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SP-1

ENZYMATIC APPROACH ON PROCESSING OF TENCEL FABRIC

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ABSTRACT

"The Earth will not continue to offer its harvest, except with faithful stewardship. We cannot say we love the land and then take steps to destroy it for use by future generations."
- John Paul II

As the Quote goes though the garment industry and recent technologies has created enormous opportunities even beyond what nature can offer but the damage and the impact it creates to the environment is beyond the acceptable level. The damage it causes starts right from the farming of cotton till the final finishing and processing of fabric. The industry use different harmful chemicals in various processes like, desizing, scouring, bleaching, mercerizing, dyeing, washing, finishing, and it utilizes large quantities of water and also large amount of waste waters in each stages of processing Biotechnology is the process of applying living organisms and their different components for industrial processes and products. In the current scenario the application of biotechnology is wide. The use of enzymes in the garment industry has found to be an ecofriendly approach in various processing and best alternative for a quality and safe product. The application of enzymes have found roots in various processing of textiles like scouring, bleaching, mercerizing, dyeing, finishing, degumming and finishing, where the alternatives used in the traditional methods where harmful chemicals whose disposal was a threat to the environment. The enzymes are not only ecofriendly alternatives but they are also very specific and efficient, working under mild condition creating less damage to the fabric. Furthermore, the application of enzymes results in reduced energy, temperature, water saving and process times, thereby improving the quality and process integration of the product. The current study aims at processing tencel fabric with enzymes and creating an eco-friendly fabric.

KEYWORDS: ***Enzymes, application, textile industries, processing, eco-friendly characteristics.***

INTRODUCTION

Enzymes have played an important role in many aspects of life since the dawn of time. In fact they are vitally important to the survival of life itself. The globalization and technology development has made new concepts and progresses in the textile industry. Eco friendliness has turned to be the major criteria for the textile products due to the awareness of environmental impact caused by various chemical processing of textiles and also the strict governmental legislation on effluent treatment and customer's interest to use eco-friendly products. Today enzymes have become a vital part in textile processing. The enzymes have two major applications. Firstly in preparatory process like desizing where amylases are majorly used and the secondly in the finishing process. In finishing process Cellulase, proteases, lipases, pectinases etc., are being used. The most common application is bio washing where the traditional pumice stone is being replaced with enzymes. There are various applications which entail enzymes included fading of denim and non-denim, bio-scouring, bio-polishing, wool finishing, peroxide removal, decolourization of dyestuff, etc.[2]

Application of enzymes

The principal enzymes applied in textile industry are hydrolases and oxidoreductases. The group of hydrolases includes amylases, cellulases, proteases, pectinases and lipases/esterases.[9] The use of enzymes in textile industry is one of the most rapidly growing field in industrial enzymology. The enzymes used in the textile field are amylases, catalase, and laccase which are used to removing the starch, degrading excess hydrogen peroxide, bleaching textiles and degrading lignin.[3] The use of enzymes in the textile chemical processing is rapidly gaining globally recognition because of their non-toxic and eco-friendly characteristics with the increasingly important requirements for textile manufactures to reduce pollution in textile

production. The application of cellulases for denim finishing and lactases for decolourization of textile effluents and textile bleaching are the most recent commercial advances.[4]

Table I : ***Enzyme Application***

Types of Enzyme	Textile Use and Effects
Cellulases	Bio polishing, anti-pilling, softness, smoothness, lustre improvement and stone-washed Effects on denim
Amylases	Standard procedure for the removal of starch warp size
Proteases	In household washing agents better removal of protein containing soil orstains. Anti-felting of wool, accompanied by high loss of weight, tearstrength and of the typical handle, degumming of silk with the problem ofsilk fibroin damage
Lipases	In detergents for the hydrolysis of lipids
Pectinases	Hydrolysis of pectins, for example in cotton 'preparation' and retting offlax and hemp
Catalases	Catalyse the decomposition of hydrogen peroxide, important beforereactive dyeing of printing of peroxide bleaching fabrics and yarn
Peroxidases	Used as an enzymatic rinse process after reactive dyeing, oxidativesplitting of hydrolysed reactive dyes on the fibre and in the liquor,providing better wet fastness, decolourised waste water and potentiallytoxic decomposition compounds (aromatic nitro-compounds)
Ligninases	Removal of burrs and other plant compounds from raw wool
Collagenases	Removal of residual skin parts in wool
Esterases	In development: polyester finish, removal of 01 igomers
Nitrilases	In development: polyacrylonitrile preparation for better coloration

