influence of *Punica granatum* against Membrane Stabilization

S.No	Concentration (mg / mL)	% Inhibition of Haemolysis
1	**1.56**	**41.71**
2	**3.125**	**42.29**
3	**6.25**	**46.28**
4	**12.5**	**62.85**
5	**25**	**83.42**
6	**50**	**89.14**

Table: 8. Influence of Acetylsalicylic acid against Membrane Stabilization

S.No	Concentration (mg / mL)	% Inhibition of Haemolysis
1	**0.078**	**85.71**
2	**0.156**	**88.57**
3	**0.312**	**90.28**
4	**0.625**	**91.42**
5	**1.250**	**93.14**

Table: 9. IC_{50} values of *Punica granatum* and Acetylsalicylic acid against Membrane Stabilization

S.No	Treatments	IC_{50} values (mg / mL)
1	***Punica granatum***	**6.752**
2	**Acetylsalicylic acid**	**0.0455**

Discussion

Among the five extracts of *Punica granatum*, the acetone extract from the mother plant seed dried powder of *Punica granatum* recorded the most effective DPPH radical scavenging activity (95.7 %) with the values being very close to synthetic antioxidant (BHT) as positive control. In each case, acetone extract recorded higher percentage of free radical scavenging activity than other extractions of *Punica granatum.* From the results in Table.3, seed dried

powder petroleum ether extraction of *Punica granatum* was recorded the least percentage of radical-scavenging activity (69.0 %).

Scavenging activity for free radicals of DPPH (1,1-Diphenyl-2-picryl hydrazyl) has widely used to evaluate the antioxidant activity of natural products from plant and natural sources. Free radicals have aroused significant interest among scientists in the past decade. Their broad range of effects in biological systems has drawn the attention of many experimental works. It has been proved that these mechanisms may be important in the pathogenesis of certain diseases and ageing. Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants (Gálvez and Cordero, 2005; Tepe, *et al.*, 2005; Mammadov 2011).

In the present study the in-vitro anti-inflammatory effect of ***Punica granatum*** was evaluated against the denaturation of egg albumin. The results are summarised in table 1. The present findings exhibited concentration dependent inhibition of protein denaturation by the test extract throughout the concentration range of **1.56** to **50** mg/ml. Diclofenac sodium (at the concentration range of 78.125 to 1200 µg/ml) was used as the reference drug which also exhibited concentration dependent inhibition of protein denaturation (Table 6) however, the effect of diclofenac sodium was found to be less as compare with that of **50**. This was further confirmed by comparing their IC_{50} values(Table 6)

The in-vitro anti-inflammatory effect of *Naringi* ***Punica granatum*** may be due to its polyphenols content. The effect may be due to synergistic effect rather than single constituent. It has been reported that one of the features of several non-steroidal anti-inflammatory drugs is their ability to stabilize (protein denaturation) heat treated albumin at the physiological pH (Williams L.A.D., et al., 2008). Therefore, form the findings of the present preliminary experiment it can be concluded that the leaf of ***Punica granatum*** had marked anti-inflammatory effect against the degeneration of protein in-vitro. It is suggested that anti-inflammatory effect of this plant should be further evaluated in other experimental models in pursuit of newer Phyto therapeutics against inflammatory diseases.

CONCLUSION

There are certain problems associated with use of animal in experimental pharmacological research such as ethical tissues and the lack of rationale for their use when other suitable methods are available and could be investigated. Hence, in the present study the protein denaturation bioassay was selected for in-vitro assessment of anti-inflammatory property of ***Punica granatum*** .Denaturation of tissue proteins is one of the well documented cause of inflammatory and arthritic disease. Production of auto-antigens in certain arthritic diseases may be due to denaturation. Therefore, would be worthwhile for anti-inflammatory drug development.

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REFERENCES

- Benjamin Jeya Rathna Kumar P and Christopher Patrick Kiladi S, Preliminary Phytochemical and Pharmacognostic Studies of Holoptelea integrifolia Roxb., 2009, Ethnobotanical Leaflets 13: 1222-31
- Galvez M, Martin-Cordero C, Houghton PJ and Ayuso MJ (2005). Antioxidant activity of methanol extracts obtained from Plantago species. J. Agric. Food Chem., 53: 1927-1933.
- George, J.A., Burke, W.D., Eickbush, T.H. (1996). Analysis of the 5' junctions of R2 insertions with the 28S gene: Implications for non-LTR retrotransposition. Genetics **142(3)**: 853--863.
- Janarthanam B & Sumathi E, 2010. Antimicrobial activity of Gymnema sylvestre leaf and callus extracts. Journal of Tropical Medicinal Plants, 11(2):143-147.
- Mammadov R, Ili P, Ertem Vaizogullar H, 2011. Antioxidant activity and total phenolic content of Gagea fibrosa and Romulea ramiflora . Iran... J. Chem. Chem. Eng. 30.
- *Marina Gálvez, Carmen Martín-Cordero,Peter J. Houghton and María Jesús Ayuso Antioxidant activity of* Plantago bellardii *All. Phytotherapy Research December 2005 Volume 19, Issue 12, pages 1074–1076,*
- Mukherjee, A., 2006. Deeper Groundwater Chemistry and Flow in the Arsenic Affected Western Bengal Basin, West Bengal, India. Dissertation. University of Kentucky, Lexington. 248 pp
- N. Savithramma, M. Linga Rao and G. Bhum, Phytochemical screening of Thespesia populnea (L.) Soland and Tridax procumbens L. J. Chem. Pharm. Res., 2011, 3(5):28-34
- Nguelefack TB, Fotio LA, Watcho P, Wansi S, Dimo T, Kamanyi A. Analgesic activities of aqueous and ethanolic extracts of the leaves of Kalanchoe crenata (Crassulaceae). Phytother Res 2004;18:385-8.
- Tepe B, Sokmen M, Akpulat HA and Sokmen A (2005). In vitro antioxidant activities of the methanol extracts of four Helichrysum species from Turkey. Food Chem., 90: 685-689.
- Toker G, Küpeli E, Memisoðlu M and Yesilada E. Flavonoids with antinociceptive and anti-inflammatory activities from the leaves of Tilia argentea (silver linden) J Ethnopharmacol 2004;95:393-97.

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ANTIBACTERIAL EVALUATION OF METALLIC SALTS AGAINST INFECTIOUS BACTERIA

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ABSTRACT

The bacteria, *Bacillussubtilis*, *Enterococcus* sp., *Escherichia coli*, *Klebsiella* sp., *Proteus* sp., *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* sp., *Vibrio cholerae*and *Vibrio parahaemolyticus*which were found to be either pathogen or opportunistic pathogen were evaluated for their sensitivity towards metallic salts. The antibacterial potency of the metallic salt solutions of Chromium, Cobalt, Mercury, Nickel, Lead, Cadmium and Zinc were evaluated. The study was conducted using Kirby-Bauer's disc diffusion method. The results in detail will be discussed.

INTRODUCTION

The prevalence of microbial infections leading to an increased rate of mortality is on the high for the past two decades, causing it a major concern globally (Tatli and Akdemir, 2005). Though the fourth generation of antibiotics have been discovered, the incidence of antibiotic resistance strains has increased owing to the misuse or overuse of the drugs and antibiotics (Diab, 2002; WHO 2015). Infection by such drug resistant strains leads to difficulties in treatment. In India, about 58,000 deaths of neonatal sepsis were attributed to microbial resistance to drugs (Laxminaraya*et al*., 2013). Subsequently, there is an increasing urge to identify potential antimicrobial agents from varying sources. Several reports inter-relate drug resistance and metal tolerance (Silver, 1996; Mgbemena*et al.*, 2012).

Metals such as copper, mercury, silver and arsenic have been conventionally used as antimicrobial agents since ancient times, until they were replaced by antibiotics. Due to the declining efficiency of antibiotics, the use of metals as nanostructures or alloys or complexes, especially those of silver, are re-emerging for their use as an antimicrobial agents. Their efficiency is supported by recent studies that indicate the oxidative stress, protein dysfunction or membrane impairment to microbial cells caused by different metals, eventually leading to cell death (Franci*et al.*, 2015). Thus, the present study is focused on the antibacterial activity of different metallic salts against pathogens/opportunistic pathogens, which are found to be associated with diseases such as bacteremia, urinary tract infections, respiratory infections, etc.

MATERIALS AND METHODS

Bacterial cultures

Laboratory cultures of 12pathogenic bacterial strains were used for the study, of which 7are Gram negative and 5 are Gram positive.The Gram positive bacteriaincludes,*Bacillussubtilis*, *Enterococcus* sp., *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus* sp. while the Gram negative strains are*Escherichia coli*, *Klebsiella* sp., *Proteus* sp., *Pseudomonas aeruginosa*, *Salmonella typhi*, *Vibrio cholerae*and *Vibrio parahaemolyticus*.The strains of bacteria mentioned above are of infectious nature and their source and infection differ according to the strain.

Kirby-Bauer disc diffusion method

The salts used for the study are sulphate salt of Chromium, nitrate salts of Cobalt, Nickel, Lead, Cadmium and Zinc and Mercuric chloride. The antibacterial activity of the metallic salts is performed by Kirby-Bauer disc diffusion method where nutrient agar plates are spread with 100 µl of 24 hour cultures of bacteria. Sterile discs (5mm diameter) loaded with 15, 20 and 25 µl of the respective metallic salt solutions corresponding to 100, 150 and 200 ppm were placed over the plates. After an incubation period of 24 h, at 37°C, the plates were observed for the zone of inhibition against the bacterial strains.

RESULTS

Among the metallic salts studied, mercuric chloride was found to be the most efficient antibacterial agent which showed zone of inhibition against all the species of bacteria studied. This was followed by the nitrate salt of Cadmium which recorded zone of inhibition against all the bacteria except that of *Escherichia coli.* The nitrate salt of Cobalt too showed similar trend in recording antibacterial nature against all the bacteria studied except that of *Escherichia coli* and *Vibrio parahaemolyticus.* It is noticed that the sulphate salt of Chromium has not recorded zone of inhibition against any of the bacteria studied.

The salt of lead recorded zone of inhibition only against the following bacteria, i.e. *Bacillus subtilis, Proteus* sp. and *Vibrio cholerae.* All other bacteria showed resistance against the salt of lead even at the concentration of 200ppm. Although, the salt of Nickel has showed wide spectrum of antibiotic against 9 out of 13 bacterial strains studied, the zone of inhibition was not so significant when compared to other metallic salts. Similar trend was observed with that of the salt of Zinc. The bacteria, *Bacillus subtilis, Enterococcus* sp. and *Escherichia coli* have showed resistance towards the nitrate salt of Zinc.

Mercury, cadmium, cobalt and zinc exhibited high antibacterial activity against *S. typhi,* with the zone of 24, 21, 20 and 20 mm respectively, at 200 ppm of respective salts, but in case of lead, a zone of only 7 mm was recorded at 200 ppm. For most of the strains, nickel and lead showed marginal antibacterial activity only at higher concentrations while cobalt, cadmium and zinc showed moderate action. Zinc was found to be the most toxic to *S. typhi*and *Proteus* sp., with zones of 12, 16, 20 and 17, 17, 19 mm respectively at 100, 150 and 200 ppm of the salt. *B. subtilis* and *E. coli* had tolerance towards zinc.In addition to chromium and zinc, *E. coli* had gained resistance against nickel, lead and cadmium, while low susceptibility, with a zone of 6 mm was recorded against cobalt. The pathogen, *S. aureus,* had gained resistance against nickel and lead.*Enterococcus* sp., *Klebsiella*sp. and *S. epidermidis* showed tolerance against lead whereas *V. parahaemolyticus*had tolerance towards cobalt and nickel at all the

concentrations studied.The zones of inhibition recorded for individual metallic salts against the bacterial strains are tabulated in Table 1.

DISCUSSION

Heavy metals, at lower concentrations, are found to be crucial for the growth and metabolic processes of microbes while at higher concentrations, they are toxic (Appenroth, 2010). In the present study, Mercuric chloride was found to be the most toxic of the metallic salts studied, followed by Zinc, Cadmium, Cobalt and Nickel, while Lead was toxic to very few of the strains studied.

Among the metallic salts studied, Chromium sulphatewas non-toxic to all the bacterial strains evaluated. Further,*B. subtilis* had acquired tolerance towards Zinc while *S. aureus* and *V. parahaemolyticus* were resistant to nickel and lead. *E. coli* was found to resistant to all the salts studied except mercury and cobalt. This can be correlated with a report by Center for Disease Dynamics, Economics & Policy, revealing the resistance exhibited by 13% of *E. coli* against carbapenems in India by the year 2013 (CDDEP 2015).

The resistance of the bacteria against the metals could be attributed to the mobilization of metals in the environment by anthropogenic sources, which has eventually led to the development of resistance factors in bacteria. The major mechanisms by which microbes tolerate heavy metals include exclusion by permeability barrier, intra- and extra-cellular sequestration, active transport efflux pumps, enzymatic detoxification and reduction in the sensitivity of cellular targets to metal ions (Bruins et al., 2000), which might be encoded in their plasmids (Mgbemena*et al.,* 2012).

It is proposed to study the other parameters that influence the toxic nature of metals such as the form of metals used, effect of media components, hydrogen ion concentration, etc., are required.It is of concern that the salts of metals studied belongs to that of heavy metals, the question of toxicity is raised. So, to avoid toxicity of these metallic salts conversion of them into non-ionic form from the ionic form will be of helpful in nature. Thus, conversion of these metallic salts to that of the oxides of metals or as nanoparticles is recommended and usage of these as an antimicrobial agent is highly proposed to combat the infectious bacteria.

CONCLUSION

The present study reveals the antibacterial activity of the metallic salts of Chromium, Cobalt, Mercury, Nickel, Lead, Cadmium and Zinc against 12 bacterial pathogens. The action of each metal differs with each bacterium. It is evident that the metallic salts are a potential agent in combating the infectious bacteria as most of them showed zone of inhibition against the infectious bacteria studied. As, the metallic salts used are reported to be that of heavy metals, it is proposed to use the metallic salts in their oxide forms or as nanostructures in combatting infectious bacteria.

REFERENCES

1. Antimicrobial resistance – Factsheet. WHO. 2015

2. Appenroth KJ. Definition of "heavy metals" and their role in biological systems. In: Sherameti I., Varma A. (eds.) Soil heavy Metals, Soil Biology. Berlin: Springer; 19: 19-29. 2010.
3. Bruins MR, Kapil S, Oehme FW. Microbial resistance to metals in the environment. Ecotoxicology and environmental safety. 45: 198-207. 2000.
4. Center for Disease Dynamics, Economics & Policy (CDDEP). 2015. ResistanceMap.
5. Diab AS. Antibacterial and antifungal effects of *Allium sativum* (garlic), *Hibiscus subdariffa*(karkade) and *Nigella sativa* (black seed) extract on some bacterial and fungal isolated from AbbassaHatchert, Proceeding of the 6th Veterinary Medicine Zagaazig Conference, Hurghada. 491- 505. 2002.
6. Cloete TE. Resistance mechanism of bacteria to antimicrobial compounds. International Biodeterioration& Biodegradation. 51(4):277-282. 2003.

7. Franci G, Falanga A, Galdiero S, Palomba L, Rai M, Morelli G, Galdiero M. Silver nanoparticles as potential antibacterial agents. Molecules. 20: 8856-8874. 2015.
8. Hartmann HT, Kester DE. Plant Propagation, Principle and Practice. 5th Ed 89:115, U.S.A, Prentice Hall. 1964.
9. Laxminarayan R, Duse A, Wattal C, Zaidi AKM, Wertheim HFL, Sumpradit N, *et al*. Antibiotic resistance-The need for global solutions. The Lancet Infectious Diseases, 13(12):1057–98. 2013.
10. Mgbemena IC,Nnokwe JC, Adjeroh LA, Onyemekara NN. Resistance of bacteria isolated from Otamiririverto heavy metals and some selected antibiotics. Current Research Journal of Biological Sciences. 4(5): 551-556. 2012.
11. Silver S. Bacterial resistance to toxic metal ions-A review. Gene. 179(1): 9-19. 1996.
12. Tatli II, Akdemir ZS. Antimicrobial and antimalarial activities of secondary metabolites from some Turkish Verbascum species. FABAD Journal of Pharmaceutical Sciences. 30: 84-92. 2005.

Table 1 : Zones of inhibition (in mm) observed for the metallic salts against pathogens/opportunistic pathogens

Bacteria / Metal			***Bacillus subtilis***	***Enterococcus* sp.**	***Escherichia coli***	***Klebsiella*sp.**	***Proteus* sp.**	***Pseudomonas aeruginosa***	***Salmonella typhi***	***Staphylococcus aureus***	***Staphylococcus epidermis***	***Streptococci sp.***	***Vibrio cholerae***	***Vibrio parahaemolyticus***
Chromium	**Concentration in ppm**	**100**	-	-	-	-	-	-	-	-	-	-	-	-
		150	-	-	-	-	-	-	-	-	-	-	-	-
		200	-	-	-	-	-	-	-	-	-	-	-	-
Mercury		**100**	12	13	15	14	18	9	19	15	16	15	15	10
		150	15	15	16	15	19	18	20	17	17	18	16	10
		200	15	16	17	17	22	19	24	19	18	20	18	11

Cobalt		**100**	8	10	-	9	11	9	17	-	9	8	10	-
		150	9	11	-	11	10	10	19	11	10	10	11	-
		200	10	11	6	12	13	10	20	12	11	12	12	-
Nickel		**100**	-	-	-	-	-	-	-	-	-	-	-	-
		150	9	8	-	9	8	11	11	-	10	6	6	-
		200	11	8	-	9	8	11	15	-	10	10	7	-
Lead		**100**	6	-	-	-	6	-	-	-	-	-	6	-
		150	11	-	-	-	7	6	-	-	-	6	7	-
		200	11	-	-	-	10	10	7	-	-	9	11	10
Cadmium		**100**	15	10	-	6	13	-	18	8	6	17	15	16
		150	17	13	-	7	17	8	20	11	9	19	18	17
		200	17	14	-	11	20	9	21	20	10	19	19	20
Zinc		**100**	-	-	-	9	17	6	12	6	6	9	11	6
		150	-	6	-	9	17	8	16	7	8	10	16	7
		200	-	9	-	10	19	9	20	11	11	10	17	11

Represents no zone of inhibition

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EVALUATION OF *CAULERPA SCALPELLIFORMIS* FOR THE PRESENCE OF PHYTOCHEMICALS AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

Marine algae were considered as the source of various bioactive compounds as they are able to produce variety of secondary metabolites. The study reveals the presence of phytochemical, and antibacterial activity of *Caulerpa scalpelliformis*. By direct extraction method the algae material was extracted with methanol. The phytochemical analysis of *Caulerpa scalpelliformis* extract reveals the presence of terpenoids, glycosides, and steroids. But the extract shows the absence tannins, phlobatannins, alkaloids, saponins, flavonoids. The antibacterial activity was carried out by using Disc Diffusion Method against the pathogenic organisms such as *Escherichia coli, Bacillus subtilis, Staphlococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa.*

KEYWORDS: *Caulerpa scalpelliformis,* phytochemicals, Antibacterial.

1. INTRODUCTION

Marine algae has a significant attraction as natural source of bioactive molecules with a broad range of biological activities,such as antibiotics,antivirals,antitumorals,antioxidant and antiinflammatories.they produce a wide variety of chemically active metabolities in their surroundings, potentially as an aid to protect themselves against the other settling organism. Since sea weeds are a good source of antimicrobial compounds,fatty acids,antioxidants and other bioactive compounds, there is an interest to utilize these products as nutraceuticals and in functionalfoods **(Yuan, 2008)**.
Ara *et al* (2002) reported brown algae to be active against a number of Gram positive and Gram negative organisms. The separation of the isomers by gas chromatography and their structural elucidation by mass spectrometry **(Youngblood *et al.,* 1973).**Now there is more current research in the phytochemical investigation in higher plants **(Peteros *et al.,* 2010).**

This fact implies that seaweed cells have some protective compounds and mechanism **(Matsukawa *et al.,* 1997).**Chloroform: methanol is the best solution for extracting the effective antibacterial materials from the brown algae species **(Rajasulochanna *et al.,* 2009).** In Phaeophyta, fucosterol is the main sterol and small amounts of cholesterol, 24-methylene-cholestrol and saringosterol were identified **(Ikekawa *et al.,* 1968).** The hot water crude extract of these algae species such as Gracilaria corticata, Gracillaria salicornia and Sargassum oligocystum showed effective anti Leishmanial activity **(Fouladvand *et al,* 2011).**

The green algae *Chlorococcum humicola* is a rich and varied source of pharmacologically active natural products. Nutraceutical and pharmaceutical content in the baseline algae strain is very less, they showed excellent effect the micobial pathogens **(Baghavati *et al*, 2011).** The most characteristic chemical property of theglycosides is their susceptibility to hydrolysis, where they yield sugar or glycone and non sugar or a glycones motieties **(Rangari, 2003)**

Evidence of phytochemical and pharmacological studies on algae is available in the literature with special reference to terpenoids and steroids. In this study, the *Caulerpa scalpelliformis* was evaluated for the presence of phytochemicals and Antibacterial activity are analysed by Disc Diffusion Method.

2. MATERIALS AND METHODS

Preparation of marine algae material

The fresh marine algae *Caulerpa scalpelliformis* were collected and it was washed thoroughly with running water to remove debris. It was shade dried in room temperature for about 7-9 days. The dried marine algae material was powdered and extracted with solvent for the analysis of the phytochemicals and antimicrobial activity in it.

Preparation of algae extract

15g marine algae were weighed and it was macerated for three days in 150ml of methanol at room temperature in an orbital shaker. After three days, the total extract were filtered and the filtrates were concentrated under reduced pressure to dryness, yielding the crude extract which was then weighed for different concentration (25mg,50 mg,75 mg,100 mg) and each concentrations was suspended in 1ml of methanol. The dried material was reconstituted in methanol for the analysis of the antibacterial activity.

PHYTOCHEMICAL TEST

Phytochemical screening for steroids, terpenoids, flavonoids, tannins, saponins, alkaloids, phlobatannins were carried out as described below.

Test for tannins

About 2ml of the extract was stirred with 2ml of distilled water and few drops of $FeCl_3$ solution were added. The formation of a green precipitate was an indication for the presence of tannins.

Test for cardiac glycosides (KELLER-KILLANI TEST)

5ml of extracts was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. A brown ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer which shows the presence of Cardiac glycosides.

Test for flavonoids

5ml of dilute ammonia solution were added to a portion of the extract followed by addition of concentrated H_2SO_4 .formation of yellow colour indicated the presence of flavonoids.

Test for steroids

1ml of extracts was dissolved in a few drops of acetic acid. It was gently warmed and cooled under the tap water and a drop of concentrated sulphuric acid was added along the sides of the test tube. Appearance of green colour indicates the presence of steroids.

Test for terpenoids (SALKOWSKI TEST)

5ml of extract was mixed in 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. Formation of reddish brown coloration at the interface shows the positive results for the presence of terpenoids.

Test for alkaloids (MAYERS TEST)

With 1ml of extract add 1ml of mayer's reagent (potassium chloride solution) whitish cream colored precipitate indicates presence of alkaloids.

Test for saponins

5ml of extract was shaken vigorously with 5ml of distilled water in a test tube and warmed. The formation of stable foam, honey comb shapes was taken as an indication for the presence of saponins.

Test for phlobatannins

About 2ml of extract was added to 2ml of 1% HCL and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins.

Antibacterial Assay

Preparation of inoculums

Bacterial inoculums were prepared by transferring bacterial strains from fresh culture plates to tubes containing 10ml of Muller Hinton Borth (Hi-Media) and incubated for 24hrs at 37°c in an Orbital Shaker. The antibacterial activity was carried out by using Disc Diffusion Method.

Antibacterial Assay

The Muller Hinton agar medium was prepared and sterilized by autoclaveat 121°c for 15mins. Then the media was poured into sterile petriplate and it was kept for solidification. After 24 hrs bacterial culture from Muller Hinton Borth was swabbed and the sterile disc with 100µl of different concentration of algal extract was placed on the swabbed and the sterile disc with 100µl of different concentration of algal extract was placed on the swabbed petridishes. The petridish was incubated at 37°c for 24 hrs. Amphicillin was used as positive control. Methanol solvent (100%) without algal extract was also used as negative control. After 24hrs the zone of inhibition was observed and measured.

3. RESULTS AND DISCUSSION

Preparation of algae materials

The algal materials were collected, shade dried, powdered and extracted with solvent for the analysis of photochemical present in extract.

Extraction of algal materials

The powdered material was extracted with methanol using direct extraction method described in materials and methods. The extract was dried and weighed for yield extract.

Phytochemicals analysis

The phytochemical analysis of the methanol algae extract revealed the presence of phytochemical compounds like glycoside, terpenoids and steroids (Table 1).A large number of algal extract products have beenfound to have antimicrobial activity,many of the structures were identify as fatty acids, glycolipids, steroids,phenolic and terpenoids**.(Thillairajasekar 2009, Luesch,2000).**

Table1: Phytochemical constituents identified in *Caulerpa scalpelliformis*

Phytoconstituents	*Caulerpa scalpelliformis*
Tannins	-
Phlobatannins	-
Terpenoids	+
Glycosides	+
Alkaloids	-
Saponins	-
Flavonoids	-
Steroids	+

Antibacterial Assay

The antibacterial activity of extracts of the *Caulerpa scalpelliformis* was presented in Table 2. The extracts of the algae shows strong inhibition in the growth of tested bacteria. The maximum zone of inhibition was observed against the other bacteria.the antibacterial effect of various concentration of extract of *Caulerpa scalpelliformis* by using standard drugs such as antibiotic (amphicillin) is also shown in Table 2 & Fig 1. The sea weeds are rich source of antibacterial compounds.the fatty acid from Broticoccus braunii has previously reported to have significant antimicrobial activity **(Benkendroff 2007, Abd El- baky 2006, Athukorale, 2002).**

Table 2: Antibacterial activity of methanol extract of *Caulerpa scalpelliformis*

S.no	Test Bacterial Pathogens	Zone of Inhibition				
		25mg/ml	50mg/ml	75mg/ml	100mg/ml	Amphicillin
1	*Escherichia coli*	10mm	13mm	14.5mm	15.5mm	10mm
2	*Bacillus subtilis*	-	9mm	11mm	14mm	16.5mm
3	*Klebsiella pneumonia*	-	-	-	-	10mm
4	*Pseudomonas aeruginosa*	-	-	12.5mm	14mm	13mm
5	*Staphylococcus aureus*	-	-	12mm	13mm	22mm

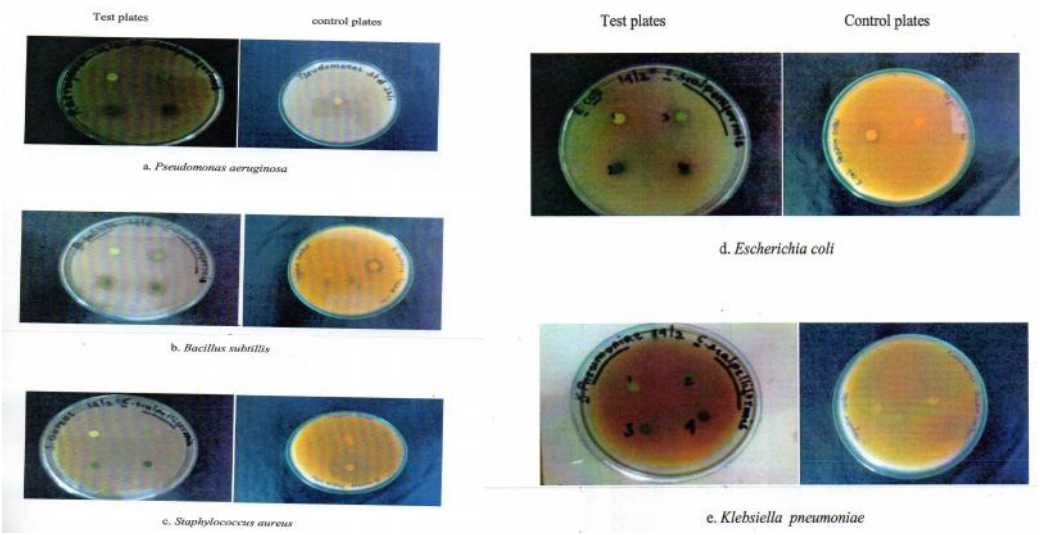

Fig 1: Showing the Antibacterial activity of methanol extract of *Caulerpa scalpelliformis*

4. CONCLUSION

Algae have been used for years in medicinal practices mostly in therapy of diverse pathogenesis. The phytochemical test of marine algae showed the presence of gylcosides, terpenoids and steroids. By using Disc Diffusion Method the antibacterial activity was carried against the pathogenic organisms such as *Escherichia coli, Bacillus subtilis, Staphlococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa.* The maximum inhibition was found at at the concentration of 100mg/ml and minimum inhibition was observed at the concentration of 25mg/ml. The extract shows maximum activity against *Escherichia coli, Bacillus subtilis.* This study is to reveal the biological production of bioactive compounds *Caulerpa scalpelliformis.* The phytochemicals in this marine algae shows various beneficial effects which are supported by variety of literature.

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6. REFERENCE

1. Ara, J., Sultana, V., Ehteshamul-Haque, S., Athar, M. and **Qasim, R**. 2002: Antibacterial activity of marine macro algae from Karachi coast. *Bull. Pol. Acad. Sci.,* **50:** 199-206**.**

2. Abd El- baky H.H., El Baz FK,and El-Baroty G.S.2006**.** Over production of lipid rich in linolenic acid by blue green alga spirulina maxima and its Inhibitory effect on carcinoma cells. *Adv Food Sci* **4:** 206-212.

3. Athukorale, D.P.2002. The health giving benefits of seaweed. Colombo: Leader Publication (PVT) Ltd.

4. Benkendroff,K., Davis, A.R., Rogers, C.N., and **Bremner, J.B.**2007.Free fatty acids 1 and 2 sterols in the benthic spawn of aquatic mollusks, and their associated antimicrobial three properties. *Journal of experimental marine biology and ecology*, **316:** 29-44.
5. Baghavathy, S., Sumathi, P. and **Jancy Sherene, B.I.** 2011. Green algae *Chlorococcum humicola* –a new source of bioactive compounds with antimicrobial activity. *Asian Pacific Journal Tropical Biomedicine*, p.S1-S7.
6. Fouladvand, M., Barazesh A., Farokhzad F., Malekizadeh H. and **K.Sartavi.** 2011. Evaluation of in vitro anti- Leishmanial activity of some brown, green and red algae from the Persian Gulf. *European Review for Medical and Pharmacologicial Sciences*, **15:** 597-600.
7. Ikekawa, N., Morisali, N. and **Kyosuke,** Tsudo.1968. Sterol compositions in some green algae and brown algae. *Steroids*, **12(1):**41-48.
8. Luesch, H., Yoshida,W.Y., Moore, R.E., Paul, V.J and **Mooberry, S.L. 2000.** Isolation, Structure determination and biological activity of Lyngbyabellin A from the marine Cyanobacterium Lyngbya majuscule.Journal of Natural Products, **63:** 611-615.
9. Matsukawa, R., D ubinsky, Z., Kishimoto, E., Masuda, Y. and **Takeuchi, T**.1997. A comparison of screening methods for antioxidant activity in seaweeds. *Journal of Applied Phycology*, 9(1): 29-35.
10. Peteros N.P and **Uy M.M**. 2010. Antioxidant and Cytotoxic activities and phytochemical screening of four philipine medicinal plants, *Journal of medicinal plants research*, **4(5)**: 407-414.
11. Rajasulochana, P., D hamotharan, R., Kirshnamoorthy, P. and **Murugern, S.** 2009. Antibacterial activity of the extracts of marine red and brown algae, Journal of Americian Science, **5(3)**: 20-25.
12. Rangari, V.D (2003), Pharmacognosy and phytochemistry part 2, career Publications 339-341.
13. Thillairajasekar .K., Duraipandian. V., Peumal. P. and Ignacimuthu .S. 2009 Antimicrobial activity of Trichodesmium erythraceum (Microalgae) from south east coast of Tamil Nadu, India. *International Journal of Integrated Biology*, **5 (3):** 167-170.
14. Yuan, Y.V. 2008. Marine algal constituents. In: C. Barrow & F.Shahidi (Eds), Marine nutraceuticals and functional foods. Boca Raton, CRC, 259-296.
15. Youngblood,W.W and **Blumer, M**. 1973.Alkanes and Alkenes in marine benthic algae,marine biology, **21(3):**163-172.

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Molecular Evolutionary Analysis On Insect Acetylcholinesterase

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Abstract: Acetylcholinesterase (AchE2 EC 3.1.1.7) encoded by acetylcholinesterase gene (*ace*) in insects can terminate neurotransmission in the postsynaptic membrane by hydrolysis of the neurotransmitter, acetylcholine (ACh). Evolutionary analysis of 75 Acetylcholine esterase gene sequences belonging to four different orders was determined. Statistical analysis was carried out using MEGA 4. This study will reveal sequence divergence between various species.

KEYWORDS: Acetyl choline Esterase, Insects, Phylogenetic analysis, MEME, MEGA4

INTRODUCTION

Acetylcholinesterase (AChE) catalyses the hydrolysis of the neurotransmitter, acetylcholine, thereby stopping transmission of nerve impulses at synapses of cholinergic neurons in the central and peripheral nervous systems in both vertebrates and invertebrates (Taylor 1991). Consequently, inhibition of AChE leads to paralysis and death. In addition, AChEs are expressed at other sites in animals, where they may act as regulators involved in cell growth and adhesion, probably unrelated to their catalytic properties (Soreq and Seidman 2001). In insects, AChE is a target of organophosphorus and carbamate compounds, which remain widely used pesticides around the world (Harel et al. 2000). Acetylcholine Esterase (AChE) is a key enzyme of the cholinergic system. It regulates the level of acetylcholine and terminates nerve impulses by catalyzing the hydrolysis of acetylcholine. Numerous studies have focused on insect AchE because it is the molecular target of the two major classes of pesticides, Organophosphates and Carbamates. Mutation is the major cause for AChE insensitivity and formation of resistance mechanism in insects. The evolution of insecticide resistance in insects tends to be rapid because selection is strong, populations are large, and generation times are short. The evolutionary analysis of AChE gene sequences among insects of various orders will form the basis for future investigations into the mechanism of OP resistance.

METHODOLOGY

The gene sequences of Acetyl choline esterase from 75 species pertaining to four different Orders were retrieved from NCBI. The sequences were subjected to cluster analysis based on each pairwise alignment score using ClustalW. ClustalW2 is a general purpose multiple sequence alignment program for DNA or proteins. It attempts to calculate the best match for the selected sequences, and lines them up so that the identities, similarities and differences can be seen. The sequence specific motifs present in the target gene were analysed using the tool-MEME (Timothy *et al*, 1994). MEME discovers novel, ungapped motifs (recurring, fixed-length patterns) in your sequences (sample output from sequences). The statistical

analysis was performed using MEGA 4 (Larkin *et al*, 2007). Various parameters such as Aminoacid frequency calculation, estimation of evolutionary divergence between the sequences, Tajima test, Disparity index test and derivation of evolutionary pattern were determined.

RESULTS AND DISCUSSION

Cluster Analysis: The cluster analysis has been done using the pair wise alignment score obtained from clustal w tool. We found possible pair wise alignment from the clustal w for 75 input sequences [Figure 1]. The multiple sequence analysis results showed that the most of the sequences analysed in this study are closely related to each other with better pair wise alignment score. The sequence *Anopheles moucheti* is closely related with *Anopheles funestus*.

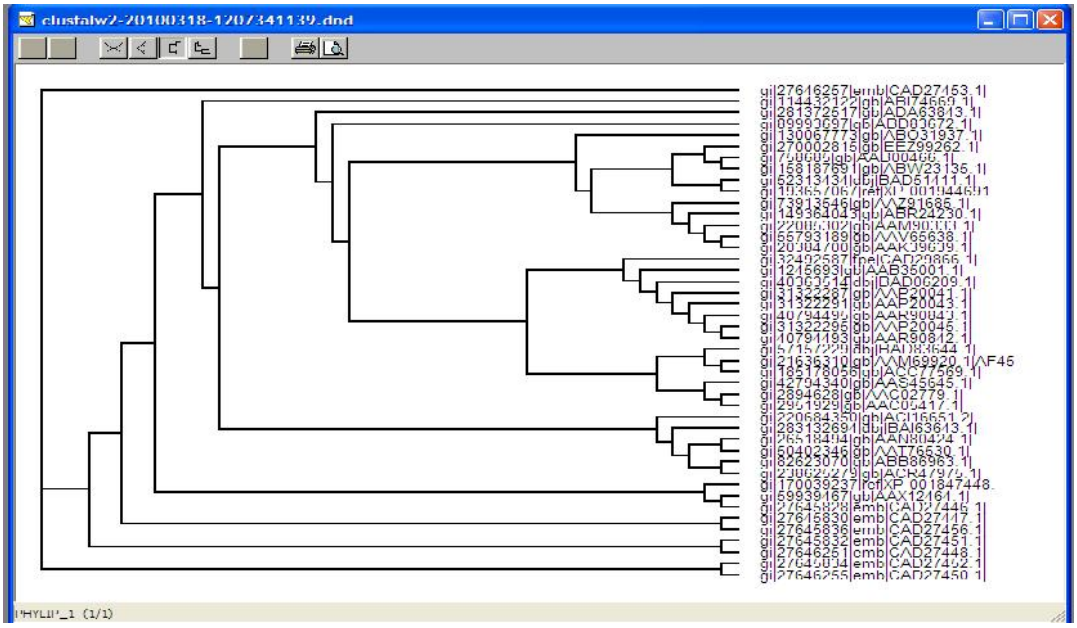

Figure 1: Phylogenetic Tree generated by ClustalW

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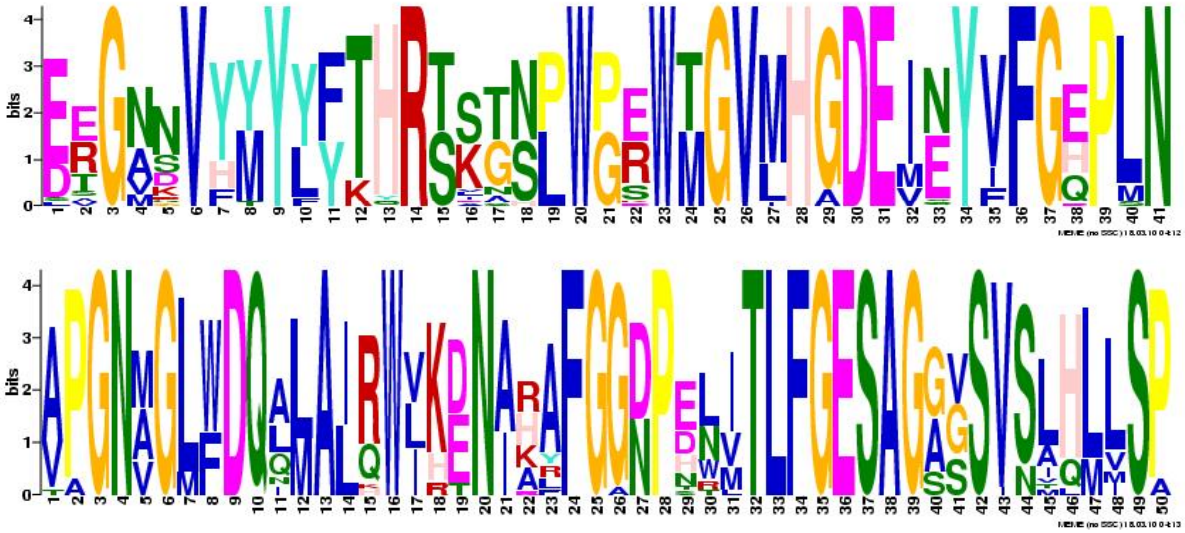

Figure 2: Three different Motifs Predicted by MEME

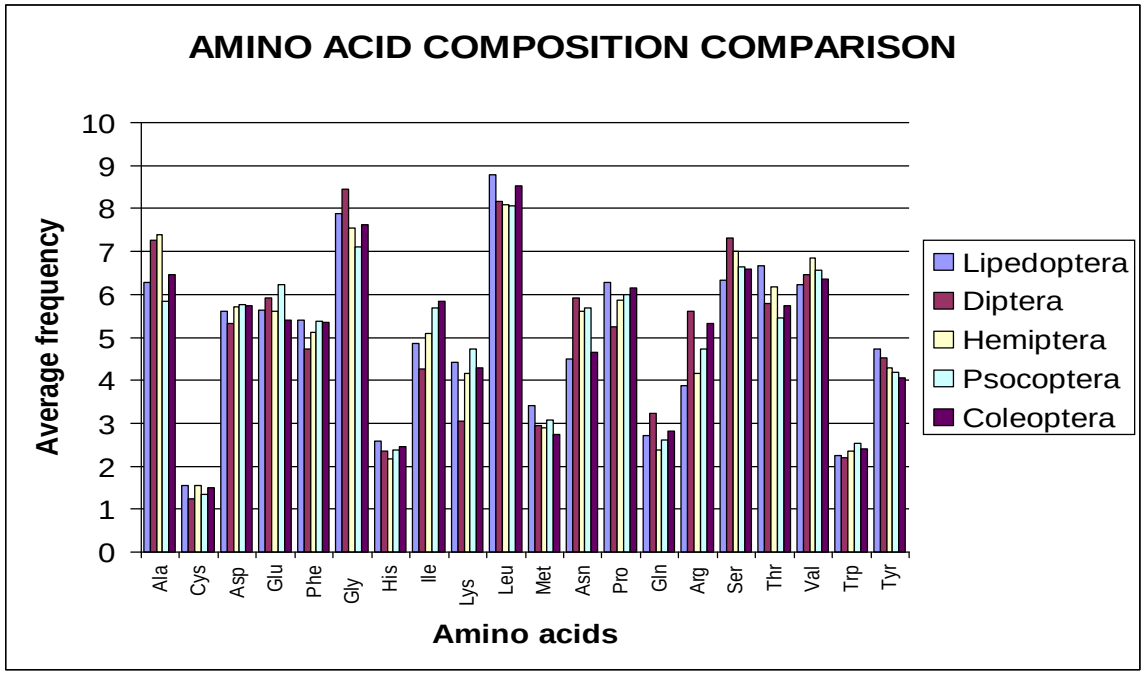

Figure 3: Aminoacid Composition Comparison Between the Different Orders

ESTIMATES OF EVOLUTIONARY DIVERGENCE BETWEEN SEQUENCES :

In the four taxonomic unit the sequence having lower value are said to be closely related sequence and the higher value sequences are said to be distantly related sequence. The statistical analysis is given in Table 1.