Source: Textile learner[10]

METHODOLOGY

Selection of sample

Tencel is a regenerated fabric of cellulosic origin which is produced from the wood pulp, making them ecofriendly and sustainable fabric. In the present study 100% tencel fabric of twill weave structure was taken. The macroscopic and stereo zoom microscopic image of the selected fabric is presented below.

Figure 1 – Stereo Microscopic View

Processing of Tencel

Desizing

Desizing of a grey fabric is a process of removing previously added size or starchy material which can be done by using water (rot steeping), acid, enzyme, oxidation chemicals and alkali.[7] The desizing process is being done to remove the starch that is being added to the fabric in the weaving process. The desizing

process involves removing the sizing agent by impregnating the fabric under a specified temperature and chemicals to degrade the sizing agent. Traditionally, desizing was carried out by treating the fabric with water or by chemicals such as acids and oxidising agents. [5]

Recipe for desizing

Fabric weight
Material:liquor ratio - 1: 10
Time duration - 1 hr
Temperature - 60^0c
Hydrochloric acid - few drops
Before Desizing - 94.544 gm
After Desizing - 92.544 gm

For this study, the above said recipe[8], was used for the desizing process. The fabricated sample was dipped in 2 liters of soft water in which few drops of hydrochloric acid was added and boiled for 60 minutes and stirred continuously. The fabric was removed and washed thoroughly with soft water and then dried.

Bleaching

The purpose of cotton bleaching is to decolourise natural pigments and to confer a pure white appearance to the fibres. The most common industrial bleaching agent is hydrogen peroxide. [4] In addition to an increase in whiteness, bleaching results in an increase in absorbency, levelness of pre-treatment, and complete removal of seed husks and trash.[6] In the case of the production of full white finished materials, the degree of whiteness is the main requirement of bleaching. Although several bleaching agents are available, hydrogen peroxide is most popular agents for bleaching of cotton. [8]

Recipe

Hydrogen peroxide (35 %) - 1gl
Sodium hydroxide (NaOH) - 2% owf
Wetting agent - 0.3 -0.8% owf
Sodium silicate - 2-3% owf
Magnesium sulphate (Epsom salt) - 0.5% owf
Temperature - 80^0C and 100^0C
Time - 60-120 minutes
Water - 2 l

Adopting the above recipe the bleaching process had been followed. The tencel fabric was bleached in a water bath containing 2 liter soft water, sodium hydroxide, hydrogen peroxide and it was boiled for an hour. After 2 hours the fabric was taken and rinsed thoroughly in running water. It was dried in the shade

Enzymatic Dyeing

Extraction of dye from Microbial source

Procurement of culture (*Serratia marcescens* and *Pseudomonas fluorescence*) (Culture, Sub culture and preservation)

Microbial pigment extraction

Procedure

Microbial pigment was synthesized, extracted from the selective bacteriological media. Test bacterial culture, (*Serratia marcescens* and *Pseudomonas fluorescence*) was used in the study based on their high pigment producing abilities naturally. The bacteria was inoculated in the Nutrient broth (culture media) and incubated for 48h to 72h till high pigment production. After observing the change in colour of the media, the entire broth culture was centrifuged at 5000 to 8000rpm for 30 min repeatedly for two times. The supernatant containing the fluorescent pigment was collected separately in a glass container and stored at 4°C prior use.

Figure II: Fluorescent Pigment Production In Plates

Figure III: Confirmation Of Pigment Production By The Bacterial Culture By Exposing In UV Light

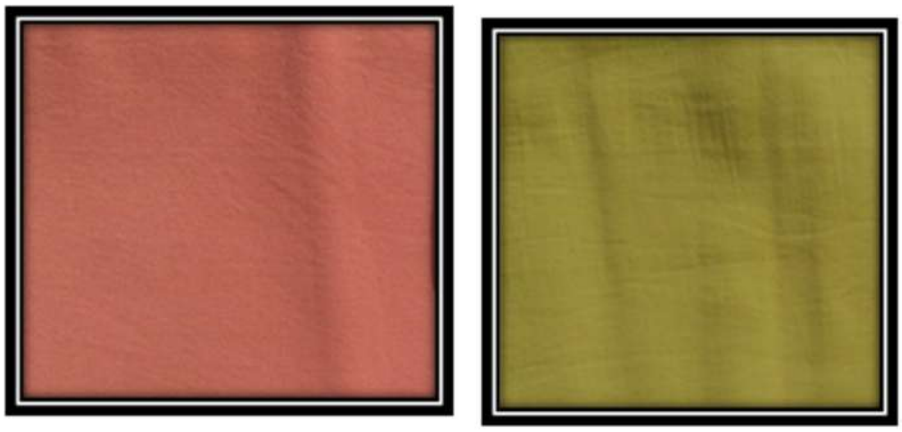
Dyeing of fabric

The tencel fabric was dyed using the *Serratia marcescens* and *Pseudomonas fluorescence*.

RESULT & DISCUSSION

The pigment dyed fabric was evaluated for colour fastness test like washing, rubbing and to sunlight, of all the samples evaluated by enzymatic treated were rated from excellent to good

Figure IV: Dyed Samples



Dyed samples of serratia marcescens and pseudomonas fluorescence

The samples were tested to various colourfastness test like colourfastness to washing, light, alkali and acids

Table II*: Colourfastness test grading points*

Fastness property	Grading	
	Serratia marcescens	*Pseudomonas fluorescence*
Alkalis & acids	4	3
Light	7	5
Washing	5	4
Perspiration	4	4

From the table II it is evident that the fabric dyed with Serratia marcescens showed better result towards light, washing and alkalis, the fastness towards perspiration was graded 4 for both the fabric dyed with *Serratia marcescens* and *Pseudomonas fluorescence.*

CONCLUSION

Textiles though an integral part of human being causes serious problems and damage to the environment. A shift towards organic and eco-friendly fabrics trade, from both consumers and producers has created an eco-friendly textiles and clothing around the world to reach the ethical customer. Enzymes are not only beneficial from ecological point of view but they are also saving lot of money by reducing water and energy consumption which ultimately reduce the cost of production. The biotechnology move will slowly replace all the process using enzymes. The use of various enzymes in the early stages of development but their innovative applications are increasing and spreading rapidly into all areas of textile processing. From the study it is evident that dyeing of textiles with enzymes is a better alternative towards the chemical dyeing.

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SP-2

SCREENING OF MARINE ACTINOMYCETES AGAINST BACTERIAL PATHOGENS

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ABSTRACT

Marine actinomycetes were isolated using actinomycete isolation agar and starch casein nitrate agar. The actinomycetes colonies were purified, subcultured and screened for antibiotic production using cross streak method and agar plug method. Also the isolates were screened using agar plug method and best six isolates were selected. The best six isolates were challenged against five different clinical pathogens (*E. coli*, *Staphylococcus aureus, Proteus species, Klebsiella species, Bacillus subtilis* and a multi drug resistant gram negative bacterium. Morphological and biochemical examinations were performed for the isolated actinomycetes. The total protein content of the best six actinomycete isolates were extracted using solubilization buffer and the protein samples were resolved using SDS PAGE.