Table 1: TAJIMA TEST (RELATIVE RATE TEST)

Queries	Equality of	Outgroup	x2	P	Null

	evolutionary rate between the sequences		**valu e**	**value**	**hypoth esis**
Lepidopt era	gi|55793189|gb|AAV6 5638.1| and gi|20384700|gb|AAK3 9639.1|	gi|82623070|gb|ABB 86963.1|	2.00	0.157 30	Not rejected
Diptera	gi|1245693|gb|AAB35 001.1| and gi|31322295|gb|AAP2 0045.1|	gi|31322291|gb|AAP 20043.1|	0.20	0.654 72	Not rejected
Hemipte ra	gi|283132694|dbj|BAI 63643.1| and gi|26518494|gb|AAN8 0424.1|	gi|89993697|gb|ABD 83672.1|	2.00	0.157 30	Not rejected
Coleopt era	gi|270002815|gb|EEZ 99262.1| and gi|281372517|gb|ADA 63843.1|	gi|758685|gb|AAB00 466.1|	244. 98	0.000 01	rejected

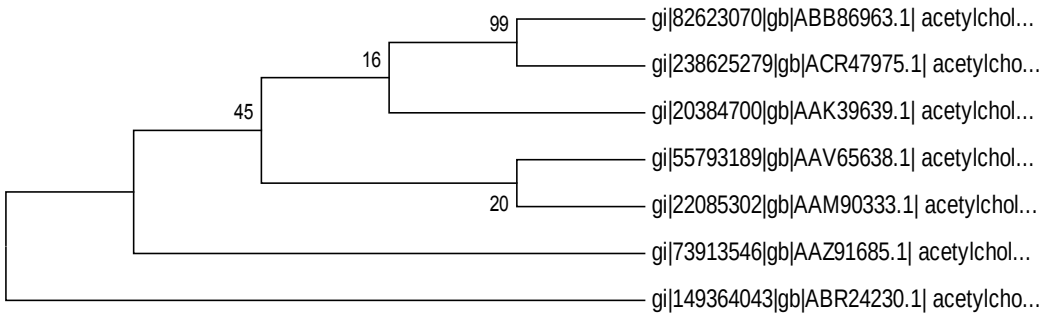

Figure 4: Bootstrap consensus Tree of Lepidopteran

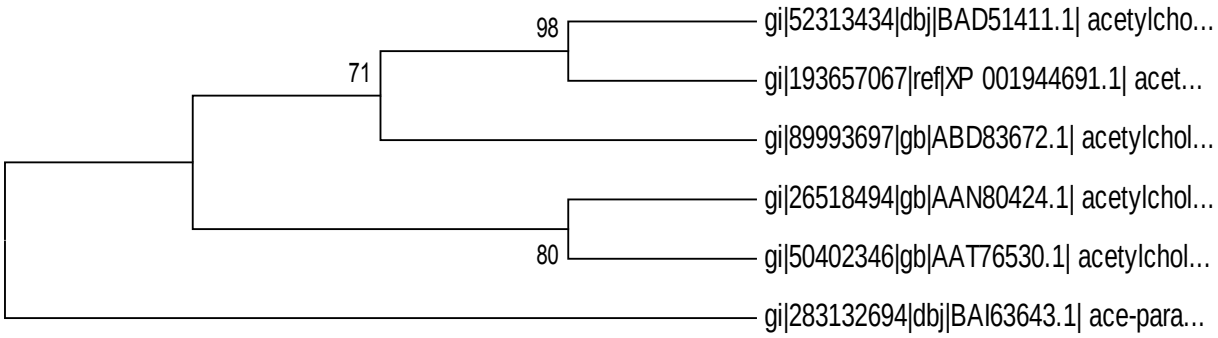

Figure 5: Bootstrap consensus Tree of Hemipteran

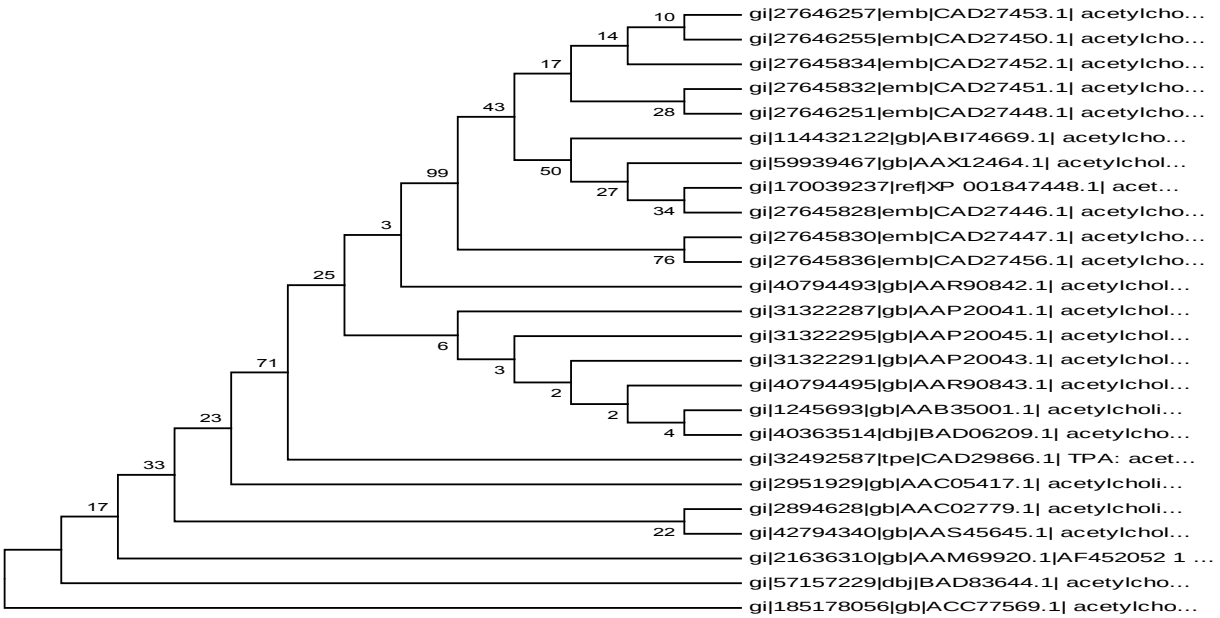

Figure 6: Bootstrap consensus Tree of Dipteran

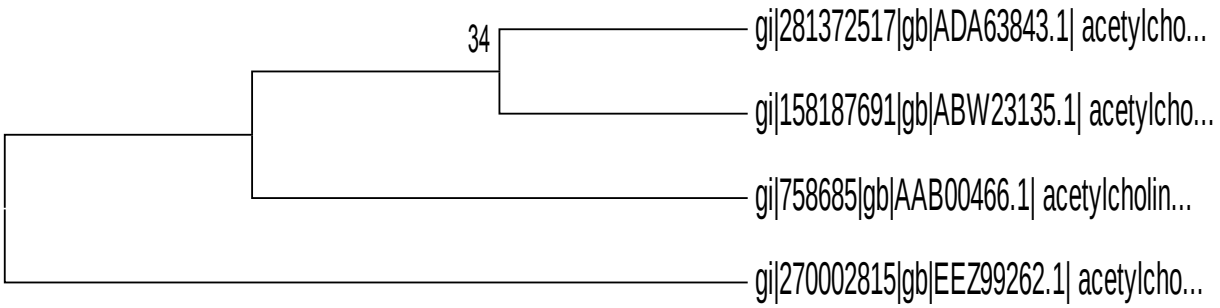

Figure 7: Bootstrap consensus Tree of Coleopteran

The motif analysis revealed that some of the insect species such as *Culex tritaeniorhynchus, Culex pipiens, Culex quinquefasciatus, Culex salinarius, Culex restuans, Culex nigripalpus* and *Culex torrentium* have common gene specific sequence pattern [Figure 2]. The comparison of Aminoacid composition [Figure 3] and Disparity Index test results of the query sets showed that the AchE sequences in some of the species such as *Spodoptera exigua* and Spodoptera litura, *Aphis gossyp* and *Myzus persicae*, *Lutzomyia longipalpis* and *Anopheles funestus* has evolved from common substitution pattern. The bootstrap consensus tree showed that *Spodoptera exigua* and *spodoptera litura* are closely related among lepidoptera whereas *Aphis gossyp* and *Acrythosiphon pisum* are closely related among hemiptera. *Anopheles pseudopunctipennis* and *Anopheles moucheti* are closely related amongst Diptera. Among Coleoptera *Lasidoderma serricorne* and *Phyllotreta striolata* are closely related [Figure 4-7].

Thus, in the present study, the molecular evolution of the AchE in insects were analyzed using the in-silico approaches and statistical methods. Phylogenetic analysis was conducted

to assess the evolutionary origin and divergence pattern. In general, sequences that have evolved with the same substitution process are expected to have similar compositions. The motif analysis and similarity in the amino acids composition explored that evolutionary pattern of Acetylcholine esterase in insects are group-specific.

REFERENCES

1. Taylor P. 1991. The cholinesterases. *Journal of Biological Chemistry* 266: 4025–4028. PubMed Soreq H, Seidman S. 2001. Acetylcholinesterase-new roles for an old actor. *Nature Reviews Neuroscience* 2:294– 302.
2. Harel M, Kryger G, Rosenberry TL, Mallender WD, Lewis T, Fletcher RJ, Guss M, Silman I, Sussman JL. 2000.Threedimensional structures of *Drosophila melanogaster* acetylcholinesterase and of its complexes with two potent inhibitors. *Protein Science* 9: 1063–1072. CrossRef, PubMed
3. Timothy L. Bailey and Charles Elkan, "Fitting a mixture model by expectation maximization to discover motifs in biopolymers", *Proceedings of the Second International Conference on Intelligent Systems for Molecular Biology*, pp. 28-36, AAAI Press, Menlo Park, California, 1994. [postscript] [pdf]
4. Larkin MA , Blackshields G , Brown NP , Chenna R , McGettigan PA , McWilliam H , Valentin F , Wallace IM , Wilm A , Lopez R ,Thompson JD , Gibson TJ , Higgins DG Bioinformatics (Oxford, England) [2007, 23(21):2947-2948]
5. **Clustal W and Clustal X version 2.0.** *(2007 November 01) Bioinformatics (Oxford, England) 23 (21) :2947-8* PMID: 17846036

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Preliminary study on isolation and characterization of dye decolorizing bacteria from lime stone soils

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ABSTRACT

Microorganisms from saline environments proved to be beneficial for commercial as well as research applications. Calcium carbonate rich lime stone fossils are one among those unfavourable conditions that supports extremely halophilic or moderately halophilic organisms. Current investigation on ariylaur lime stone samples is a preliminary initiative to unearth microorganisms possessing beneficial applications. Four distinct isolates were selected from pool of colonies and characterized for their ability to degrade following commercial dyes - reactive deep red, diazone green and reactive blue G. Results showed that all these isolates are found to be decolorizing the dyes in effective manner and they can be considered as potential candidates to address the menace of dyes as environmental pollutant.

INTRODUCTION

Microorganisms are ubiquitous and are considered as 'masters of the biosphere'. Every process in the biosphere is touched by the seemingly endless capacity of microbes to transform the world around them. They underpin most geochemical cycles and many human health conditions that were previously thought to be driven by inorganic processes and stress. (Reysenbach And Hamamura, 2008). Soil is considered a storehouse of microbial activity (Tilak *et al*., 2005). Understanding the biodiversity of the soil is difficult because of the heterogeneity that exists on both local and geological scales (Horner-Devine *et al*., 2004; Fierer and Jackson, 2006; Kang and Mills**,** 2006). Texture, temperature, salinity, pH, contaminants and other characteristics allow a unique microbial community to establish a niche (Zwolinski, 2007). Extreme environments with variable salinity, temperatures, underground environments (ice and rocks), and deep sea vent (extremely high temperature and pressure) contain specialized microbial communities, mostly known as Halophiles. They provide important clues on the origin, evolution of life and geological time scales. They also provide beneficial genomic, protein resources. Functional characterization of microorganisms for specific functions such as salt tolerance, antibiotic production, or enzyme activity (hydrolysis, bioremediation) helps to identify microbes that have potential applications in medicine, agriculture or industry (Oren, 2002).

Ariyalur, youngest district in Tamil Nadu, is famous for limestone resources. According to the previous archaeological study, about 100 million years ago, spontaneous transformation phenomenon of sea into land caused burial of aquatic organisms and subsequent formation of fossil prints of those creatures as soil layers of the site. These soil layers still hold the same aquatic nature; hence researchers have opined that microorganisms of ancient times might still be existing along with the fossils underground (Manivel 2005; Seshachur 1932).

Thus analysis of microbial communities by traditional microbiological and recently developed metagenomic approach would offer evidence of the rare microbial communities and their evolutionary pathways. Additionally, beneficial genes and biochemical pathways can also be prospected from them.

In the study, soil samples collected 150 ft below ground off the Ariyalur site were investigated for cultivable microbial diversity along with their possible beneficial genomic and protein contents. Cultivable microorganisms were isolated and subjected to preliminary characterization with routine microbiological techniques. Their functional property was assessed for degrading hazardous chemicals in commercial dye solutions.

MATERIALS AND METHODS

To isolate the culturable bacteria from soil, a portion of the soil samples was overlaid with sterile distilled water in a sterile falcon tube and were left undisturbed for about a week. This procedure allows the bacterial population to get concentrated in the overlaid water.

1) Isolation of single colony

To separate mixed population of microorganisms, 100µl of the overlaid liquid from each sample was serially diluted (10^{-1} to 10^{-10}). An aliquot of 50µl was spread on Luria-Bertani (LB) agar (pH 7.5). Colonies obtained by were selected based morphology and were quadrant streaked on LB agar and single colonies were isolated and inoculated in an LB broth (pH 7.2).

2) Bacterial growth kinetics

One ml of the overlaying aqueous sample was inoculated into 50ml LB broth and incubated at 37ºC in a shaker. The cultures were allowed to grow overnight. This served as the inoculum. An aliquot of 100µl inoculam was added to 30ml LB broth and kept in a shaker at 37ºC. Sampling was done every 2 hours for 12 hours. Wet cell mass concentration and turbidity were measured.

3) Morphological & Biochemical characterization

Isolates were then subjected to standard morphological characterization & biochemical characterization. Gram's staining, Capsular staining, Endospore staining, Flagellar staining, motility assay were performed to reveal the morphological organization of fossil isolates. 25 Biochemical tests (name of tests) were done to characterize functional properties of isolates.

4) Genome extraction and 16S Amplification

In view of standardization and molecular characterization purposes, genome of isolated cultures and soil microorganisms were extracted using Mo-Bio power soil DNA kit. 16S rRNA gene amplification for all the samples was standardized with repeated PCR using 25pM each of universal primers 16F27 (5´-CCA GAG TTT GAT CMT GGC TCA G-3´) and 16R1525 (5´-TTC TGC AGT CTA GAA GGA GGT GWT CCA GCC-3´) (Pidiyar *et al*., 2004).Concentrations of other components are dNTPS (10mM each), Buffer (1X), Taq polymerase (1U), sterile milliQ water (15.8 µl/ 25 µl) and finally 1 µl template DNA.

5) Dye degradation study

Three dyes, diazone green, reactive deep red and reactive blue G were selected for study. Four falcon tubes were filled with 20ml of broth and 500µl of 0.1% of each dye was added separately. The tubes were then inoculated separately with 1ml of pure culture. The tubes were incubated in a shaker (120 rpm at 37ºC) along with a positive control. The samples that

were found to decolorize the dye were subcultured onto fresh medium containing the dye to confirm decolorization.

6) Dye degradation kinetics

An aliquot of 100µl of the overnight culture was inoculated into 30ml LB broth containing reactive deep red, diazone green and reactive blue G. The flasks were incubated at 37ºC in a shaker. Sampling was done every 2 hrs for duration of 12 hours. Decolorizing rate of the culture was determined by reading sample absorbance at 493nm, 821nm and 831nm respectively

.

Results and Discussion

Growth kinetics of culturable population

Four distinct colonies were selected from the preliminary cultivable step in LB agar condition. In order to study the growth pattern of the obtained cultures, bacterial growth kinetics was carried out. An overnight culture was used as inoculum.

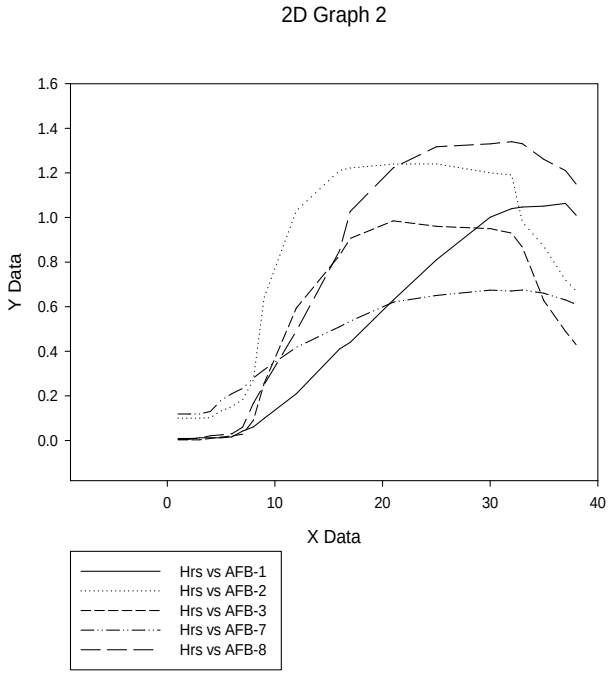

Figure 1 Graph representing bacterial growth kinetics for 40 hrs.

The growth kinetics of all the four samples followed a similar pattern with maximum growth at 6 hours

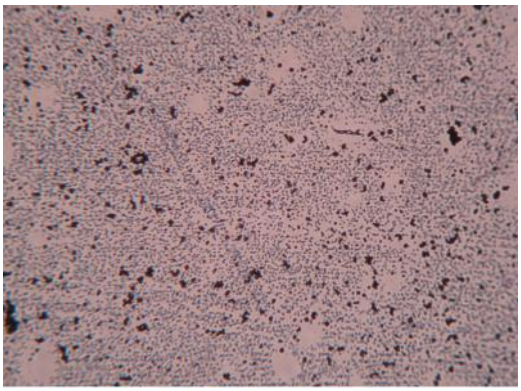

Figure 2 Microscopic view of gram stained slide containing sample AFB 1 which represents the presence of gram negative cocci

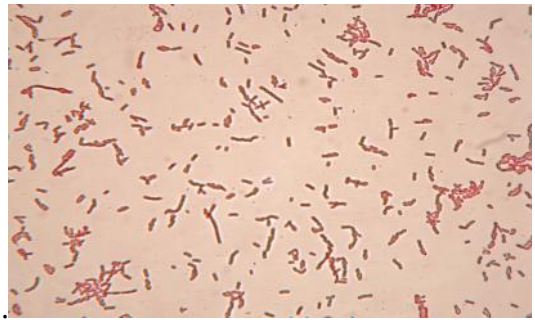

Figure 3 Microscopic view of gram stained slide representing gram negative bacilli. AFB2,AFB3 and AFB 4 were gram negative cocci.

Characterization

Gram staining was done to distinguish the bacteria based on its cell wall composition. All the cultures obtained from samples all took up Saffranin and appeared pink suggesting that they all are gram negative. Among those four samples, Sample AFB1 was gram negative cocci, whereas the rest were gram negative bacilli. None of the four samples gave a positive result for indole production test which indicated that the test organisms do not produce indole by tryptophan breakdown.

All four samples gave a negative MR test since they convert glucose to non acidic products such as ethanol or butanediol which elevated the pH approximately up to 6. The samples gave a negative result for the VP test too, in which Barrits reagent indicates the presence of acetoin. Two of the test samples, sample AFB 2 and AFB 7, utilized citrate and hence the medium turned blue. But samples AFB 1 and AFB 3 failed to utilize citrate.

Bacterial cultures that utilize diatomic oxygen, super oxide and peroxide were tested by tracing one of their oxidizing enzyme 'Catalase'. All the cultures showed positive result, that emphasizes the aerobic respiration nature of those cultures.

16S rRNA Amplification

DNA isolation using *Mo-Bio DNA kit* yielded pure DNA within 2 to 3 hours, but the amount was comparatively low. The DNA isolated samples were used for PCR amplification. After repeated PCR with varying primer concentrations and temperatures, the annealing temperature for the 16S rRNA primers (16F27 and 16R1525) was found to be 55ºC. Thus standard condition to amplify 16S bacterial gene from pure culture was developed.

Microbial decolorization of dyes

Microbial dye degradation was carried out to know if these isolates had the ability to utilize complex chemicals such as dyes as sole source of carbon, nitrogen. Three dyes were used for the investigation: Diazone green, reactive deep red and reactive blue G.

All three dyes were used at a concentration of 0.1%. Samples AFB 1, AFB 2 and AFB 7 decolorized reactive deep red. Out of the three, sample AFB 7 performed significantly high decolorization. Diazone green was decolourised by all four samples. Maximum decolorization was observed in sample AFB 7, followed by AFB 1, AFB 2 and AFB 3. Reactive blue G was decolorized by sample AFB 7 alone.

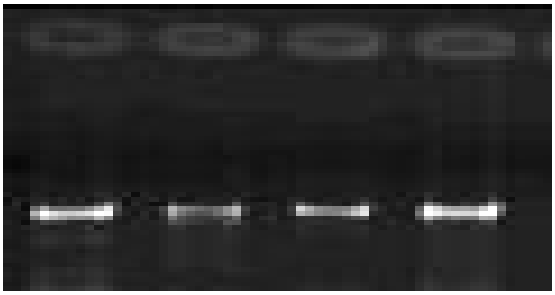

Figure 4 AGE of amplified 16S rRNA gene product. Lane 1- sample AFB 1; Lane 2- sample AFB 2; Lane 3- sample AFB 3; Lane 4- sample AFB 7

These interesting results drove us to study the kinetics of decolorization. So dye degradation kinetics was carried out in a similar way as that of the growth kinetics. The decolorizing ability of each of the four samples on the dyes was noted by measuring the absorbance every 2 hours for 12 hours. Prior to this, the maximum absorbance of each dye was determined by spectral scanning. According to this, the maximum absorbance of Reactive Deep Red was found to be 280nm, Diazone Green was 483nm and reactive Deep Blue was 822nm.

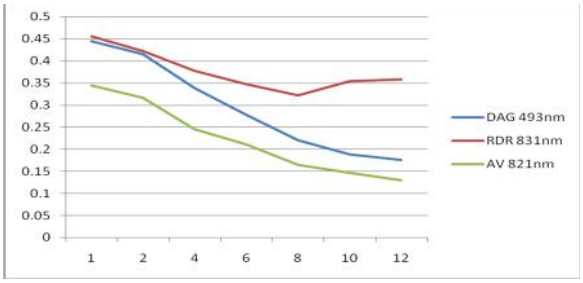

Figure 5 Degradation kinetics of sample AFB1 for the dyes Diazone Green, Deep Red, Reactive Deep Blue.

The work was started as an attempt to detect and bio-prospect bacterial population from moderate halophilic condition. The cultivable bacterial population was isolated by the conventional spread plates and streak plates, yielding method which yielded four distinct

organisms. Gram staining and biochemical tests were helped to characterize organisms. It was further supplemented with 16s rRNA gene data to get an authenticated species identification. All the four cultures gave a positive result for catalase test and is hence suspected to be aerobic. To understand the growth kinetics of the bacteria, bacterial growth kinetics was performed, followed by dye degradation with pure bacterial population. This was to know if the limestone derived organisms had the ability to utilize the dye for its growth. All the four samples degraded one or the other dye. Dye degradation kinetics was also performed to understand the rate of degradation. Absorption values (OD) at respective wavelength showed the conversion of product into various by products, generally thought to be aromatic amines, along with the increasing hours.

The future perspectives of the investigation include following aspects. Since the area of investigation has evolutionary significance and most of the sites there in are unreached by humans, expanded sequencing of the microbial population may reveal some novel organisms, genes and property. Since the organisms were found to degrade few dyes, the work can be extended to many other dyes. This would prove beneficial since textile effluent treatment remains a colossal problem in the state of Tamilnadu and India, because of the highly expensive chemical effluent treatment methods. Microbial dye degradation can be used as an alternative since it is a cheap source for the degradation of textile effluent.

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References

Claire Horner-Devine M, Karen Carney M and Brendan. Bohannan JM. 2004. An ecological perspective on bacterial biodiversity Proc. R. Soc. Lond. B 271, 113–122

Noah Fierer and Robert Jackson B. 2006. The diversity and biogeography of soil bacterial communities. PNAS. 103(3): 626–631

Sanghoon Kang, Aaron Mills L. The effect of sample size in studies of soil microbial community structure. 2006. Journal of Microbiological Methods. 66: 242– 250

Courtois, S., C. M. Cappellano, M. Ball, F. X. Francou, P. Normand, G. Helynck, A. Martinez, S. J. Kolvek, J. Hopke, M. S. Osburne, P. R. August, R. Nalin, M. Guerineau, P. Jeannin, P. Simonet, and J. L. Pernodet. 2003. Recombinant environmental libraries provide access to microbial diversity for drug discovery from natural products. Appl. Environ. Microbiol. 69: 49–55.

Handelsman J. 2004. Biotechnology prospects from metagenomics. *Current Opinion in Biotechnology* **14**: 303-310.

Handelsman J. 2005. Metagenomics for studying unculturable microorganisms: cutting the Gordian knot. *Genome Biology* **6**: 229.

Hoff KJ, Tech M, Lingner T, Daniel R, Morgenstern M, Meinicke P. 2008. Gene prediction in metagenomic fragments: a large scale machine learning approach. BMC Bioinformatics 9:217

Oh, H.J., Cho, K.W., Jung, I.S., Kim, W.H., Hur, B.K., & Kim, G.J. 2003 Journal of Molecular Catalysis. 26, 241-250

MacNeil IA, Tiong CL, Minor C, August PR, Grossman TH, Loiacono KA, Lynch BA, Phillips T, Narula S, Sundaramoorthi R, Tyler A, Aldredge T, Long H, Gilman M, Holt D, Osburne MS. 2001. Expression and isolation of antimicrobial small molecules from soil DNA libraries. J. Mol. Microbiol. Biotechnol. 3: 301-308

Manivel, M., Sakthivel, R., Muthu, M.S.P., and Kamalakkannan,R 2005 Environmental Impact Assessement Of Cretaceous Limestone Mining Area Of Dalmiapuram Of Tiruchirappalli, India Using Remote Sensing And Gis Map India 2005 Geomatics 2005

Oren A. 2002. Diversity of halophilic microorganisms: Environments, phylogeny, physiology, and applications. Journal of Industrial Microbiology & Biotechnology 28: 56-63

Riesenfeld CS, Goodman RM, and Handelsman J. 2004. Uncultured soil bacteria are a reservoir of new antibiotic resistance genes. *Environ. Microbiol.* **6**: 981–989.

Reysenbach A.L. and Hamamura. N. 2008. A geobiological perspective on metagenomics. Geobiology 6:337-340

Seshachur B, Narayana Rao A, Ramasamy LS 1932 Further notes on Ariyalur fossils 1: 39-40

Simu K, and Hagstrom A. 2004. Oligotrophic bacterioplankton with a novel single-cell life strategy. *Appl. Environ. Microbiol.* **70**: 2445–2451.

Stein JL, Marsh TL, Wu KY, Shizuya H, and DeLong EF. 1996. Characterization of uncultivated prokaryotes: isolation and analysis of a 40- kilobase-pair genome fragment front a planktonic marine archaeon. *J. Bacteriol.* **178**: 591–599.

Tilak KVBR, Ranganayaki N, Pal KK, De R, Saxena AK, Nautiyal CS,Mittal S, Tripathi AK, Johri BN. 2005. Diversity of plant growth and soil health supporting bacteria. Curr. Sci. 89:136-150

Venter, J. C., K. Remington, J. F. Heidelberg, A. L. Halpern, D. Rusch, J. A. Eisen, D. Wu, I. Paulsen, K. E. Nelson, W. Nelson, D. E. Fouts, S. Levy, A. H. Knap, M. W. Lomas, K. Nealson, O. White, J. Peterson, J. Hoffman, R. Parsons, H. Baden-Tillson, C. Pfannkoch, Y. H. Rogers, and H. O. Smith. 2004. Environmental genome shotgun sequencing of the Sargasso Sea. Science 304:66–74.

Vyankatesh Pidiyar J, Kamlesh Jangid, Milind Patole S and Yogesh Shouche S .2004. Studies on cultured and uncultured microbiota of wild culex quinquefasciatus mosquito midgut based on 16s ribosomal rna gene analysis. Am. J. Trop. Med. Hyg., 70:597–603

Zwolinski MD. 2007. DNA sequencing: strategies for soil microbiology. *Soil Sci. Soc. Am. J.* **71**: 592-600.

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EFFICIENT MANAGEMENT STRATEGIES OF COMBATTING FUNGAL INFECTIONS IN COPRA (*Cocos nucifera*)

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ABSTRACT
Coconut oil or copra oil is used in the country as a cooking fat, hair oil, body oil and industrial oil. It is extracted from the kernel or meat of mature coconuts harvested from the coconut palm (*Cocos nucifera)* having maximum moisture content of six per cent. Refined coconut oil is mainly used in the manufacture of biscuits, chocolates and other confectionery items, ice cream, pharmaceutical products and costly paints. Coconut copra is susceptible to fungal and insect attacks during storage. *Aspergillus and Penicillium* sp. infections are commonly reported in copra deterioration. Most farmers who cultivate coconut palms stand at loss during harvest as fungi and insect infect the copra. Hence management strategies are indispensible to eliminate fungal infections and ensure high quality yield of coconut oil.

Keywords
Cocos nucifera, copra oil, *Aspergillus and Penicillium* sp., moisture

INTRODUCTION
Coconut (*Cocosnucifera*) is a very important tree crop grown in many parts of the world. World production of coconuts has been steadily increasing during the last several years due mainly to the increased planting of the palm in the form of better managed large plantations in many of the major coconut-growing countries. More than 90 per cent of area and production of coconut in India is from the four southern states of Kerala, Karnataka, Tamil Nadu and Andhra Pradesh.

Dried coconut has umpty number of applications. Copra is the dried meat, or kernel, of the coconut. Coconut oil is extracted from it and has made copra an important agricultural commodity for many coconut-producing countries. It also yields coconut cake which is mainly used as feed for livestock. Coconut cake is the residue left after the extraction of oil from copra which is mainly used as a cattle feed. Coconut cake contains 4-5 per cent oil which is extracted by solvent extraction process. This oil is generally used for industrial purpose and de-oiled cake is used to make mixed cattle feed. There are a few such units in the country especially in Kerala.

In India, most farmers who cultivate coconut palms stand at loss during harvest as fungi and insect infect the copra. A number of pests including scale insects, bruchids, aphids, spider mites, mealybugs, palm weevils and some caterpillars are occasionally found on coconut. *Aspergillus and Penicillium* sp. infections are commonly reported in copra deterioration.(*Nathaneal,* et al). The present study is aimed at studying storage problems in copra and find good methods for high recovery of coconut oil production.

MATERIALS AND METHODS
1.Estimation of moisture content in coconut:
The moisture content of coconut is important during drying to prevent the fungal infection. The moisture content of the coconut was estimated by oven dry method. Ten

gram of the coconut sample was weighed and placed in the moisture box. The cover of the moisture box was removed and kept in a hot air oven previously heated to standard temperature of 105°C. The moisture box was removed from oven exactly seven hours of standard temperature, 105°C and transferred to desiccators for cooling till it reaches the room temperature. Then the final weight of sample was observed.

Calculation

$$\text{Moisture per cent} = \frac{(\text{Empty Wt} + \text{Sample Wt}) - \text{Final Wt}}{\text{Sample Wt}} \times 100$$

The fresh coconut was kept in room temperature for drying. The moisture content of fresh coconut and 24 hrs interval up to 5 days was measured as mentioned above.

2. Identification of fungal infection in the coconut during Copra preparation

The fungal infection was observed during the drying process for copra. The fungus were isolated and cultured in suitable media as mentioned below.

2.a. Procedure for preparation of potato dextrose agar media

The potato dextrose agar media was prepared for culturing of fungus for further studies.

Procedure

Weigh 3.9g of potato dextrose agar and mix 100ml of distilled water. After that add 2gm of agar-agar to dissolve shake nicely. The conical flask is to be capped (or) cotton plugged. Sterilize the conical flask in an autoclave at 121 C under 15 lbs. pressure for 15 hour.

ISOLATION OF FUNGAL CULTURE

PROCEDURE:

Label sterilized plate with name and dates liquidly the potato dextrose agar and pour into the sterile plates using aseptic procedure. After pouring place on a plate tube and make sure that media is overly distributed in plate failure of cooling the media result in condensation of moisture on corner of plate which is considerable. These droplets will fail on the colonies spreading a detecting the entire isolation technique. Heats the inoculation loop to red hot, allow cooling and taking the fungal culture from infected coconut by using inoculation loop. Inoculate the fungal culture sample on potato dextrose agar in petriplate. Allowed for overnight culture in room temperature.

ISOLATION OF FUNGUS

Identification of fungus from colony was done by the Lactophenol cotton blue method.

PROCEDURE: Place a small drop of lactophenol cotton blue on a clean glass microscope slide before clean the laminar air low by using ethanol.Remove a small portion of the yeast colony and placed it into the drop of lactophenol by using inoculation loop and suspend the cells. Place a clean cover glass over the suspension and observe microscopically.

3. Effect of salt solution on the growth of fungus.

Salt solution method in fresh Copra. Prepare the salt solution at 1%, 2% and 5%.

1% SALT SOLUTION

1g of salt was weighed and dissolved in 100ml of distilled water.Take a cotton scroll, it can be soaked in the salt solution and the solution was applied in the fresh open

coconut.Leave it for 24hrs or few days for the fungal growth.Then finally go for fungal identification.

2% SALT SOLUTION

2g of salt was weighed and dissolved in 100ml of distilled water.Take a cotton scroll, it can be soaked in the salt solution and the solution was applied in the fresh open coconut.Leave it for 24hrs or few days for the fungal growth.Then finally go for fungal identification.

5% SALT SOLUTION

5g of salt was weighed and dissolved in 100ml of distilled water.Take a cotton scroll, it can be soaked in the salt solution and the solution was applied in the fresh open coconut.Leave it for 24hrs or few days for the fungal growth.Then finally go for fungal identification.

4.1 ACETIC ACID TREATMENT

In this method for avoid the fungal infection in fresh open coconut.Take a 250ml of beaker Prepare the acetic acid concentration at 5%, 7% and 10% by using micropipettes.

4.2 SULFURIC ACID TREATMENT

Take a 250ml of beaker Prepare the sulfuric acid concentration at 5%, 7% and 10% by using micropipettes.

4.3 ACETIC ACID WITH SULFURIC ACID TREATMENT

Prepare the acetic acid 10% with sulfuric acid 10% and acetic acid 15% with sulfuric acid 5%.

4.4 SPRAY TECHNIQUE

Using sprayer for spray in open coconut in above different concentration of acetic acid and sulfuric acid.

4.5 DIPPING TECHNIQUE

In the method, a piece coconut dipped in above the different concentration of acetic acid and sulfuric acid.

RESULTS AND DISCUSSION

MOISTURE

It was observed that the intial moisture content of freshly opened coconut is 40.91%. The high moisture content of fresh coconut and slow reduction in moisture content of copra to 16.56% both favors fungal growth during ordinary storage.

Days	Percentage (%)
1	40.91
2	33.45
3	30.32
4	28.77
5	21.71
6	16.56

Effect of Treatment (Dipping method) on the fungal infection

The effect of slat solution has less protection for the first day, but infection occurs from 2nd day. The use of 15% Acetic acid and 5% Sulfuric acid solution have the best effect on fungal infection on dipping method.

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Salt solution 1%	No infection	Aspergillus	Aspergillus, Penicillium	Aspergillus, Penicillium	Aspergillus, Penicillium
Salt solution 3%	No infection	Aspergillus	Aspergillus, Penicillium	Aspergillus, Penicillium	Aspergillus, Penicillium
Salt solution 5%	No infection	Aspergillus	Aspergillus, Penicillium	Aspergillus, Penicillium	Aspergillus, Penicillium
Acetic acid 5%	No infection	Aspergillus	Aspergillus, Penicillium	Aspergillus, Penicillium	Aspergillus, Penicillium
Acetic acid 10%	No infection	Aspergillus	Aspergillus, Penicillium	Aspergillus, Penicillium	Aspergillus, Penicillium
Acetic acid 15%	No infection	No infection	No infection	No infection	No infection
Sulfuric acid 5%	No infection	No infection	No infection	No infection	No infection
Sulfuric acid 10%	No infection	No infection	No infection	No infection	No infection
Sulfuric acid 15%	No infection	No infection	No infection	No infection	No infection
Acetic acid with $H_2SO_4$10%+10%	No infection	No infection	No infection	No infection	No infection
Acetic acid with $H_2SO_4$15%+5%	No infection	No infection	No infection	No infection	No infection

Effect of Treatment (Spray method) on the fungal infection

The use of 15% Acetic acid and combined treatment of 10% Acetic acid and 10% Sulfuric acid solution have the best effect on fungal infection on spraying method.

	DAY 1	DAY 2	DAY3	DAY 4	DAY 5
Acetic acid 5%	No infection	Aspergillus	Aspergillus, Penicillium	Aspergillus, Penicillium	Aspergillus, Penicillium
Acetic acid 10%	No infection	Aspergillus	Aspergillus, Penicillium	Aspergillus, Penicillium	Aspergillus, Penicillium
Acetic acid 15%	No infection	Aspergillus	Aspergillus, Penicillium	Aspergillus, Penicillium	Aspergillus, Penicillium
Sulfuric acid 5%	No infection	Aspergillus	Aspergillus, Penicillium	Aspergillus, Penicillium	Aspergillus, Penicillium
Sulfuric acid 10%	No infection	No infection	No infection	No infection	No infection
Sulfuric acid 15%	No infection	No infection	No infection	No infection	No infection
Acetic acid with $H_2SO_4$10%+10%	No infection	No infection	No infection	No infection	No infection

Acetic acid with $H_2SO_4$15%+5%	No infection	No infection	No infection	No infection	No infection

Treatment with sundry

After sundrying, treatment with 10% acetic acid, 10% sulphuric acid and combining 5%Acetic acid with 5% sulphuric acid solution have best fungicidal effects

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Acetic acid 10%	No infection	No infection	No infection	No infection	No infection
Sulfuric acid 10%	No infection	No infection	No infection	No infection	No infection
Acetic acid with H2SO4 5%+5%	No infection	No infection	No infection	No infection	No infection

CONCLUSION

The higher moisture content of fresh coconut and the slow reduction of moisture favours the microbial growth during the period of ordinary storage.Copra can be affected by various fungi like Aspergillus, Penicilium, Mucor sp. In order to prevent infections, many chemical and physical methods were standardised. Sundrying reduces moisture content in copra by 16.56% in 6 days. Sun drying and treatment with combined 5% Acetic acid and 5% Sulfuric acid gives the best protection from fungal infections during storage. These findings can be taken as basic step and suggested for Oil industries for better storage and best ways of high quality oil recovery which will enhance the ptoduction of copra oil in future.

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DETERMINATION OF FUNGAL QUALITY OF MARKETED HONEY SAMPLES

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ABSTRACT

Honey is widely used as medicine and as a nutritional source. The anti-oxidant nature of honey favours its utility in nutraceuticals. Honey is also widely used as a medicine in Indian Medical System such as Siddha and Ayurveda. However, the quality of honey is a concern, as microorganisms are found to affect the health of the consumers as well as the quality and shelf life. Thus, the knowledge on the presence of microbes and their diversity is necessary. Though, the bacteria and the presence of yeasts are widely studied, the same related to the presence of fungi is scarce. Hence the present study is conducted. Altogether, 33 honey samples were procured from the markets of Chennai. The samples were serially diluted to 10^{-2} and 1 ml of the diluted sample was plated with Potato Dextrose Agar and the plates were maintained in triplicates. A total of 813 colonies belonging to 20 species were isolated. *Aspergillus flavus*was the most dominant species isolated, followed by *Aspergillus niger*and*Penicilliumoxalicum.* The results showed that few of the samples were highly contaminated and failed to comply with the industrial standards.

INTRODUCTION

Honey is used as medicine and as a nutritional source. Honey has long been used as a natural sweetener in foods. The anti-oxidant nature of honey favours its utility in nutraceuticals. Honey is also widely used as a medicine in Indian Medical System such as Siddha and Ayurveda which is now used for treatment of wounds, burns and even cancer due to its rich antioxidant property (Layflurrie 2008, Brady et al 2008, Khalil et al 2011). Honey is a super saturated solution of sugars composed mainly of monosaccharides, fructose and glucose, apart from other di- and trisacchardies, which gets eventually broken down into monosaccharides by enzymes produced by bees (Martins et al. 2003). Honey is characterized by low water activity, low protein content, natural acidity and high viscosity. The contamination of such highly used food or the product of pharmaceutical importance is a major issue in the field of food safety. The quality of honey in terms of flavor, aroma or taint should not be altered by the presence of objectionable or foreign matter or by the addition of any food ingredient or removal of its constituent (CODEX STAN 12-1981 and European Union Legislation DLgs 179/2004). These guidelines suggest that honey that is available for the consumption or for addition in other food products as ingredients must lack pathogenic microorganisms or microorganisms that causes illness (Popa et al 2009). These microorganisms cause spoilage of honey by fermentation (Jimenez et al 1994).

Microorganims inhabiting honey include bacteria, fungi and yeasts. They introduce antibiotics, enzymes, mycotoxins and other growth factors into honey (Goerzan 1991) thus causing infections in consumers. The presence of such microorganisms also affect the

stability and thereby the shelf life of honey. Studies are extensively focused on *Clostridium botulinum* due to the risk of child botulinum, while industrial standards appreciate the testing of honey for pathogenic bacteria such as *Salmonella* and *Staphylococcus.* Yeasts and spore forming bacteria are commonly found in honey (Farris et al 1986, Snowdon and Cliver 1996, Carvalho et al 2006, Rozanska 2011). Pertaining to fungi, reports are limited and the available literature has exposed several filamentous fungi especially of *Aspergillus* and *Penicillium*genera (Kacaniova et al 2009, Sinacori et al 2013). These potentially harmful species though in dormant state at low water activity precluding their mycotoxin production, improper processing render them active toxin producers. Such infestation of toxins into honey by fungi can be harmful to consumers. Thus, owing to the lack of data on fungal quality of honey, and to compare the difference in quality of processed and unprocessed honey, the present study was instigated.

MATERIALS AND METHODS

Collection of Honey samples:

Altogether 33 samples of honey were collected from the local markets of Chennai, India. Based on the manufacturing details, the samples are classified into branded and unbranded honey. Branded honey consist of 25 samples and unbranded honey consisting of 8. The product name, manufacturer details, lot number and packaging details are presented in Table 1.

Table 1: Name of the Product, Manufacturer, Lot no and Packing details of honey samples

No.	Product name, Manufacturer, Lot no and Packing details.
1	Unbranded Local vendor
2	Amrutham Honey, Nature food products, Chennai, Tamil Nadu, Lot No: 04, Packed on: 07/2013
3	Agmark Kashmir Honey,NaturalShameethaBeeo, Viruthunagar, Tamil Nadu, Lot No:674, Packed on: 09/2013
4	Natural Honey J.J Agmark, Johnson Honey, Mumbai, Maharashtra, Lot No:38, Packed on:08/13
5	KodaikanalAgmark Honey, Sri Vignesh Cottage Industry, Chennai, Tamil Nadu, Lot No:5, Packed on:08/13
6	Unbranded Local vendor
7	Unbranded VSR Honey
8	Dabur Honey,Dabur India Ltd. Himachal Pradesh, Lot No: BDO 365, Packed on:07/13
9	SGS Honey Rex,SGS Cottage Industries, Chennai, Tamil Nadu
10	Unbranded Local Vendor
11	100% Natura Agmark Honey, New Indian Apiary Industry, Marthandam, Tamil Nadu, Lot No:380/2013, Packed on:09/2013
12	SwastikHoney,D.S.T.(Devi Swarna) Trade mark, Chennai, Tamil Nadu, (No other details)
13	Unbranded Local Vendor
14	100% Natural Marthandam Co-operative Agmark Honey, Co-operative Society Ltd, Marthandam, Tamil Nadu, Lot No:302/2013, Packed on:06/2013
15	AgmarkAnnai Honey, M/S Muruhan Co, Chennai, Tamil Nadu, Packed on:12/2012
16	Khadi Alocory Natural Kashmir Honey, Ashwin Khadi GramodyogSamitiModinagar, Uttar Pradesh, Packed on:09/2013
17	Devi's Honey Taste, Devi Packaging, Chennai, Tamil Nadu. (No other details)

18	Jay Honey, J G S Cottage Industries, Chennai, Tamil Nadu, S.No:06, Packed on:06/2013
19	New gem Agmark Honey, Natural Honey Zone, Kuzhithurai, Tamil Nadu, Lot No:217/2013, Packed on:05/2013
20	Unbranded S.K Swastik Honey
21	Lion Honey, Lion Dates Imper Pvt. Ltd., Trichy, Tamil Nadu, Batch No:H105, Packed on:08/2013
22	Coorg Honey, The Coorg Honey & Wax Product Co-operation Marketing Society Ltd., Kodagu, Karnataka. Lot No:21,
23	Royal Agmark honey grade Natural Multi floral Honey, Royal Food Products, Chennai, Tamil Nadu,Packed on:09/2013
24	Marthandam Star Agmark Honey, Marthandam Star Honey Traders, Kanyakumari, Tamil Nadu, Lot No:172/ 2013, Packed on: 07/2013
25	Hills KodaikanalAgmarkHoney,SriBalamurugan Cottage Industries, Chennai, Tamil Nadu, Lot No:02, Packed on:06/2013
26	R.R Honey Agmark Honey, R.R Honey enterprises, Chennai, Tamil Nadu,Lot No:12, Packed on: 10/2013
27	Reliance Healthy Life, Reliance Group of Companies, Mumbai, Maharastra, Lot No:Rl-045, Packed on:04/2014
28	Unbranded Khadi kraft
29	SKM Honey, SKM Siddha and ayurvedha Company(India) Ltd., Erode , Batch No:H0A13050, Mfg dt:10/2013
30	Unbranded Local Vendor
31	Sagar Forest Hill Nilgiri Honey, SagarNilgiri Oil Distillery, Ooty, Nilgiris, Tamil Nadu, Code 152
32	Heritage Agmark Honey, Prakruthi Health products, Banglore, Karnataka, Batch No:004, Packed on: 04/2013
33	HeavenaAgmark Honey, M/S Heavenly Fuel Pvt Ltd. (No other details)

Isolation and identification of Fungi:

The samples procured were stored in room temperature until processing. The samples were processed within a week from the collection of samples. The microorganisms were isolated by diluting with sterilized distilled water. The honey samples were serially diluted to achieve 10^2 concentrations and 1 ml of the diluted sample was plated into petridishes containing Potato Dextrose Agar (PDA, HiMedia, Mumbai) supplemented with Streptomycin (0.06g/L) and maintained as triplicates at room temperature (28±2^o C). The fungal colonies were allowed to grow for 4-7 days and identified upto species level wherever possible based on the morphological, macro and microscopical observations with reference to standard identification manuals (Ellis, 1971, Onions et al, 1981, Subramaniam, 1971, Udayaprakash, 2004).

Presentation of data:

$$\text{Percentage contribution} = \frac{\textit{Number of isolates of a particular species}}{\textit{total number of isolates}} \times 100$$

$$\text{Frequencyoccurrence} = \frac{\textit{Number of samples infected by a particular species}}{\textit{total number of samples}} \times 100$$

RESULTS AND DISCUSSION

Fungal counts observed on PDA ranged from 0 to 88 x 10^2CFU ml-^{1} of honey, with an average of 8.2 x 10^2 CFU ml^{-1}. Altogether, 813 isolates belonging to 20 fungal species representing 11 generaincluding non-sporulatingcolonies were recovered in the present study. The number of colony forming units recorded per ml. of honey samples and the number of species recorded from individual honey samples is presented in Fig. 1. The incidence, frequency occurrence and total number of isolates of individual fungal species in honey samples is presented in table 3.

Fig.1. The total CFUg^{-1}and number of species isolated from the honey samples.

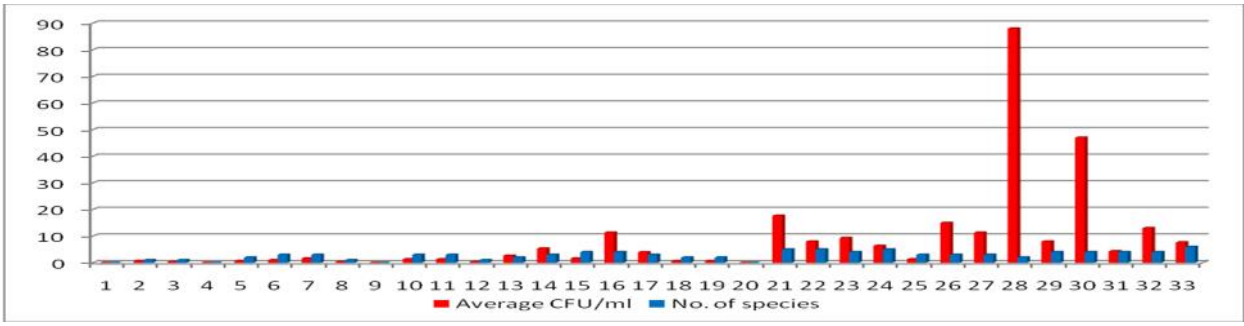

Table 2: Total number of isolates, % Contribution and Frequency occurrence of Individual fungal species

S.No.	Species	No. of Isolates	% Contribution	Frequency Occurrence
1	*Absidiacorymbifera*	7	0.86	6.06
2	*Alternariaalternata*	4	0.49	9.09
3	*Aspergillus flavus*	461	56.70	39.39
4	*Aspergillus fumigatus*	39	4.80	21.21
5	*Aspergillus niger*	193	23.74	57.57
6	*Aspergillus tamarii*	2	0.25	6.06
7	*Aspergillus terreus*	17	2.09	18.18
8	*Aspergllusnidulans*	2	0.25	6.06
9	*Chrysosporium sp.*	1	0.12	3.03
10	*Curvulariabrachyspora*	1	0.12	3.03
11	*Curvularialunata*	1	0.12	3.03
12	*Drechsleraaustraliensis*	1	0.12	3.03
13	*Drechslerahawaiiensis*	3	0.37	3.03
14	*Emericillanidulans*	1	0.12	3.03
15	*Fusarium moniliforme*	3	0.37	3.03
16	*Moniliasitophila*	4	0.49	12.12
17	Non-sporulating colonies	67	8.24	54.54

18	*Penicilliumcitrinum*	3	0.37	6.06
19	*Penicilliumoxalicum*	1	0.12	3.03
20	*Rhizopusstolonifer*	2	0.25	6.06
		813	100	

Of the 33 samples analyzed, it was observed that *Aspergillus niger*colonized 57% (19 out of 33) of the samples followed by Non-sporulating colonies (54%) and *Aspergillus flavus*(39%). On the whole, 87.87% of the samples were contaminated with atleast fungal species and 75.75% samples contaminated with two or more fungal species. Of the 20 species recovered, Zygomycetes was represented by 2 genera (*Absidia* and *Rhizopus*), Ascomycetes was represented by singlegenus (*Emericella*)and Deuteromycetes by 8 genera (*Alternaria, Aspergillus, Chrysosporium, Curvularia, Drechslera, Fusarium, Monilia* and *Penicillium*) and other non-sporulating fungi were placed together under Mycelia Sterilia. The most dominant genera was *Aspergillus* represented by6 species and occurred in 72% of the samples, followed by *Curvularia, Drechslera* and *Penicillium* each represented by 2 species. *Aspergillus flavus*was the highest contributor with 461 isolates (57%) followed by *A. niger*(24%) and Non-sporulating colonies (8%).

The genera *Aspergillus* and *Penicillium*are usual inhabitants of honey. Kacaniovaet al, (2009)reported *Alternaria*and Mycelia sterilia to be the most frequently encountered microscopic fungi. In this study, similar results were observed, with *Aspergillus* being the most dominant genera followed by Mycelia Sterilia. The present study reported *Aspergillus flavus,A.niger, A. fumigatus*as the most prevalent species, which was observed similar to Nasser (2004) and Finola et al (2007). Finola et al (2007) reported that 46 of 80 honey samples from southern Argentina was infected by microscopic fungi while, Nasser (2004) reported 40 of 45 honey samples from the retail markets in Saudi Arabia was infected.Kacaniova et al. (2012)from Portugal reported 71 of 80 samples as infected. The present study revealed that 29 of 33 samples were infected. The samples had high $CFUg^{-1}$of fungi with an average of 8200 $CFUg^{-1}$. Such high $CFUg^{-1}$ was reported previously by Kacaniova et al. (2009) who reported a total of 81000 $CFUg^{-1}$ in 30 samples of honey examined from various areas of Slovakia.

The source of the microfungi can be termed innumerable with pollen being the major source (Gilliam et al., 1983). Honey bees get infected mostly on consumption of pollens and spreads the infection to other bees in the colony through food exchange (Snowdon and Cliver, 1996). Thus, making honey bees in the hive environment more exposed to a high diversity fungi with *Aspergillus* sp. being the highly prevalent (Foley et al 2014). The honey from infected hives will thus contain high quantities of fungi. Vojvodic et al.(2011) reported that *A. flavus* from honey bee larvae showed high virulence. In the present study *A. flavus* was found to be the most dominant species, which could be attributed to the study that *A. flavus* is the most common Aspergillus species infecting insects (St. Leger et al., 1997).
In the present study, it was observed that unbranded honey had high $CFUg^{-1}$of47 and88 $CFUg^{-1}$in 2 of the samples, neglecting which the average was found to be 0.75. The high $CFUg^{-1}$in 2 honey samples could have been due to infection of the bee hive. While the branded honey showed an average of 5.17 $CFUg^{-1}$. According to industrial standards, honey must lack the presence of pathogenic organisms, however in the present study, it was

observed that pathogenic organisms such as *A. flavus, A.fumigatus*were present in high number in few of the samples studied.

It was reported that all the processed honey samples from Nigeria was contaminated with fungi (Ayansola 2012) while the unprocessed honey from Nigeria was not reported with significant quantities of fungi. Popa et al. 200 reported microbial contamination in honey samples during or after processing. However, samples collected from primary sources (honey from bee hives) showed absence of microbial contamination. The contamination in processed honey indicate improper or inadequate hygiene conditions during collecting, manipulating, processing or storing (Tchoumboue et al. 2007)

The present study also experienced similar results, with branded processed honey samples showing high contamination than unbranded honey samples. Also, it has been reported that the fungi produced Aflatoxin in all apiarian substrates (unprocessed honey, pollen, brod comb, whole larvae and whole bees) in low levels except the unprocessed honey (Hilldrup et al. 1977).Wellford et al. (1978) also reported that though toxigenic strains of *A. flavus* NRRL 5862 and *A. parasiticus* NRRL 2999 colonized unprocessed honey samples, detectable amounts of aflatoxin production was not observed. Thus, the present study shows that the processed or branded honey samples provided the habitat for abundance of fungal colonies compared to unbranded honey. Also, it was observed that many of the samples had abundance of pathogenic fungi and hence fail to comply with the industrial standards.

References:

Ayansola, A.A. (2012). Fungal Isolates from the Honey Samples Collected from Retail Outlets in Southwestern Nigeria. Journal of Biology and Life Science. 3 (1), 189-199

Bardy, J., Slevin, N.J., Mais, K.L. and Molassiotis, A. (2008). A systematic review of honey uses and its potential value within oncology care. J. Clin. Nurs., 17, 2604-2623.

Bogdanov, S., Jurendic, T., Sieber, R., and Gallmann, P. (2008). Honey for nutrition and

Carvalho, M.C., Rocha, A., Estevinho, L., and Choupina, A.B. (2006). Identification of honey yeast species based on RFLP analyses of the ITS region. Cienc. Tecnol. Alimentos., 5 (1), 11-17.

Ceausi, C., Togoe, L., Tudor, L., and Furnaris, F. (2009). The evaluation of microbiological quality of some bee honey sorts. Bull. UASVM Animal. Scie. Bio., 66 (1), 273e277

Codex Alimentarius Commission (2001). Revised Codex Standard for Honey. Codex STAN, 12e1981, Rev. 1 1987, Rev. 2.

Ellis, M.B. (1976). More Dematiaceous Hyphomycetes. CAB. International Mycological Institute, Kew, UK. 507 pp.

Farris, G.A., Fatichenti, F., Deina, P., and Agostini, F. (1986). Aerobic and anaerobic sporeforming bacteria in sardinian honey. Annalidellafacoltà di Agrariadell'Università di Sassari., 32, 173-179.

Finola, M.S., Lasagno, M.C., and Marioli, J.M. (2007). Microbiological and chemical characterization of honeys from central Argentina. Food Chem., 100(4), 1649–1653.

Gilliam, M., Taber, S., and Richardson, G.V. (1983). Hygienic behavior of honey bees in relation to chalkbrood disease. Apidologie, 14, 29–39.

Goerzen D.W. (1991). Microflora associated with the alfalfa leaf cuting bee, Egachilerotundata (Fab) (Hymenoptera:Megachilidae) in Saskatchewan, Canada. Apidologie., 22, 553-561.

health: A review. J. Am. Coll. Nutr., 27 (6), 677-689.

Hilldrup J.A., Eadie T. and Liewellyn G.C. (1977): Fungal growth and aflatoxin production on apiarian substrates. J.Ass. Off. Anal. Chem., 60, 96-99.

Jiménez, M., Mateo, J. J., Huerta ,T. and Mateo, R. (1994). Influence of the storage conditions on some Physicochemical and Mycological Parameters of Honey. J. Sci. Food Agric., 64, 67-74.

Kacaniova, M., Knazovicka, V., Felsociova, S., and Rovna, K. (2012). Microscopic fungi recovered from honey and their toxinogenity, Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, 47(11), 1659-1664

Kacaniova, M., Sudzina, M., Sudzinova, J., Fikselova,M., Cubon, J., and Hascik, P. (2007) Microbiological and physico-chemical quality of honey collected from different Slovak habitats. Slov. J. Ani. Sci., 40(1), 38–43.

Khalil, M.I., Sulaiman, S.A., and Boukraa, L. (2010). Antioxidant Properties of Honey and Its Role in Preventing Health Disorder. The Open Nutraceuticals Journal, 3, 6-16

Kirsten Foley, Fazio, G., Jensen, A. B., and Hughes, W. O.H. (2014). The distribution of Aspergillus spp. opportunistic parasites in hives and their pathogenicity to honey bees. Veterinary Microbiology, 169, 203–210

Lay-Flurrie, K. (2008). Honey in wound care: effects, clinical application and patient benefit. Br. J. Nurs., 17, 32-36.

Martins, H. A., Martins, M. L., and Bernardo, F. M. A. (2003). Bacillaceae spores, fungi and aflatoxins determination in honey. Rev. Portug. Cienc. Veter., 98, 85–88.

Nasser, L.A. (2004) Isolation and characterization of fungi contaminating packaged honey commonly consumed in Saudi Arabia. Ass. Univ. Bull. Environ. Res., 7(1), 1–7.

Onions, A.H.S., Allsopp, D. and Eggins, H.O.W. (1981). Smith's Introduction to Industrial Mycology. 7th Ed. Edward Arnold, London.

Popa, M., Vica, M., Axinte, R., Glevitzky, M., and Varvara, S. (2009). Study concerning the honey qualities in transylvania region. Ann. Universe Apulensis Series Oecon., 11(2), 1034–040

Rozanska, H. (2011). Microbiological quality of Polish honey. B. Vet. Pulawy., 55 (3), 443-445.

Sinacori. M., Francesca. N., Alfonzo. A., Cruciata. M., Sannino. C., Settanni. L., and Moschetti G. (2013) Cultivable microorganisms associated with honeys of different geographical and botanical origin. Food Microbiol., 38, 284-294.

Snowdon, J. A. and Cliver, D. O. (1996). Microorganisms in honey. Review article. Int. J. Food Microb., 31, 1-26.

St. Leger, R.J., Joshi, L., and Roberts, D.W. (1997). Adaptation of proteases and carbohydrases of saprophytic, phytopathogenic and entomopathogenic fungi to the requirements of their ecological niches. Microbiology, 143, 1983–1992.

Subramaniam, C.V., (1971). Hyphomycetes (An account of Indian species, except carpospores)", ICAR, New Delhi.

Tchoumboue, J., Awah-Ndukum, J., Fonteh, F.A., Dongock, N.D., Pinta, J., and Mvondo, Z.A. (2007). Physico-chemical and microbiological characteristics of honey from sudan-guinean zone of West Cameroon. Africa J. Biotech., 6, 908-913.

Udaya Prakash, N.K. (2004). Indoor molds-Isolation and Identification. Colour wings (M) Pvt. Ltd. Madras. pp- 99.

Vojvodic, S., Jensen, A.B., James, R.R., Boomsma, J.J., and Eilenberg, J. (2011). Temperature dependent virulence of obligate and facultative fungal pathogens of honeybee brood. Vet. Microbiol., 149, 200–205.

Wellford T.E., Eadie T. and Liewellyn G.C. (1978): Evaluation the inhibitory action of honey on fungal growth sporulation. In aflatoxin production. Z. LebensmUntersForsch., 28, 280-283.

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Cytotoxic effect of *Semecarpusanacardium*nut extract in B16F10 melanoma cell lines

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ABSTRACT
This study was focused on the activity of methanolic extract and the fractions of *S. anacardium*nut against B16F10 melanoma cell lines.Caspase3 and caspase 9 activities of B16F10 melanoma cell lines were determined.Cell viability assaywas performed for B16F10 melanoma cellto determine the effect of *S.anacardium*crude methanolic extract and its fractions.DNA fragmentation assaywas also carried out in B16F10 melanoma cell treated with 100 µg/ml of crude extract and n-hexane fraction. Hexane fraction treated B16F10 melanoma cell line showed a highest activity for caspase-3 (145 ± 11.5pg/ µg)and caspase-9 (154 ± 5.5pg/ µg)than crud extract. The hexane fraction and the crude methanolic extract showed a comparatively higher cytotoxic effect in the B16F10 melanoma cell lines. B16F10 melanoma cell line DNAtreated with *S. anacardium*crude extract and n-hexane fraction showed a marked DNA fragmentation. From this study it is concluded that the *S.anacardium*methanolic extract has a potential anticancer activity against B16F10 melanoma cell lines. Yet further investigation is necessary for identifying its mode of action for the cytotoxic effect.
KEYWORDS: B16F10 cell lines, Cell viability, *Semecarpusanacardium*, Skin cancer.

I. Introduction

Skin acts as a barrier and gives protection against exogenous influence like UV radiations, toxic chemicals and infectious or mechanical stress [1]. Skin regulates body temperature, involves in vitamin D production and also actively takes part in immune system. Skin cancers generally develop in the epidermis and tumors are easily visible. This helps in the early identification of tumor. Skin cancer contributes 30% of total cancer diagnosed worldwide and 90% of skin cancer is due to solar UV radiation [2]. The World Health Organization estimates that as many as 65,161 people a year worldwide die from too much sun, mostly from malignant skin cancer.In India there are relatively higher incidence of skin cancer in Rajasthan, Tamil nadu and Kerala.

Free radicals are substances which are formed during metabolism or through the action of ionizing radiation which are very short lived with half lives of milli/micro/nanoseconds(Eg: superoxide, hydrogen peroxide and nitric oxide). Increased production of reactive oxygen species (ROS)is associated with the onset of a variety of diseases including cancer[3]. A free radical can damage the specific site of DNA leading to breaking of strands, or it might delay the repair before replication occurs, leading to mutations [4].

Semecarpusanacardium is a potent drug and traditionally used for neuritis, arthritis, leprosy, helminthic infections and it is capable of anticancer, antibacterial and anti-inflammatory property [5, 6]. Hembree*et al.*[7] found that a fraction of the aqueous methanolic extract of the nuts was active against Eagles 9KB nasopharynx carcinoma cell cultures.Studies also have reported cytotoxic effect of *S. anacardium* in colo-320 tumor cells [8] and toxic at dose dependent levels in animal studies.

Even though the anticancer property of *S.anacardium* was reported earlier for various cancer types, study against B16 melanoma cell line is scanty. Hence, the present study is focused on the activity of methanolic extract and the fractions of *S. anacardium*nut against B16F10 melanoma cell lines.

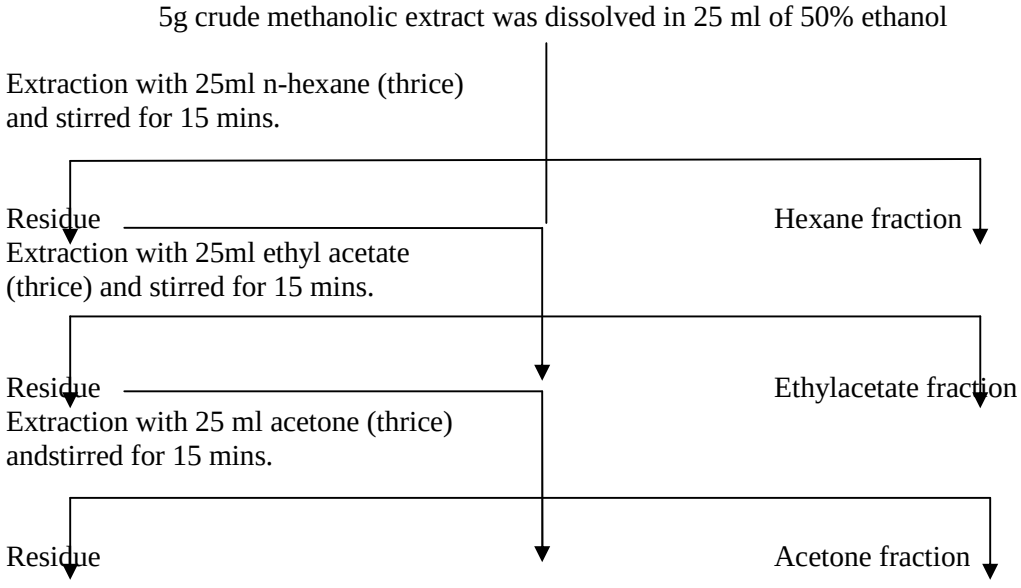
2. Materials and methods

Collection of plants

The *Semecarpusanacardium*nuts was bought from local market and authenticated by Dr. S. Karupasamy, Assistant Professor, Dept. of Botany, The Madura College, Madurai, Tamil Nadu, India.

Preparation of extract

Extraction of*S. anacardium* was carried out by the method of Sowmyalakshmi*et al.* [9] and Weimin*et al.* [10].The dried nuts of *S. anacardium*were washed in distilled water and crushed well. By cold extraction method the plant material was kept in hexane for 24 hours. Then it was filtered and the residues were dried. 25 gof the dried residue was then used for methanolic extraction in soxhlet apparatus for 48 hours. The extract was then dried in air over night and in the next day it was collected and stored at 4°C. Various fractions of the crude methanolic extract were prepared as per the following figure [11];



All the fractions were collected and solvents were evaporated at room temperature. The dried fractions were collected and stored in 4°C.

Effect of S.anacardiummethanolic extract and its fractions on B16F10 melanoma cancer cell lines

Cell lines

B16F10 Skin cancer melanoma cell lines were obtained from National centre for cell sciences (NCCS), Pune, India. The cell were maintained in DMEM with 10% FBS. The culture environment was maintained as 5% CO_2 with optimum humidity at 37°C. The cells were passaged for every 4 days or after 100% confluency.

Cell viability assay

Cell viability assaywas performed for B16F10 melanoma cell in triplicate as per the method of Kang *et al.* [12].The mean of the cell viability values was compared to the control to determine the effect of the extract on cells. A graph was plotted against the % cell viability (B16F10 cells) Vs dilution of the extract. The minimum concentration of extract that was toxic to cells was recorded as the effective drug concentration with compare to positive control (cyclophasphamide).

Determination of caspase activity

Caspase3 and caspase 9 activities of B16F10 melanoma cell lines were determined by chromogenic substrate based colorimetric assays [13] and expressed as pg μg^{-1} protein.

DNA fragmentation assay

DNA was extractedfrom B16F10 melanoma cell without treatment and treated with 100 µg/ml of crude extract and n-hexane fraction and separated in agarose gel electrophoresis using the method of Burton [14] and Perandones*et al*. [15].

3. Results

Cell viability assay

The anticancer activity of *S. anacardium*methanolic crude extract and its fractions were analyzed in B16F10 melanoma cell lines using MTT assay. Percentage of viable cell after treating the cells with various concentrations of the extract is shown in Figure 1.Viability of the cells decreased in dose dependent manner. Among the samples, the hexane fraction and crude extract showed a high cytotoxicity compared with ethyl acetate and acetone fractions.

Caspase activity

Expression of caspase-3 and caspase-9 is given in Table 1. The activity of caspases showed an increase in expression in the cell lines with increase in concentration of the extract. Hexane fraction treated B16F10 melanoma cell line showed a higher activity for caspase-3 and caspase-9 followed by the crud extract.

DNA fragmentation assay

*S.anacardium*crude extract and n-hexane fraction treated B16F10 melanoma cell line DNA is shown in Figure 2. Lane 1 loaded with B16F10 melanoma cell line DNA without any treatment showing no fragmentation and the Lane 2 and 3 loaded with *S. anacardium*crude extract and n-hexane fraction showed a marked DNA fragmentation.

4. Discussion

In recent years, the natural and plant derived compounds are been in greater attraction in the discovery of drugs for cancer treatment. Cancer chemoprevention by phytochemicals appears to be one of the most feasible approaches for cancer control. Vegetables, fruits, spices, teas, herbs, and medicinal plant metabolites such as carotenoids,

phenolic compounds, and terpenoids, have been proven to suppress experimental carcinogenesis in various organs [16, 17].

*S.anacardium*crude methanolic extract and its hexane fraction were tested efficient against B16F10 melanoma cell lines, showing its high cytotoxicity. Weimin*et al.*[10] reported that the hexane fractions of *S. anacardium*lehyam showed high cytotoxic activity and caspase activity in breast cancer cell line. They isolated the active compounds (7:Z, 10:Z)-3-pentadeca-7,10-dienyl-benzene-1,2,-diol and (8:Z)-3-pentadec-10-enyl-benzene-1,2-diol found to be in large concentration in the hexane fraction and responsible for the anticancer property. Similarly, Raveendran*et al.* [18]isolated an anticancer catechol i.e., 3-[8'(Z),11'(Z)-pentadecadienyl]catechol, from *S.anacardium*, which actively inhibited tumor cells and the cytotoxicity was due to induction of apoptotic pathway.

Caspases level was found to increase in a dose dependent level in the B16F10 cell line. DNA fragmentation assay of B16F10 cell line treated with the hexane fraction and crude extract showed DNA fragmentations which confirmed the cell are killed by apoptotic pathway. Similar study reported by Soumya*et al*. [19] in the anticancer potential in DMBA induced skin cancer of a synthetic coumarinwas due to induction of caspase mediated apoptosis.

In conclusion, the *S.anacardium*nut methanolic extract has significant application against B16F10 melanoma cell lines. The anticancer property is due to its rich antioxidant contents and free radical scavenging activity. Yet further investigation is necessary for identifying its mode of action for the cytotoxic effect.

References

[1] Fuchs E, Ragavan S. Getting under the skin of epidermal morphogenesis. Nat. Rev. Genet2002; 3: 199-209.
[2] Armstrong BK, Kricker A. The epidemiology of UV induced Skin cancer. J PhotochemPhotobiol B2001; 63: 8-18.
[3] Leboritz RL, Zhang H, Vogel H, CatwrightJ, Dionne L, Lu N, et al.Neurodegeneration myocardial injury and perinatal death in mitochondrial superoxide dismutase- deficient mice. Proc. Natl. Acad. Sci. USA 1996; 93: 9782-87.
[4] Cheesman KH, Slater TF. An introduction to free radical biochemistry.British Med. Bull 1993; 49: 481-93
[5]Chopra RN, Chopra IC, Handa KL, Kapur LD. Indigenous drugs of India, Calcutta: UN Dhur and Sons; 1958.p.408.
[6]Sharma PV,Chatuvedi C.*Clinical observation*on the*effect*of *Semicarpusanacardium*Linn. inantylostomiasis.Antiseptic 1965; 62: 845-48.
[7] Hembree JA, Chang CJ, McLaughlin JL, Peck G, Cassady JM. The anticancer activity of *Semecarpusanacardium*L. 9 KB ativepentadecylaccatechols.Lloydia 1978; 41: 491.
[8] Smit HF, Woerdenbag HJ, Singh RH, Meulenbeld J, Labadie RP,Zwavin JH. Ayurvedic herbal drugs with possible cytostatic activity.J. Ethanopharmacol 1995; 47: 75-84.
[9] Sowmyalakshmi S, Nur EAM, Akbarsha MA, Thirugnanam S, Rohr J,Chendil D. Investigation on *Semecarpus* Lehyam – a Siddha medicine for breast cancer. Planta 2005; 220: 910-18.
[10] Weimin Z, Zhu L, Srinivasan S, Damodaran C,RohrJ. Identification of urushiols as the major active principle of the Siddha herbal medicine *Semecarpus*Lehyam: Anti-tumor agents for the treatment of breast cancer.Pharm Biol 2009; 47: 886–93.

[11] Raj KS,Dhall A. Antiomicrobial and free radical scavenging ativity of extracts of some Indian medicinal plants. J. of Med. Plants Res 2010; 4: 2313-20.
[12] Kang BK, Chon SK, Kim SH, Jeong SY, Kim MS, Cho SH, et al.Controlled release of paclitaxel from microemulsion containing PLGA and evaluation of anti-tumor activity *in vitro* and *in vivo*. Int. J. of Pharmaceutics2004; 286: 147-56.
[13] Das RK, HossainSkU, BhattacharyaS. Diphenylmethylselenocynate inhibits DMBA-croton oil induced two stae skin carcinogenesis by inducing apoptosis and inhibiting cutaneous cell proliferation. Cancer Lett 2005; 230: 90-101.
[14] BurtonK. A study of conditions and mechanisms of diphenylamine reaction for the estimation of deoxyribonucleic acid.Biochemical J 1956; 62: 315-23.
[15] Perandones CE, Illera VA, Peckham D, Stunz LL, Ashman RF. Regulation of apoptosis *in vitro* in mature murine spleen T-cells. J. Immunol1993; 151: 3521-29.
[16] Wattenberg LW. Chemoprevention of cancer.Cancer Res 1985; 45: 1-8.
[17] Borek C. Antioxidant health effects of aged garlic extract. J Nutr2001; 131: 1010-15.
[18] Raveendran PKN, Melnick SJ, Wnuk SF, Rapp M, Escalon E, RamahandranC. Isolation and characterization of an anticancer catechol compound from *Semecarpusanacardium.* Jof Ethanopharmacol 2009; 122: 450-56.
[19] Soumya SB, Paul S, Mandal SK, Banerjee A, Boujedaini N, Khuda-Bukhsh AR. A synthetic coumarin (4-Methyl-7 hydroxycoumarin) has anti-cancer potentials against DMBA-induced skin cancer in mice. European J. of Pharmacol 2009; 614: 128-36.

Table 2. Activity of caspase 3 and caspase9 in B16F10 melanoma cell lines after treatment.Values are mean ± SD (n=3).

Samples (µg/ml)	Caspase – 3 (pg/ µg)	Caspase – 9 (pg/µg)
Control	56 ± 4.2	65 ± 3.4
Hexane		
10	68 ± 4.7	78 ± 4.6
100	127 ± 9.2	135 ± 4.9
500	145 ± 11.5	154 ± 5.5
Crude		
100	72 ± 3.1	82 ± 1.9
500	99 ± 8.8	111 ± 7.1

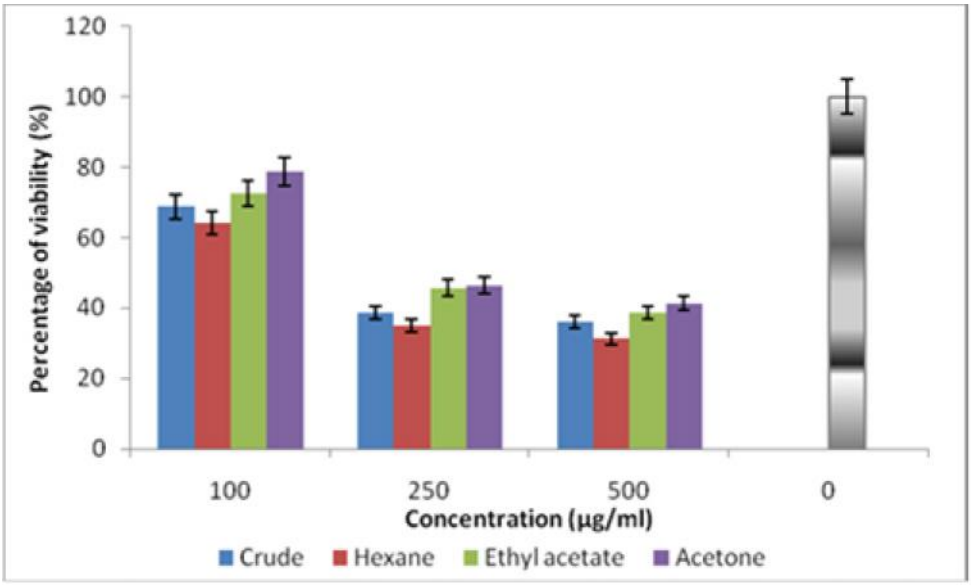


Figure 2. Percentage of viable B16F10 melanoma cells treated with *S.anacardium*nut crude methanolic extract and its fractions. Error bars indicate standarddeviation.

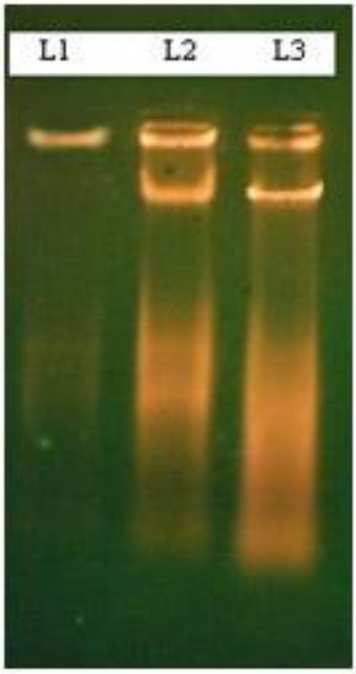


Figure 3.DNA fragmentation assay.
L1 –B16F10 melanoma cell DNA without treatment; L2 –B16F10 melanoma cell DNA treated with 100µg/ml of crude extract; L3 –B16F10 melanoma cell DNA treated with 100µg/ml of n-hexane fraction.

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Herbo-mineral salts from Marine halophytes against human bacterial pathogens

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ABSTRACT

Marine halophytes are also proven to have rich source of structurally diverse bioactive compounds with valuable pharmaceutical potential. In the present study antibacterial activity was carried out with herbo mineral salts of marine halophyte (*Sesuvium portulacastrum, Salicornia brachiata, Suaeda maritima* and *Suaeda monoica*) against antibiotic resistant (ABR) bacterial pathogens, Urinary tract infectious pathogens and human eye pathogens. Antibiotic sensitivity test was performed by agar well diffusion method. Of the selected salt extracts, the *Sesuvium portulacastrum* has potential antibacterial property. The results of the present study will open a new way for the development of alternative therapeutic sources for several infectious diseases.

KEY WORDS: Marine Halophytes; Infectious Pathogens; Salts;

1. INTRODUCTION:

Herbalism is the study and use of medicinal properties of plants and plant extracts. Many of the herbs and spices used by humans as medicinal compounds (Lai and Roy, 2004). The use of herbs to treat disease is almost universal among non-industrialized societies (Edgar *et al*., 2002; Ragunathan and Kitto, 2009; Saiman and Siegel, 2004)). In India, Ayurveda medicine has used many herbs such as turmeric possibly as early a In Tamil Nadu, Tamils have their own medicinal system now popularly called the Siddha medicinal system. The Siddha system is entirely in Tamil language. It contains roughly 300,000 verses covering diverse aspects of medicine such as anatomy, sex ("Kokokam" is the sexual treatise of par excellence), herbal, mineral and metallic compositions to cure many diseases that are relevant even to-day 1900 BC (Agarwal *et al*., 2007). Many infectious diseases are known to be treated with herbal remedies through the history of mankind. Marine halophytes are also proven to have rich source of structurally diverse bioactive compounds with valuable pharmaceutical potential. Scientific reports suggested that, the herbo-minerals extracts from terrestrial plants proved to have antibacterial, anti viral, anti fungal, antioxidant and immune modulator properties (Agarwal *et al*., 2007). But the studies related to salt extraction from the marine halophytes are still lacking. Though mangrove plants are classified as salt extruders and salt intruders and hence the intruders are having a special adaptation of accumulating several therapeutically important salts at an optimum level are still unexplored. In view of this the present study was carried out to perform the salt extraction and mineral composition analysis with mangrove associates.

2. Materials and Methods:

2.1. Bacterial pathogens

Four antibiotic resistant (ABR) human pathogens *viz*., *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumoniae* and *Klebsiella pneumoniae* were obtained from the

culture collections of Institute for Microbial Technology (IMTECH), Chandigarh, India. All the bacterial strains were suspended in nutrient broth and incubated at 37ºC for 48 hrs. Five urinary tract infectious pathogens *viz.*, *Pseudomonas aeruginosa*, *Enterobacter* sp. *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus* sp., were obtained from Jeyasekaran hospital, Nagercoil, Tamil Nadu, India. Five eye pathogens namely *viz*., *Acinitobacter* sp., *Proteus* sp., *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pyogenes* were obtained from the Aravind Eye Hospital, Madurai, Tamil Nadu, India

2.2. Extraction of salts from halophytes

The green, succulent leaves of the four halophytes were collected by hand picking. Then the cleaned specimens were dried in a hot air oven at 100°C. When they were completely dried, they were burnt into ash by using muffle furnace (450°C). The ash was collected in a large mouthed big vessel. The salt was extracted from that ash by dissolving it in deionized water. Then the supernatant was heated and dried in hot air oven (150°C) to produce flaks.

2.3. Antimicrobial sensitivity assay:

2.3.1. Well diffusion method (Pandey *et al*., 2004)

Antibiotic sensitivity test was performed by agar well diffusion method, which is designed to determine the smallest amount of the bioactive secondary metabolites needs to inhibit the growth of the microorganism. The medium of choice is Mueller-Hinton agar with a pH of 7.2 to 7.4, which is poured into plates to a uniform depth to 5 mm and allowed to solidify. Prior to use, the plates were transferred to an incubator at 37ºC for 10-20 minutes to dry the moisture that develops on the surface. Overnight growth of chosen bacterial broth culture was swabbed on the surface of the agar media and further four wells were prepared by using sterile well cutter. To each well, 70 µg, 80 µg, 90 µg and 100 µg mineral flake was added and incubated in a thermostat incubator at 37ºC for 24 hours. After incubation, the zone of inhibition around the well was calculated and expressed as zone of inhibition in millimeter in diameter.

3. Results

The antibacterial activity of the salts extracted from four salt marsh marine halophytes *viz.*, *Sesuvium portulacastrum*, *Salicornia brachiata*, *Suaeda maritima* and *Suaeda monoica* against antibiotic resistant (ABR) bacterial pathogens were carried out by the present study and represented in Table 1. It revealed that, the salt extracted from the *S. portulacastrum* showed the maximum sensitivity (10.0 mm dia. and 11.0 mm dia.) against *Staphylococcus aureus* and *Streptococcus pneumoniae* respectively at 100µg concentration. Likewise, the salt extracted from *S. monoica* showed the maximum sensitivity (10.0 mm dia. and 11.0 mm dia.) against *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* respectively at 100µg concentration. Whereas, the other two salts from *S. brachiata* and *S. maritima* did not show any activity against any of the antibiotic resistant human bacterial pathogens. The antibacterial activity of the salts against urinary tract infectious (UTI) pathogens were carried out by the present study and represented in Table 2. It reveals that, the salt extracted from the *S. portulacastrum* showed the maximum sensitivity (12.0 mm dia.) against *Pseudomonas* sp., *Enterobacter* sp., and *Klebsiella* sp. at 100 µg concentration. The salt from *S. monoica* showed the maximum sensitivity (10.0 mm dia. and 11.0 mm dia.) against *Pseudomonas* sp. and *Escherichia coli* at 100 µg concentration. The salt from *S. brachiata* showed maximum sensitivity (9.0 mm dia.) against the *Klebsiella* sp. at 100µg concentration and the salt from *S. maritima* did not show any activity against UTI bacterial pathogensThe antibacterial activity of the salts extracted from salt marsh marine halophytes against human ophthalmic pathogens were carried out by the present study and represented

in Table 3. It reveals that, the salt extracted from *S. portulacastrum* showed maximum sensitivity (10.0 mm dia. and 13.0 mm dia.) against *Streptococcus pyogens* and *Escherichia coli* respectively at 100 µg concentration. The salt extracted from *S. monoica* showed maximum sensitivity (7.0 mm dia. and 9.0 mm dia.) against *Acinitobacter* sp., *Proteus* sp. and *Streptococcus pyogens* respectively at 100 µg concentration. The salts extracted from *S. brachiata* and *S. maritima* did not show any activity against ophthalmic pathogens.