INTRODUCTION

Isolation of actinomycetes from marine sediments, suggested that this source may be valuable for the isolation of novel actinomycetes with the potential to yield useful new products (Goodfellow M, and J A Haynes, 1984). The actinomycetes found in the marine and coastal ecosystem was viewed as a rich gene pool possibly containing isolates capable of producing used metabolites (Okami, 1986; Goodfellow M and Haynes J A, 1984). (Newman *et al.,* 1989 and Okami 1986) reported that compared to terrestrial species marine actinomycetes are important sources of novel antibiotics. The actinomycetes found in the marine and coastal ecosystem was viewed as a rich gene pool possibly containing isolates capable of producing used metabolites (Okami, 1986; Goodfellow M and Haynes J A, 1984). Multiple drug resistance or Multidrug resistance is a condition enabling a disease-causing organism to resist distinct drugs or chemicals of a wide variety of structure and function targeted at eradicating the organism. Excessive use of antibiotics resulted in the emergence of bacterial resistance. The resistant strains had a survival advantage, and under the selective pressure of antibiotics propagated and spread throughout the world. Research reveals that in 2005, MRSA infections caused 18,650 deaths in United States. Meanwhile, the far more publicized scourge of AIDS killed 12,500 in the same time period. It's thought that nearly 32 out of 100,000 U.S. residents get MRSA infections each year – that's 94,360 infections – more than meningitis, bacterial pneumonia, and flesh-eating strep combined. In view of the potential importance of marine actinomycetes as a source of novel bio active compounds there are methods to improve the isolation of actinomycetes from marine and estuarine environment has been practiced. Due to the wide spread interest in antibiotic substances in the world, several groups of workers have studied on newer antibiotics. Thus the search for antibiotic producing microbial strains and studying their conditions for maximum production is of greater importance now-a-days. The present study deals with the preliminary studies on the isolation of marine actinomycetes showing broad spectrum antibiotic activity against clinical pathogens.

MATERIAL AND METHODS

Collection of samples and test organisms

Sediment samples were collected from the marine environment of Chennai, Chavakkad, Mumbai, Allapuzha and Ernakulam. The sediment samples were taken to the laboratory in sterile screw cap bottles and used for isolation. The clinical pathogens used for the screening of actinomycetes were collected from Kovai Medical Centre and Hospital, Coimbatore. The organisms like *Escherichia coli, Staphylococus aureus, Proteus spp, Klebsiella spp and Bacillus subtilis* used for the screening of actinomycetes.

Isolation of actinomycetes

One gram of the sediment sample was weighed and added to 9 ml of sterile distilled water. It was mixed vigorously and then allowed to settle for few minutes. The supernatant was taken and serially diluted up to 10^{-5} dilution. The dilutions were aseptically plated on to the surface of the Isolation media (Actinomycetes isolation agar [Himedia labs], Starch casein nitrate agar and Malt Yeast Extract Agar). The isolation plates were incubated at 28±1º C for 5 – 8 days. Several isolated actinomycetes colonies with different morphological characters like size and colour were subcultured for screening.

Screening and selection of antibiotic producing actinomycetes

The activities of antibiotics were carried out by cultivating the actinomycetes on agar plates by Cross Streak Method (Waksman S A, 1968) and Agar Plug Method (Sen *et al.*, 1995). In Cross Streak Method the malt yeast extract agar was prepared and it was inoculated at one side of the plate with a single straight streak of the actinomycete culture under study. The plates were incubated at 30ºC for 3-5 days. When the growth of the actinomycetes was noted, the various test organisms (Bacterial forms) were seeded against the actinomycetes colony and were again incubated at 33ºC for 20 – 24 hours. After incubation, the antibiotic producing Actinomycetes and their antibacterial spectrum (zone of clearance) was recorded. In Agar plug method the actinomycetes isolates were selected over the entire surface of the malt yeast extract agar plate. The medium used not only favored the growth of organisms but also the production of the antibiotic. The plates were incubated at 30ºC for 3-5 days. After the actinomycetes has developed well, agar blocks were cut using sterile syringe with a puncture (3 mm) and was transferred to another agar plates swabbed with test organism. Wells were already prepared in Nutrient agar plates to insert in the agar blocks. The plates were incubated for 20-24 hrs at a suitable temperature for the growth of the test organism. The plates were observed for the zone of inhibition around the agar plugs and were measured.