4. Discussion

It was observed that, the salt extracted from the *S. portulacastrum* showed maximum sensitivity against the tested microbial pathogens. Whereas, the other two salts from *S. brachiata* and *S. maritima* did not show any activity against any of the bacterial pathogens. It was already reported that, if a whole plant is required to be given at a dose of 10gm for a particular disease, the same plant's uppu can have the same therapeutic value in a dose of 50 to 100 mg (Sambasivapillai dictionary P.1170. Vol.I).The present study found that, for effective killing of the bacterial pathogens from different sources required 100µg of salt. Comparatively, the salt extracted from other terrestrial plants required more concentration to kill the bacterial pathogens whereas the salts extracted from marine halophytic plants required very less (100 fold less) concentration to kill the bacterial pathogens. Earlier findings reported that, the salt extracted from erukku *Calotropis gigantia* at a dose of 10-20 mg has been used for the treatment of spasmodic pains, bronchial asthma, toxic bites, the salt extracted from kuppaimeni *Acalypha indica* at a concentration of 50-100mg has been used for the treatment of vatha and kabha diseases, the salt extracted from thuti *Abutilon indicum* at a dose of 50-100 mg has been used for the treatment of piles and ulcers, the salt extracted from vaazhi *Musa paradisiaca* at a concentration of 50-100 mg has been used for the treatment of diuretic, burning micturition, kidney stones, the salt extracted from murungai *Moringa tinctoria* at a dose of 50-100 mg has been used for the treatment of diuretic, breaks kidney and bladder stones, strengthens teeth, the salt extracted from naauruvi *Achyranthes aspera* at a dose of 50-100 mg has been used for the treatment of diuretic, the mineral extracted from thennai oolai leaves of *Cocos nucifera* at a dose of 50-100 mg has been used for the treatment of diuretic, the salt extracted from arugu *Cynodon dactylon* at a dose of 50-100 mg has been used for the treatment of skin diseases, the salt extracted from pirandai *Cissus quadrangularis* at a dose of 50-100 mg has been used for the treatment of indigestion, piles and diarrhea, the salt extracted from ellu *Sesamum indicum* at a dose of 50-100 mg has been used for the treatment of diarrhea and pitha diseases, the salt extracted from poovarasu *Thespesia populnea* at a dose of 50-100 mg has been used for the treatment of intestinal worms, the neer mulli *Hydrophila auriculata* at a dose of 50-100 mg has been used for the treatment of anemia, drowsy, ascites and kidney stones, the salt extracted from panai aan poo *Borassus flabellifer* (male flowers) at a dose of 50-100 mg has been used for the treatment of diuretic, liver and spleen disorders, the salt extracted from pugaiilai *Nicotiana tabacum* at a dose of 50-100 mg has been used for the treatment of ascites (Venkatappan and Malliga, 2004). It is concluded from the present study that, among the salts extracted from salt marsh marine herbals, the *Sesuvium portulacastrum* has potential antibacterial property. This will open a new way for the development of alternative therapeutic drug for the treatment of several infectious and non- infectious diseases.

5. References:

Aggarwal, B. B., C. Sundaram, N. Malani and H. Ichikawa, 2007. Curcumin: the Indian solid gold. *Advanced Experimental Medical Biology*, **595**: 1–75.

Edgar J. DaSilva, Elias Baydoun and Adnan Badran, 2002. Biotechnology and the developing world". *Electronic Journal of Biotechnology*, **5** (1):12-18.

Lai, P.K. and J. Roy, 2004. Antimicrobial and chemopreventive properties of herbs and spices. *Curent Medical Chemistry*, **11** (11): 1451–60.

Pandey, B. P., P. Ghimire and V. P. Agarwal, 2004. Studies on the antibacterial activity of actinomycetes isolated from the Khumbu region of Mt. Everest. A paper presented in the international conference on great Himalayas: Climate, Health, Ecology, Management and conservation, kathmandu, January 12-15, 2004. Organized by Kathmandu University and the Aquatic Ecosystem health and Management Society, Canada.

Raghunathan and Kitto, 2009. Drugs from the Indian Seas More expectations. *Current Science*, **97 (12**): 1705-1706.

Ravi Kumar, S., G. Ramanathan, M. Subhakaran and S. Jacob Inbaneson,2009. Antimicrobial compounds from marine halophytes for silkworm disease treatment. *International Journal of Medical Science*, **1(5):** 184–191.

Saiman, L. and J. Siegel, 2004. Infection control in Cystic fibrosis. Clinical microbiology Reviews, **17:** 57-71.

Sambasivam Pillai, T.V., 1938. A Tamil - English Dictionary of Medicine, Chemistry, Botany and Allied Sciences. Published by Department of Indian Medicine and Homeopathy, Chennai, Vol.I: p. 1170.

Venkatappan, **N. and K. Malliga, 2004.** Uppu: a novel medicinal plant extract preparation in siddha medicine. In: Rajabudeen.E. (Ed.) Proceedings of State level seminar on medicinal plants in India-Prospects and Perspectives, December,15-16,2004, Department of Biology, Dr. Zakir Husain College, Illayangudi -630 702, Tamilnadu, India, pp: 24-27.

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Table 1. Antibacterial activity of the salts extracted from halophytes against antibiotic resistant (ABR) human pathogens

Name of the Pathogen	Zone of Inhibition (mm in diameter)															
	S. portulacastrum (g.ml^{-1})				*S. brachiata* (g.ml^{-1})				*S. maritima*(g.ml^{-1})				*S. monoica* (g.ml^{-1})			
	70	**80**	**90**	**100**	**70**	**80**	**90**	**100**	**70**	**80**	**90**	**100**	**70**	**80**	**90**	**100**
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-	-	9 ± 2.25	10 ± 1.73	10 ± 0.69
Staphylococcus aureus	-	-	10 ± 0.89	10 ± 0.73	-	-	-	-	-	-	-	-	-	-	-	-
Streptococcus pneumoniae	10 ± 1.56	10.5 ± 1.97	11 ± 1.78	11 ± 1.86	-	-	-	-	-	-	-	-	9 ± 1.86	10 ± 0.98	10 ± 1.22	11 ± 1.75
Klebsiella pneumoniae	7 ± 1.48	7 ± 0.73	7 ± 0.83	7 ± 0.73	-	-	-	-	-	-	-	-	-	-	-	-

(-) indicates no activity

Table 2. Antibacterial activity of the salts extracted from halophytes against urinary tract infectious (UTI) pathogens

(-) indicates no activity

Name of the Pathogen	Zone of Inhibition (mm in diameter)															
	S. portulacastrum (g.ml^{-1})				*S. brachiata* (g.ml^{-1})				*S. maritima*(g.ml^{-1})				*S. monoica* (g.ml^{-1})			
	70	**80**	**90**	**100**	**70**	**80**	**90**	**100**	**70**	**80**	**90**	**100**	**70**	**80**	**90**	**100**
Pseudomonas sp.	-	10 ± 1.36	10 ± 1.78	12 ± 1.81	-	-	-	-	-	-	-	-	8 ± 0.45	8 ± 0.69	9 ± 1.86	10 ± 1.9
Enterobacter sp.	9 ± 1.73	12 ± 1.96	12 ± 1.63	12 ± 1.98	-	-	-	-	-	-	-	-	-	-	-	-
E.coli	-	-	-	-	-	-	-	-	-	-	-	-	6 ± 0.65	8 ± 0.96	10 ± 1.36	11 ± 1.52
Klebsiella sp.	10 ± 1.13	10 ± 0.93	12 ± 1.97	12 ± 1.86	-	-	6 ± 0.53	9 ± 1.09	-	-	-	-	-	-	-	-
Proteus sp.	-	7 ± 1.62	7 ± 1.48	7 ± 0.69	-	-	-	-	-	-	-	-	-	-	-	-

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Table 3. Antibacterial activity of the salts extracted from halophytes against ophthalmic pathogens

Name of the Pathogen	Zone of Inhibition (mm in diameter)															
	S. portulacastrum (g.ml^{-1})				*S. brachiata* (g.ml^{-1})				*S. maritima* (g.ml^{-1})				*S. monoica* (g.ml^{-1})			
	70	**80**	**90**	**100**	**70**	**80**	**90**	**100**	**70**	**80**	**90**	**100**	**70**	**80**	**90**	**100**
Acinitobacter sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7 ± 0.63
Proteus sp.	-	-	-	-	-	-	-	-	-	-	-	-	6 ± 1.01	6 ± 0.93	6 ± 0.71	7 ± 0.93
Staphylococcus aureus	-	6 ± 1.2	7 ± 0.76	7 ± 1.06	-	-	-	-	-	-	-	-	-	-	-	-
E.coli	10 ± 1.68	10 ± 2.36	12 ± 1.96	13 ± 1.99	-	-	-	-	-	-	-	-	-	-	-	-
Streptococcus pyogenes	7 ± 0.65	8 ± 1.53	8 ± 1.1	10 ± 1.78	-	-	-	-	-	-	-	-	6 ± 0.36	6 ± 0.98	8 ± 1.5	9 ± 1.79

(-) indicates no activity

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Biosynthesis and characterization of Alkaline protease from *Bacillus subtilis*

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Abstract:

In the present study, alkaline protease producing *Bacillus subtilis* was isolated and identified by various biochemical tests and by 16S rDNA sequencing. Agricultural waste was used as a solid substrate for enzyme production in solid state culture. The process parameters such as fermentation period (72 h), pH (9.0), moisture content (120%), inoculum (12%), carbon source (maltose), nitrogen source (casein) and in-organic ion (sodium di-hydrogen phosphate) were optimized for maximum enzyme production. The crude enzyme was concentrated by ammonium sulphate precipitation and further purified by ion exchange and gel filtration chromatography. This enzyme was highly stable and active at 50 °C and at pH 8.0, respectively. The molecular weight of protease was estimated to be 42.5 KD by SDS-PAGE.

Introduction:

Microbial proteases are among the most important hydrolytic enzymes and have been studied extensively since the advent of enzymology. Microorganisms elaborate a large array of proteases, which are intracellular and/or extracellular. Intracellular proteases are important for various cellular and metabolic processes, such as sporulation and differentiation, protein turnover, maturation of enzymes and hormones and maintenance of the cellular protein pool. Extracellular proteases are important for the hydrolysis of proteins in cell-free environments and enable the cell to absorb and utilize hydrolytic products (Kalisz, 1988). At the same time, these extracellular proteases have also been commercially exploited to assist protein degradation in various industrial processes (*Kumar* and *Takagi* 1999; *Outtrup* and *Boyce*, 1990).
Among bacteria, there are number of genera which produce proteases but *Bacillus* remains the organism of choice (*Nomoto et al.,* 1984; *Kumar and Takagi*, 1999; *Mehrotra et al.,* 1999). And, the proteases produced by *Bacillus* are mostly alkaline in nature and are highly stable against extreme temperature, pH and other conditions (Sharma *et al.,* 1980; Yang *et al.,* 2000). Proteases account for 40% of the total enzyme sales worldwide and this trend is expected to increase in the near future. This has led to increasing attention towards the exploitation of potent microbial strains for the production of alkaline proteases from an industrial point of view (*Ellaiah et al*., 2002). Alkaline proteases are a physiologically and commercially important group of enzymes used primarily as detergent additives. They play a specific catalytic role in the hydrolysis of proteins. In 1994, the total market for industrial enzymes accounted for approximately $400 million, of which enzymes worth $112 million were used for detergent purposes (*Hodgson*, 1994). In Japan, 1994 alkaline protease sales were estimated at 15000 million yen (equivalent to $116 million) (*Horikoshi*, 1996). There is expected to be an upward trend in the use of alkaline proteases so that by the turn of the decade the total value for industrial enzymes is likely to reach $700 million or more (*Hodgson*, 1994).

It is essential that these organisms be provided with optimal growth conditions to increase enzyme production. The culture conditions that promote protease production were found to be significantly different from the culture conditions promoting cell growth (*Moon* and *Parulekar*, 1991). In the industrial production of alkaline proteases, technical media were usually employed that contained very high concentrations (100–150 g dry weight/litre) of complex carbohydrates, proteins, and other media components (*Aunstrup*, 1980). With a view to develop an economically feasible technology, research

efforts are mainly focused on: (i) improvement in the yields of alkaline proteases; and (ii) optimization of the fermentation medium and production conditions.

As part of the search for a cheap alternative production system which utilizes simpler agro industrial wastes as substrates, solid state fermentation (SSF) has gained importance in the production of microbial enzymes. Production of biocatalyst using agro biotech substrates under SSF conditions provides several advantages in productivity, cost effectiveness in labour, time and medium components, use of simple machinery, use of an inexpensive substrate, simple downstream processing, in addition to environmental advantages like less effluent production, waste minimization etc (Lonsane *et al*., 1985; Pandey *et al*., 2000).

Materials and Methods:

Screening of protease producing strain: The soil samples were collected near to Nagercoil, Kanyakumari District, Tamilnadu for the screening of protease producing organism. The organisms were grown in the minimal medium containing (g l^{-1}): KH_2PO_4, 10; $NaHPO_4.12H_2O$, 3.0; $MgSO_4$, 0.2; peptone, 10; trisodium citrate, 3.0 and skim milk, 10, agar, 15. The media pH was adjusted to 7.0 with 1N HCl/NaOH for initial experiments. The sample was serially diluted and streaked on skimmed milk agar plates. The plates were incubated for 48 h at 37 °C and enzyme activity was observed. After incubation the colonies formed were counted and respective colonies which differ in morphology and colour were subcultured. Positive colonies those degraded skim milk were studied for further protease production. The organism was identified based on morphology and biochemical characteristics (Holt *et al.*, 1994).

Protease production in Submerged Fermentation

The organism was grown in a nutrient broth medium (g l^{-1}) [peptic digest of animal tissue-5, beef extract-1.5, yeast extract-1.5 and sodium chloride-5]. It was incubated at 37 °C in an orbital shaker at 150 rpm for 48 h. Five ml of culture medium was withdrawn for every 24 h of incubation and centrifuged at 10,000 rpm for 10 min. The supernatant was assayed for protease activity. Briefly, one ml of crude enzyme solution was added to one ml of casein substrate (1%, w/v), prepared in 50 mM Tris buffer (pH 8.0) and incubated at 37 °C for 30 min. The reaction was terminated by adding 100 mM trichloroacetic acid and the content was filtered through Whatman No. 1 filter paper. The filtrate absorbance was read at 280 nm using UV–visible spectrophotometer (Ultraviolet visible spectrophotometer) and the protease activity was calculated using tyrosine standard curve. One unit of protease activity was defined as 1 µmol of tyrosine liberated ml^{-1} under the assay condition. The total protein content of the sample was determined as described by Lowry *et al*. (1951).

Solid state fermentation (SSF)

A mass of 2.5 gram of wheat bran and agricultural waste (dried vegetable wastes) were taken in 100 ml Erlenmeyer flask. To maintain the moisture level, 50 mM of Tris buffer (pH 8.0) was added. The content of the flasks were mixed thoroughly and autoclaved at 121 °C for 20 min. After sterilization, 0.25 ml of seed culture was inoculated on the substrates aseptically and incubated for 48 h at 37 °C. The substrate that supported to produce higher amount of proteolytic enzyme was used for further studies.

Optimization of culture conditions for the production of proteases

Various process parameters affecting enzyme production during SSF were optimized. The strategy was to optimize each parameter independently of the others and subsequently optimal conditions were employed in all experiments. In a sequential order, the various process parameters were optimized for maximal enzyme production by using the agro-waste medium. The various process parameters tested were incubation period (24, 48, 72 and 96 h, respectively), pH (5.0, 6.0, 7.0, 8.0, 9.0 and 10.0, respectively), moisture (80%, 100%, 120%, 140%, and 160%,) content, inoculum concentration (3%, 6%, 9%, 12%, 15% and 18% respectively), carbon sources (maltose, sucrose, starch, glucose, trehalose and xylose), nitrogen sources (peptone, beef extract, yeast extract, casein, gelatin and urea) and inorganic salts (ammonium sulphate, ferrous sulphate, ammonium chloride, di-sodium hydrogen phosphate, sodium di-hydrogen phosphate, sodium nitrate and calcium chloride).

Purification of alkaline protease from *Bacillus subtilis*

The crude extract was subjected to ammonium sulphate fractionation. The pellet recovered by centrifugation at 10,000 rpm for 10 min was resuspended in sodium phosphate buffer (pH 7.4, 100 mM). It was dialysed for overnight against double distilled water and sodium phosphate buffer. It was stored at 2 to 8 °C for further studies. Then it was purified by a two step purification by DEAE cellulose column chromatography followed by gel filtration chromatography.

SDS- PAGE and Molecular weight determination

Twenty micro liters of the active fractions were loaded on 12% SDS - PAGE. The molecular weight of the alkaline protease was estimated by SDS - Polyacrylamide gel electrophoresis with phosphorylase b (97,400 Dalton), Bovine Serum Albumin (66,000 Dalton), Ovalbumin (43,000 Dalton), Carbonic Anhydrase (29,000 Dalton), Soyabean Trypsin Inhibitor (20,100) and Lysozyme (14,300) molecular markers.

Zymography analysis

Activity gel was prepared according to the method of Schmidt *et al.* (1988) with some modifications. A 12% polyacrylamide gel was co-polymerized with 1% casein. The samples were applied in non-reducing condition without denaturation and run at 50 V. The gel was rinsed twice in 2.5% Triton X-100 for 30 min, to remove SDS, and was incubated in 50 mM sodium phosphate buffer (pH 7.4) for 1 h at 30 °C. The gel was stained in 0.5% Coomassie brilliant blue R-250 for 30 min followed by destaining in a solution containing methanol, acetic acid, water in ratio 40:7:53.

Results and Discussion:

The protease secreting *Bacillus subtilis* was isolated from the soil sample using skimmed milk agar plates. Of the tested isolates, few were found to have the ability to produce proteases. Among the positive isolates, *Bacillus subtilis* which produced a larger halo zone in response to the colony diameter was selected (Fig. 1).

Fig 1: The clear zone indicated the result of protease production by *Bacillus subtilis*

The selected isolate was identified as *Bacillus* sp. on the basis of various microscopic and biochemical investigations. The organism was a Gram-positive rod, spore-producing, MR, VP-, catalase- and gelatin-positive. It fermented glucose, lactose and sucrose. It reacted negatively in the indole, methyl red, citrate,

and starch and nitrate reduction test. It was negative to gas production. All these results suggest that it belongs to the genus *Bacillus.* Moreover, the organism was confirmed by its 16S rRNA gene sequence. The 779-bp sequence was submitted to GenBank (accession number: 688989).

GCAGTCGAGCGGACCAAAGAAGAGCTTGCTCTTCGGCGGTTAGCGGCGGACGGGTGAGTAAC
ACGTAGGCAACCTGCCTGTACGACTGGGATAACTCCGGGAAACCGGAGCTAATACCGGATAC
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GGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGG
AATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAACGATGAAGGTTTTCGG
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ACGAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTG
TCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGCTTCTTAAGTCAGGTGTGAAAGCCCAC
GGCTCAACCGTGGAGGGCCACTTGAAACTGGGAGGCTTGAGTGCAGGAGAGGAGAGCGGAA
TTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCCGTGGCGAAGGCGGCTCT
CTGGCCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGG
TAGTCCACGCCGTAAACGTTGAGTGCTAGGTGTTGGG

Of all the alkalophilic microorganisms that have been screened for use in various industrial applications, members of the genus *Bacillus* were found to be predominant and a prolific source of alkaline proteases.

Evaluation of agricultural waste as a cheap substrate for alkaline protease production:

Alkaline proteases are of considerable interest in view of their activity and stability at alkaline pH. In the present study, an alkaline protease was produced using *Bacillus subtilis* under SSF using wheat bran and vegetable wastes as solid substrate. The agricultural waste (vegetable wastes) supported more enzyme production than the wheat bran. Hence, vegetable waste was selected for further studies.

Effect of fermentation period and moisture on enzyme production

To evaluate the effect of fermentation period on protease production, the fermentation experiment was carried out for a period of 96 h. Results of this study showed that protease production increased with incubation time up to 72 h. Maximum alkaline protease production was achieved around 72 h of fermentation (6740 U/ml) at 37 °C (Fig. 2). The incubation time is governed by the characteristics of the culture and is also based on the growth rate and enzyme production. Similar findings have been reported with other *Bacillus* sp. (Ravindran *et al.,* 2011). The reduction in enzyme yield after the optimum period was probably due to the depletion of nutrients available to the microorganisms. The maximum enzyme production was observed with 120% moisture content (10321 U/ml). The enzyme production decreased thereafter and it was 7863 U/ml at 160% moisture (Fig. 3). This could be attributed to low microbial growth and anchoring on the surface of the solid medium at higher moisture content. Among the several factors that are important for microbial growth and enzyme production under SSF, moisture content is a critical factor (Pandey *et al.*, 2000; Nigam and Singh, 1994). Moisture is reported to cause swelling and thereby facilitating better utilization of the substrate by the organisms (Kim *et al.,* 1985) which in turn resulted in enhanced level of metabolic activity by the organism and increased level of proteins including secretory proteins.

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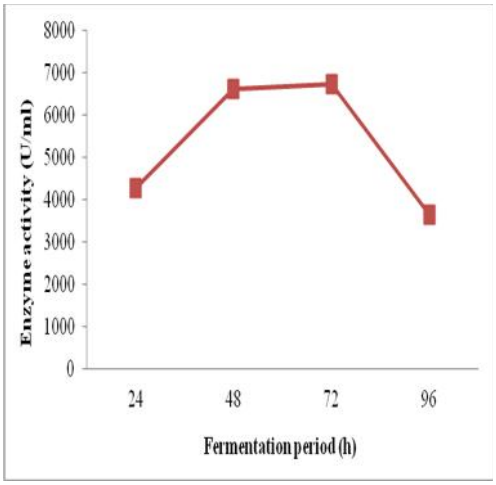

Fig. 2

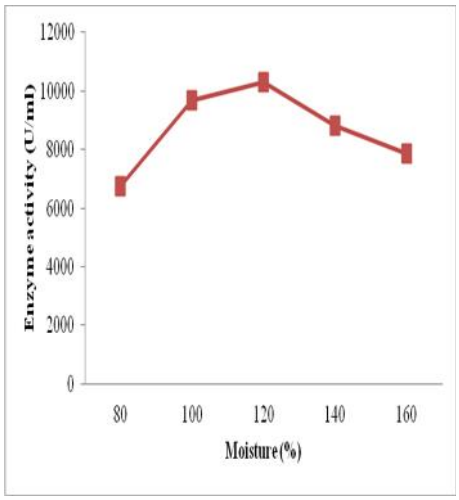

Fig. 3

Effect of pH and inoculum on protease production

The important characteristic of most alkalophilic microorganisms is their strong dependence on the extracellular pH for cell growth and enzyme production. For increased protease yields from these alkalophiles, the pH of the medium must be maintained above 7.5 throughout the fermentation period (*Aunstrup*, 1980). It was found that the production rate of proteases enzyme varies according the change in pH and was found to be maximum at pH 9. (Fig. 4). This result was in accordance with the observations made with other alkaliphilic protease-secreting *Bacillus* sp. (*Uyar* and *Baysal*, 2004). Alkaline protease production by microbial strains strongly depends on extracellular pH because culture pH strongly influences many enzymatic processes and transport of various components across the cell membranes, which in turn support cell growth and product production (*Ellaiah et al*., 2002). There was a significant increase in alkaline protease production with an increase in inoculum size upto 12% (Fig. 5). Increase of the inoculum level after 12% adversely affected enzyme production. This result was in accordance with the results observed with other *Bacillus* sp. (*Rajkumar et al.*, 2011).

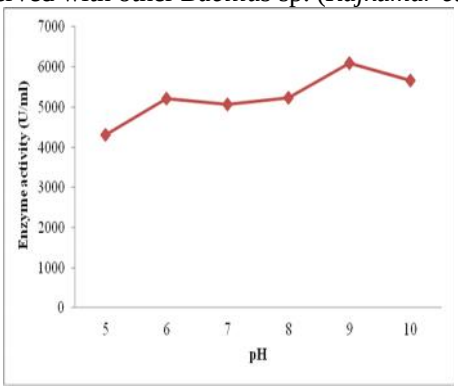

Fig 4

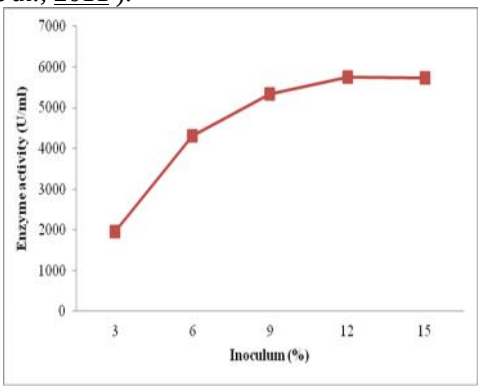

Fig 5

Effect of carbon and nitrogen sources on enzyme production

Protease production is an inherent property of all organisms and these enzymes are generally constitutive, although, at times, they are partially inducible (*Beg et al*., 2002; *Kaliaz*, 1988). Although complex nitrogen sources are usually used for alkaline protease production, the requirement for a specific nitrogen supplement differs from organism to organism (*Kumar* and *Takagi*, 1999). In SSF, addition of carbon sources increased enzyme production (Fig. 6). When different concentrations of maltose were added, 1.0% maltose supported the maximum production enzyme yield (6472 U/ml) (Fig. 7). These results are in

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accordance with those of another study in which different sugars were supplemented (*Ellaiah et al.*, 2002). In the present study, among the nitrogen sources, casein supported more enzyme production (Fig. 8). When different concentrations of casein were added, casein at 1.5% supported maximum protease production (5867 U/ml) (Fig. 9). Similar observations were noticed in the case of protease production by different microbial species (*Pandey et al*., 2000).

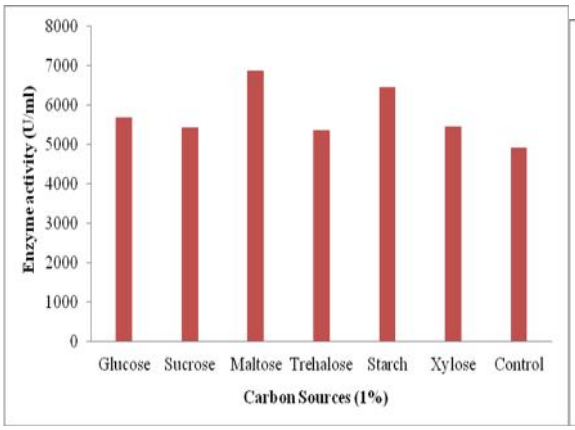

Fig 6

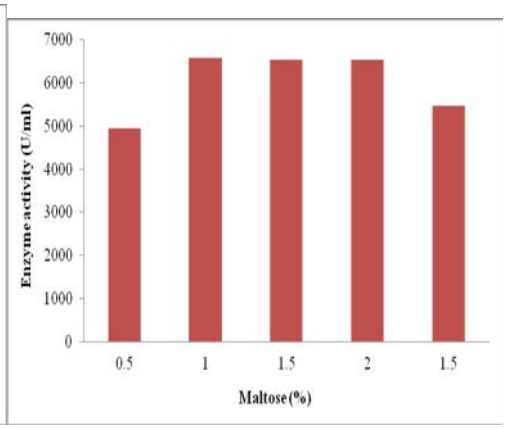

Fig 7

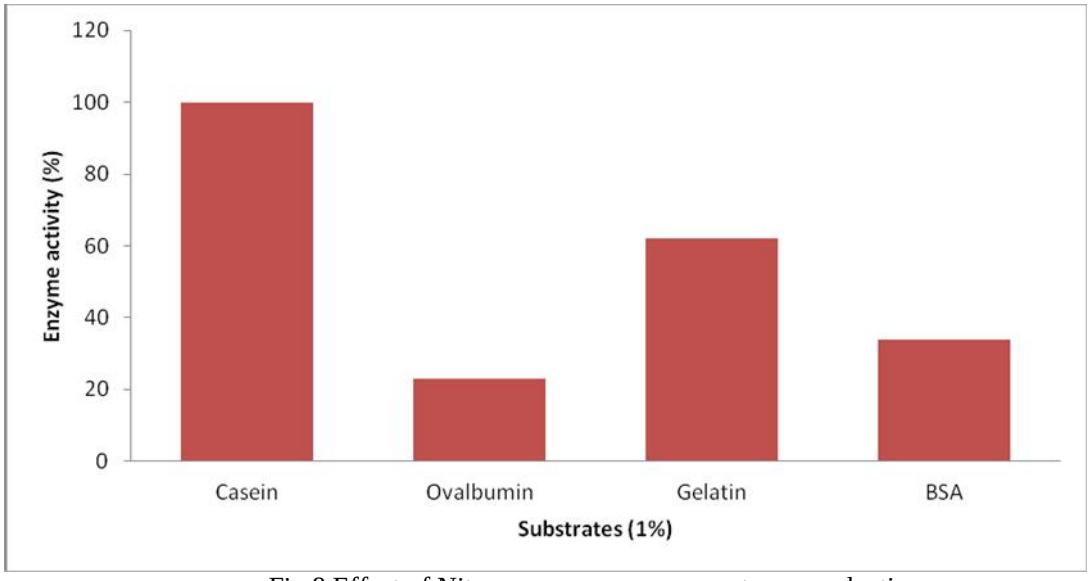

Fig 8 Effect of Nitrogenous sources on protease production

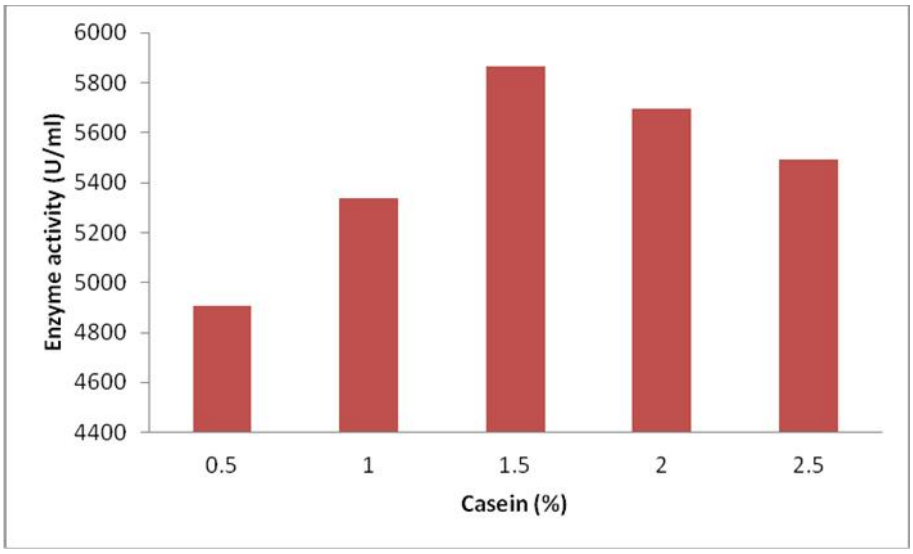

Fig 9 Effect of concentration of casein on protease production

Various inorganic salts were incorporated with the solid substrate to investigate their effect on protease production by *Bacillus subtilis*. Among the supplemented salts, ammonium chloride (1203 U/ml), ferrous sulphate (479 U/ml) and sodium nitrate (385 U/ml) suppressed protease production. Calcium chloride (2425 U/ml), disodium hydrogen phosphate (2412 U/ml) and sodium di-hydrogen phosphate (3072 U/ml) supported protease production (Fig. 10). Among the different concentrations of sodium di-hydrogen phosphate evaluated, 1% concentration supported more proteolytic enzyme secretion (Fig. 11)

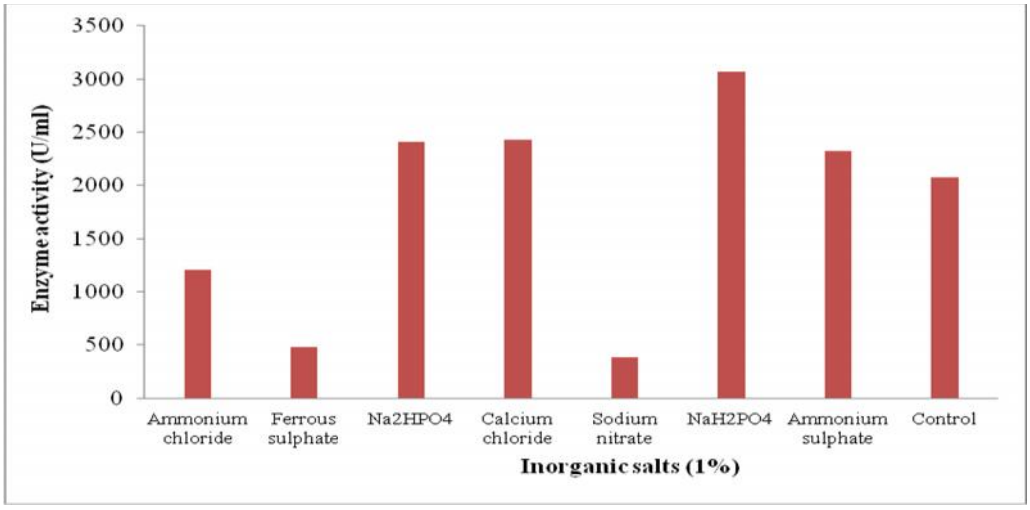

Fig 10: Effect of inorganic ions on enzyme production

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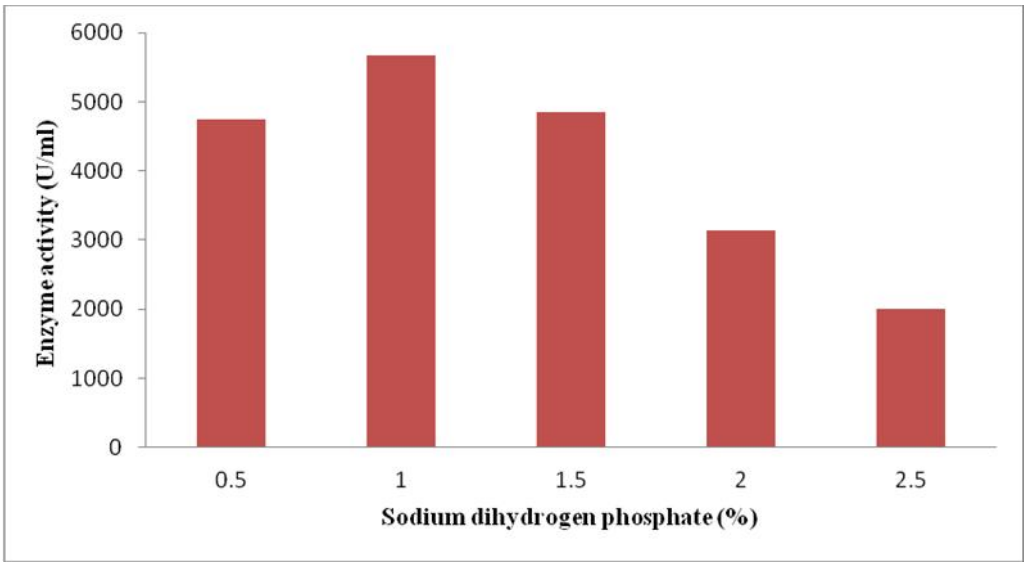

Fig 11 Effect of concentration of sodium dihydrogen phosphate on protease activity

Substrate specificity of the protease

Proteolytic activity of the enzyme was measured using 1.0% (w/v) casein, Bovine Serum Albumin (BSA), ovalbumin and gelatin (Fig. 12). This enzyme showed more activity towards casein substrate (100% activity).

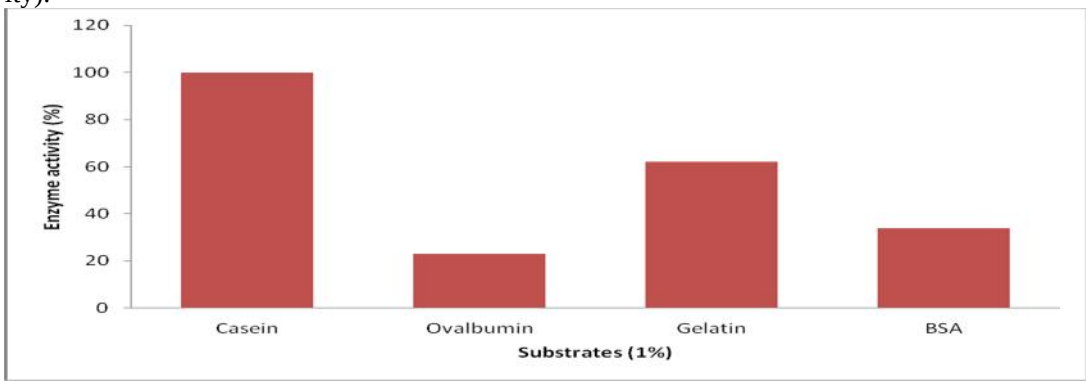

Fig 12: Effect of various substrate on protease activity

Purification of alkaline protease

In the present study, the protease was purified by the combination of ammonium sulphate precipitation, ion exchange and gel filtration column chromatogrpahy.

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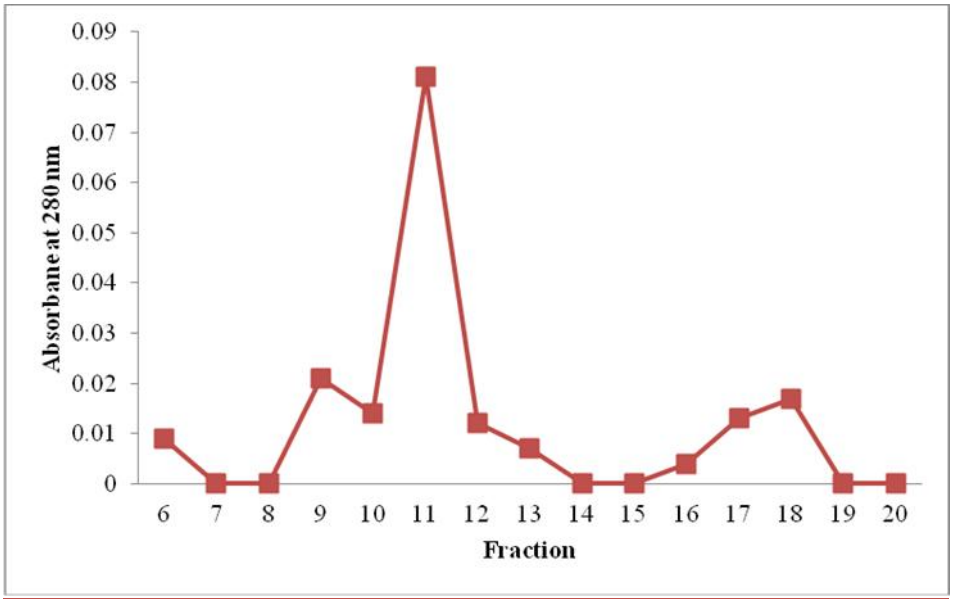
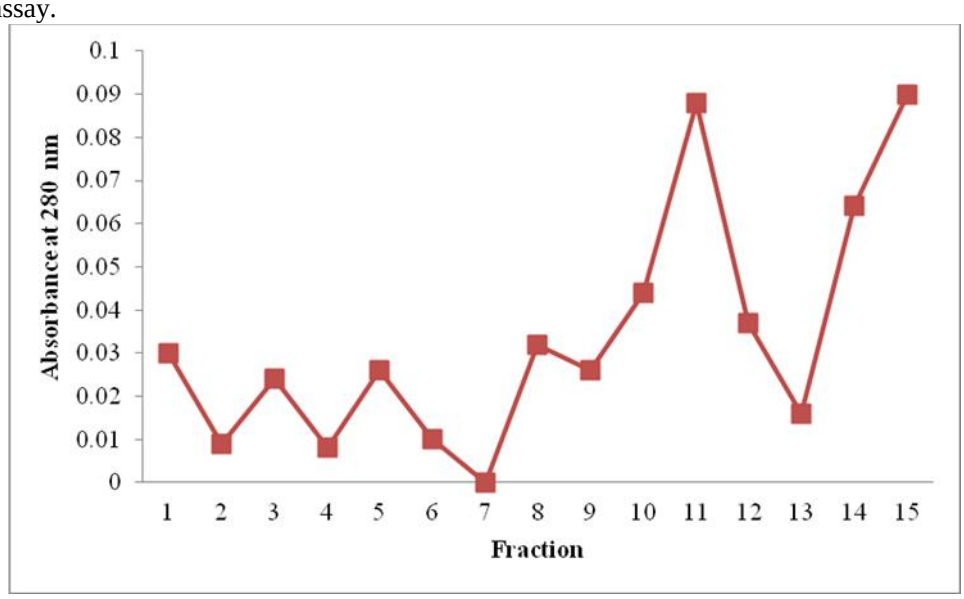


Elution profile of protease by Gel filtration column chromatography

Three milli litre of ammonium sulphate precipitated fraction was loaded on the column and was eluted with buffer containing 0.1 - 1.0 M NaCl. 15 fractions were collected and all fractions were subjected to protease assay.



Elution profile of protease by DEAE-cellulose column chromatography.
Among all fractions, fraction 11 showed more enzyme activity.

SDS-Polyacrylamide gel electrophoresis of the protease

Purified fraction was subjected to SDS-PAGE analysis yielded a single band, which confirms the homogeneity of enzyme. The molecular weight of protease was found to be approximately 42.5 kDa; this was also the case with the zymography analysis. These results are in accordance with literature reports

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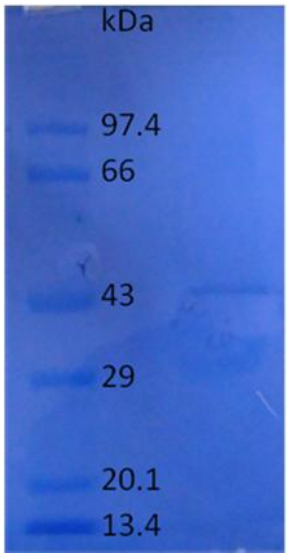
where molecular masses of most proteases derived from *Bacillus* sp. are less than 50 kDa (*Sousa et al*., 2007).



Conclusion:

In the present study, an alkaline protease was produced using *Bacillus subtilis* under SSF using wheat bran and vegetable wastes as solid substrate The agricultural waste (vegetable wastes) supported more enzyme production than the wheat bran. The enzyme produced was purified and its molecular weight determined.