Challenging against multidrug resistant bacteria

The multidrug resistant bacterium (gram negative rod) was picked from a medium containing different antibiotics (Streptomycin, cefatoxamine and ampicillin). The screened actinomycetes were then challenged against this multidrug resistant bacteria using agar plug method and zone of inhibition formed by the actinomycetes against this multidrug resistant bacteria was noted. The selected antibiotic producing actinomycetes could be identified by the following procedures. They are morphological method, staining Procedure (Simple staining and Gram's staining) and biochemical tests (Carbohydrate utilization test, starch hydrolysis test and lysozyme broth utilization) (Kawato and Sinobu, 1979).

Total protein extraction from actinomycetes

The total protein content of actinomycetes was extracted by centrifuging the culture broth. After centrifugation, the pellets were obtained, required amount of solubilization buffer (62.5 mM Tris HCl, pH 6.8 containing 10 % glycerol, 5 % β. Mercaptoethanol, 3 % SDS, 0.01 % bromophenol blue) was added. This can be used as the sample for SDS PAGE (U K Laemmli, 1970). The total protein extract of actinomycetes (antibiotic producers) were resolved using SDS PAGE.(4 % stacking gel and 12 % separating gel). After electrophoresis the gel stained with coomassie brilliant blue and destained for overnight. Then the gel was analyzed for its banding pattern.

RESULT

Isolation and identification of actinomycetes from sediment samples

Sediment samples collected in sterile containers from different places were analyzed. The sediment samples were serially diluted up to 10^{-3} and plated on to actinomycetes isolation agar or starch casein nitrate agar. The growths of the actinomycetes were observed from the fourth day of incubation and about 16 isolates were obtained. The suspected colonies of actinomycetes were subcultured in starch casein nitrate agar plates. The colonies which appeared on the isolation plates were milky white, with hard texture, the reverse side in some cases changed from yellow to brown and some species appeared black and showed the cultural characteristics of Actinomycetes. The actinomycetes were given positive results for carbohydrate utilization test, starch hydrolysis test and lysozyme broth utilization.

Screening and challenging of isolated actinomycetes against clinical pathogens

The isolated and identified isolates of actinomycetes were subcultured and screened for Antibiotic production using cross streak method and Agar plug method and the few isolates were selected. The isolated

actinomycetes were challenged against the test organisms and best six isolates were selected based on the zone of inhibition against the test organisms and designated as actinomycetes isolate No. 1, 2, 3, 4, 5 & 6. The results are presented in bar diagram 1. The potency was compared in terms of the inhibition zone. Among the isolates of actinomycetes only six isolates listed in the bar diagram 2 were chosen from final screening for further studies.

Challenging against multidrug resistant bacteria

The screened isolates were then challenged against the multidrug resistant bacteria (gram negative rod) and the best isolates were selected based on the zone of inhibition. The results are presented in bar diagram 1. Among the isolates only one isolate showed antibacterial activity.

Morphological and biochemical examination

In the simple staining the isolates were identified as curved rods with slender filaments. Also in the gram staining gram positive results were observed for all isolated. In the Carbohydrate utilization test the utilization of the Ammonium salt by the isolates the colour of the medium changed to yellow which is a positive result for the Streptomyces. In the Starch hydrolysis test the hydrolysis of the starch by the isolate a zone of clearance was obtained by performing iodine test which is a positive result for *Streptomyces*. Also in the lysozyme broth utilization, all the isolates showed good growth in the basal glycerol broth control tube and no growth in lysozyme broth which is a positive result for *Streptomyces* species.

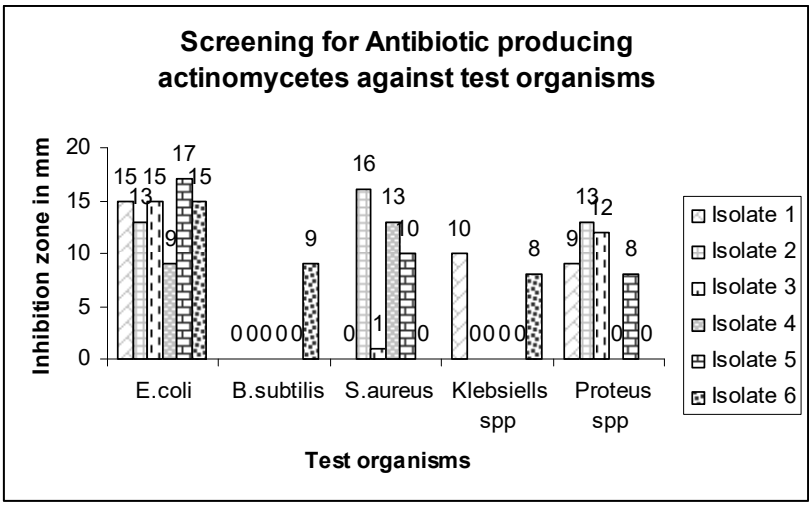
The total cellular proteins were extracted from all six isolates and resolved through SDS PAGE. The gel resulted as, the bands were not similar to one another. When it is compared with marker protein lane, the all six lanes contain protein with different molecular weight.

DISCUSSION

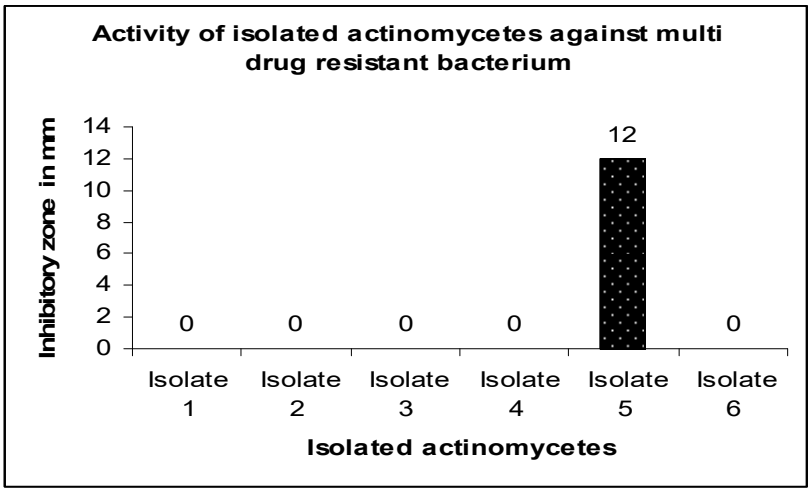
Assumption has persisted despite evidence that actinomycetes can be recovered from deep ocean sediment and that marine derived actinomycetes can be metabolically active and physiologically adapted to growth in seawater. (Jensen *et al.,* 1991). The isolation of actinomycetes was found luxuriant in starch casein nitrate agar media prepared using sterilized distilled water and similar method was carried out by (Nadaraj, 1996) for the isolation of Actinomycetes. The actinomycetes isolates were screened for the antibiotic production using cross streak and agar plug diffusion technique. Six active isolates of Actinomycetes were selected and subjected for further analysis. The name technique has been recommended by (Sen *et al.,* 1995). In the present study only 50 % of the isolation gave positive results in producing antibacterial substance and among them 20 % only of the isolates showed positive results against both gram positive and gram negative bacteria. The results also supported the view that those small quantities of different antibiotics are produced by many micro organisms but their effectiveness against both gram positive and gram negative organisms is comparatively a rare event. The result and abbreviation of our work related to the isolation and screening of antibiotic producing actinomycetes are in agreement with (Hugo *et al.,*1983). Most of the isolates obtained belong to the genus *Streptomyces*, the member of *Actinomycetales*. Similar fact was also reported by (Hopwood *et al.,*1985) stating that 80 % of the total secondary metabolites were produced by *Streptomyces*. When the actinomycete isolates challenges against clinical pathogens, all the isolates shown such remarkable activity against gram negative bacteria such as *E.coli* and *Proteus* spp., also it has shown such considerable activity against gram positive bacterium like *Staphylococcus aureus*. But these isolates completely failed against *Bacillus subtilis* except isolate number 6. Comparing the obtained results with those of other scientists, it could be varied that exhibited activity against *B. subtilis* (Moncheva *et al.,* 2002). But when the isolates challenged against multi drug resistant bacteria only the isolate number 5 shown the considerable result, when all other organisms failed to perform. So the isolate number 5 can be a potential actinomycete for the multi drug resistant bacteria. The further studies on the isolate number 5 may reveal some solution for the treatment strategies against multi drug resistant bacteria. In the morphological examination, the isolates give a clear white colored colony with brownish pigmentation on the starch casein nitrate agar. Also in the microscopic examination all the isolates were gram positive. The biochemical examinations results were suggesting that the isolates purely may be the *Streptomycetes* species (Viswanath P. Kurup, 1975). Antibiotics are chemotherapeutic agents. They cause cytostatic effect. Antibiotics may also be toxic to tumour cells. Antibiotics have found use in medical and vetenary applications, treatment of plant diseases, as an aid in animal nutrition when mixed with feeds or water and in the preservation of food e.g. Nisin. The antibiotic substances produced in culture by Actinomycetes are able to inhibit the growth (or)