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Modulatory effect of Polydatin on in vivo carbohydrate metabolism in streptozocin-induced type II diabetic rats.

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PG and Research Department of Biochemistry[1], Mohamed Sathak College of Arts and Science, University of Madras, Sholiganallur, Chennai

Abstract

The study was designed to investigate the antihyperglycemic effect of polydatin on streptozotocin-diabetic rats. Diabetes was induced in adult male albino rats of the wistar strain, weighing 170-210g, by administration of streptozotocin (40mg/kg of body weight) intraperitoneally. Diabetic rats showed increase in plasma glucose and glycosylated hemoglobin and a decrease in plasma insulin and hemoglobin. Activities of gluconeogenic enzymes such as glucose 6-phosphatase, fructose 1, 6-bisphosphatase incresed and glucokinase, glucose 6-phosphatase dehydrogenase decreased in the liver of diabetic rats along with liver glycogen. Oral administration of Polydatin (150mg/kg of body weight) or glibenclamide (600 mg/kg of body weight) in saline, for 45 days, prevented the above changes and improved towards normal. In against body weight loss of diabetic rats by polydatin was also observed. No significant effect was observed in normal rats treated with polydatin (150mg/kg of body weight). These results showed that polydatin has potantial antihyperglycemic activity at a dose of 150mg/kg of body weight in streptozotocin induced diabetic rats.
Keywords: streptozotocin, polydatin, antihyperglcemia, glibenclamide, insulin

Introduction

The Health Equity and Accountability Act of 2012 will improve diabetes research, treatment and education in minority populations, and address the unique circumstances faced by minority populations and women. This bill is critical to our nation's fight to Stop Diabetes. Statistical projection about the diabetes affects nearly 26 million people in the U.S. and another 79 million Americans have prediabetes, placing them at higher risk for developing type 2 diabetes. While 7.1 percent of non-Hispanic white adults have been diagnosed with the disease, the statistics are much higher for minority populations in the U.S (American diabetes association. 2012). Type 2 diabetes mellitus, also known as noninsulin dependent diabetes mellitus, develops in middle or later life and affects 2–6% of adults in most Western societies (Bailey, 2000). The pharmacological agents currently used for the treatment of type 2 diabetes include sulfonylurea, biguanide, thiazolidinedione and α-glycosidase inhibitors. These agents, however, have restricted usage due to several undesirable side effects and fail to significantly alter the course of diabetic complications (Rang and Dale, 1991). The control of blood glucose in diabetic patients is achieved mainly by the use of oral hypoglycemic/antihyperglycemic agents and insulin. However, all these treatments have limited efficacy and have been reported to be associated with undesirable side effects (Harrower, 1994). Hyperglycemia, due to uncontrolled glucose regulation is considered as the causal link between diabetes and diabetic complications. A number of studies emphasize that alteration in glucose metabolism leads to hyperglycemia-induced cell damage by four key metabolic pathways. Protein kinase C (PKC) isoforms (Rolo and Palmeira 2006). An increase in the biosynthesis and or a decrease in the metabolism of glycoproteins attributed to the deposition of these materials in the basal membrane of pancreatic cells. In recent times, many traditionally important medicinal plants have been tested for their efficacy against impaired glycoprotein levels in diabetes (Ramkumar et al. 2007). β- Cells are affected by many immunological and chemical agents leading to local inflammations producing IL-6 and glucocorticoids. IL-6/glucocorticoid stimulation produces an active transcriptional complex for Reg, a β-cell regenerating

factor gene, in which poly (ADP-ribose) synthetaseypolymerase (PARP) is involved. In the presence of PARP inhibitors such as nicotinamide when PARP is not itself poly (ADP-ribosyl)-ated, the transcriptional complex is stabilized and Reg gene transcription and subsequent Reg protein formation occurs in β -cells. This protein acts as a growth factor on β -cells via Reg receptor. DNA replication in β -cells takes place and β-cell regeneration is accomplished. DNA damaging substances such as superoxide (O°2) and nitric oxide (NO°) are produced in inflammatory processes by cytotoxic agents such as STZ. When DNA is damaged, PARP senses the nicks and autopoly (ADP-ribosyl)-ates itself for DNA repair. Autopoly (ADP-ribosylation) of PARP inhibits the formation of Reg gene transcriptional complex and transcription of this gene stops (Akiyama et al., 2001). Nowadays, clinical treatment of diabetes targets both insulin deficiency and resistance and more recently the prevention of pancreatic β cell function decline (Hansotia and Drucker, 2005)

Polydatin 3, 4', 5-trihydroxystilbene-3-β-mono-D-glucoside is a monocrystalline compound isolated from a traditional Chinese herbal medicine named *polygonum cuspidatum* sieb. It is a glucoside of reveratrol and has also been named piced. Both polydatin and reveratrol are the main effective compounds of *polygonum cuspidatum. In plants,* reveratrol can quickly be glycosylated into piced. Previous studies have shown that polydatin resist free radical, kill or inhibit tumor cells, antibacterial activity (Shu, 2002; shu et al., 2004;Zhu and jin, 2005), exhibited anti-platelet aggregation, anti-oxidative activities (Zhang et al., 1995; Du et al., 2009; Kerem et al., 2006), antifatigue activity (Ding et al.,2009), reduce lipd oxidation (Pan et al., 2007), Polydatin attenuate the development of ventricular remodeling induced by isoproterenol in mice and by pressure overload in rats, and it can decrease BP in high dose (Chang Xun Chen et al., 2010). Very little information is available on the absorption, distribution, metabolism or excretion of polydatin (Lin J.H et al., 2001). The genartion of preclinical pharmacokinetic data of polydatin requires the development of an analytical method in different biological matrices (Shouhong gao et al., 2006). Polydatin markedly lowers the serum levels of total cholesrerol, triglyceride and low-density lipoprotein cholesterol in hyperlipidamic rabbits and hamster in hepatic triglycerides decreased levels (Qin et al., 2008 and Qin et al., 2009).

Hence, the present study was conducted in order to find the therapeutic potential of polydatin as oral hypoglycemic agent and exhibits additional pharmacological effects on plasma glucose, insulin, total hemoglobin, glycosylated hemoglobin, hepatic glycogen, and tissue (Liver and Kidney) carbohydrate metabolic enzymes in both normal and STZinduced diabetic rats. The effects produced by these treatments are compared with standard drug glipenclamide an anti-diabetic drug (Andrade-Cetto and Wiedefeld, 2004).

Materials and Methods

Animals

Female albino (9 week - old) rats of wistar strain with a body weight ranging from 180-210g, were procured from central animal house, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and were maintained in an air conditioned room (25±1 ºC) with a 12 h light/ 12 h dark cycle, feed and water were provide ad libitum to all the animals. The study was approved by the institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital (Reg No. 160/1999/CPCSEA), Annamalai University, Annamalainagar.

Chemical

Streptozotocin and Polydatin were purchased from Sigma-Aldrich (St, Louis, Missouri, USA). All other chemicals used in this study were of analytical grade obtained from E.Merck or Himedia, India.

Experimental induction of non insulin dependent diabetes mellitus

The animals were rendered diabetic by a single intraperitoneal injection of Streptozotocin (40mg/kg body weight) in freshly prepared citrate buffer (0.1 M, pH 4.5) after an overnight fast. Streptozotocin-injected animals were given 20% glucose solution for 24 h to prevent initial drug–induced hypoglycemic mortality. Streptozotocin-injected animals exhibited hyperglycemia within a few days.

Diabetes in Streptozotocin rats was conformed by measuring the blood glucose (by glucose oxidase method) 96 h after injection with Streptozotocin. The animals with blood glucose above 230 mg/dl were considered to be diabetic and used for the experiment.

Experimental design

The animals were randomly divided into seven groups of six animals each as given below. 50,100,150 mg/kg body weights of polydatin were dissolved in water. The administered post orally by intragastric intubations, once in a day in the morning for 45 days.

Group I : Normal control rats receiving 0.1 citrate buffer (pH 4.5).
Group II : Diabetic control rats.
Group III : Normal control administered with polydatin (150mg/kg/body weight).
Group IV : Diabetic rats administered with polydatin (50mg/kg/body weight).
Group V : Diabetic rats administered with polydatin (100mg/kg/body weight).
Group VI : Diabetic rats administered with polydatin (150mg/kg/body weight).
Group VII : Diabetic rats administered with glibenclamide (600µg/kg/body weight).

At the end of 45 days, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in tubes containing potassium oxalate and sodium fluoride mixture for the estimation of blood glucose. Plasma was separated for the estimation of insulin. Liver and kidney were immediately dissected out, washed in ice-cold saline to remove the blood.

Biochemical Assays

Estimation of plasma glucose and hepatic glycogen

Fasting plasma glucose was estimated using glucose oxidase–peroxidase method (Trinder, 1969). Hepatic glycogen was estimated by the method (Morales et al., 1973).

Determination of plasma insulin

Plasma insulin level was assayed by enzyme-linked immunosorbent assay kit (ELISA) (Boehringer Mannheim kit).

Determination of total hemoglobin and glycosylated hemoglobin

Total hemoglobin was estimated by cyanmethaemoglobin method (Drabkin and Austin, 1932) and Glycosylated hemoglobin (HbA1C) was estimated by the method of Sudhakar Nayak and (Pattabiraman., 1981), as modified by (Bannon., 1982).

Carbohrdrate metabolic enzmes

Estimation of hexokinase

Hepatic hexokinase activity was assayed by the method (Brandstrup et al., 1957). The reaction mixture a total volume of 5.3 ml cotained the following, 1 ml of glucose (5mM) solution, 0.5 ml of ATP (0.072 M) solution, 0.1 ml of megnesium chloride (0.05M) solution, 0.4 ml of potassium dihydrogen phosphate (0.0125 M), 0.4 ml of potassium chloride (0.1M), 0.4ml of sodium fluoride (0.5 M) and 2.5 ml of tris Hcl buffer (0.01 M, pH 8.0). the mixture was preincubated at 37°C for 5 min. the reaction was initiated by the addition of 2 ml of tissue homogenate. One millilitre of the reaction mixture was immediately transferred to the tubes containing 1 ml of 10% TCA that was considered as zerotime. A second aliquot was removed and deproteinised after 30 min of incubation at 37°C. The protein precipitate was removed by centrifugetion and the residual glucose in the supernatant of tissue homogenate was estimated by the method (Bolzan AD., 2003) as described previously.

Estimation of glucose-6-phosphate dehydrogenase

Glucose-6-phosphate dehrdrogenase was assayed by the method (Ellis and kirkman., 1961). The incubation mixture contained 1 ml of tris-Hcl buffer (0.05 M, pH7.5), 0.1 ml of magnesium chloride (0.1 M), 0.1 ml of NADP + (0.1 M), 0.5 ml of phenazine methosulphate, 0.4 ml of the dye solution and the requisite amount of the enzyme extract. The mixture was allowed to stand for 10 min at romm temperature to permit the oxidation of endogenous materials. The reaction was initiated by the addition of 0.5 ml of

glucose-6-phosphate. Absorbence of the sample was read at 640 nm against water blank at 1-min intervals for 3-5min. The enzyme was expressed in units by mutiplying the change in OD/min by the factor 6/17.6, the molar extinction coefficient of the reduced co-enzyme.

Estimation of glucose-6-phosphate

Glucose-6-phosphate was assayed by the mothod (Koide and Oda., 1959). Incubation mixture contained 0.7ml of citrate buffer (0.1 M, pH 6.5), 0.3 ml of substrate (0.01M), and 0.3 ml of tissue homogenate. The reaction mixture was incubated at 37°C for 1 h. addition of 1 ml of 10% TCA to the reaction tubes terminated the reaction of the enzymes. The suspension was centrifuged and the phosphorous content of the supernatent the tissue homogenate was estimate by the method (Fiske and subbarow., 1925). The supernatent was adjusted to known volume. To this, 1 ml of ammonium molybdate was added followed by 0.4 ml of ANSA. After 20 min, the absorbance aws raed at 680 nm.

Estimation of fructose-1, 6-bisphosphatase

Fructose-1, 6-bisphosphatase activity was measured by the method (Gancedo and Ganced., 1971). The assay mixture in a final volume of 2ml contained 1.2 ml of tris-Hcl buffer (0.1 M, pH 7.0), 0.1 ml substrate (0.05 M), 0.25 ml of magnesium chloride (0.1M), 0.1 ml of potassium chloride solution (0.1 M), 0.25 ml of EDTA (0.001 M) solution, and 0.1 ml of enzyme homogenate. The incubaton was carried out at 37°C for 5 min. The reaction terminated by the addition of 10% TCA. The suspension was centrifuged and the supernatant was used for phosphorus determination by the method (Fiske and subbarow., 1925). The supernatant was made up to known volume. To this, 1ml of ammonium molybdate was added followed by the 0.4ml of ANSA. After 20 min, the absorbance was read at 680nm. The activities of glucose-6-phosphate and fructose-1, 6-bisphosphatase were expressed as µmol of Pi liberated per minute. Total protein content of tissue homogenate was estimated by (Lowry et al., 1951).

Determination of liver glycogen

Glycogen content was determined as described by (Morales et al., 1975). The alkali extract of the tissue was prepared by digesting 50 mg of fresh tissue with 3 ml of 30% potassium hydroxide solution in boiling water bath for 15min. The tubes were cooled and a drop of 1 M ammonium acetate was added to precipitate glycogen and left it in freezer overnight for complete precipitation. Glycogen was collected by centrifuging at 3,000 rpm for 20 min. The precipitate was dissolved with aid of heating and again the glycogen was reprecipitated with alcohol and 1M ammonium acetate and centrifuged. The final precipitate was dissolved in saturated ammonium chloride solution by heating in a boiling water bath for 5min. Aliquots of glycogen solution solution were taken up for suitable dilution and 4ml of anthrone reagent was added by cooling the tubes in an ice bath. The tubes were shaken well, coverd with marble caps, and heated in boiling water bath for 20 min. After cooling, the absorance was read at 640 nm.

Statistical analysis

The experimental results are expressed as the means ±SD were subjected to one way analysis of variance, using a computer software package (SPSS version 16.0, SPSS Inc, Cary, NC) and the comparison of significant groups were performed using the Duncan Multible Range Test at $P<0.05$.

Results

The effect of Polydatin on plasma glucose, bodyweight and insulin in diabetic rats in depicted in Table 1. The plasma glucose level elevated and body weight, insulin decreased significantly in diabetic rats. Treatment with polydatin at a dose of 50, 100 and 150 mg/kg/body weight lowered plasma glucose and elevated body weight and insulin level significantly; 150 mg dose restored the plasma glucose level to near normalcy than the other two doses.

Disscusion

This study evaluates the antidiabetic effect of polydatin by measuring the level of plasma glucose, insulin, Hb, HbA1c and hepatic glycogen the activities of key enzymes involved in carbohydrate metabolism in the liver of non-diabetic and streptozotocin-diabetic rats. Streptozotocin is well known for its selective pancreatic islet β-cell cytotoxicity, and the drug interferes with cellular metabolic oxidative mechanism (Papaccio et al., 2000) and has been extensively used to induce diabetes mellitus in animals. Streptozotocin-induced hyperglycemia in animals is considered to be a good model for the preliminary screening of agents active against diabetes and is widely used (Ivorra et al. 1989). Streptozotocin, N-(methylnitrocarbamoyl)-d-glucosamine, is a potent DNA methylating agent and acts as a nitric oxide donor in pancreatic cells. At appropriate doses, STZ allows the production of a diabetic state with mild, moderate or severe hyperglycemia. The use of a lower dose of STZ produced an incomplete destruction of pancreatic β-cells (Aybar et al. 2001).

Hyperglycemia generates abnormally high levels of free radicals by autoxidation of glucose and protein glycation, and oxidative stress has been reported to be a causal factor of cardiovascular complications in STZ-induced diabetes mellitus (Okutan et al., 2005). Hyperglycemia is associated with the generation of reactive oxygen species (ROS) causing oxidative damage particularly to heart, kidney, eyes, nerves, liver, small and large vessels and gastrointestinal system (Tunali and Yanardag, 2006). Intraperitoneal administration of streptozotocin effectively induced diabetes in normal rats, as observed by hyperglycemia when

Compared with normal rats. Persistent hyperglycemia, the common characteristic of diabetes can cause most diabetic complications and it is normalized by the action of insulin (Gayathri and Kannabiran, 2008).

Hence we observed an increase in the level of, plasma glucose and decrease in the level of body weight and insulin. The ability of polydatin to significantly reduce fasting plasma glucose levels in diabetic rats is due to its potential to secrete insulin from existing islet β-cells. The decrease in plasma glucose in diabetic rats treated with polydatin and standard drug glibencalmide might be due to an elevated secretion of incresease the body weight and insulin which in turn, increases the utilization of glucose by the tissues. Insulin generally has an anabolic effect on protein metabolism so that it stimulates protein synthesis and retards protein degradation (Murray et al., 2000) and thus, decreasing the synthesis of hemoglobin. Increased glycation of protein has been found to be a consequence of diabetic complications. Polydatin, standard drug glibencalmide treated groups showed a significant reduction in glycosylated hemoglobin and an increase in total hemoglobin which was due to improved glycemic control. HbA1C was found to increase in patients with diabetes mellitus to approximately 16% and the amount of increase was directly proportional to the fasting blood glucose levels (Pari and Saravanan, 2002). In uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of proteins, including Hb (Alberti and Press, 1982). HbA1c was found to increase in diabetic patients up to 16% (Koeing et al., 1976). The level of HbA1c is a reliable index of glycemic control in diabetes mellitus (Gabbay, 1976)

Glycogen is the primary intracellular storable form of glucose and its level in various tissues is a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase (Golden et al., 1979) and it has been previously demonstrated that glycogen deposition from glucose is impaired in diabetic animals (Bollen et al., 1998) in proportion to the severity of insulin deficiency (Gannon and Nuttall, 1997). Insulin is a stimulator of glycogen synthase system and when insulin is lacking this enzyme is not activated. On the other hand insulin inhibits glycogenolysis and, if there is a lack of insulin glycogenolysis, it is not under inhibition of insulin and, therefore, glycogen content of the liver decreases (Vats et al., 2004). The diabetic rats treated with polydatin increased muscle and liver glycogen contents significantly when compared to the diabetic control which could be due to increased secretion of insulin.

In experimental diabetes Glycolytic enzymes of glucose metabolism are markedly altered. One of the key enzymes in the catabolism of glucose is hexokinase, which phosphorylates glucose and converts it into glucose-6-phosphate (Laakso et al., 1995), Decreased enzymatic activity of hexokinase has also been reported in diabetic animals, resulting in depletion of liver and muscle glycogen (Murray et al., 2000) .

Table 1
Effect of Polydatin on body weight, plasma glucose and insulin in streptozotocin-diabetic rats.
Values are given as means ±S.D.from six rats in each group.
a,b,c,d,e,f values sharing a common superscript (a,b,c,d,e,f) do not differ significantly at p<0.05 (DMRT).

Name of the group	Body Weight (g)		Plasma glucose(mg/dl)	
	0^{th} day	45^{th} day	0^{th} day	45^{th} day
Normal control	182.83±3.32	207.23±2.42a	84.15±5.68	91.13±5.39a
Normal control and Polydatin	177.60±6.60	175.56±4.06a	89.35±3.78	83.10±3.89a
Diabetic control	183.07±2.09	132.89±1.36b	246.28±7.89	290.39±2.48b
Diabetic+Polydatin(50mg/kg body weight)	181.36±3.76	192.28±1.89c	239.56±2.65	193.64±3.89c
Diabetic+Polydatin(100mg/kg body weight)	180.57±4.26	186.30±3.89d	242.09±3.64	163.62±2.65d
Diabetic+Polydatin(150mg/kg body weight)	179.78±3.90	181.51±2.06e	236.83±1.24	156.38±5.04e
Diabetic+glibenclamide(600µg/kg body weight)	181.09±2.08	198.45±4.07f	243.12±4.87	96.29±5.18af

Table 2 shows the level of Hb, HbA1c and hepatic glycogen in streptozotocin diabetic rats. Hb decreased and HbA1c increased significantly
in diabetic rats and when diabetic rats were treated with polydatin, these values were brought towards normal level.

Table 2

Effect of Polydatin on total hemoglobin, glycosylated hemoglobin and hepatic glycogen in streptozotocin-diabetic rats.
Values are given as means ±S.D.from six rats in each group.
a,b,c,d,e,f values sharing a common superscript (a,b,c,d,e,f) do not differ significantly at p<0.05 (DMRT).

Name of the group	Total hemoglobin(g/dL)	Glycosylated hemoglobin (mg/g of Hb)
Normal control	15.06±1.46a	0.54±0.30
Normal control and Polydatin	14.56±1.78a	0.52±.0.07
Diabetic control	7.15±0.57b	1.67±0.09
Diabetic+Polydatin(50mg/kg body weight)	8.24± 0.71c	1.28±0.04
Diabetic+Polydatin(100mg/kg body weight)	10.06±1.03c	0.96±0.09
Diabetic+Polydatin(150mg/kg body weight)	12.30±0.56d	0.63±0.04
Diabetic+glibenclamide(600µg/kg body weight)	13.15±0.49a,d	0.58±0.08

Table 3 shows the activities of carbohydrate metabolic enzymes and the level of glycogen in the liver of control and streptozotocin-diabetic rats.
The activities of glucokinase, glucose 6-phosphate dehydrogenase and the level of glycogen decreased in the liver of diabetic rats.
The values improved towards normality in diabetic rats on treatment with polydatin.

Table 3
Effect of Polydatin on carbohydrate metabolic enzyme activities in the liver of streptozotocin - diabetic rats.

Name of the group	Carbohydrate metabolic enzymes	
	Glucokinase (U*/h/mg protein)	Glucose 6-phoaphate dehydrogenase (U#/mg protein)
Normal control	0.49±0.02a	5.18±0.26a
Normal control and Polydatin	0.47±0.04a	5.24±0.38a
Diabetic control	0.20±0.01b	2.21±0.17b
Diabetic+Polydatin(50mg/kg body weight)	0.26±0.03c	2.89±0.29c
Diabetic+Polydatin(100mg/kg body weight)	0.34±0.02c	3.67±0.41c
Diabetic+Polydatin(150mg/kg body weight)	0.42±0.05c	4.12±0.19c
Diabetic+glibenclamide(600µg/kg body weight)	0.47±0.03d	4.67±0.57d

Values are given as means±S.D. for six rats in each group.
Values not sharing a common superscript differ significantly at pb0.05. Duncan's Multiple Range Test (DMRT).
U* — µmol of glucose phosphorylated per hour.
U# — nmol of NADPH formed per minute.

Table 4 shows changes in the activities of gluconeogenic enzymes in the liver and kidney of diabetic rats. Increased activities of glucose 6-phosphatase and fructose 1, 6-bisphosphatase were observed in the liver and kidney of diabetic rats and treatment with polydatin brought the activities of these enzymes towards normality.

Table 4
Effect of polydatin on gluconeogenic enzyme activities in the liver and kidney of streptozotocin - diabetic rats.

Name of the group	Glucose 6-phosphatase (Unit@/min/mg protein)		Fructose 1, 6-b (Unit$/h/m
	kidney	Liver	kidney
Normal control	0.28±0.02a	0.23±0.02a	0.85±0.34a
Normal control and Polydatin	0.26±0.03a	0.23±0.02a	0.87±0.39a
Diabetic control	0.40±0.06b	0.49±0.04b	1.51±0.12b
Diabetic+Polydatin(50mg/kg body weight)	0.34±0.01c	0.41±0.01c	1.30±0.09c
Diabetic+Polydatin(100mg/kg body weight)	0.30±0.02c	0.34±0.02c	1.22±0.10c
Diabetic+Polydatin(150mg/kg body weight)	0.27±0.04c	0.30±0.03c	1.09±0.18c
Diabetic+glibenclamide(600µg/kg body weight)	0.24±0.03d	0.28±0.03d	0.93±0.05d

Values are given as means±S.D. for six rats in each group.
Values not sharing a common superscript differ significantly at pb0.05. Duncan's Multiple Range Test (DMRT).
U@—µmol of Pi liberated per minute. U$—µmol of Pi liberated per hour.

Glucokinase insufficiency in diabetic rats can cause decreased utilization of glucose for energy production (Vats et al., 2003). The insulin-dependent and insulin-sensitive enzyme and are almost completely inhibited or inactivated in diabetic rat liver in the absence of insulin (Gupta et al., 1997). Administration of polydatin and glibenclamide standard drug to diabetic rats resulted in a significant reversal in the activity of hexokinase. The increased plasma insulin and decreased glucose in diabetic rats given polydatin may also be as a result of increased hepatic hexokinase activity, thereby increased glycolysis.
The activity of glucose-6-phosphate dehydrogenase was found to be decreased in our study. The decreased activity of this enzyme in diabetic condition may result in the diminished functioning of hexose monophosphate shunt and thereby decreasing the production of reducing equivalents such NADH and NADPH. A decrease in the activity of glucose 6-phosphate dehydrogenase has been shown to slow down the pentose phosphate pathway in diabetic conditions (Abdel-Rahim et al., 1992). In our study, administration of polydatin increased the activity of glucose 6-phosphate dehydrogenase significantly by enhancing insulin secretion. As an increased the influxes of glucose into the pentose monophosphate shunt resulting in an increased production of the reducing agent NADPH with concomitant decrease in oxidative stress (Ugochukwu and Babady, 2002).
The activities of the gluconeogenic enzymes such as glucose 6- phosphatase and fructose 1,6-bisphosphatase increased significantly in the liver and kidney of diabetic rats (Berg et al., 2001), Hepatic glucose production through gluconeogenesis significantly contributes to hyperglycemia in diabetes mellitus (Ishikawa et al. 1998). It has been demonstrated that in diabetes mellitus, the increased rate of gluconeogenesis is related to the increased expression of key gluconeogenic enzymes such as phosphoenol pyruvatecarboxykinase (PEPCK), glucose 6-phosphatase, fructose 1,6-bisphosphatase in hepatic tissues (Van deWerve et al. 2000). Glucose 6 phosphatase is an important enzyme for the final step of

gluconeogenesis or glucogenolysis in which it catalyzes the hydrolysis of glucose 6 phosphate to glucose and phosphate. Glucose is transported out of the liver to increase blood glucose concentration. Normally insulin inhibits the hepatic glucose production by suppressing glucose 6 phosphatase and fructose 1, 6-bisphosphatase activity (Chen et al., 2000; Wiernsperger and Bailey, 1999). The hepatic glucose-6-phosphatase catalyses the terminal step of glucose production and it plays a key role in the maintenance of blood glucose homeostasis. Increased activities of glucose-6-phosphatase in diabetic rats provide hydrogen which binds with NADP+ in the form of NADPH and enhances synthesis of fats from carbohydrates, lipogenesis (Bopanna et al., 1997). Fructose1-6-phosphatase is the important regulatory enzyme of the gluconeogenic pathway (Minnassian and Mitheux, 1994). The diabetic rats treated with polydatin brought these enzyme activities to near normal levels, which might be due to increased secretion of insulin. The possible mechanism by which polydatin bring about the normalization of enzyme activity may be by potentiation of insulin release from β-cells of the islets of Langerhan's which might enhance glucose utilization.

Conclusion

In conclusion the investigation depicts that oral administration of polydatin has beneficial effect in reducing blood glucose, decreased Hb, increased level of HbA1c, hepatic glycogen and increased insulin levels improving glycemic control as well as carbohydrate metabolism.

Reference

1. American diabetes association. 2012
2. Bailey, Agardh CD, Bjorgell P, Nilson EP (2000). The effect of tolbutamide on lipoproteins, and lipoproteinlipase and hormone sensitive lipase. Diabetes Res Clin Pract 1999; 46:99-108.
3. Rang and Dale, (1991), Diuretic, and antiinflammatory principle from Scoparia dulcis. Pharmazie 56:657-660.
4. Shu SY, Wang XY, Ling ZY, Lu ZY (2004) Effect of polydatin on phospholipase A2 in lung tissues in rats with endo toxic shock. Chin J Traumatol7 (4):239–43.
5. Son.s. Lewis B.A (2002) Free radical scavenging and antioxidative activity of caffeic acid amide and ester analogues; structure activity relationships. J. Agric. Food Chem 30: 468-472.
6. Sundaram, R.K., Bhaskar, A., Vijayalingam, S., Viswanatthan, M., Moha, R.,Shanmugasundaram, K.R. (1996)Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. Clinical Science 90, 255–260.
7. The WHO Expert Committee on Diabetes Mellitus (1980) Technical Reports series 646. World Health Organization, Geneva.
8. Trinder P (1969) Determination of Glucose in Blood using glucose oxidase with an alternative oxygen acceptor. Ann. Cli. Biochem 6: 24-25.
9. Bourgeois. Wiggins, D., Hems, R., Gibbons, G.F (1995) VLDL output by hepatocytes from obese Zucker rats is resistant to the inhibitory effect of insulin. American Journal of Physiology 269, E208–E215.
10. Bernal J, Mendiola JA, Ibanez E, Cifuentes A (2010) Advanced analysis of nutraceuticals. J pharm and Biomed Anal 55:758-74.
11. Betteridge J (1997) Lipid disorder in diabetes mellitus. In: pickup, J.C., Williams, Text book of Diabetes, second ed. Blakwell science 1-35.
12. Blades, B., Garg,A (1995) Mechanisms of increase in plasma triacylglycerol concentration as a result of high carbohydrate intakes in patients with noninsulin-dependent diabetes mellitus. American Journal of Clinical Nutrition 62, 996-1002.
13. Bopanna KN, Kannan J, Sushma G, Balaraman R, Rathod SP (1997) Antidiabetic and antihyperlipidemic effect of neem seed, kernel powder on alloxan diabetic rabbits. Ind J Pharmacol 29:162-167.
14. Papaccio et al., 2000, Lipid lowering and laque regression: new insights into prevention events of plaque disruption and clinical in coronary disease. Circulation 81:1781.

15. Ivorra et al. 1989, Nutritional approaches to combat oxidative stress in Alzheimer's disease. J Nutr Biochem 13:444-61.
16. Deliorman Orhan, D., Aslan, M., S¸ endo˘gdu, N., Ergun, F., Yes¸ ilada, E (2005)Evaluation of the hypoglycemic effect and antioxidant activity of three Viscum album subspecies (European mistletoe) in streptozotocin-diabetic rats. Journal of Ethnopharmacology 98, 95–102.
17. Du, J., Sun, L.N., Xing, W.W., Huang, B.K., Jia, M., Wu, J.Z., Zhang, H., Qin, L.P (2009) Lipid-lowering effects of polydatin from Polygonum cuspidatum in hyperlipidemic hamsters. Phytomedicine 16, 652–658.
18. World Health Organization (2012),
http://www.who.int/features/factfiles/diabetes/en/index.html.
19. World Health Organization 2002, Launches the first global strategyontra-ditional and alternative medicine. WHO Press release p.38:
http://www.who.int/mediacentre/news/release/release38/en.
20. Y.W.Bynes, R.Thrope (1999) Role of oxidative stress in diabetic complication, diabetes 481-9.
21. Zhang PW, Yu CL, Wang YZ, Luo SF, Sun LS, Li RS (1995) Infuence of 3, 4', 5-trihydroxystibene- 3-beta -mono-D-glucoside on vascular endothelial epoprostenol and platelet aggregation. Zhongguo Yao Li Xue Bao 16(3):265–8.
22. Zilversmit DB,Davis AK (1950) Micro determination of phospholipids by TCA precipitation. J Lab Clin Med 35:155–9.
23. Zlatkis A, ZakB, BoyleGJ (1953) A simple method for determination of serum cholesterol. Clin J Med 41:486–92.
24. Bopanna et al., 1997 Micro determination of phospholipids by TCA precipitation. J Lab Clin Med 35:155–9.
25. Minnassian and Mitheux, 1994, Polydatin ameliorates experimental diabetes induced fibronectin through inhibiting the activation of NF-kB signaling pathway in rat glomerular mesangial cells, Mol and cellu Endocrine, 362; 183-193.

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MOLECULAR DIAGNOSIS OF ISONIAZID DRUG RESISTANT *Mycobacterium tuberculosis* IN HIV PATIENTS

Dr.B.Bharathi* and Kavitha.S

Abstract

Tuberculosis is an infectious disease caused by the bacillus *Mycobacterium tuberculosis* affecting nearly one-third of the global population. Transmission of multidrug – resistant strains of *Mycobacterium tuberculosis* (MDR-TB) presents a serious problem for TB control, particularly in the context of co-infection with the human immunodeficiency virus (HIV). MDR-TB has been well studied in outbreaks in settings of low endemicity in developed countries. However, the characteristics of MDR-TB in the community with high endemicity such as India have not been well investigated. This study was designed to isolate and characterize *inhA* and *katG* gene from a clinical isolate of isoniazid drug resistant TB patients co-infected with HIV. The blood sample obtained was first subjected to CD4 analysis using Partec Flow Cytometry instrument, RT-PCR analysis to confirm HIV positive. The serum albumin was separated by 10% SDS – PAGE and then the suspected protein spot was sequenced using Nano LC/MS. The DNA was isolated from the isoniazid resistant *Mycobacterium tuberculosis* culture, which was used for the amplification of the *kat G* and *inhA* genes using primers by PCR technique. The PCR product was subjected to electrophoresis on 2% agarose gel and desired gene product was eluted by the gel cleanup kit. The *kat G* and *inhA* gene PCR purified products were analysed in the bioanalyser electropherogram to detect the molecular weight and was directly sequenced at Bioserve at Bangalore. The BLASTn and BLASTx search tool was used to compare the suspected DNA sequence for detecting the mutation to confirm the drug resistance. Then the pure culture of *Mycobacterium tuberculosis* were then subjected to phylogenetic analysis using 16s rRNA primer by PCR technique. The 16s rRNA PCR purified products was sequenced in the automated DNA sequencer. The MEGA search tool was used to compare and differentiate the 16s rRNA sequence from H_{37}Rv wild strain and other *Mycobacterium* sp.

Introduction

Drug resistant strains of MTB are also increasing at an alarming rate which is a devastating threat to TB control particularly in HIV patients. HIV/AIDS and TB are so closely connected that the term "co-epidemic" or "dual epidemic" is often used to describe their relationship. Drug-resistant tuberculosis poses a significant problem for treatment. The mechanisms of resistance to the front-line drug isoniazid (INH) are complex and can be mediated by katG, inhA and other unknown genes. Isoniazid (INH) is an important first-line tuberculosis drug. INH is a pro-drug that requires activation by the M. tuberculosis catalase-peroxidase enzyme (katG) to its active form (Zhang *et al*., 1992). Mutation of the katG gene, which leads to loss of or reduced catalase-peroxidase activity, is a major mechanism of INH resistance in *M. tuberculosis* (Zhang *et al*., 2000). Although various mutations in the katG gene have been reported in INH-resistant isolates, the most common mutation is the KatG Ser315Thr mutation, which is present in approximately 50–90% of all INH-resistant isolates and is associated with relatively high-level resistance to INH (Zhang *et al*., 2000). Mutations in inhA or its promoter region can cause INH resistance, with promoter mutations being more frequent than mutations in the structural gene (Miesel *et al*., 1998). Mutations in inhA cause not only INH resistance, but also resistance to the structurally related second-line drug ethionamide (ETH) (Banerjee *et al*., 1994). The TB bacteria are virulent enough that they can make even healthy adults and children sick, who in turn can spread it to others. If there are a lot of HIV-infected people in the community, the vicious cycle results in more and more people with TB. In this study, we performed a detailed characterization of a panel of primarily INH-resistant M. tuberculosis strains in terms of their mechanism of INH resistance in order to shed light on the frequency of such strains.

METHODS

Isolation,Identification and Characterization of *M. tuberculosis* isolates:

HIV positive patient attending the HIV Counselling and testing center of Government Hospital at Puducherry enrolled in this study. HIV positive patient in age group of 15-49 presenting with cough more than 3 weeks or enlargement o lymph nodes. For Mycobacterial examination, Morning-Spot specimens were collected. Early morning coughed up sputum specimens were collected after rinsing the mouth with plain water in a sterile sputum container. The sputum samples were processed by Modified Petroffs method (Allen *et al*, 1968). The isolates were stained by fluorescent (Blair *et al*., 1970) and Ziehl Neelson staining(Bishop *et al*., 1970). M. tuberculosis strains were grown in Lowenstein Jensen medium. The *M.tuberculosis* strains identified by using biochemical tests namely niacin and catalase and PNB tests. To determine the MICs of INH and ETH for the INH-resistant strains, the agar proportion method was performed in 7H11 plates containing varying concentrations of INH (0.2, 0.4, 1 and 5µ g /ml).

Bacterial genomic DNA isolation, PCR and DNA sequencing:

Genomic DNA was isolated as described previously (Zhang *et al*.,1992).Primers for *inhA* and *katG* gene.The isolated template DNA was amplified using IS6110 primer in an authorized thermal cycler (Eppendorf). This confirms the template DNA as *Mycobacterium tuberculosis.*

The PCR cycling parameters were 94^0Cfor 5 minutes; followed by 40 cycles of 94^0C for 1 minute, 57^0C for 1 minute and 74^0C for 1 minute; and a final extension of 74^0C for 5 minutes. The PCR was then kept at hold at 4^0C for 15 minutes.

inhA 1-5'CCTCGCTGCCCAGAAAGGGA3' (10pmol/µl)
inhA 2-5'ATCCCCCGGTTTCCTCCGG3' (10pmol/µl)
katG 1-5'GAAACAGCGGCGCTGATCGT3' (10pmol/µl)
katG 2-5'GTTGTCCCATTTCGTCGGGG3' (10pmol/µl)

The purified PCR product was directly sequenced in an automated DNA Sequencer at Bioserve in Bangalore.The nucleotide sequence obtained was analyzed using BLASTn Bioinformatics tool available at NationalCenter for Biotechnology Information (Altschul *et al*., 1997.) to know the specificity of PCR amplification and to identify the nucleotide variation. The sequence was further subjected for BLASTx to know the amino acid changes in comparison with the wild type *Mycobacterium tuberculosis* (H_{37}Rv).The nucleotide sequence obtained was analyzed using CLUSTAL W algorithm.

RESULTS

Small, rough and cream coloured colonies were observed on the inoculated L.J.slants after 4 weeks of incubation, indicating the presence of *M.tuberculosis* in the isolate. Various biochemical tests were performed to confirm the *M.tuberculosis* in the LJ medium slants and the results are tabulated in table 1.

Table: 1 Biochemical tests for identification of *M.tuberculosis*

S.No.	Name of the tests	Results
1.	Ziehl Nielsen staining	Appearance of pink coloured bacilli with beaded in blue back ground (Fig 2)
2.	Fluorescent staining	Appearance golden yellow coloured bacilli in dark back ground (Fig 3)
3.	Niacin test	Pink to red colour formation
4.	Catalase test	No appearance of bright effervescence
5.	PNB test	No Growth on L.J medium supplemented with PNB
6.	PCR- IS6110 PRIMER	Positive

The suspected defaulter sputum sample was subjected to isolation and identification, culture and conventional drug susceptibility test using first line TB drugs (SHRZE). The DNA was extracted from

well grown culture for the amplification of *inh A* and *kat G* gene and for the further molecular characterization.

PROPORTIONAL SENSITIVITY TEST METHOD

Isoniazid drug resistance:

Pure culture of *Mycobacterium tuberculosis* isolated from LJ medium were subjected to drug sensitivity testing using the proportion sensitivity test method. Individual colonies on Isoniazid drug slants, and drug free L.J slants were counted and tabulated as in table -2.

Percentage of Isoniazid drug resistant bacilli present in the bacterial population

$$= \frac{\text{No. of CFU on drug slopes x Dilution Factor}}{\text{No. of CFU on drug-free slopes x Dilution factor}} \times 100$$

$$\text{Percentage of isoniazid resistant bacilli} = \frac{26 \times 10}{18 \times 1000} \times 100$$

$$= 1.44\ \%$$

Since the percentage of resistance is greater than 1%, the strain was considered to be resistant to Isoniazid drug. The growth of *M.tuberculosis* in isoniazid and drug free slants shown in table 2.

TABLE- 2 GROWTH OF *Mycobacterium tuberculosis ISONIAZID* DRUG SLANTS AND DRUG FREE L.J SLANTS

DILUTION FACTOR	NO.OF CFU IN NORMAL L.J SLANT	NO.OF CFU IN DRUG CONTAINING L.J SLANT
S1	TNTC	62
S2	124	26
S3	78	-
S4	18	-

Polymerase Chain Reaction:

In PCR, A clear band was formed at 123bp region confirming the presence of *M. tuberculosis* in the culture. Mycobacterial DNA was isolated from the Mycobacterium tuberculosis clinical isolate and $H_{37}Rv$ wild type strain. The templates DNA were amplified using primers inhA1-5'CCTCGCTGCCCAGAAAGGGA3' and inhA2-5'ATCCCCCGGTTTCCTCCG G3'. The amplified PCR product of $H_{37}Rv$ and isoniazid-resistant clinical isolates were run on a 2% agarose gel. A clear band was formed at 247bp and 206bp region confirming the amplification of *inhA and katG* region of *Mycobacterium tuberculosis* respectively.

Bioanalyzer:

The amplified PCR products were subjected to Bioanalyzer to know the exact molecular size and purity of amplified products. The molecular size of PCR products of *kat G* and *inh A gene* are 206 and 247 respectively

DNA sequencing:

Sequenced DNA of mutant strain was compared with $H_{37}Rv$ (wild type) strain DNA sequence using Bioinformatics tool BLASTn available at National Centre for Biotechnology Information (Altschul*et al*,1997) for detecting the mutation. The mutation and the amino acid changes pattern are shown in table 4.