cause the elimination of population of bacteria, yeasts, fungi of many taxonomic categories. The percentage of antibiotics produced by actinomycetes varies with the marine sediments and the season of the year. In the SDS PAGE banding pattern suggested that the all six isolates were may be different actinomycetes. Because it may vary in their cell wall composition. Mostly many of the actinomycetes cell wall is made up of purely proteins and some time that may be carbohydrate. In the gel the position of bands suggesting each lane may contains a different species of actinomycete. This kind of experiments can be used to find out the differences of our isolates and its confirming we are not used the single species as six isolates. Different strains of actinomycetes isolated from marine sediments were found to be varying in the ability to produce antibiotic substances and in their total protein content. Also the deep research in this area may provide more possibilities for the new antibiotics. It may help to combat the newly emerging antibiotic resistant organisms.

Bar diagram 1: ***Screening for Antibiotic producing actinomycetes against test organisms***



Bar diagram 2: ***Challenging against multi drug resistant bacteria***



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SP-3

EFFICACY OF AQUEOUS LEAF EXTRACT OF *ANNONA MURICATA* ON CYPERMETHRIN INDUCED HEPTOTOXICITY IN RATS

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ABSTRACT

This study aims to elicit the potential efficacy of aqueous extract of *Annona muricota* leaf on induction of cadmium chloride on liver of rats, and compare with usage of Methotrexate, a known drug. Six groups of rats were maintained, one as control given saline, next given Cypermethrin (CYP) (0.05ml/100gm ,BW/week), third given aqueous extract of *Annona muricata* leaf (AMLE) (0.05ml/100gm BW/week), fourth given both CYP and AMLE, fifth given methotrexate (MTX) (1.0mg/100gm BW/week) and sixth given CYP and MTX, for a duration of 60 days. After collection of blood for estimation of liver enzymes, liver tissues were collected and used for histo pathological studies. An increase in body weight and a decrease in liver weight seen in all the treatment groups, except MTX treated group. The activities of SGOT, SGPT, and ALP were significantly increased in all the treatment groups, except MTX supplemented group. Hepatoarchitecture is deranged on CYP treatment and partial reversal observed on AMLE supplementation; while near normal reversal can be observed on MTX supplementation.

KEYWORDS: ***Liver, Cypermethrin, Methotrexate, Aqueous extract, Annona muricata leaf***