TABLE 4:NUCLEOTIDE AND AMINO ACID CHANGES IN DRUG TARGET GENES

GENE	NUCLEOTIDE CHANGES	AMINO ACID CHANGES
inhA	Substitution at 9, 14, 16, 24, 25,212 Deletion at 35	No changes
katG	Substitution at 118, 133 Deletion at 11	Serine→Threonine at 41 and 52 codon

>25INH_F
CCTTGGGTGGAAGGGGGCTGAGTTCCACCGACAACGTCACGAGCGTAACCCCAGTGCGAAAGTTCCCGCCGGAAA
TCGCAGCCACGTTACGCTCGTGGACATACCGATTTCGGCCCGGCCGCGGCGAGACGATAGGTTGTCGGGGTGACT
GCCACAGCCACTGAAGGGGCCAAACCCCCATTCGTATCCCGTTCAGTCCTGGTTACCGGAGGAAACCGGGGGATCA
>25INH_R
AGAGGAAAAACGGTTCGAATGGGGGTTTGGCCCCTTCAGTGGCTGTGGCAGTCACCCCGACAACCTATCGTCTCGC
CGCGGCCGGGCCGAAATCGGTATGTCCACGAGCGTAACGTGGCTGCGATTTCCGGCGGGAACTTTCGCACTGGGG
TTACGCTCGTGACGTTTGTCGGTGTGACTCAGCACACTTCGACCATGACGGATCCCTTTATGGGCAGCGAGGA
>V8_KatG2_KatGL
ATGGCGCCGGCCCGGCCGATCTGGTCGGCCCCGAACCCGAGGCTGCTCCGCTGGAGCAGATGGGCTTGGGCTGGA
AGAGCTCGTATGGCACCGGAACCGGTAAGGACGCGATCACCACCGGCATCGAGGTCGTATGGACGAACACCCCGA
CGAAATGGGAC
>V8_KatG1_KatGR.
ACTTTCGGTAGACCCATGGCGCCGGCCCGGCCGATCTGGTCGGCCCCGAACCCGAGGCTGCTCCGCTGGAGCAGAT
GGGCTTGGGCTGGAAGAGCTCGTATGGCACCGGAACCGGTAAGGACGCGATCACCACCGGCATCGAGGTCGTATG
GACGAACACCCCGACGAAATGGGACA

SDS- PAGE Electrophoresis

The serum of HIV, TB co infected with HIV, TB and Normal patients were subjected to SDS PAGE analysis. The band size of particular molecular weight protein of TB co infected with HIV was highly diminished than others.

AMPLIFICATION OF *rrs*GENE

Mycobacterial DNA was isolated from the Mycobacterium tuberculosis clinical isolate and H_{37}Rv wild type strain. The templates DNA were amplified using the primers *rrs* gene:*rrs* Forward Sequence: CCATGCCGC GTGTGTGAAGA and *rrs*Reverse Sequence: TGCAGACTGCGATCCGGACT. The amplified PCR product of H_{37}Rv andisoniazid-resistant clinical isolates were run on a 2% agarose gel. Clear bands were formed at 500bp region to confirm the amplification of*rrs*gene of *Mycobacterium tuberculosis.* (Fig: 26)

PHYLOGENETIC ANALYSIS:

The primer for *rrs*gene of *Mycobacterium tuberculosis* was designed from *rrs* gene obtained from the NCBI gene bank using epperndorf primer designing software as per following parameters.

DISCUSSION:

MTB strains exhibit resistance to both isoniazid and rifampicin, they are termed multidrug-resistant MTB (MDR-TB). These MDR-TB strains have been shown to be increasingly associated with infections in AIDS patients. HIV is considered to be the most potent risk factor for progression to active TB among those infected both with TB and HIV; as a result, TB is the most common life threatening opportunistic infection associated with HIV, and biggest cause of death among patients with acquired immunodeficiency

syndrome (AIDS). Currently the detection of drug resistance in *Mycobacterium tuberculosis* is primarily based on phenotypic drug susceptibility testing, which involves time-consuming culture of the slow-growing *Mycobacterium tuberculosis* in the presence antibiotics (Canetti and Khomenko, 1969; Libonati *et al.,* 1988; Laszlo *et al.,* 1997). The prevalence of drug resistant *Mycobacterium tuberculosis* isolates among HIV seropositive tuberculosis patients was similar to that of HIV seronegative TB patients, indicating HIV infection may not be associated with drug resistant tuberculosis. However, considering the results from other studies and a high prevalence of HIV-TB infection in the country, monitoring of drug resistance in *M. tuberculosis* isolates needs prioritization to ensure success in national tuberculosis control programme (Pereira *et al., 2005)*.

Bostanabad *et al.,* (2008) investigated the Standard PCR method for detection of isoniazid resistance associated mutations was performed by *katG* gene amplification and DNA sequencing. Most mutations were found in *katG* gene codons 315,316 and 309. Four types of mutations were identified in codons 315. One type of mutation was found in codon 316. Four types of mutations were detected in codon 309. The highest frequency of mutations sharing between primary and secondary infections was found in codon 315 (Cardoso et al, 2007). It was found that a particular protein concentration in HIV-TB patient has declined than the normal patient. When compared with the HIV-TB patient the protein concentration in HIV patient was found to be low, followed by PTB patient.

Design Parameters	
Amplicon Range (bp)	900 - To - 1000
Formula	SantaLucia's

Sequence No (Raw Sequence): 1

Primer Pair: 1

Forward Sequence: CCATGCCGCGTGTGTGAAGA

Reverse Sequence: TGCAGACTGCGATCCGGACT

Primer Properties		
Parameters	**Forward**	**Reverse**
Amplicon Position	370-1294	
Primer Length	20	20
Position	370-389	1275-1294
Tm (°C)	59.35	59.33
GC (%)	60.0	60.0
3' end stability (kcal/mol)	-5.49	-9.76
Hairpin loop energy (kcal/mol)	2.4	0.1
3' end free energy (kcal/mol)	No Self-Dimer formed at 3'	-13.0
Duplex free energy (kcal/mol)	-10.3	-13.0
Molecular Weight (g/mol)	6158.03	6118.01
Extn. Coeff. (L/mol'cm)	192100	186600
nmole/OD260nm	5.21	5.36
µg/OD260nm	32.08	32.79

>rrs4-F sequence exported from OciSeq_rrs4-F_081.ab1
GCACAAGACCACTAAGGTTTGGCCTCACATTCGGCTGTGTAGCAAACCCTTTGTACCAACCAT
TGTAGCACGTGTGTAGCCCTGGTCGTAAGGGCCATGATGACTTGACGTCGTCCCCGCCTTCCT
CCAGTTTGTCACTGGCAGTATCCTTAGAGTTCCCGACCGAGTCGCTGGTAACTAAGGAAAAG
GGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCA
GCACCTGTATGTGAATTCCCGAAGGCACTCTCGCATCTCTGCAAGATTCTCACTATGTCAAGA
CCAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCC

CGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCTACTTATTGCGTTAA
CTGCGTCACTAAGTCTTTAAAAGACCCAACGACTGGTAGACATCGTTTACGGCGTGGACTACC
AGGGTATCTAATCCTGTTTGCTACCCACGCTTTCGCACCTCAGTGTCAGTATGATGCCAGGGA
GCTGCCTTCGCCATCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTA
CTCCCCTCTCACCTACTCTAGTTATCCAGTATCAGATGCAGTTCCCAGGTTAAGCCCGGGGCT
TTCACATCTGACTTAAATAACCACCTACGCGCGCTTTACGCCCAGTAATTCCGATTAACGCTT
GCACCCTCTGTATTACCGCGGCTGCTGGCACAGAGTTAGCCGGTGCTTATTCTGTGGGTAACG
TCAGGGCTTATGGGTATTAACCATAAGCTTTTCCTCCCCACTTAAAGTGCTTTACAACCAAAA
GGCCTTCTTCACAACCCCCGGCATGGAATA

>rrs4-R sequence exported from OciSeq_rrs4-R_096.ab1

CCTTTGGTTTTAAAACTTTTAGTGGGGAGGAAAGCTTATGGTTAATACCCATAAGCCCTGACG
TTACCCACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCA
AGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTATTTAAGTCAGATGTGAA
AGCCCCGGGCTTAACCTGGGAACTGCATCTGATACTGGATAACTAGAGTAGGTGAGAGGGGA
GTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGATGGCGAAGGC
AGCTCCCTGGCATCATACTGACACTGAGGTGCGAAAGCGTGGGTAGCAAACAGGATTAGATA
CCCTGGTAGTCCACGCCGTAAACGATGTCTACCAGTCGTTGGGTCTTTTAAAGACTTAGTGAC
GCAGTTAACGCAATAAGTAGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAA
TTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCT
TACCTGGTCTTGACATAGTGAGAATCTTGCAGAGATGCGAGAGTGCCTTCGGGAATTCACATA
CAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTTTCCTTAGTTACCAGCGACTCGGTCGGGAACTCTAAGGATACTGCCAGTGACAA
ACTGGAGGAAGGCGGGGACGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGT
GCTACAATGGTTGGTACAAAGGGTTGCTACACAGCGATGTGATGCTAATCTCAAAAAGCCAA
TCGTAGTCCGGTGCCCACTTCTTGTGAAAAAAAAGATTCTC

BIBLIOGRAPHY

Allen B.W and Baker F.J. Mycobacteria Isolation, Identification and Sensitivity testing. Butterworths. 1968**;** 10.

Altschul, Stephen F, Thomas L, Madden, Alejandro A, Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J.Lipman. "Gapped Blast and PSI-BLAST: A new generation of protein database search programs", *J Nucleic acids Res.* 1997; 25:3389-3402

Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um K.S, Wilson T, Collins D. de Lisle. G and Jacobs W.R. *inhA*, a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis*.Science.1994; 263:227-230.

Bishop P.J and Neuman G. The History of the Ziehl Neelsen Staining. 1970; 196:51.

Blair E.B, Weiser O.L and Tull N. H. Micro bacteriology laboratory methods- Lab report No.323 U.S. Army Med Res and Nutr Laboratory, Fitrzsimmoun General Hospital, Denver, Colorado, USA. 1970.

Bostanabad S.Z, Titov L.P, Barhrmand A and Nojoumi S.A. Detection of mutation in isoniazid-resistant *Mycobacterium tuberculosis* isolates from Tuberculosis patients in Belarus. *Indian J Med Microbiol*. 2008; 26 (2): 143-147.

Canetti G, Fox W, Khomenko A,Mahler H.T,Menon N.K and Mitchinson A.Advances in techniques of testing Mycobacterial drug sensitivity and the use of sensitivity tests in tubercuosis control programmes.Bull World Health Organ,Supp 41.1969.

Cardoso R.F, Cardoso M.A, Leite C.Q.F,Sato D.Q, Mamizuka E.M,Hirata R.D.C, Mello F.F.D,Hirata M.H. Characterization of ndh gene of isoniazid resistant and susceptible *Mycobacterium tuberculosis* isolates from Brazil.*Mem Inst Oswaldo Cruz, Rio de Janeiro.*2007;102(1): 59-61

Lazzlo A, Rahman M, Raviglione M and Bustreo F.Quality assurance program for drug susceptibility testinf of Mycobacterium tuberculosis in the WHO/IUATLD Supranational Laboratory Network; First round of proficiency testing. *Int J Tuberc Lung Dis*.1997;1:231-238.

Libonati J.P, Stager C.E, Davis J.R and Siddiqi S.H. Direct anti microbial drug susceptibility testing of *Mycobacterium tuberculosis* by the radiometric method. *Diagn Microbiol Infect Dis*.1988; 10:41-48.

Mani C, Selvakumar N, Kumar V, Narayanan S, Narayanan P.R. Comparison of DNA sequencing, PCR-SSCP and PhaB assays with indirect sensitivity testing for detection of rifampcin resistance in *Mycobacterium tuberculosis*. *Int J Tuber Lung Dis.* 2003; **7:**625-659.

Miesel L, Weisbrod T.R, Marcinkeviciene J. A, Bittman R and Jacobs W. R. NADH dehydrogenase defects confer isoniazid resistance and conditional lethality in *Mycobacterium smegmatis*. *J Bacteriol*. 1998; 180: 2459-2467.

Pereira M, Tripathy S, Inamdar V, Ramesh K, Bhavsar M, Date A, Iyyer R, Acchammachary A, Mehendale S and Risbud A. Drug resistance pattern of Mycobacterium tuberculosis in seropositive and seronegative HIV-TB patients in Pune, India.Indian J Med Res.2005; 121:235-239

Zhang Y.B, Heym B, Allen D, Young and Cole S.T. The catalseperoxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. *Nature*. 1992; 358: 501-593.

Zhang Y, Telenti A. Genetics of drug resistance in Mycobacterium tuberculosis. Washington, *ASM Press*. 2000.

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PHYTOCHEMICAL SCREENING AND ANTICANCEROUS ACTIVITY OF RHIZOME EXTRACT *COLEUS FORSKHOLI* ON HEP G2 CELL LINE

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ABSTRACT
To evaluate the presence of Photochemicals, and anticancerous activities of *Coleus forskohlii*. The plant material was extracted by using acetone and aqueous solvents. The phyotochemical analysis of *Coleus forskohlii* reveals the presence of tannin, flavonoids, terpenoids, alkaloids, cardiac glycosides, coumarin and steroids. The different acetone extract of *Coleus forskohlii* were prepared 40mg/ml, 80mg/ml, 120mg/ml, 160mg/ml, and Cyclophasphamide (Positive control) 180 µg/ml and their activity were determined by MTT Assay using Hep G2 Cell line (liver).Among these concentration the maximum anticancerous activity were observed at 120 mg/ml and it determined by using MTT Assay.
KEYWORDS - *Coleus forskohlii*, Phytochemicals, MTT assay, Anticancerous activity.

INTRODUCTION

Herbs are widely exploited in the traditional medicine and their curative potentials are well documented **(Dubey et al., 2004).** About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful especially in the areas of infectious disease and cancer **(Cragg, 2005).** Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. In many developing countries, traditional medicine is one of the primary health care systems (Houghton, 1995).

Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action **(Runyoro et al., 2006; Shahidi et al., 2004).** The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world **(Reddy, 2001).**
Coleus forskohlii belonging to Lamiaceae family (Mint family) has been selected. The leaves of the green type of country borage are often eaten raw with bread and butter. The chopped leaves are also used as substitute for sage (Salvia officinalis L) in stuffing. Coleus forskohlii is used for seasoning meat dishes and in food products, while a decoction of its leaves is administered in cases of chronic cough and asthma **(Kusumoto et al., 1995).** It is considered to be an antispasmodic, stimulant and stomachic and is used for the treatment of headache, fever, epilepsy and dyspepsia. It is used to treat conditions such as indigestion, diarrhea, nervous tension, insect bites, toothache, earache, rheumatism, whooping cough, and bronchitis **(Warrier et al., 1995).**
Nearly 25,000 effective plant based formulations are used in folk medicine by rural communities in India **(Ramakrishnappa et al., 2002).** Both plant species and traditional knowledge are important to the herbal medicine trade and the pharmaceutical industry, whereby plants provide raw materials and the traditional knowledge prerequisite information **(Tabuti et al., 2003).** The present study aims to investigate the presence of phytochemicals and anticancerous activity of *Coleus forskohlii* rhizome extract.

2. MATERIALS AND METHODS

Collection of Plant material

The rhizome of *Coleus forskohlii* was collected from Chennai, Tamil Nadu. And it was washed thoroughly in distilled water to remove to dirt and air dried.

Preparation of plant powder

The air dried and powdered roots were extracted with solvent like acetone and aqueous extract using a mortar and pestle. The extraction mixture was filtered through Whatman No.1 filter paper. The extract was evaporated under reduced pressure at 50° C using a rota vapour apparatus. Finally 10 mg of residue was dissolved in 10 ml of each solvent and these extract used for anticancerous activity.

Phytochemical Screening

Phytochemical screening for tannins, flavonoids, terpenoids, alkaloids, glycosides, cardiac glycosides**,** coumarins and steroids were carried out as described below.

1. Test for tannins

About 0.5 g of plant rhizome extract was boiled in 20 ml of distilled water in a test tube and then filtered. 0.1% FeCl3 was added to the filtrate. Appearance of brownish green or blue black coloration showed the presence of tannins.

2. Test for flavonoids

5 ml of dilute ammonia solution were added to a portion of the extract followed by addition of concentrated H_2SO_4.Formation of yellow colour indicates the presence of flavonoids.

3. Test for Terpenoids

To 0.5 ml of the rhizome extract, 2 ml of chloroform was added and concentrated Sulphuric acid was added carefully. Formation of red brown colour at the interface indicates the presence of Terpenoids.

4. Test for alkaloids

Plant extract (0.5 to 0.6 g) was mixed with about 8 ml of 1% HCl, warmed and filtered. 2 ml of filtrate were treated separately with Mayer's reagent / Dragendorff's reagent. Turbidity or precipitation was observed to indicate the presence of alkaloids.

5. Test for Glycosides

To 2ml of the rhizome extract, 3ml of chloroform and 10% ammonium solution was added. Formation of pink colour indicates the presence of glycosides.

6. Test for cardiac glycosides

To 0.5 ml of the rhizome extract, 2 ml of glacial acetic acid and few drops of 5 % ferric chloride were added. This under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at interface indicates the presence of cardiac glycosides.

7. Test for coumarins

Moistened plant rhizome extract (0.5 g) was taken in a small test tube and covered with filter paper moistened with 1 N NaOH. The test tube was placed for few minutes in boiling water. Then the filter paper was removed and examined in UV light for yellow florescence to indicate the presence of coumarins.

8. Test for Steriods

To 0.5 ml of the rhizome extract, 2 ml of chloroform and 1 ml of Sulphuric acid were added. Formation of reddish brown ring at interface indicates the presence of steroids.

Anticancer activity from rhizome extracts of *Coleus forskohlii*

Cell line and culture

Hep G2 cell line (Liver) was obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS (Foetal Bovine serum), penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

Reagents

MEM was purchased from Hi Media Laboratories, Foetal bovine serum (FBS) was purchased from Cistron laboratories. Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT), and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

In vitro assay for Cytotoxicity activity (MTT assay)

The Cytotoxicity of samples on Hep G2 cell line (Liver) was determined by the MTT assay (*Mosmann et al*., 1983). Cells (1×10^5/well) were plated in 1ml of medium/well in 24-well plates (Costar Corning, Rochester,NY). After 24 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 24h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 200µl/well (5mg/ml) of 0.5% 3-

(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide cells (MTT) phosphate -buffered saline solution was added. After 4h incubation, 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The absorbance at 570 nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of Hep G2 cell line (Liver) was expressed as the % cell viability, using the following formula:

% cell viability = A570 of treated cells / A570 of control cells × 100

3. RESULTS AND DISCUSSION

Preparation of plant material

The *Coleus forskohlii* rhizome materials were collected and extracted with acetone and aqueous for the analysis.

Preparation of rhizome powder

The dried and powdered roots were extracted with acetone and aqueous extract.

Phytochemical analysis

The phytochemical analysis revealed the presence of tannin, flavonoids, terpenoids, alkaloids, cardiac glycosides, coumarin and steroids (Table 1).

Table 1: Phytochemical analysis from rhizome extract of *Coleus forskohlii*

Phytochemical composition	Rhizome extract	
	Acetone	Aqueous
Tannins	+	-
Flavonoids	+	-
Terpenoids	+	+
Alkaloids	+	-
Glycosides	+	-
Cardiac glycosides	+	+
Coumarins	+	-
Steroids	+	+

IN VITRO Assays (Cytotoxic studies)

MTT Assay

The IC50 value for Hep G2 cell line (Liver) (120 mg/ml) acetone rhizome extract was found to be effective, the reduction percentage of MTT at 24Hrs also estimated for Hep G2 cell line (Liver). When incubated with the extract, it induced cytotoxicity in a significant manner which implicit the damage to the member integrity of the cell when contributed with control. The cytotoxicity was minimized in the extract treated cells and near normal level was attained at various concentrations (40mg/ml, 80 mg/ml, 120mg/ml and 160mg/ml) and maximum effect was found when treated at 120 mg/ml(Table 2, Fig 1). From the above results, it was confirmed that *Coleus forskohlii* Acetone rhizome extract at 120 mg/ml seems to offer significant protection and maintain the structural integrity of the hepatocellular membrane and this active concentration was followed for further studies.

Tryphan Blue assay

Tryphan blue is one of the several stains recommended for use in dye exclusion procedure for viable cell counting. This assay is based on the principle that live cells do not take up blue, where as dead cells do and appear as blue under microscope - depicts the viability of cells by Tryphan Blue assay. The

viability is measured in terms of percentage was found to decreased 98% in drug treated hepatic cell line (Table 3, Fig 2). The cell treated with *Coleus forskohlii* Acetone rhizome extract at various concentrations (40mg/ml, 80 mg/ml, 120mg/ml and 160mg/ml) showed protective nature of the extract act against the deleterious effects and the maximum effect was observed at 120 mg/ml (Table 4, Fig 3).

Table 2: MTT reduction % on Hep G2 cell line (Liver)

S.No	Concentration(µg)	MTT reduction (%)
1	Control	98.17
2	400	84.12
3	80	71.47
4	**120**	**46.3**
5	160	22.67
6	Positive Control	11.13

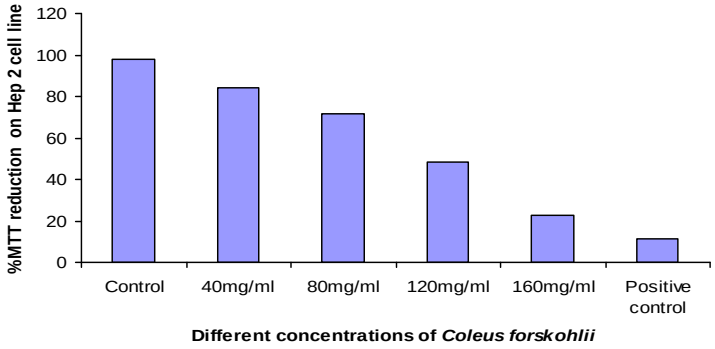

Fig 1: Showing the graphical representation of MTT reduction % on Hep G2 cell line (Liver) at different concentrations of extract.

Table 3: Tryphan blue viability assay

S.No	Concentration(µg)	% Viability
1	Control	99.0
2	40	87.0
3	80	71.3
4	**120**	**51.4**
5	160	36.3
6	Positive control	10.67

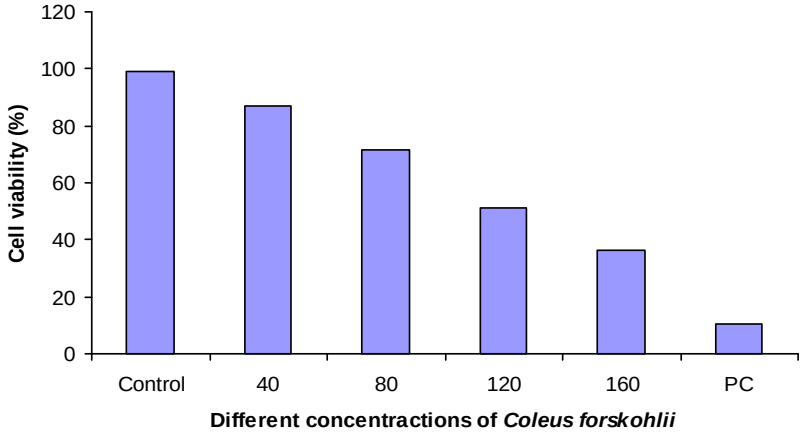

Fig 2: Showing the graphical representation of Cell viability (%) on Hep G2 cell line (Liver) at different concentrations of extract.

Table 4: Showing the IC50 value of cell line

S.No	Cell line	IC50 Value
1	**Hep G2 cell line (Liver)** Acetone rhizome extract of *Coleus forskohlii*	120 mg/ml

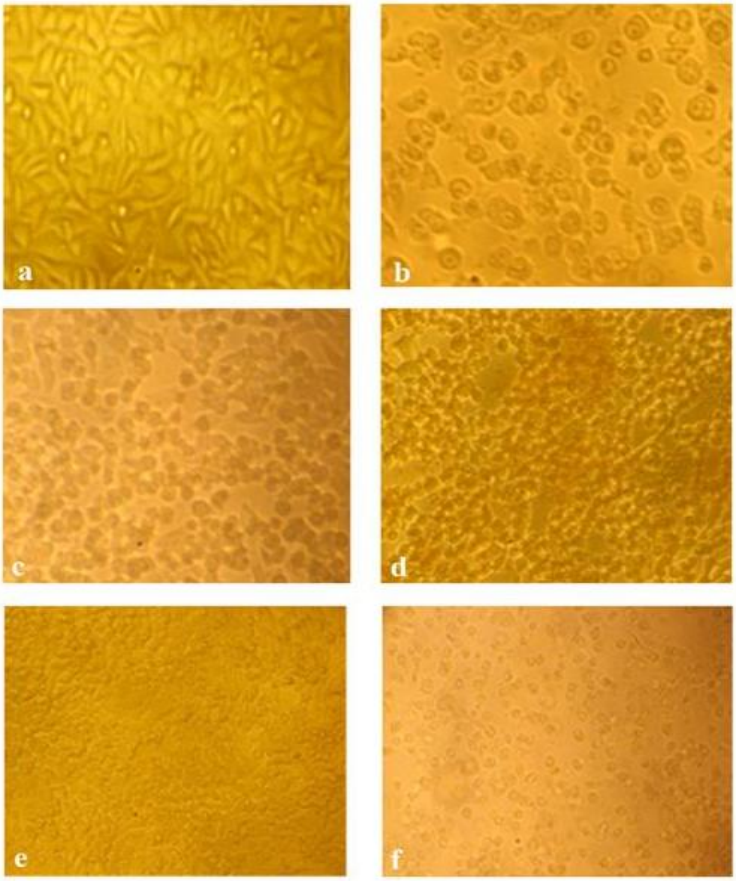

Fig 3: Showing the effect Acetone rhizome extract on Hep G2 cell line

a: Control cells (Untreated), **b:** Acetone rhizome extract 40mg/ml, **c:** Acetone rhizome extract 80 mg/ml, **d:** Acetone rhizome extract 120mg/ml, **e:** Acetone rhizome extract 160 mg/ml, **f:** Cyclophasphamide (Positive control) 180 µg/ml.

4. CONCLUSION

In the present study the phytochemical analysis of *Coleus forskohlii* reveals, the presence of tannin, flavonoids, terpenoids, alkaloids, cardiac glycosides, coumarin and steroids. MTT Assay, using Hep G2 Cell line (liver) at 120 mg/ml acetone rhizome extract was found to be effective, the reduction percentage of MTT and cytotoxicity were also determined.

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7. REFERENCES

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1. Cragg GM, Newman DJ (2005). Biodiversity: a continuing source of novel drug leads. *Pure Appl Chem* **77**: 7–24.

2. Dubey NK, Kumar R, Tripathi P (2004). Global promotion of herbal medicines: India's opportunity. *Current Science* **86:** 37- 41.

3. Houghton PJ (1995). The role of plants in traditional medicine and current therapy. *J Altern and Compl Med* **1:** 131- 143.

4. Kusumoto IT, Nakabayashi T, Kida H (1995). Screening of various plant extracts used in ayurvedic medicine for inhibitory effects on human immunodeficiency virus type 1 (HIV- 1) protease. *Phytotherapy Res* **9:** 180- 184.

5. Ramakrishnappa K. (2002) Impact of cultivation and gathering of medicinal plants on biodiversity: Case studies from India, In: *Biodiversity and the Ecosystems Approach in Agriculture, Forestry and Fisheries (Online)*, FAO.

6. Reddy PS, Jamil K, Madhusudhan P (2001). Antibacterial activity of isolates from Piper longum and Taxus baccata. *Pharmaceutical Biol* **39:** 236- 238.

7. Runyoro D, Matee M, Olipa N, Joseph C, Mbwambo H (2006). Screening of Tanzanian medicinal plants for anti- Candida activity. *BMC Complement Altern Med* **6**(11).

8. Shahidi BH (2004). Evaluation of antimicrobial properties of Iranian medicinal plants against Micrococcus luteus, Serratia marcescens, Klebsiella pneumoniae and Bordetella bronchoseptica. *Asian J Plant Sci* **3**: 82-86.

9. Tabuti JRS, Lye KA, Dhillon SS. (2003) Traditional herbal drugs of Bulamogi, Uganda: Plants, use and administration. *J. Ethnopharmacol*, **88,** 19-44.

10. Warrier PK, Nambiar VP, Ramankutty (1995). Indian medicinal plants. 1 st Ed, *Orient longman limited, Madras*. 315.

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STUDIES ON PHYSICO – CHEMICAL PROPERTIES, NODULATION PATTERN AND RHIZOBIAL POPULATION OF BLACK GRAM CULTIVATED SOIL

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ABSTRACT

Black gram (*Vigna mungo* L.) is one of the important pulse crops gaining importance all over the world in recent years. It is rich in proteins and contains amino acids higher quantities than any other cereals and pulses. Black gram is an annual food legume. Black gram seeds are boiled and eaten whole or after splitting into dhal. The dried seeds contain approximately 9.7 % water, 23.4 % protein, 1 % fat, 57.3 % carbohydrate, 3.8 % fibre and 4.8 % ash. It is very nutritious and is recommended for diabetics. The nutrient status of soil is more essential for root nodulation. The black gram soil samples were collected from Nagapattinam and Cuddalore belonged to 3 textural group viz., Red loam, Clay loam and Block soils. The soil samples were analysed for its pH, Electrical conductivity, soil organic carbon availabe N, P and K content (Tab-1). The nodulation pattern and its rhizobial population on the rhizosphere soil of black gram plants were also studied. The nodulation pattern was ranged from 5.00 to 21.00 plants (Tab-2). Native rhizobial populations (Tab-3) were also estimated for the 30 locations from the above mentioned Districts of saline areas in Tamil Nadu. Among the 30 locations , 13 locations were recorded more than 3×10^3 g^{-1}.

Key words*:* Physico-chemical, nodulation, rhizobial population, Black gram, Saline areas.

INTRODUCTION

Black gram is a member of the *Vigna* Asiatic crop group. It is an annual pulse grown mostly as a follow crop in rotation with cereals. Similar to the pulses, black gram, being a legume, it enriches the soil nitrogen content and has relatively a short (90-120 days) duration. It is widely cultivated in India, Pakistan and other Asian countries. It is part of diet for millions of people in these countries and a cheap source of protein with 17-34% of protein in seeds (Gour, 1993). Black gram is very responsive to in organic nutrition. Some bacterial strains are also considered to be very effective in augmenting the growth and yield of black gram. Black gram is one of the important pulse crops in India. It has been reported that black gram has been cultivated in India since ancient times. It disbelieved that black gram is a native of India and Central Asia and grown in these regions since prehistoric times. It is widely cultivated throughout the Asia, including India, Pakistan, Bangladesh, Sri Lanka, Thailand, Laos, Cambodia, Vietnam, Indonesia, Malaysia, south China, and Formosa. In Africa and U.S.A. it is probably recent. Black gram is a protein rich staple food. It contains about 25 percent protein, which is almost three times that of cereals. It supplies protein requirement of vegetarian population of the country. Black gram is a protein rich staple food. It contains about 25 per cent protein, which is almost three times that of cereals. It supplies protein requirement of vegetarian population of the country. It is consumed in the form of split pulse as well as whole pulse, which is an essential supplement of cereal based diet. (Sureshkumar *et al*., 2011 and Kanchana *et al*., 2013). The moong dal Khichdi is recommended to the ill or aged person as it is easily digestible and considered as complete diet. Roti with Moong dal and Moong dal chawal is an important ingredient in the average Indian diet.

The environment has long been known to influence symbiotic nitrogen fixation. The clear sensitivity of nitrogen fixation to water availability (Vincent 1965) raises serious questions about the potential contribution of legumes to the nitrogen economy of semiarid grasslands. Vincent (1965) reviewed the subject of micro-environmental effects on nitrogen fixation by legumes and reported inhibition of nodule formation and nitrogen fixation by combined nitrogen and stimulation of bacterial populations, nodule formation, and nodule functioning with increased soil water availability. The delicate balance between the host plant and the symbiont is disturbed even by mildly adverse conditions that have no effect on plant growth supported by soil nitrogen. Salt stress is one of the major types of environmental stress adversely affecting legume production in arid and semi- arid regions, particularly because these plants depend on symbiotic N_2 fixation for their nitrogen requirements (El- sheikh and Wood 1995). High soil salinity can limit productivity by adversely affecting the growth of the host plant, the symbiotic development of root-nodule bacteria. Several workers have also observed that salinity increases oxygen diffusion resistance in legume nodules (James *et al*, 1993; Georgiev and Atkins, 1993; Serraj *et al*, 1994) and, finally, the nitrogen-fixation capacity (Delgado *et al*., 1993). In addition to these physiological responses, saline stress accelerates greening of the nodules and lowers the leg hemo-globin content (Delgado *et al.* 1993), which is considered an index of nodule senescence. This process usually involves increased production of toxic-activated oxygen species such as H_20_2, 0_2, and OH (Becana *et al.*, 1986). India's national program for organic production (NPOP) requires atleast a 2 years pose many challenges, because the changes in the chemical, physical and biological properties of the soil take time to reach an ecological balance (Willer and Yussefi, 2005).

In legumes, salt stress can limit plant growth and available photosynthates (Brignoli and Lauteri, 1991; Georgiev and Atkins, 1993), reduce nodulation (Sprent and Zahran, 1988; Qifu and Murray, 1993) and decrease nodule N2 fixation and respiration (Georgiev and Atkins, 1993; Delgado *et al*, 1993, 1994; Serraj *et al*, 1994). In the present study, we choose to examine the individual bacterial species, *Rhizobium leguminosarum* by *trifolii*. Despite being recognized primariliy its ability to form symbiotic associations with species of *Trifolium*, the bacterium is a succesfull soil saprophyte with the ability to support soil population ranging from 10^8 cell g^{-1} in rhizosphere to 10 cells g^{-1} in the prolonged absence of the host plant. It has been recognized for many years that plant rhizosphere is a zone where microbial activity is greater than in bulk soil and that legume rhizospheres are particularly active (Bottomley, 1992). Like most legumes, soyabeans performs N_2 fixation by establishing a symbiotic relationship with the *Rhizobia.* Numerous abiotic and biotic factors are known to influence the competitiveness of specific rhizobial inoculants. (Bottomley, 1992; Turco and Sadowsky, 1995). Semi Arid Tropics (SAT) regions spread over 11.6 million square kilometers in the developing world, and they did not benefit greatly from the green revolution and there is a need for "Grey to Green Revolution" in the SAT to feed and provide proper nourishment to the ever increasing population of the developing world (Singh, *et al.*, 1999). The crop growing environment in the semiarid tropics is highly variable due to erratic spacing and timing of season rainfall. High yields of top quality crops require an abundant supply of nutrients. Soil is the best source for the most of the essential nutrients required by the crop. So soil analysis became important criteria to be done before cultivation. The present study aims to analyze physico-chemical properties of soil, existing rhizobial population and nodulation pattern of black gram cultivated soil.

MATERIALS AND METHODS

Soil Analysis (pH)

Soil pH was determined in Elico Model LT-10T pH meter by preparing 1:2:5 soil: water suspension and stirring by means of a glass rod (Jackson 1973).

Estimation of Electrical Conductivity (EC)

Soil suspension was prepared and used to determine the EC using Conductivity Bridge and expressed in dsm^{-1} (Jackson, 1973).

Estimation of Organic Carbon

The organic carbon content of the soil sample was estimated by Walkely and Black method (1947). One gram of finely ground soil sample was transferred to 500 ml conical flask to which 10 ml of 1N $K_2Cr_2O_7$ solution and 200 ml of concentrated H_2SO_4 were added and allowed to stand for 30 min. After 30 minutes, 10 ml of NaF solution and 2ml of diphenylamine indicator were added. The solution was titrated with the standard $FeSO_4$ solution to brilliant green colour from dark blue colour. A blank without soil was run simultaneously; organic carbon in the soil was calculated as per the following formula.

Determining P in Soil by Ascorbic acid method

The method was found to be accurate for determining P in soil extracts. This method is based on reduction of the ammonium molybdiphosphate complex by ascorbic acid in the presence of antimony. The color produced is stable for 24 hours. It is less subject to interfering substances than are methods involving reduction by $SnCl_2$. A loopful of fresh bacterial culture was grown in Pikovskaya broth at 30°C for 10 days. After 3 h incubation in the dark at 30°C, the absorbance at 630 nm by calorimetrically was recorded with non-inoculated supernatant as reference. The strain was analysed for inorganic phosphate solubilization using the ascorbic acid method (Watanabe and Olsen, 1965).

Estimation of N in soil available nutrients

Available nitrogen was estimated by Alkaline permanganate method (Subbiah and Asija, 1956). Subbiah and Asija (1956) and modified by Stanford (1978), has been the most preferred method of estimating available N in soils, including the acidic soils which are mostly concentrated in the north-eastern region of India. It may therefore be hypothesized that the most commonly used alkaline permanganate method may not really be giving a reliable assessment of N availability in acidic soils of India and other regions with similar soils.

Table 1:
Physico-chemical characteristics of black gram soil samples of saline Tropics of Tamil Nadu

S. No	Location	Soil Type	Soil pH	EC (dsm^{-1})	Organic Carbon (%)	N kg/ha^{-1}	P kg/ha^{-1}	K kg/ha^{-1}
1	Konayampatinnam	Red loam	7.9	0.12	0.62	98.65	17.20	158.50
2	Poompuhar	Red loam	7.80	0.17	0.73	97.35	17.80	180.00
3	Mangai madam	Red loam	7.79	1.00	0.58	126.00	18.00	98.00
4	Thiruvargadu	Red loam	6.64	0.30	0.59	68.00	17.00	77.00
5	Melaiyur	Red loam	6.84	0.39	0.62	67.00	12.00	178.2
6	Poraiyar	Red loam	6.89	0.86	0.68	100.50	11.00	130.10
7	Tharangampadi	Red loam	6.89	0.50	0.70	68.00	11.00	169.85

8	Neithavasal	Red loam	8.15	0.39	0.62	67.00	13.00	178.25
9	Peruthottam	Red loam	7.10	0.16	0.72	96.35	17.00	184.00
10	Anna panpattai	Red loam	8.41	0.69	0.55	128.00	11.50	116.00
11	Kuravallur	Loam	7.14	0.88	0.72	122.00	12.00	145.10
12	Semmangudi	Clay loam	7.40	1.75	0.67	100.50	11.00	98.00
13	Kadavasal	Red loam	6.90	1.70	0.57	11.50	11.89	100.00
14	Edamanal	Red loam	8.30	1.00	0.70	98.00	11.00	152.70
15	Varusapatu	Red loam	7.80	0.85	0.69	114.00	13.80	127.10
16	Pichavaram	Red loam	7.80	1.69	0.56	117.00	9.00	97.00
17	Killai	Clay loam	8.19	0.10	0.85	112.0	14.00	140.00
18	C.Mutlur	Clay loam	7,59	1.38	0.79	110.00	14.89	119.00
19	Vallampadugai	Clay loam	7.00	0.90	0.88	138.80	9.00	199.00
20	Sivapuri	Red loam	7.12	1.30	0.89	110.25	11.85	120.00
21	Portonova	Red loam	8.61	1.28	0.88	100.20	17.89	117.00
22	Sethiathope	Red loam	7.10	0.16	0.64	98.14	17.00	189.30
23	Vadalur	Clay loam	7.60	0.98	0.69	100.20	11.85	130.00
24	Anna malainagar	Black loam	7.89	0.80	0.49	113.35	17.89	127.00
25	Pinnathur	Red loam	8.34	0.80	0.59	95.17	18.00	118.00
26	Devanampattinam	Clay	8.33	0.90	0.50	98.15	9.00	128.00
27	Nellikupam	Red loam	8.32	0.16	0.65	75.10	18.00	170.00
28	Pundiakupam	Clay loam	8.30	0.17	0/66	68.20	16.10	160.00
29	Samiar patt	Red loam	8.23	0.55	75.10	18.00	190.00	175.4
30	Allivilagam	Clay loam	6.90	0.41	0.66	68.20	16.10	170.38

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Table 2:
Survey of the Nodulation pattern of black gram in Saline area of Tamil Nadu

S. No	Locations	No. of green Nodules	No. of pink Nodules	Total No. of Nodules
1	Konayampatinnam	4	5	9.00
2	Poompuhar	7	5	12.00
3	Mangaimadam	3	4	7.00
4	Thiruvargadu	4	7	11.00
5	Melaiyur	5	7	12.00
6	Poraiyar	2	5	7.00
7	Tharangampadi	3	10	13.00
8	Neithavasal	7	3	10.00
9	Peruthottam	12	4	16.00
10	Annapanpattai	7	4	11.00
11	Kuravallur	3	4	7.00
12	Semmangudi	6	8	14.00
13	Kadavasal	8	4	12.00
14	Edamanal	4	8	12.00
15	Varusapatu	5	8	13
16	Pichavaram	3	2	5.00
17	Killai	2	4	6.00
18	C.Mutlur	3	5	8.00
19	Vallampadugai	14	7	21.00
20	Sivapuri	2	5	7.00
21	Portonova	2	6	9.00
22	Sethiathope	10	9	19
23	Vadalur	7	10	17.00
24	Annamalainagar	4	5	8.00
25	Pinnathur	4	6	10.00
26	Devanampattinam	3	7	10.00
27	Nellikupam	2	5	7.00
28	Pundiakupam	3	5	8.00
29	Samiar patt	2	5	7.00
30	Allivilagam	4	5	9.00

Organic Carbon Percent = 10/S (S-T) × 0.003 × 100 / Weight of Soil × M.F

Where,
S = ml $FeSO_4$ solution required for blank
T = ml $FeSO_4$ solution required for sample
M.F = Moisture percent factor of the soil sample

Table 3:
Native populations of Rhizobium in black gram fields of thirty different locations of saline area of Tamil Nadu

S. No	Locations	Populations 1×10^3/g of moisture free soil
1	Konayampattinam	4.10
2	Poompuhar	5.10
3	Mangaimadam	2.98
4	Thiruvengadu	4.80
5	Melaiyur	5.80
6	Poraiyar	2.25
7	Tharangampadi	6.85
8	Neithavasal	6.80
9	Peruthottam	6.79
10	Annapanpattai	6.20
11	Kuravallur	2.80
12	Semmangudi	5.72
13	Kadavasal	0.81
14	Edamanal	7.10
15	Varusapat	5.20
16	Pichavaram	0.65
17	Killai	0.97
18	C.Mutlur	1.20
19	Vallampadugai	8.90
20	Sivapuri	1.25
21	Portonova	1.85
22	Sethiathope	7.19
23	Vadalur	6.84
24	Annamalainagar	2.20
25	Pinnathur	2.52
26	Devanampattinam	2.25
27	Nellikupam	1.75
28	Pundiakupam	1.25
29	Samiar patti	0.98
30	Allivilagam	2.15

Determination of Rhizobial population

The rhizosphere soil samples from different locations were collected; 10g of the soil samples were serially diffused upto 10-16 dilution and 1ml were transferred to sterile YEMA petriplates. The plates were incubated at room temperature and the colonies were counted by using colony counter.

Nodulation pattern of black gram

The nodulation pattern of the green gram is not uniform, so the green gram plants were collected from each location at random without damaging the roots. The averages of ten plants were taken to give the nodulation pattern of that place. The total number of nodules as well as the number of pink and white nodules were counted and recorded.

RESULTS AND DISCUSSION

In the present study of soil samples were collected from 30 different saline area locations of 2 districts of Tamil nadu, namely Nagapattinam, Cuddalore and they were subjected to physio-chemical analysis. In our findings most of the soils from these regions were belonged to 3 textural groups, namely Red loam, Clay loam and Black soils. Soil pH ranged from 6.65-7.85, EC ranged from 0.10-1.75 dsm^{-1}. The nutrient content of the soil is most important for root nodulation and nitrogen fixation. In our study, soil organic carbon contents ranged from 67.00-138.00 kg ha^{-1}, phosphorous and potassium contents ranged from 9.00-19.00, 77-200.00 kg ha-1 EC respectively and concluded that organic carbon N, P and K status of saline areas were low. This was proved by earlier workers that the wide variation in rhizobial cell counts and nodule mass in green gram are relapsed to variation in physio- chemical characteristics of the soil. The organic matter content, salinity, texture and pH were found to affect mycorrhizal development in soils (Saif *et al.*, 1975).

The nodulation pattern of black gram and native rhizobial populations of the above 30 locations were also studied. Among the 30 locations, 4 locations were recorded less than 1×10^3 g^{-1}, 13 locations were recorded 1×10^3 to 3×10^3 g^{-1} and 13 locations recorded more than 3×103 g^{-1} of rhizobial populations from the rhizosphere of green gram plants. The number of nodules / plant was also recorded. The total number of nodules ranged from 5.00 to 21.00.$plant^{-1}$. Vallampadugai location recorded highest number of nodules of 21.00 $Plant^{-1}$. The lowest nodules number was recorded in Pichavaram i.e. 5.00 $Plant^{-1}$ in Cuddalore District.

REFERENCE

Becana M, Aparicio-Tejo P, Irigoyen JJ, Sanchez-Diaz M (1986). Some enzymes of hydrogen peroxide metabolism in leaves and root nodules of *Medicago sativa.* Plant Physiol. 82: 1169-1171.

Bottomley**,** P.J. 1992. Ecology of *Bradyrhizobium* and *Rhizobium*. In: Stacey, G., Burris, R.H. & Evarts, H.J. editors, Biological Nitrogen fixation, New York: Chapman and Hall. pp. 293-348.

ISBN 0-41202421-7. Cultivations as oilseed crop in China. *J. Agric. Sci.,* 28: 281-283.

Brignoli E, Lauteri M. 1991. Effects of salinity on stomatal conductance, photosynthetic capacity, and carbon isotope discrimination of salt-tolerant (*Gossypium hirsutum* L.) and salt-sensitive (*Phaseolus vulgaris* L.) C_3 non-halophytes. *Plant Physiology* 95, 628-35.

Delgado MJ, Garrido JM, Ligero F, Lluch C. 1993. Nitrogen fixation and carbon metabolism by nodules and bacteroids of pea plants under sodium chloride stress. *Physiologia Plantarum* 89, 824-9.

Delgado MJ, Ligero F, Lluch C. 1994. Effect of salt stress on growth and nitrogen fixation by pea, Faba-bean, common bean and soybean plants. *Soil Biology and Biochemistry* 26, 371-6.

Elsheikh, E.A.E. and Wood, M. (1995) "Nodulation and N_2 fixation by soybean inoculated with salt-tolerant rhizobia or salt-sensitive Bradyrhizobia in saline soil", *Soil Biol. Biochem*., 27, 657-661.

Georgiev GI, Atkins CA. 1993. Effects of salinity on N_2 fixation, nitrogen metabolism and export and diffusive conductance of cowpea root nodules. *Symbiosis* 15, 239-55.

Gour, Y.D., 1993. Microbiology, physiology and agronomy of nitrogen fixation: Legume-*Rhizobium* symbiosis. Proc. Indian Nat. Sci. Acad., B(59): 333-358.

Jackson, M.L. 1973. Soil chemical analysis. Prentice-Hall of Indian Pvt. Ltd., New Delhi: India

James EK, Sprent JI, Hay GT, Minchin FR. 1993. The effect of irradiance on the recovery of soybean nodules from sodium chloride induced senescence. *Journal of Experimental Botany AA*, 997-1005

Kanchana, D., M. Jayanthi, G. Usharani, P. Saranraj and D. Sujitha. 2013. Prevelence of *Azotobacter* sp. in Chilli (*Capsicum annuum* L.) Rhizosphere Soil of Cuddalore District, Tamil Nadu, India. *International Journal of Microbiological Research*, 4(3): 296 - 299.

Quifu MA, Murray AF. 1993. Effects of SO2 and salinity on nitrogenase activity, nitrogen concentration and growth of young soybean plants. *Environmental and Experimental Botany* 33, 529-37.

Saif. S.R., Sheikh, M.A. and Khan, A.G. 1975. Ecology of endogone. I. Relationship of Endogone Spore Populations with physical soil factors. *Islambad. J. Sci*., 2: 1-5.

Serraj R, Roy G, Drevon JJ. 1994. Salt stress induces a decrease in the oxygen uptake of soybean nodules and their permeability to oxygen diffusion. *Physiologia Plantarum* 91, 161-8.

Singh, H.P., Sharma, K.L., and Venkateswarlu, B., 1999. Fertilizer use in rainfed areas. *Fert. News* 44: 27-30 & 33-38.

Sprent JI, Zahran HH. 1988. Infection, development and functioning of nodules under drought and salinity. In: Beck DP, Materon LA, eds. *Nitrogen fixation by legumes in Mediterranean agriculture.* Dordrecht: Martinus Nijhoff, 145-51.

Stanford G. 1978. Evaluation of ammonium release by alkaline permanganate extraction as an index of soil nitrogen availability. Soil Science, *126:* 244–253.

Subbiah, B.V. and C.V. Asija. 1956. A rapid, procedure for estimation of available nitrogen in soil. *Curr. Sci.,* 25: 259-260.

Suresh Kumar, R., P. Ganesh, K. Tharmaraj and P. Saranraj. 2011. Growth and development of black gram (*Vigna mungo. L*) under foliar application of Panchagavya as organic source of nutrient. *Current Botany*, 2 (3): 9 – 11.

Turco, R.F. and Sadowsky, M.J. 1995. The microflora of Micromediation, In: Bioremediation: Science and Applications, Soil Science Special Publication No. 43 eds Skipper, H.D. and Turco, R.F. PP. 87-102.

Vincent, J.M. 1965. Environmental factors in the fixation of nitrogen by the legume In: Soil nitrogen. Eds. W.V. Bartholomas and F.E. Clark. Pp. 384-435. *Am.Soc. Agronomy Inc. Publ. Madison*, USA.

Walkey A. and Black, 1.4.1947. Chronic acid titration method for determination of soil organic matter. *Soil. Sci.,* 63:251

Watanabe, I. and Olsen, S.R. 1965. Test of an ascorbic acid method for determining P in H_2O and $NaHCO_3$ extracts from the soil. *Soil. Sci. Soc. Am. Proc.*, 29: 678-679

Willer H, Yussefi, M. 2005. The world of organic agriculture statistics and emerging trends 2005. International federation of organic agriculture movements (IFOAM), Bonn, Germany.

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Evaluation of phytochemical screening and *in vitro* bioactivity of leaf and stem extracts of *Solanum xanthocarpum* (Solanaceae)

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Abstract

The present study was carried out for phytochemical screening of principle bioactive compounds and antibacterial activity in *Solanum xanthocarpum* Schrad and Wendl. Phytochemical analysis revealed the presence of Alkaloids, Flavonoids, Phenols, Phlobatannins, Saponins, Steroid, Tannins and Triterpenoides. The ethanol and acetone extracts were subjected for antibacterial activity against nine bacterial strains using agar well diffusion method. Ethanol extracts of Leaf inhibited *Bacillus* sp, *E. faecalis, K. pneumoniae*, *Micrococcus sp*, *P. mirabilis* and *S. epidermidis*. Acetone extracts of stem possessed antimicrobial activity against *Bacillus* sp, *K. pneumonia*, *Micrococcus sp*, *P. mirabilis*, *P.aeruginosa, S. aureus* and *S. epidermidis*. In stem extracts, the ethanol extracts exhibit anti bacterial activity against *Bacillus* sp, *E. coli*, *K. pneumonia*, *Micrococcus sp* and *P. mirabilis.* The acetone extracts of stem gave antibacterial activity against *Bacillus* sp, *E. faecalis, E. coli, K. pneumonia, Micrococcus sp, P. mirabilis* and *S. epidermidis. K. pneumoniae* showed significant sensitivity to both leaf and stem extracts. The results suggested that ethanol and acetone extracts of leaf and stem extracts were highly potent against *K. pneumoniae* and *P. mirabilis* can be used in treatment of nosocomial infections such as pneumonia, urinary tract infections (UTIs) and bacteremia. Extensive animal studies may be required before investigating the role of *Solanum xanthocarpum* for treating RTI and UTI

Keywords: *Solanum xanthocarpum,* Phytochemical analysis, antibacterial activity, agar well diffusion method.

Introduction

Solanum xanthocarpum (Solanaceae) smoke is inhaled through mouth to led through mouth to treat toothache has profound use in Ayurveda and folkore medicine. It is supposed that the plant has solasonine and solasomargine (Yoshida *et al*., 2006) sapogenins (Khanna *et al*., 1976) and solasodine (Oudhia, and Kadu Pani, 2007) which are responsible for medicinal effect. The whole plant extracts of S. xanthocarpum have a larvicidal defect (Rajkumar, and Jebanesan, 2005) hypoglycemic activity (Kar *et al*., 2006) bronchitis and antitussive response (Govindan *et al*., 1999). Dried or fresh fruits are kept in fire and smoke is inhaled through mouth to led through mouth to treat toothache. The present investigation is aim to focus the phytochemicals of *Solanum xanthocarpum* leaf and stem and its antibacterial action against Gram-positive and Gram-negative bacteria.

Materials and methods

Leaves and Stems of *S. xanthocarpum* were collected from the Medicinal plant garden, Idaya College of Arts and Science, Tiruvannamalai, Tamil nadu, India.
The plants were dried and broken into small pieces under sterile conditions, and 20 g of each plant part were extracted with 100 ml of ethanol and acetone solvent (Merck, Darmstadt) for 24 h by using Soxhlet equipment (Bradshaw, 1992).
Phytochemical tests of each extracts were carried out to establish the presence of Alkaloids, Flavonoids, Phenols, Phlobatannins, Saponins, Steroid, Tannins and Triterpenoides using standard procedure (Jigna *et al*., 2006).

Antibacterial activity of ethanol and acetone extracts (25, 50, 75 and 100 µl/l) of leaf and stem were tested against Gram positive and Gram negative bacteria like *Bacillus* sp, *Enterococcus faecalis, Escherchia coli, Klebsiella pneumonia, Micrococcus sp, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus* and *S. epidermidis* by using the method of agar well diffusion method (Perez *et al*.,1990).

Results and discussion

Table 1 shows the results of the preliminary phytochemical analyses of the different leave and stem extracts of *S. xanthocarpum.*

Table 1: Phytochemical constituents of *S. xanthocarpum* Leaf and stem extract

Phytochemical	Leaf extract		Stem extract	
	Ethanol	Acetone	Ethanol	Acetone
Alkaloids	+ +	+ +	+	+
Flavonoids	+ +	+ +	+	+ +
Phenols	-	-	+	-
Phlobatannins	+	-	-	+
Saponins	-	+ +	+	-
Steroid	+	-	-	-

Antimicrobial activity recorded in terms of average zones of inhibition in millimeter (mm) is reported in Table 2 and 3. The leaf extractives showed a range of activity against all the tested bacteria. The ethanolic extracts of *S. xanthocarpum* exhibited maximum activity against the bacteria compared to acetone extracts. The extracts failed to exhibit any significant anti-bacterial activity against on *S. aureus*. The results suggested that ethanol and acetone extracts of leaf and stem extracts were highly potent against *K. pneumoniae* and *P. mirabilis* can be used in treatment of nosocomial infections such as pneumonia, urinary tract infections (UTIs) and bacteremia. Extensive animal studies may be required before investigating the role of *Solanum xanthocarpum* for treating RTI and UTI.

Table 2. Antibacterial activities of ethanol extract of leaf and stem

Name of bacteria	25 µg/l		50 µg/l		75 µg/l		100 µg/l	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Bacillus sp	5.33	0.00	6.99	2.33	12.33	4.66	16.00	9.66
E. faecalis	0.00	0.00	0.00	0.00	3.0	0.66	4.60	1.00
E. coli	0.00	1.33	0.00	3.00	0.00	3.66	1.3	5.33
K. pneumonia	0.66	2.00	4.00	3.00	7.33	8.66	13.33	12.66
Micrococcus sp	1.99	1.00	1.66	4.33	3.33	6.66	4.63	10.00
P. mirabilis	5.00	1.66	8.33	4.66	12.66	9.33	17.00	14.00
P.aeruginosa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.33
S. aureus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S. epidermidis	0.00	0.00	0.66	0.00	2.66	0.00	3.33	0.66

Table 3. Antibacterial activities of acetone extract of leaf and stem

Name of bacteria	25 µg/l		50 µg/l		75 µg/l		100 µg/l	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Bacillus sp	0.00	0.00	3.33	0.00	6.00	1.66	10.00	2.33
E. faecalis	0.00	1.33	0.00	2.66	0.00	2.66	0.00	2.66
E. coli	0.00	2.66	0.00	3.33	0.00	3.33	0.00	4.66
K. pneumonia	1.66	3.33	5.33	4.00	9.33	5.66	13.33	6.33
Micrococcus sp	0.33	0.66	1.33	4.66	2.66	7.00	2.66	7.33

P. mirabilis	2.66	0.66	4.66	1.00	8.33	6.66	10.66	8.66
P.aeruginosa	0.33	0.00	0.33	0.00	2.66	0.00	3.00	0.00
S. aureus	1.00	0.00	1.66	0.00	1.66	0.00	3.33	0.00
S. epidermidis	0.00	0.00	1.33	1.33	2.66	1.33	5.36	1.66

References

Yoshida, A., Yokote, F., Yahara, S., Nohara, T., Sajio, R., Murakami, K. and Tommatsu, T. 2006. *Phytochem.* 23(4): 743- 746.

Khanna, P., Uddin, A., Sharma, G. L., Manot, S. K. and Rathore, A. K. 1976. *Indian J. Exp. Biol*. 14(6): 694-696.

Oudhia, P. and Kadu Pani. 2007. A Specially Prepared Herbal Decoction for Body Wash Used by the Natives of Chhattisgarh, India. www.botanical.com (Accessed November 16, 2010.)

Rajkumar, S. and Jebanesan, A. 2005. *Tropical Biomed*. 22(2): 139-142.

Kar, D.M., Maharana, L., Pattnaik, S. and Dash, G.K. 2006. *J. Ethnopharmacol.* 108(2): 251-256.

Govindan, S., Viswanathan, S., Vijyayasekaran, V. and Algappan, R. 1999. *J. Ethnopharmacol*. *66*(2): 205-210.

Bradshaw, L.J. 1992.Laboratory Microbiology. Fourth Edition, Saunders College Publishing. Fort Worth.

Jigna, P., Nehal, K. and Sumitra, C. 2006. Evaluation of antibacterial and phytochemical analysis of *Bauhinia variegate* L. bark. *Afr. J. Biomed. Res*. 9(1): 53-56.

Perez, C., Paul, M. and Bazerque, P. 1990. Antibiotic assay by agar well diffusion method. *Acta Biol. Med. Exp*., 15: 113-115.

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Phytochemical Screening and Anti-inflammatory activity of *Citrus Limetta* peel. Oil extract

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ABSTRACT

Medicinal plants offer alternative remedies with incredible opportunities. It can be used to prevent and cure several diseases & disorders. *Citrus limetta (Rutaceae)* fruit is commonly called as sweet lime a medium sized shrubs or tree which is distributed in all over the world. It has vast medicinal properties. The objective of this study is to analysis the phyotoconstituents and to evaluate the anti inflammatory activity of essential oil extracted from *citrus limetta* peel. The medicinal plant has more chemical components which gives definite physiological actions to human bodies . Significantly high amount of secondary metabolites like steroids, cardio glycoside, phenol, terpenoids and saponins are present in peel oil extract. Inflammation is the trait of several human diseases and disorders like Atherosclerosis and Rheumatoid arthritis. Inhibitions of protein denaturation of extracted oil showed a better ability compared to standard Aspirin. The oil extract exhibited a significant activity of human RBC membrane stabilization. Thus the current studies expose that peel oil extract of *citrus limetta* fruit has better anti- inflammatory activity.
Key words: Phytoconstituents, Medicinal plant, Inflammation, Anti-inflammation, Protein Denature, Membrane Stabilization, *Citrus limetta*.

1. Introduction

The traditional drug gives an alternate source of remedies with tremendous opportunities. It can be used to prevent and cure several diseases & disorders. Modern medicine shows undesirable side effects to humans (Henry David). Even today majority of people depend on herbal medicinal practice because it provides safe and affordable primary healthcare to the people. Phyto chemical compounds are naturally available in all plants and it plays a major role in metabolic activity of humans. The major phyto chemical compounds includes, Steroids are capable in decreasing the inflammation and reduce the immune activity of immune system which is responsible for inflammation and also have anti-bacterial activity, Cardiac glycoside is regulating endothelial tissue factor expression in culture and many phyotoconstituents are present in all medicinal plants each has a different properties and functions. Inflammation is first line of defence mechanism to protect from body's tissue injury, microbial agents, and toxic chemicals, and other allergens (Shodhganga). In olden days people considered inflammation is a part of healing process but nowadays, inflammation is recognized as a complex disorder and a major response of immune system to infection and tissue damage (Harley I. et al., 2006). Nowadays the medicinal plants are containing anti inflammatory constituent to cure many inflammatory diseases and disorders. The plant *Citrus limetta* , *Rutaceae* family was used as a medicine to treat many diseases. The citrus fruit juice used to treat jaundice, cold and cough and gastrointestinal problems and also the peel oil has antiseptic properties (Riyaz ahmad et al., 2010) The present work is to determine the anti inflammatory effects of *citrus limetta* oil extracted from peel.

2. Materials and Methods

2.1 Collection and Preparation of extract

The fresh *Citrus limetta* fruits (figure 1) were collected around Poonamallee area. The peels were removed and cut into small pieces and washed twice using distilled water. Then added an equal volume of distilled water and it was boiled using steam distillation unit. The extract contains both water and oil mixture. To separate the oil and water added 20 ml of diethyl ether and was separated using separating

funnel (Zarina, Z. and Tan S. Y. 2013) . After separation the extract was evaporate at 34^0c in water bath to evaporate the diethyl ether.

2.2 Preliminary and Quantitative determination of phyto constituents:

The extracted oil of *citrus limetta* was subjected to preliminary phtochemical screening includes steroids, cardio glycoside, phenol, tannins terpinoids , saponins and the total phenol and flavonoid content was determined in a quantitative way (Sriparna KunduSen et al. 2012) .

2.3 Anti inflammatory activity:

2.3.1 Inhibition of Protein denaturation method

The inhibition of protein denaturation activity of extract was determined properties (Riyaz ahmad et al., 2010) The decreased settling of denatured protein in a tube indicates the inhibition potent of extract. The inhibition of denatured protein was calculated by the formula,

(%) inhibition = [100- (test OD - product control OD) / test control OD]*100

2.3.2 Human Red Blood Cell membrane stabilization

The membrane stabilization activity of extracted oil was identified towards the tonicity induced membrane lysis and it was evaluated (Kavita S. Mundhe et al.2009). It was calculated by the formula,

(%) stabilization = [(control-test)/control]*100

2.3.3 Hemolysis method

Hemolysis method is used to determine the membrane stabilization properties of extracted oil. RBC was subjected to pre and post treatment using hypotonic solutions.

3. Results and Discussions

The preliminary phyto constituent's screenings confirms the presence of steroids, cardio glycoside, phenol, terpenoids and saponnins. A very low amount of tannin was screened in the extracted oil. The results are shown in table 1. The appreciable amount of phenol and flavonoid content present in extracted oil which is determined by quantitative estimations of phyto constituents. The results are shown in figure 2.

The oil extract of *Citrus limetta* showed high anti inflammatory activity. The inhibition concentration of protein denaturation is shown in table 2. The IC50 value of 100µg/ml showed a higher inhibition activity compare to standard.

The Human Red Blood Cell membrane stabilization and Hemolysis method showed a better anti inflammatory activity. The results are shown in table 3 and figure 3. The above observations reveal that the *Citrus limetta* oil extracted from peel has a high anti inflammatory activity due to the secondary metabolites and various bioactive components present in the extracted *Citrus limetta* oil have ability to stabilize the membranes of human Red Blood cells.

4. Conclusion

The present study has been proven that the oil extract of *Citrus limetta* has responsible for anti inflammatory activity. Therefore, the further study is to investigate the anti-atherosclerosis activity and bioactive components present in the oil through GCMS.

5. Acknowledgement

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6. Reference

1. Ammar , A.haj, Bouajila, j. Lebrihi, A. Mathieu, F. Romdhane, M.zagrouba, 2012, Chemical composition and in vitro antimicrobial and activities of citrus aurantium L. flower essential oil (Neroli oil), Vol. 15, Issue 21, p1034-1040.
2. Henry David- Thoreau Woodwife's journal inspiring herbal quotes.
3. Harley I. manner, Richard S. buker, Virginia Easton smith, Deborah ward, and craig R.Elevitch 2006 Species profiles for pacific island agroforestry.
4. Kavita S. Mundhe, Asha A. Kale, Sucheta A. Gaikwad, Nirmala R.Deshpande, Rajashree V. Kashalkar 2009. Detection of Amino Acids from the seeds of Polyalthia longifolia. Vol.1, No.2, pp 298-299
5. Mitha (sweet lime) peel in Pakistan Shabnam Javed, Rauf Ahmad, Khurram Shahzad, Shaista Nawaz, Salman Saeed and Yasar Saleem- 2013. Chemical constituents, Antimicrobial and Antioxidant activity of essential oil of *Citrus limetta* var, Vol.7(24),Pg.3071.
6. Naseer unnisa1, hajera tabassum, mir naiman ali, and kritika ponia, 2012, Evaluation of antibacterial activity of five selected fruits on bacterial wound isolates, Vol3 (4): (P) 531 – 546).
7. Riyaz ahmad rather,swetha.c and rajgopal advance in bioresearch, , 2010, Screening of peel extract as anti oxidant,anti cancer agent and anti microbials vol1[1], pg:29-33.
8. Sriparna KunduSen, Asis Bala, Biswakanth Kar, Sanjib Bhattacharya, Upal K. Mazumder, Malaya Gupta, Pallab K. Haldar, 2012. Antitumor potential of Citrus limetta fruit peel in Ehrlichascites carcinoma bearing Swiss albino mice, Volume 2:e10.
9. Shodhganga - Introduction to traditional plants review article
10. Zarina, Z. and Tan S. Y. 2013, Determination of flavonoids in Citrus grandis (Pomelo) peels and their inhibition activity on lipid peroxidation in fish tissue, Vol 20(1), Pg 313-317.

Table 1: Phyto chemical screening of *Citrus limetta* oil Extract

S.NO	PHYTOCHEMICALS	RESULTS
1	Steroids	++
2	Cardio glycoside	++
3	Phenol	++
4	Tannins	+
5	Terpenoids	++
6	Saponins	++

Anti inflammatory activity

Table 2: Inhibition of protein denaturation of *Citrus limetta* oil Extract

S.NO	CONCENTRATION (µg/ml)	% OF PROTEIN DENATURATION INHIBITION BY STANDARD (ASPIRIN)	% OF PROTEIN DENATURATION INHIBITION BY *CITRUS LIMETTA*
1	10	11.31	12.4
2	25	22.39	23.22
3	50	33.26	40.1
4	75	49.67	52.3
5	100	57.87	60.23

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Table 3: Human Red Blood Cell Membrane Stabilization of *Citrus limetta* oil Extract

S.NO	CONCENTRATION (mg/ml)	% OF HEMOLYSIS BY STRANDARD (ASPIRIN)	% OF HEMOLYSIS BY *CITRUS LIMETTA*
1	10	33.2	20.9
2	25	45.5	33.34
3	50	61.21	45.8

Figure 1 : *Citrus limetta*

Figure 2 : Total phenol and flavonoid content of *Citrus limetta* oil Extract

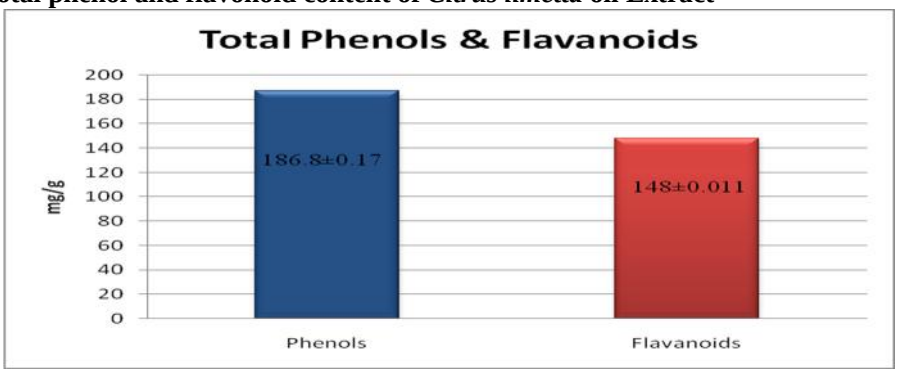

Figure 3: Hemolysis of *Citrus limetta* oil Extract

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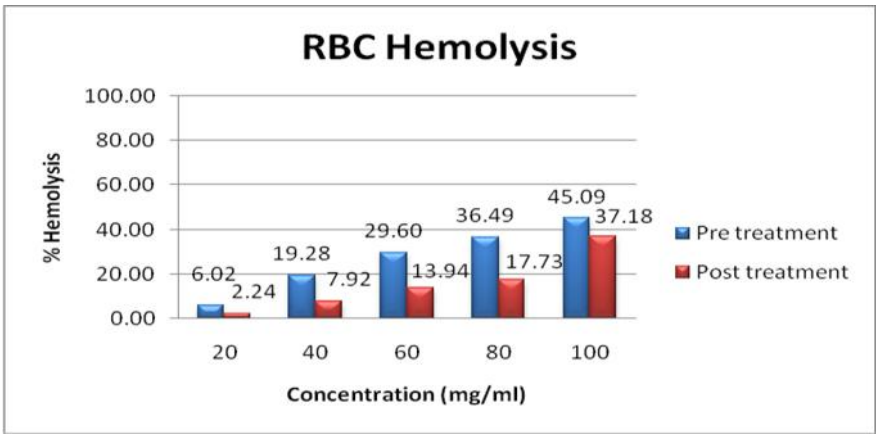

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Galactose mediated synthesis of silver nanoparticles (AgNPs) and its antimicrobial activity

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ABSTRACT

Nanotechnology has drawn significant attention in recent years due to their unique and exceptional applications. The sustainable chemical methods of silver nanoparticles (Ag-NPs) synthesis have significant interest in the current scenario due to high demand. Silver nanoparticles were synthesized in solutions of galactose in DMSO and Milli-Q water at high temperature (80^oC) under continuous stirring. Galactose acted as a reducing agents and stabilizing agents simultaneously for the synthesis of silver nanoparticles. Silver nitrate ($AgNO_3$) was used as the metal precursor for the preparation of Ag-NPs over the reaction time. The Plasmon resonance kinetics and their activation energy were determined by UV – visible spectroscopy. The Milli-Q water solution of galactose was better reductive activity than the DMSO solution of galactose. The synthesized silver nanoparticles were subjected to characterized antimicrobial activity against selected bacterial and fungal pathogens. The Milli-Q water solution of galactose mediated silver nanoparticles were exhibited better antimicrobial activity against tested bacterial pathogens. Further characterization studies are under progress to pinpoint the size, morphology and actual constituents responsible for the antibacterial activity.
[**Key words**: galactose, silver nanoparticles, antimicrobial activity, pathogens]

INTRODUCTION

Nanotechnology is an emerging and important field of modern nanoscience dealing with design, synthesis, and manipulation of particles ranging from 1-100 nm. Nanoparticles (NPs) have wide range of applications in health care, cosmetics, food and feed, environmental health, biomedical sciences, chemical industries, drug-gene delivery, energy science, catalysis applications. (Colvin et al 1994; Wang et al 1991; Schmid et al 1991). Nanobiotechnology is an important area of research for the synthesis of NPs with different chemical compositions, sizes and morphologies (Hoffman et al 1992). Several methods have been devised to prepare metallic nanoparticles. For instance, the most important current method is crystallization of nanoparticles in micro-emulsions using a variety of chemicals as precursors and surfactants as stabilizing agents (Leon et al 2013). Nowadays, there is a growing need to develop eco-friendly processes, which do not use toxic chemicals in the synthesis protocols. Silver NPs were prepared using water as an environmentally friendly solvent and polysaccharides as capping/reducing agents. For instance, synthesis of starch-silver NPs was carried out with starch (capping agent) and β-D-glucose (reducing agent) in a gently heated system (Raveendran et al 2003).

Although biosynthesis of Ag-NPs are considered to be more eco- friendly (Krishnakumar et al 2015), chemical or physical method of synthesis of preparation of silver particles are normally used because they are readily available and can be used to synthesize in large amounts in relatively less time. Chemical methods may be divided into those using in non-deleterious solvent and naturally occurring reducing agent such as polysaccharides or plants extract or microorganism such as bacteria and fungi as a reducing agents (Dokuchaev et al., 1997). Chemical reduction is the most frequently applied method for the preparation of AgNPs as stable, colloidal dispersions in water or organic solvents. Commonly used reductants are borohydride, citrate, ascorbate elemental hydrogen and polysccharides. The reduction of silver ions (Ag^+) in aqueous solution generally yields colloidal silver with particle diameters of several nanometers (Merga et al., 2007). In this research, present results on the synthesis of silver nanoparticles using galactose dissolved in DMSO and Milli-Q water. Galactose acts as reducing agent and stabilizing agent in silver

nitrate solutions. The synthesized nanoparticles were subjected to antimicrobial activity against selected pathogens. The nanoparticles were characterized by ultraviolet-visible (UV-Vis) spectroscopy and further characterization analysis under progress.

MATERIALS AND METHODS

Chemical mediated synthesis of silver nanoparticles (Ag-NPs)

Two different solvents of DMSO and Millii-Q water have been used for the synthesis of silver nanoparticle using galactose as a reducing and stabilizing agent in order to obtain nanoparticles with potential biomedical application. Galactose of analytical grade (AG) as a reducing agent and silver nitrate ($AgNO_3$) as starting material were used for the synthesis of nanoparticle under constant heating. In first set, 25 mg of galactose dissolved in 50 ml of DMSO solution in 250ml of Erlenmeyer flask by continuous stirring to dissolve completely. In second set, 25mg of galactose dissolved in 50 ml of Milli-Q water in 250ml of Erlenmeyer flask by continuous stirring to dissolve completely. Now 16.987 mg of silver nitrate was added into each flask separately. The reaction mixture was continuously heated to 80^{o}C until colour change was observed to yellowish brown indicate that the synthesis of nanoparticles. Characterization of synthesised silver nanoparticles was performed by the following standard technique of UV-visible spectroscopy analysis.

UV-Visible spectral analysis

The chemical reduction of silver ions was monitored by measuring the UV-Vis spectrum of the reaction mixture.UV-Vis spectral analysis was carried by using Systronics type 118 UV-Vis spectrophotometer.

Antimicrobial assay

The antimicrobial susceptibility assay of silver nanoparticles was evaluated by standard disc diffusion method against selected pathogens. Different concentrations (10 µl, 20 µl, 30 µl, 40 µl, 50 µl per disc) of silver nanoparticles were impregnated with commercially available sterile empty disc (Hi-media) with the size of 6mm diameter for antibacterial assay. Muller Hinton agar (MHA) plates was prepared and swabbed with broth cultures of each test pathogens (10^8 cells) separately. Nanoparticle impregnated disc was placed at the center of the plate aseptically. Triplicates were maintained for each test pathogens to obtain mean zone of inhibition. The disc impregnated with galactose solution alone (25µl/disc) was used as a negative control to compare the antibacterial efficiency. The zone of inhibition was measured after 24 hrs of incubation at 37^{o}C. The zone of inhibition around the discs were measured and recorded in mm in diameter for each tested pathogen.

Test pathogens and chemicals

Silver nanoparticles were subjected to antibacterial assay against selected bacterial pathogens *viz., E. coli, Pseudomonas aeruginosa, Salmonella paratyphi* A, *Bacillus subtilis, S. aureus,* and yeast *C. albicans*. The test pathogens were maintained as pure culture in our microbiology laboratory. All chemicals, media components and Hi media were procured form Hi media Laboratory Private Limited (Mumbai, India) for the present silver nanoparticle synthesis.

Statistical analysis

The antibacterial assay results of silver nanoparticle synthesized by two different techniques were calculated as mean diameter of zone of inhibition in mm ± standard deviation (mean ± SD).

RESULTS AND DISCUSSION

Nanobiotechnology is the most important emerging discipline in the field of both nanotechnology and biological science. In this study chemical mediated silver nanoparticles were produced by DMSO and Milli-Q water as described in the previous section for characterization and antibacterial efficacy against selected pathogens. The reaction mixture containing galactose induced the colloidal solution which turned yellowish brown for both the solvent indicating that silver nanoparticles were formed. The absorption spectrum of yellowish brown colloids prepared using galactose by DMSO and Milli-Q water is portrayed in fig.1&2 respectively. The reaction mixture showed a surface plasmon resonance absorption band with a

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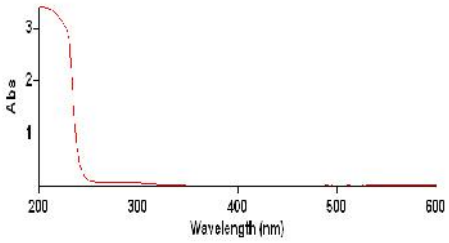
maximum peak of 380 nm and 400 nm respectively. This result shows that the silver nanoparticles were formed over a period of reaction time and are spherical in shape.



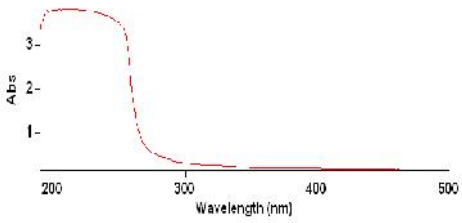


Fig. 1 UV-vis spectroscopy of Ag-NPs synthesized by DMSO Fig. 2. UV-vis spectroscopy of Ag-NPs synthesized by Milli-Q water

Table 1 Antimicrobial activity of silver nanoparticles produced by galactose with DMSO

S.No	Tested pathogens	Zone of inhibition in different concentration of nanoparticles (mm)				
		10 µl	20 µl	30µl	40 µl	50 µl
1	*E.coli*	NA	NA	NA	NA	NA
2	*Pseudomonas aeruginosa*	7	7	8	8	10
3	*Salmonella paratyphi A*	8	10	12	14	16
4	*Bacillus subtilis*	NA	NA	NA	NA	NA
5	*Staphylococcus aureus*	-	6	6	8	8
6	*Candida albicans*	NA	NA	NA	NA	NA

Values are the average of three replicates; NA – no activity

Table 2 Antimicrobial activity of silver nanoparticles produced by galactose with Milli-Q water

S.No	Tested pathogens	Zone of inhibition in different concentration of nanoparticles (mm)				
		10 µl	20 µl	30µl	40 µl	50 µl
1	*E.coli*	NA	7	8	8	10
2	*Pseudomonas aeruginosa*	8	10	12	14	16
3	*Salmonella paratyphi A*	6	8	8	10	10
4	*Bacillus subtilis*	-	6	6	8	8
5	*Staphylococcus aureus*	6	8	8	10	10
6	*Candida albicans*	NA	NA	NA	NA	NA

Values are the average of three replicates; NA – no activity

Antibacterial properties of silver are documented since 1000 B.C., when silver vessels were used to preserve water. The first scientific papers describing the medical use of silver report the prevention of eye infection in neonates in 1881 and internal antisepsis in 1901. Silver strongly inhibit bacterial growth through suppression of respiratory enzymes and electron transport components through interference with DNA functions (Li *et al*. 2006). Silver nanoparticle exhibit antimicrobial activity against human pathogens (Krishnakumar et al 2015) and plant pathogens (Krishnakumar and Dooslin Mercy Bai, 2015). Antimicrobial activity of galactose mediated silver nanoparticle synthesized by using DMSO and Milli-Q water to assess the efficiency of nanoparticles is portrayed in table 1 &2 respectively. Among the bacterial pathogens tested *Pseudomonas aeruginosa* exhibited maximum inhibition zone of 16mm by using silver nanoparticle mediated by galactose as a reducing and stabilizing agent by Milli-Q water. None of the activity was reported by silver nanoparticles produced by DMSO and Milli-Q water against *Candida albicans.*

CONCLUSION

The present study mainly focused on galactose mediated synthesis of silver nanoparticles by the reduction of silver salt using DMSO and Milli-Q water. Ag-NPs were successfully synthesized by Milli-Q water at 80^{o}C using galactose as a reducing and stabilizing agent. The formation of Ag-NPs reduced by galactose was determined by UV–visible spectroscopy where surface plasmon absorption maxima can be observed at 400 nm from the UV–vis spectrum. These NPs have significant inhibitory effects against selected microbial pathogens except *Candida albicans*, and could be widely used as antimicrobial agents in a diverse range of products to treat bacterial diseases.

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REFERENCE

1. Colvin VL, Schlamp MC, Alivisatos A. Light emitting diodes made from cadmium selenide nanocrystals and a semiconducting polymer. Nature. 1994; 370:354–357.
2. Dokuchaev, A., Myasoedova, T., Revina, A., 1997. Chem. High Energies (Russia) 31, 353.
3. Hoffman AJ, Mills G, Yee H, Hoffmann M. Q-sized cadmium sulfide: synthesis, characterization, and efficiency of photoinitiation of polymerization of several vinylic monomers. J Phys Chem. 1992; 96:5546–5552.
4. Krishnakumar S and V. Dooslin Mercy Bai Extracelluler biosynthesis of silver nanoparticles using terrestrial *Streptomyces* sp-SBU3 and its antimicrobial efficacy against plant pathogens. Int J. TechnoChem Research. 2015; 1(2), 112-118.
5. Krishnakumar S, Sindu Divakaran, G Uma Shankar, Prakash G Williums and M Sasikumar Extracellular biosynthesis of silver nanoparticles (Ag- NPs) using *Fusarium oxysporium* (MTCC-2480) and its antibacterial efficacy against gram negative human pathogens, Journal of Chem and Pharmaceutic Res., 2015; 7(1): 62-67.
6. León ER, R I Palomares, R ENavarro, R HUrbina, J Tánori, C I Palomares and A Maldonado Synthesis of silver nanoparticles using reducing agents obtained from natural sources (*Rumex hymenosepalus* extracts) Nanoscale Research Letters 2013; 8:318.
7. Li, Y., Leung, P., Yao, L., Song, Q. W., & Newton, E. Antimicrobial effect of surgical masks coated with nanoparticles. *J. Hosp Infect*, 2006*;* 62, (58-63).
8. Merga, G., Wilson, R., Lynn, G., Milosavljevic, B., Meisel, D., J. Phys. Chem. C 2007; 111, 12220.

9. Raveendran P, Fu J, Wallen SL. Completely "green" synthesis and stabilization of metal nanoparticles. J Am Chem Soc. 2003; 125:13940–13941.
10. Schmid G. Large clusters and colloids. Metals in the embryonic state. Chem Rev. 1992; 92:1709–1027.
11. Wang Y, Herron N. Nanometer-sized semiconductor clusters: materials synthesis, quantum size effects, and photophysical properties. J Phys Chem. 1991; 95:525–532.

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Antimicrobial Activity Of *Bougainvillea spectabilis* And Identification Of Its Active Compounds By GC- MS

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INTRODUCTION

Microbial infections can cause severe health effects and can even lead to death among the residing population, especially in the developing regions of the world. Selection of an effective antimicrobial agent for a microbial infection requires knowledge of the potential microbial pathogen, an understanding of the pathophysiology of the infectious process and an understanding of the pharmacology and pharmaco kinetics of the intended therapeutic agents (1). Urinary tract infection represents one of the most common diseases encountered in medical practice today and occurring from the neonate to the geriatric age group (2,3) Enteric fever (typhoid) caused by *Salmonella typhi* is a global bacterial infection with an annual infection rate of 21.6 million and 10% fatality rate (4). In developing countries, typhoid is more severe due to poor hygiene, indiscriminate use of antibiotics and a rapid rise in multidrug resistance. Resistance to the first line drugs chloramphenicol, ciprofloxacinand amoxycillin has been reported.(5).

The continual emergence of antibiotic-resistant microorganisms has prompted researchers world over to search for new antimicrobial agents that are more effective against the resistant microbial pathogens .With the increase in resistance to antityphoid drugs, medicinal plants have gained popularity among both urban and rural dwellers in the treatment of the ailment. In the scope of searching new antimicrobial agents, antimicrobials derived from plant materials are often regarded as natural and safe compared to industrial chemicals

Ornamental plants offer aesthetic value and ecological balance in ecosystems. Most flowering ornamental plants however are utilized more for their beauty as they impart different colourless to their surroundings. As their flower blooms, they serve this purpose and when they wilt, they fall off as trash. These flowers could be still used as pharmaceuticals or nutraceuticals. *Bougainvillea spectabilis* belonging to the family Nyctaginaceae is a very common ornamental plant grown almost all over the world in tropical and sub-tropical gardens. It grows as a shrub or thorny, woody vine reaching upwards of 12 meters tall and 7 meters wide. Recent research has shown *B. spectabilis* to possess some potentially useful antiviral and antibacterial compounds (6,7) as well as anti-diabetic properties (8).

Phytochemical characterization of plant material is important as it relates to the therapeutic actions. Phytochemical evaluation of a herbal drug is an essential criteria, before proceeding for its pharmacological and toxicological studies (9). Gas chromatography separates the components of a mixture and mass spectroscopy characterizes each of the components individually. By combining the two techniques, an analytical chemist can both qualitatively and quantitatively evaluate a solution containing a number of chemicals.(10)

There had been a limited screening of phytochemicals, antioxidant and antimicrobial from flowers of ornamental plants Keeping in view the above idea and considering the vast potentiality of *Bougainvillea spectabilis* as sources for antimicrobial drugs, a systematic investigation was undertaken to evaluate the antibacterial and antifungal activity from its flower extracts against clinical pathogens ,screen for phytochemicals and separate its active constituents by GC MS analysis.

MATERIALS AND METHODS:

Sample Collection and Processing :

Blood, urine and pus samples were collected in sterile containers as per standard procedures and processed for the isolation of bacterial pathogen. Skin scrapings were collected and processed for the isolation of dermatophytes. The bacterial pure culture obtained was further subjected to Gram staining, motility and various biochemical tests and streaked on selective media for the identification of each isolate

The fungal culture isolated was further subjected to KOH mount and Lacto Phenol Cotton Blue Staining method

ANTIBIOTIC SEITIVITY TEST BY KIRBY BAUER DISC DIFFUSION METHOD

The sensitivity pattern of isolates to standard antibiotics was tested by the Kirby Bauer(11) disc diffusion method as follows Four or five colonies of the test organisms were transferred to Muller Hinton broth The turbidity was adjusted to McFarland standard (0.5) A sterile cotton swab was immersed in the bacterial suspension and excess fluid was compressed against the wall of the test tube. The surface of the sterile Mueller Hinton Agar plates was swabbed by passing the swab three times over the entire surface. The plates were allowed to dry for ten minutes. The discs containing antimicrobial agents were placed in such a way that it adheres perfectly to the surface of the agar by gentle pressing. The plates were incubated at 37^{o}C for 24hours. After incubation the size of the zones were measured and the results were interpreted and compared with the ATCC control strains by standard zone size interpretative chart as per CLSI.

ANTIMICROBIAL ACTIVITY OF FLORAL EXTRACTS OF *Bougainvilla Spectablis*

Collection of B. spectabilis flowers

B. spectabilis flowers were collected from surrounding areas of Besant nagar, Chennai and their identity was verified and authenticated from Prof. P. Jayaraman, Director Plant Anatomy Research Centre, Tambaram.

Extraction & Drying

The extracts of the flower samples were prepared in a direct method by soaking 25 g of flower samples in 75 ml of different solvents (chloroform, ethyl acetate and ethanol) for 48 h. At the end of each respective extraction, the extracts were filtered using Whatma1 filter paper. The filtrate was concentrated under reduced pressure in vacuum at 40°C for 25 min using a rotary evaporator (Super fit-ROTAVAP, India). The percentage yield of extracts was calculated.using the formula:-

$$\text{Percent yield} = \frac{\text{Weight of the crude extract obtained in gram} \times 100}{\text{Total weight of plant powder in gram}}$$

SCREENING OF THE EXTRACT FOR ANTIBACTERIAL ACTIVITY

PREPARATION AND STANDARDISATION OF INOCULUM

The invitro susceptibility of any bacteria to potential drug or standard drug will vary depending on number of organism in the given inoculums increases.. Four to five colonies from pure growth of each test organism were transferred to5ml of MHB. The broth was incubated at 35-37^{o}c for 18 hours. The turbidity of the culture was compared to 0.5 Mcfarland Nephelometer standard to get 150X10 CFU/ml. The standardized inoculums suspension was inoculated within 15-20 minutes (12). For fungi, Sabaurouds dextrose broth(SDB) was used.

4.AGAR WELL DIFFUSION METHOD

Screening of antibacterial activity was performed by well diffusion technique (13). The Muller Hinton Agar plates were prepared and then seeded with 0.1ml of standardized inoculums of each test organism. The inoculum was spread evenly over plate with sterile glass spreader or sterile cotton swab. The seeded plates were allowed to dry for 10 minutes. A standard cork borer of 8mm was used to cut uniform wells on the surface of the MHA and 5µl(3.9mg), 10µl(7.8mg), 15µl(11.7mg), 20µl(15.7mg) of flower extract dispensed into well separately and each plate was labeled with the test organism.

The dissolution of the organic extract was aided by 1% DMSO, which did not effect the growth of microorganisms, in accordance with the control experiments. The plates were then incubated at 37^{o}C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 2000). The experiments were performed in duplicate and repeated thrice

MINIMUM INHIBITORY CONCENTRATION (MIC) OF THE FLORAL EXTRACTS

The Minimum Inhibitory Concentration(MIC) of the flower extract was determined by tube dilution techniques Muller Hinton Broth(Merck) according to NCCLS(2000). The concentration ranged from

3.9mg to 195mg. 1ml of nutrient broth was taken in each of sterile test tubes and the ethanolic extract of the flowers were added to all the tubes serially. 500µl of bacterial suspension that was previously grown in nutrient broth were added to all tubes and incubated at 37ºC for 24 hours. The lowest concentration that exhibited no visible growth were recorded as the MIC of the extract.

PHYTOCHEMICAL ANALYSIS OF FLOWER EXTRACT OF *Bougainvillea spectabilis*

Specific qualitative tests were performed for the presence and absence of phytochemicals viz, alkaloids, tannins, flavanoids, saponins, glycosided etc., in flower extract to identify the constituents using standard procedures.(14)

GAS CHROMATOGRAPHY – MASS SPECTRUM ANALYSIS (GC-MS)

GC-MS analysis of this extract was performed using GC SHIMADZU QP2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column (Length : 30.0 m, Diameter : 0.25 mm, Film thickness : 0.25 is Composed of 100% Dimethyl poly siloxane). An electron ionization energy system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.51ml/min and an injection volume of 2ìl was employed (split ratio: 20). Injector temperature 200°C; Ion-source temperature 200°C. The oven temperature was programmed from 70°C (isothermal for 2 min.), with an increase of 300°C for 10 min. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds with scan range of 40 – 1000 m/z. Total GC running time was 35 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas

Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST08s), WILEY8 and FAME having more patterns.. The active components of the extract were identified. By comparing the spectrum of the unknown component with the spectrum of the known components stored in the NIST08s, WILEY8 and FAME library

RESULTS

Samples of blood urine and pus were processed for the isolation of pathogen. Based on the morphological and biochemical characteristics the isolates from blood was identified as *Salmonella typhi. Pseudomonas aeruginosa* and *Staphylococcus aureus* were isolated from pus *E.coli* and *Enterococcus faecalis.*from urine sample. *Trichophyton rubrum* and *Epidermophyton flocossum* were the dermatophytes isolated from skin scrapings

ANTIBIOTIC SUSCEPTIBILITY:-

The resistance / susceptibility pattern of the clinical isolates to various antibiotics have been assessed by disc diffusion method. Of all the 5 isolates it was found that. *E.coli* isolated from urine sample exhibited multidrug resistance .It was sensitive to Amikacin, Nitrothrantoin, Imipenem, Co- trimoxazole and was resistant to Amoxylin, Genofloxacin, Amoxyclav, Cefotaxime, Ciprofloxacin, Ampicillin, Ceftriaxone, Astreonam, Ceftazidime, Gentamycin, Cefepime, Cefaloxin and Nalidixic acid.

ANTIMICROBIAL ACTIVITY OF FLORAL EXTRACTS OF *B. spectabilis*

Ethanol, chloroform and ethyl acetate extracts of the plant were prepared, filtered and evaporated as per standard methods Maximum percentage yield was observed in ethanolic extract of flowers 5% for 25g of fresh flowers used for extraction, followed by ethyl acetate (2.2%) and chloroform (1.2%).

The various solvent extracts of the plant *B. spectabilis* were observed for antibacterial activity against the pathogens isolated from clinical samples. Their antibacterial potency was assessed by the presence or absence of inhibition of zones and zone diameters (mm).(Table1)

ANTIFUNGAL ACTIVITY OF SOLVENT EXTRACTS OF B. spectabilis:-

The antifungal activities of the various solvent extracts of flowers of *B. spectabilis* were tested by well diffusion method .*Trichophyton rubrum* exhibited maximum zone of inhibition 24mm at a concentration of 20mg of ethanolic extract of flowers of *B. spectabilis* and *Epidermophyton* exhibited 23mm zone of inhibition.(Table2)

MINIMUM INHIBITORY CONCENTRATION OF SOLVENT EXTRACTS OF B. spectabilis FLOWERS FOR BACTERIAL ISOLATES:-

The minimum inhibitory concentration of various solvent extracts of *Bougainvillea spectabilis* flowers was measured by broth dilution method. The MIC value of ethanolic and ethyl acetate solvent extract for *E. coli* at lowest concentration 11.7mg/ml. The minimum inhibitory concentration of ethanolic flower extract was 19.5mg, chloroform extract 15.6mg and ethyl acetate extract 30mg for *Salmonella typhi*

PHYTOCHEMICAL ANALYSIS

The major phytochemicals found were saponins, carbohydrates, phlobotannins, flavanoids, trepenoids and alkaloids. All extracts showed the absence of sterols, quinines, glycosides and tannins. Ethyl acetate extract yielded maximum phytochemicals

GC-MS ANALYSIS:-

GC-MS analysis was carried out on ethanolic extract of B. spectabilis flowers and four different compounds were identified. The chromatogram revealed four peaks in the retention time range 10.63 minutes to 14.5 minutes. The largest peak at13.18 min was identified as 2(3H)Furanone-dihydro5-tetradecyl with 25%. The second less prominent peak at 13.57 minutes corresponds to the compound Dasycarpidan-1-methanol, acetate (ester) with 16%. The third prominent peak at 10.63% minute with 12% area was due to the presence of Ergoline-8-carboxylic acid, 10-methoxy-6-methyl-,methyl ester. The fourth prominent peak at 11.9 minute corresponds to the compound Ascorbic acid 2,6-dihexadecanoate with peak area 5% (Table3).

DISCUSSION:

There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This medical challenge has made man to depend on his immediate environment taking advantage of nature's provisions for life and survival the present study was undertaken to screen for antimicrobial activity of floral extracts of *Bougainvillea spectabilis*, and identify the bioactive components responsible for antimicrobial activity by GC-MS analysis.

. The present study demonstrated *E. coli* and *E. faecalis* be the predominant aetiological agent as the causative agent of UTI. These findings are similar to studies conducted by(15). E. coli exhibited a high rate of resistance to many antibiotics used in the present study. This pattern of resistance also has been reported by(16). *S.aureus* and *P. aeruginosa* were identified as aetiological agent from wound infections.Similar results were reported by(17). , *Salmonella typhi* was found to be the aetiological agents of blood samples collected from enteric fever patients. Anthropophilic dermatophytes are the main agents of *Tinea cruris* throughout the world in particular, *Trichophyton rubrum* and *Epidermophyton flocossum*. In the present study *Trichophyton rubrum* was the predominant against isolated followed by *E. flocossum*, thus confirming the results of earlier studies by(18)

There is a global interest in non-synthetic natural drugs derived from plant sources, because of low cost, non-toxic nature and availability. The research for medicinal plants having dictinguishable medicinal properties, among which one is *Bougainvillea spectabilis*. It is an ornamental flowering plant, belonging to th family Nyctaginaceae. It is used in herbal combination for the treatment of diabetes. The incidence of screening *B. spectabilis* flowers for pharmacological evaluation was very less and hence the present study was aimed to investigate the antimicrobial activity of different solvent extracts of *Bougainvillea spectabilis* flowers and identify the bioactive components.

Different solvent extracts of *B. spectabilis* were prepared by Soxhlet extraction method and evaluated for antimicrobial activity by disc diffusion method. The percentage yield of various solvents was calculated and it was found to be less. Ethanolic extract yield was only 5% per 25g of flowers the antibacterial activity of 3 solvent extracts of flowers of *Bougainvilla spectabilis* were studied against Gram positive and Gram negative pathogens. Ethanolic extract showed inhibitory effect on all pathogens isolated from clinical samples. *Escherichia coli* and *Enterococcus* the aetiological agent of U.T.I. were highly

susceptible to ethanolic and chloroform floral extracts of *Bougainvillea spectabilis*. *E. coli* was found to be resistant to most antibiotics and was found to be significant multidrug resistant bacteria.

All the three solvent extracts were able to inhibit both the dermatophytes *Trichophyton rubrum* and *Epidermophyton flocossum*. Chloroform extracts were more efficient in controlling dermatophyte followed by chloroform and ethyl acetate extract. The study showed that Bougainvillea spectabilis extracts have antimicrobial potential against *S. typhi*. Chloroform extracts and ethanolic extracts were effective against *S. typhi*. This was in consistent with the results observed by(19). The organic solvents exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted only through organic solvent medium.

Phytochemical analysis revealed the presence of various phytochemicals like flavanoids, saponins, alkaloids and phlobotannins. Ethyl acetate extract showed more number of phytochemicals than other extracts. Higher activity of ethanol and chloroform extracts may be due to the presence of flavanoids and saponins.(20)

The bioactive compounds present in the ethanolic extract was identified by GC-MS studies.. The major component identified are "Ergoline-B-carboxylic 10-methyl-methyl ester, Ascorbic acid 2,6-dihexadecanote, Dasycarpidan-1-methanol acetate(ester), Furanone, dihydro-5-tetradecyl. The antifungal activity of the plant *Warburgia salutaris* is due to one of the active component Furanone, dihydro-5-tetradecyl) which was identified as a major component with maximum peak height in the present study. The antifungal activity of the flower extract against dermatophytes was mainly due to the presence of furan compounds which is similar to the earlier findings (21) Ergoline is a chemical compound whose structural skeleton is contained in a diverse range of alkaloids. Ergoline derivatives are used clinically for the purpose of vasoconstriction (5-HT_1 receptor agonists—ergotamine) and in the treatment of migraines (used with caffeine) and Parkinson's disease. (22)

The effectiveness of flower extracts against a wider spectrum of pathogenic microorganisms needs to be investigated before being used for food preservation or medicinal purposes. In addition, clinical effects of the floral extracts and their essential oils under "*in vivo*" conditions as well as in food systems need to be studied to evaluate in detail their potential effectiveness as antimicrobial agents as well as presence of any acute or chronic effect

The present study is a preliminary evaluation of antimicrobial activity of the plant *Bougainvillea spectabilis* Future investigations will focus the research on the invitro antimicrobial activities and on chemical identification of the antimicrobial ingredients in the screened efficacious extracts of Bougainville spectabilis. Based on these conclusions, further purifications and testing of efficacious plant extracts of *B. spectabilis* is recommended, in order to identify the major active ingredients responsible for the antimicrobial and antioxidant activities of these extracts.

REFERENCES

1 Kelwin W.S. 1999. Anti microbial therapy for diabetic foot infections, *Post Grad. Med*., **106**: 22-28.

2 Raju C.B, Tiwari S.C 2004. Urinary tract infections: A suitable approach. *J. Ind. Acad. Clin. Med.*, **2**(4): 331-334

3 Kunin, C.M., 1994. Urinary tract infections in females. *Clin. Infect. Dis.*, **18**: 1-10

4 John A.C, Fouad G.Y, Stephen P, Maha T, Said A.O, Frank J.M 2003. Estimating the incidence of typhoid fever and other febrile illnesses indeveloping countries. *Emerg. Infect*. Dis **9**(5): 539-544.

5 Benoit D, Renand L, Daniele M, Ame B, David B, Michael R.M, Elisabeth C.,Anel C 2003 Variant *Salmonella* genomic island 1antibiotic gene resistance cluster in Salmonella enteric Albany. *Emerg. Infect. Dis*. **9(5),** 585-591.

6.Balasaraswathi R, Sadasivam,S Ward M Walker J.M. 1998 An antiviral protein from *Bougainvillea spectabilis* roots; Purification and characterization. *Phytochemistry*.; **47**(8): 1561- 1565.

7 Umamaheshwari.A, Shreevidya.R, Nuni.A 2008 Invitro antibacterial activity of *Bougainvillea spectabilis* leaves extracts. *Advances in Biological Research* **2**(1-2): 01-05

8 Adebayo J.O, Adesokan A.A, Olatunji L.A, Buoro D.O, Soladoye A.O 2005. Effect of Ethanolic extract of *Bougainvillea spectabilis* leaves on haematological and serum lipid variables in rats. *Biochem.* **17**: 45
9Eslavath.R.,Venkateswarulu.G..,Manoranjansabath.,HarikrishnaV.,JyothiC.H.,Latha K.2013.Phytochemical screening and analytical studies of *Tridax parviflora* UJP,**2(3)**144-147
10 Maria J.R.P, Kannan P.S.M., Kumaravel S 2011.GC-MS Analysis of *Lantana camara* L. Leaves. *JPRD*; **2(11***):* 63- 66
11 Bauer AW, KirbyW.M.M, SherrisJ.C, Turck,M. 1966.Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol*.;**45**: 493-496
12 Saeed,S.,Tariq,P.,Fatima,N.2005. Antibacterial activity of rhizome of *Zingiber officinale* against species of oral viridans streptococci. *Int. J. Biotechnol*., **2**: 913-916
13 Saeed,S., Tariq,P 2005. Antibacterial activities *of Mentha piperita*, *Pisum sativum* and *Momordica charantia. Pak. J. Bot.*, **37**(4): 997-1001.
14 Sonali J.,G.S. Shekhawat, 2010. Phytochemical Analysis and Antibacterial Screening of in vivo and in vitro Extracts of Indian Medicinal Herb: Anethum graveolens. *Research Journal of Medicinal Plant*, **4**: 206-212.
15 Nerurkar, A.,Solanky,P. Naik.S.S .2012. Bacterial pathogen in urinary tract infection and antimicrobial susceptibility pattern. *J.Pharm Biomed Sci*., **21**:1-3
16 Tankhiwale S.S, Jalgaonkar S.V, Ahamad S, Hassani U 2004. Evaluation of extended spectrum β lactamase in urinary isolates. *Indian J. Med. Res*. **120(6)**: 553-556
17 Kaftandzieva,Z.C, Igo, K., Milena, P., Nikola P.2012 Bacteriology of Wound - Clinical Utility of Gram Stain Microscopy. *Macedonian Journal of Medical Sciences*. **5(1):**72-77
18 Chakrabarti A, Sharma SC, Talwar P. 1992.Isolation of dermatophytes from clinically normal sites in patients with tinea cruris. *Mycopathologia.* ; **120**:139–141
19 Sharif.N, Ali.I, Sharif.S, Nisa.U.F2013. Phytochemical analysis and inhibitory activity of ornamental plant *Bougainvillea spectabilis. Asian journal of plant science and Research* **3**(2): 1-
20 Hegazi A.G,Abd E.l.,Hady,F.K,Abdullah F.A. 2000 Chemical composition and antimicrobial activity of European *Propolis*. Z. *Naturforsch.* C.; **55**: 70–75
21 Mohanlall .V., Odhav B .2009 Furans and Furanones with antimycotoxigenic activity isolated from Warburgia salutaris (Canellaceae) *Journal of Medicinal Plants Res* **3**(4), 231-240.
22 Schardl C.L, Panaccione D.G, Tudzynski P. 2006. Ergot alkaloids—biology and molecular biology. *Alkaloids* **63**:45– 86.

Table 2. ANTIFUNGAL ACTIVITY OF VARIOUS SOLVENT EXTRACTS OF B. spectabilis:-

S.No	Solvent	Organism	Diameter of zone of inhibition in mm			
			5mg	10mg	15mg	20mg
1.)	Ethanol	*Trichophyton rubrum*	20	22	23	24
		Epidermophyton flocossum	16	17	21	23
2.)	Chloroform	*Trichophyton rubrum*	21	23	24	26
		Epidermophyton flocossum	17	19	22	23
3.)	Ethyl acetate	*Trichophyton rubrum*	16	18	20	22
		Epidermophyton flocossum	13	14	16	17

SI. NO	Retention time	Peak area %	Name of the compound
1	10.63	12%	Ergoline-8-carboxylic acid, 10-methoxy-6-methyl-,methyl

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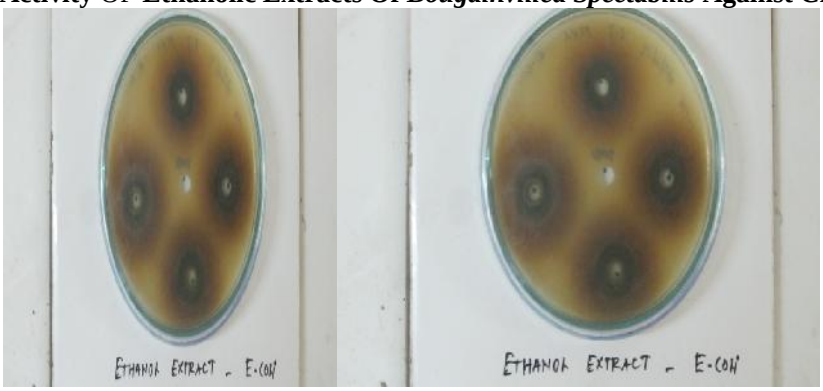
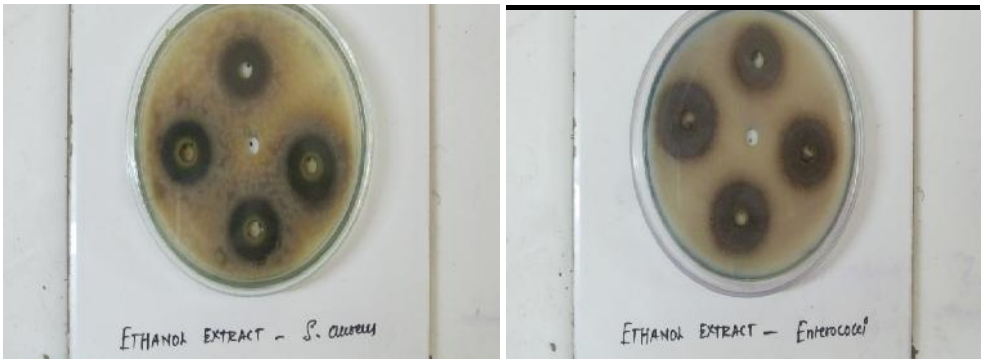
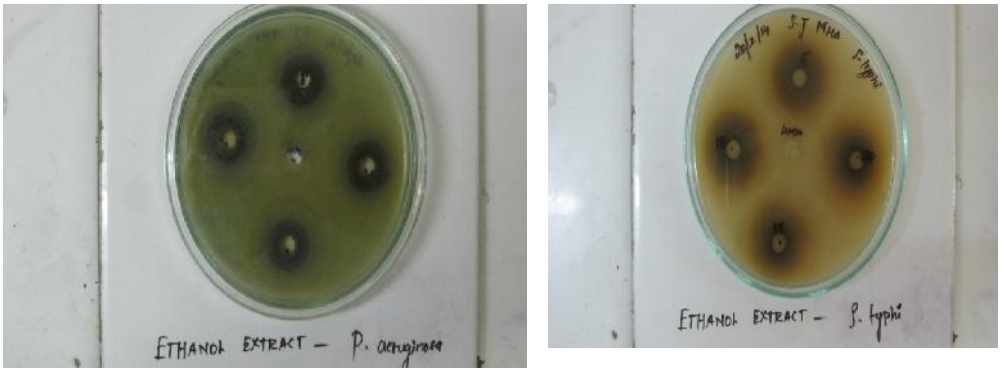
			ester,
2	11.9	5%	Ascorbic acid 2,6-dihexadecanoate
3	13.18	25%	2(3H)-Furanone, duhydro-5-tetradecyl
4	13.57	16%	Dasycarpidan-1-methanol, acetate (ester)

TABLE 3 GC-MS ANALYSIS OF ETHANOLIC FLOWER EXTRACT OF *B. spectabilis*

Antibacterial Activity Of **Ethanolic Extracts Of *Bougainvillea Spectabilis* Against Clinical Isolates**







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Effect of Monocrotophos and Azadiractin on germination of *Pisum sativum* and *Zea mays*

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ABSTRACT: The use of pesticides has greatly improved agricultural yield through inhibition of disease caused by many soil pests. Also the pesticide residues in the soil affect the crop seed germination and growth. The extensive use of insecticide in agricultural and their potential carcinogenicity strongly suggest the need to extend the cytotoxic evaluations. The active ingredients of the pesticides bind to the cell cycle enzymes and inhibit the mitotic cell division of the plants. The objective of the present study focuses on the effect of Monocrotophos and Azadirachtin on green peas (Pisum sativum) and maize (Zea mays) seed germination and on root tip mitotic index. The difference between Botanical pesticide and Chemical pesticides on the growth of plant was also assessed. The molecular interaction studies were also done using bioinformatics tools.

Keywords: Monocrotophos, Azadiractin, Seed germination, Mitotic index, Molecular interaction.

INTRODUCTION

In developed agricultural system inorganic fertilizers are applied to the soil to supply the essential nutrients required for plants. The use of pesticides has greatly improved agricultural yield through inhibition of disease causing and by acting against pest in the field and during storage of agricultural products (Taylor *et.al.,* 1997). The killing insect "Pest," insecticides obviously have the potential to harm non-target insects such as beneficial natural predators and pollinators. Less obviously, weed-killers can also be harmful to beneficial insects (Hassan, *et al.* 1988). It is estimated that often than 0.1 percent of an applied pesticide reaches the target pest, leaving 99.9 percent an unintended pollutant in the environment, including in soil, air and water, or on nearby vegetation (Pimentel, 1995).

Hence, the study on the effect of insecticides on plant seedling growth and germination along with root tip analysis is an essential tool to derive the best insecticide which will provide both disease resistance and improves the crop yield.

MATERIALS AND METHODS

Different concentration of Monocrotophos and Azadirachtin 100,200 and 400ppm is taken to study their effect on germination and growth of Green pea and Maize seeds. The test Green pea and Maize seeds are sterilized (to avoid fungal infestation) with 0.1% Mercuric chloride solution for 5minutes, followed by several washing under running tap water to remove the residues. Three replicates, each containing 10seeds of Green pea and Maize are arranged in circles on a two layered Whatman No.1 filter paper placed in 9 cm diameter Petri dishes and to this 3ml of insecticide at different concentrations is added. Thereafter, 2ml of insecticide is added on alternate days till the end of the experiment. Distilled water is used as control. The germination of seeds is recorded at 3, 5, 7 and 9 days and the seedling length was also measured.

In this experiment, Green pea and Maize seeds were placed in a 9cm diameter Petri dishes containing the double layered filter paper. Different concentrations ranging from 100, 200 and 400 ppm of the insecticide solution are applied in respective Petri plates and distilled water

was used for control. After three days, the root tips (1-2mm) were excised, transferred to 1N hydrochloric acid and maintained at 80°C for 5minutes, then fixed overnight in 3:1 (v/v) ethanol acetic acid and stained with fuelgan stain. A squash preparation is made and an average of 1000 cells is scored by using a LEICA phase contrast microscope for different phases of mitotic division (interphase, prophase, metaphase, anaphase, and telophase). At each treatment, from at least five root tips of different seeds, the mitotic index is arrived by using the formula:

$$\text{MITOTIC INDEX (\%)} = \frac{\text{Total number of dividing cells}}{\text{The total number of observed cells}} \times 100$$

Data was subjected to two ways ANOVA and the turkey's pair wise comparison is carried out for all the treatments.

Insilico analysis: Molecular Modeling:

The protein sequences of MAPK (Mitogen-activated protein kinases) of the targets (Green pea, Maize) were collected from the sequence database NCBI (http://www.ncbi.nlm.nih.gov/). The chemical 3D structures of the ligands (Monocrotophos and Azadirachtin) were collected from PUBCHEM through NCBI search. The MAPK sequences of Green pea and Maize were modeled with the homology modeling server GENO3D (http://geno3d-pbil.ibcp.fr). The target sequences were submitted to the GENO3D tool to find the template using PSI-BLAST method against Protein Data Bank (PDB). From the PSI-BLAST results the correct template were selected based on the sequence similarity and submitted again for modeling the structure. The structures were modeled by extracting geometrical restraints (dihedral angles and distances) for corresponding atoms between the query and the template and the 3D construction of the protein by using a distance geometry approach (Combet et.al., 2002). The modeled structures were validated using the Ramachandran plot produced by PROCHECK.

Molecular Docking

The docking studies on MAPK of Green pea and Maize with Monocrotophos and Azadirachtin were done using the iGEMDOCK software, A graphical-automatic drug discovery system for integrating docking, screening, post-analysis, and visualization. iGEMDOCK provides the post-analysis tools by using k-means and hierarchical clustering methods based on the docked poses (i.e. protein-ligand interactions) and compound properties (i.e. atomic compositions). Atomic composition (AC), which is similar to the amino acid composition of a protein sequence, is a new concept for measuring compound similarity.

RESULTS AND DISCUSSION:

The effects of Monocrotophos and Azadiractin on the germination and seedling growth were determined. Three concentrations of the Monocrotophos treated were proved to be inhibitory over seed germination and mitotic division of Green peas and Maize [Fig.1a & 1b]. The root length of the seedling decreased as increasing concentrations of Monocrotophos treatments whereas Azadirachtin treatments significantly increased the root length [Fig.2a, 2b, 2c & 2d].

Fig-1a: Effect of monocrotophos treatment on *P. sativum* and *Z.mays* seed germination

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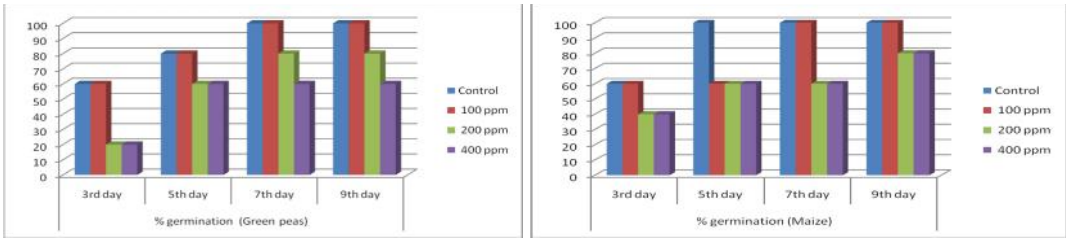


Fig-1b: Effect of azadirachtin treatment on *P. sativum* and *Z.mays* seed germination

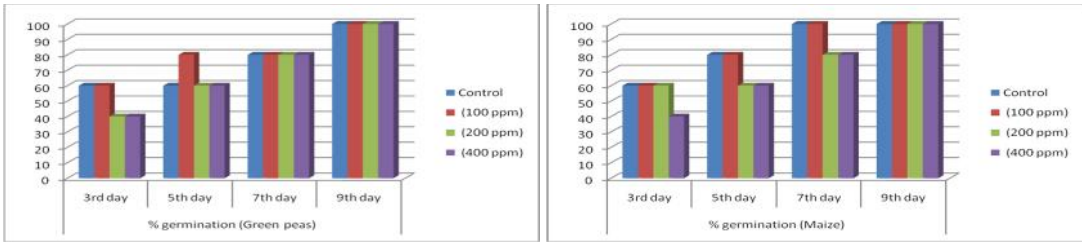
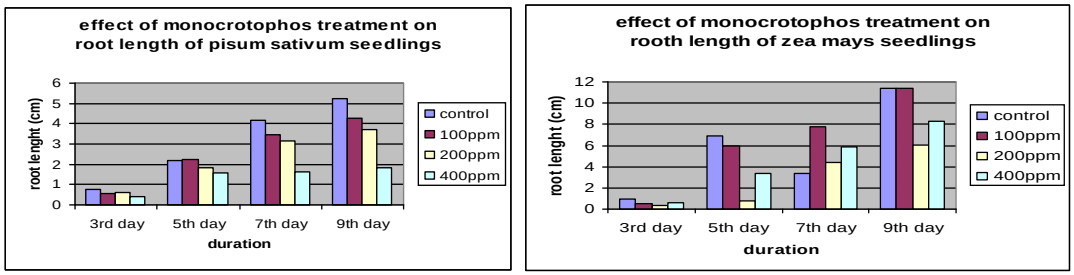


Fig-2a and 2b: Effect of monocrotophos treatment on root length of *P. sativum* and *Z.mays* seedlings



***The mean difference between control and treatment is significant at the .05 level.**

Cabrera *et al.* (1994) have validated the use of plants for evaluation of environmental pollutants such as pesticides because plants are direct recipients of agrotoxins. So plants are important study material for environmental monitoring of places affected by such pollutants. Effects of pesticides on the germination of weed seeds were explored by Gange et al. (1992) where the effects of three pesticides, chlorpyrifos (a contact insecticide), dimethoate (a systemic insecticide) and iprodione (a contact fungicide) on seed germination of 20 weed species were examined in the laboratory.

Chlorpyrifos reduced germination in the annual grass and one annual forb and iprodione in one perennial forb. In 2007, Wang et al. examined the fate of chlorpyrifos in soil-crop system where concentrations of chlorpyrifos in wheat seedlings at harvest correlated positively with the initial concentrations spiked in the soil.

Figure-2c and 2d: Effect of azadiractin treatment on root length of *P.sativum* and *Z. mays* seedlings.

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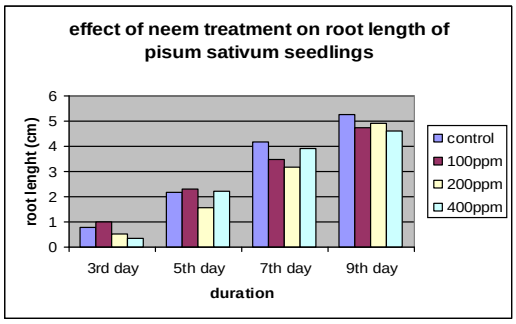

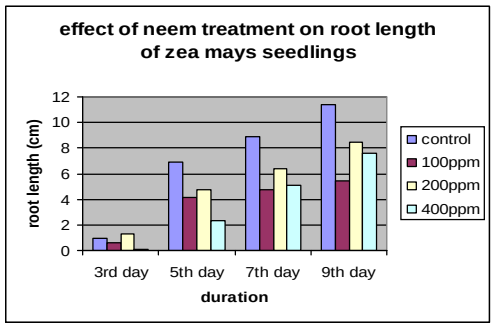

***The mean difference between control and treatment is significant at the .05 level.**

The Mitotic cell division results showed that the Monocrotophos treatment inhibit the growth of Green peas and Maize (Table-1). It was calculated by quantifying the number of dividing cells in the root tip of the seedlings. Mitotic analysis showed gradual decrease in the mitotic index either by increasing the concentrations of Monocrotophos similar to the same trend reported by Pandy *et al*., 1994 and Adam *et al*., 1990.

HOMOLOGY MODELING:

The PSI-Blast results were short listed based on the alignment file provided by the tool. The template for modeling the 3D structure of the targets was selected based on the sequence identity (Table 3).

Table-1: Effect of Monocrotophos on Mitotic cell division of *Pisum sativum* and *Zea mays*

Treatment	Prophase		Metaphase		Anaphase		Telophase	
	P. sativum	*Zea mays*	*P. sativum*	*Zea mays*	*P. sativum*	*Zea mays*	*P. sativum*	*Zea mays*
Control	28.46±0.25*	33.63±0.2*	35.1±0.10*	33.7±0.1*	42.2±0.26*	26.6±0.10*	46.1±0.2*	28.06±0.057*
100 ppm	25.03±0.25*	29.23±0.2*	28.2±0.20*	28.6±0.1*	36.53±0.2*	24.6±0.15*	37.03±0.15*	25.36±0.20*
200 ppm	17.28±0.076*	25.56±0.15*	25.4±0.15*	27.16±0.15*	29.1±0.26*	19.3±0.10*	19.26±0.25*	19.33±0.15*
400 ppm	11.73±0.20*	20.6±0.15*	10.13±0.13*	19.23±0.15*	11.4±0.10*	18.2±0.20*	9.46±0.25*	17.66±0.25*

***The mean difference between control and treatment is significant at the .05 level.**

The selected templates were submitted to the GENO3D tool for modeling. Ten models were generated for each target. Among the three models, the top was filtered based on the Energy score provided by the tool. The top model of the MAPK of Green pea and Maize has the energy value of -6042.14 kcal /mol and -15288.30 kcal /mol respectively. The modeled 3D structure of the target was visualized using SWISS PDB-viewer

Table -2: Effect of Azadirachtin in Mitotic cell division of *Pisum sativum* and *Zea mays*

Treatment	Prophase		Metaphase		Anaphase		Telophase	
	P. sativum	*Zea mays*	*P. sativum*	*Zea mays*	*P. sativum*	*Zea mays*	*P. sativum*	*Zea mays*

Control	28.46±0.25	33.63±0.2	35.1±0.1	33.7±0.1	42.2±0.26 *	26.6±0.1	46.1±0.20	28.06±0.057
100 ppm	28.60±0.1	33.2±0.15	35±0.1	33.36±0. 15	41.4±0.11 *	26.36±0. 20	45.76±0.1 1	27.83±0.057
200 ppm	25.4±0.1*	29.3±0.17 *	28.7±0.20 *	28.6±0.1 *	36.53±0.2 0*	24.1±0.1 *	37.16±0.1 5*	24.56±0.30*
400 ppm	18.3±0.057*	27.4±0.1*	23.4±0.20 *	28.03±0. 15*	27.56±0.1 5*	2106±0.1 *	19.66±0.1 5*	20.60±0.20*

*The mean difference between control and treatment is significant at the .05 level.

. **Table 3: Template used for modeling the 3D structure of the targets.**

Target	Name of the obtained significant template	% of sequence identity between target and template	E-Value	PDB ID of the template
Mapk of *Pisum sativum*	Putative uncharacterized protein of *Oryza sativa* subsp. indica	75	7e-59	A2X0W6
Mapk of *zea mays*	Mitogen-activated protein kinase of *Dunaliella salina*	71	8e-67	A2TDK2

Table 4: Ramachandran plot of the top model produced by PROCHECK

Target	% of residues present in		
	Core	Allowed	Disallowed
Mapk of *Pisum sativum*	65.6	31.2	0.8
Mapk of *zea mays*	74.6	22.5	1.0

MOLECULAR DOCKING: The iGEMDOCK results showed that the intermolecular complex formed between the MAPK of *Pisum sativum, zea mays* and Monocrotophos, Azadirachtin are stable with lower energy values (Table 6). Since molecules in nature have a tendency to be found in their low energy form, the final configuration of intermolecular complex should also be of low energy. The interaction between the target and the ligand is due to van der waals and hydrogen bond interaction.

The post dock analysis explored the amino acids involved in the intermolecular complex formation. The results showed that Monocrotophos and Azadirachtin probably binds to the same site of MAPK of *Pisum sativum*, where the amino acids, "ARG", "GLU", "SER", "ASP" are present. In the case of *zea mays*, the binding pocket amino acids are ARG, PRO, GLU, LYS and ILE (Table 6, Figure-4, 5).

Table 5: Interaction profile of Monocrotophos, Azadirachtin with the MAPK of *Pisum sativum, zea mays*

Intermolecular complex	**Total energy value**	**VDW**	**H bond**	**Binding pocket residues**
MAPK of *Pisum sativum* – Monocrotophos	-91.65	-71.52	-20.13	ARG97, ARG121, GLU125, SER124, SER126, ASP127

MAPK of *zea mays* – Monocrotophos	-88.2	-84.7	-3.5	ARG251 PRO233, GLU235, LYS247, ILE250
MAPK of *Pisum sativum* – Azadirachtin	-93.82	-75.73	-18.09	ARG121, SER126 ARG97, SER124, GLU125, ASP127
MAPK of *zea mays* - Azadirachtin	-90.12	-83.4	-6.72	GLU183, LEU238, SER239, LEU241, LEU282, ASN283, PRO284

Figure-4: A. Intermolecular complex of MAPK of *Pisum sativum* and Monocrotophos.
B. Intermolecular complex of MAPK of *zea mays* and Monocrotophos

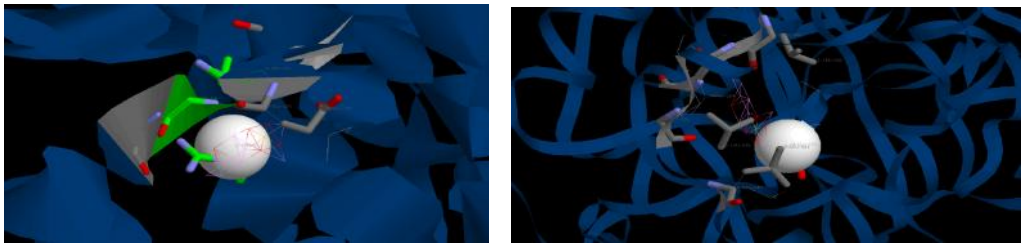

Figure-5: A. Intermolecular complex of MAPK of *Pisum sativum* and Azadirachtin
B. Intermolecular complex of MAPK of *zea mays* and Azadirachtin

Molecular docking has become an increasingly important tool for studying the interaction of target and the ligand. The molecular docking approach can be used to model the interaction between a small molecule and a protein at the atomic level, which allow us to characterize the behavior of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes (McConkey, 2002). Thus, the present study proved the efficiency of using experimental evidence together with insilico analysis in determining the toxicology of pesticides or insecticides upon the quality of crops and will pave way to derive disease resistance crops with better yield.

REFERENCES

Adam, S.M., L.R. Shugart, G.R. Southworth and D.E. Hinton. 1990. Application of bioindicators in assessing the health of fish populations experiencing contaminant stress. In: Biomarkers of environmental contamination (Ed: J.F. McCarthy and L.R. Shugart). Lewis Publishers, Boca Raton. pp 333-353.

Cabrera MTG, Cebulska-wasilewska A, Chen R, Loarca F, Vandererg AL Salamone MF, 1994. Tradescantia-Stamen-Hair-Mutation Bioassay-A Collaborative Study on Plant Genotoxicity Bioassays for the International Program on Chemical Safety, WHO, The United Nations. 310: 211- 220

Combet C., Blanchet C., Geourjon C. and Deleage G. 2000, NPS@: Network Protein Sequence analysis.

Gange AC, Brown VK, Farmer LM, 1992.Effects of Pesticides on the Germination of Weed Seeds:Implications for Manipulative Experiments. Journal of Applied Ecology,29(2):303-310.

Hassan SA, Bigler F, Bogenschütz H, Boller E, Brun J, Chiverton P, Edwards P, Mansour F, Naton E, Oomen PA, Overmeer PJ, Polgar L, Rieckmann W, Samsøe-Petersen L, Stäubli A, Sterk G, Tavares K, Tuset JJ, Viggiani G, Vivas AG. 1988, Results of the fourth joint pesticide testing programme carried out by the IOBC/WPRS-Working Group "Pesticides and Beneficial Organsisms". J Appl. Entomol 105: 321-329.

McConkey BJ, Sobolev V, Edelman M. 2002, The performance of current methods in ligand-protein docking. Current Science; 83:845–855

Nwachukwu E. O. and Umechuruba C. I. 2001. Antifungal activities of some leave extracts on seed-borne fungi of African yam bean seeds. Journal of Applied and Natural Sciences 1:29-31

Pandey N. D., Mathur K. K., Pandey S. and Tripathi R. A. 1986. Effects of some plant extracts against pulse beetle, Callosobruchus chinensis Linnaeus. *Indian J. of Entomology* 48(1): 85-90.

Pimentel D., Harvey C., Resosudarmo P., Sinclair K., Kurz D., McNair M., Crist S., Sphpritz, L.,Fitton, L., Saffouri, R. and Blair, R., 1995, 'Environmental and economic costs of soil erosion and conservation benefits',Science 267,1117–1123.

Taylor D, Green N, Stout G. 1997. Biological Science. 3rd edition Cambridge University Press, Australia.Trends Biochem.Sci.,25, 147–150.

Wang L, Jiang X, Yan D, Wu J, Bian Y, Wang F, 2007. Behavior and fate of chlorpyrifos introduced into soil-crop systems by irrigation. Chemosphere, 66:391-396.

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