INTRODUCTION

Pesticides constitute the major source of potential environmental hazards not only for animals but also to human when they become part of food chains[1]. Intensive agriculture activities require the application of large quantities of pesticides annually. The long term application of these pesticides is always expected to induce pesticide residue accumulation in soil, water, and in the general environment, thereby posing a serious threat to public health[2]. Cypermethrin **(**CY**) (**TYPE II synthetic pyrethroid**),** lipophilic in nature is considered to be less toxic due to its speedy insect killing properties and having low toxicity to mammalian tissues. However, it is fairly toxic when applied dermally or administered orally[3]**.** Mammalian liver, by virtue of its unique relationship with the gastro intestinal tract and its role in xenobiotic metabolism, is a target organ of xenobiotic stress. Disturbed liver homeostasis under such stress is sufficient to alter normal body physiology of any organism. Liver is a hub for protein synthesis, regulating cell functions such as maintenance of cellular rigidity, flow management of material through cell membranes, catalysis of an extraordinary range of chemical reaction, regulation of metabolic concentration, and arrangement of nuclear material to control gene function. Detoxification of drug metabolites can induce various and varying degrees of injury to the liver. *Annona muricata* **(**Linn.**)** commonly called soursop, is a small erect evergreen tropical fruit tree plant belonging to the family annonaceae , growing 5 to 6 metres in height .The leaves of *A. muricata* have been reported to contain several groups substances collectively called annonaceous acetogenins including murihexocin and annocuricin[4]**.**The high potency selectivity, wide chemical and biological diversity, and effectiveness of these compounds against microbial resistance could well make them the next class of useful natural antitumor and pesticidal agents and other pharmacological effects[5]. The leaves of *A .muricata* have essential oils with parasiticidal, anti-diarrhoeal, rheumatological and anti-neuralgic properties[6]**.** Methotrexate formerly known as amethopterein , is an antimetabolite and antifolate drug**.** It is used in treatment of cancer, autoimmune disease, ectopic pregnancy, and for the induction of medical abortions. It acts by inhibiting the metabolism of folic acid[7]**.** Methotrexate is thought to affect cancer and rheumatoid arthritis by two different pathways. For cancer, methotrexate competitively inhibits dihydrofolate reductase **(**DHFR**),** an enzyme that participates in the tetrahydrofolate synthesis**.** The affinity of methotrexate for DHFR is about one thousand –fold that of folate . DHFR catalyses the conversion of dihydrofolate to active tetrahydrofolate. Folic acid is needed for the *denovo* synthesis of the

nucleosidethymidine, required for DNA synthesis[8]. Also, folate is essential for purine and pyrimidine base biosynthesis , so synthesis will be inhibited. Methotrexate, therefore, inhibits the synthesis of DNA,RNA, thymidylates, and proteins. Plant derivatives have been used for medicinal purposes for centuries and also being used in our daily food intake. At present, it is estimated that about 80% of the world population relies on botanical preparation as medicines to meet their health needs[9]. Recently, considerable research has been carried out in the search for natural or synthetic compounds as means of chemically important compounds in cancer therapy, in which the occurrence of cancer can be entirely prevented, slowed or reversed[10]. This study thus aims to find out the level of protection of liver given by the herb *Annona muricata* and comparison with Methotrexate, the anticancer drug from the destructive role of cypermethrin as a mode of cancer treatment.

MATERIALS AND METHODS

Selection of the animal model

Albino rats, which had comparable absorption, tissue metabolism and excretion of test compound, to that of human beings, weighing about 130-250 grams were housed in a well ventilated, temperature and humidity controlled animal house, with a light schedule of fourteen hours and ten hours darkness. They were fed with standard diet and drinking water was made available *ad libitum.*

Preparation of cypermethrin

Cypermethrin was purchased from the M.Ramaswamy Mudaliar and Sons Chemical Company. Cypermethrin dissolved in corn oil **(**6 unit Cypermethrin and 6 unit oil) and dosage used is 0.05ml /100 gm BW once a week /rat.

Prearation of annona muricata leaf extract

60 gms ground *Annona muricata* leaf powder was soaked in 400 ml of hot water (88˚C) in water bath for 6 hours, then filtered by carbon silica cloth (150µ) and the filtrate were stored in dark bottles in refrigerator at (45˚C). These procedures were repeated each week.

Experimental design

Healthy male albino rats were divided into 6 groups of 4 animals and received the following regimen of treatments for 60 days.

Group I (C) - Animals received normal saline 1ml/100gm BW/week for used as control.
Group II (CYP) – Animals were given cypermethrin 0.05ml/100gm BW/ week orally.
Group III (AMLE) - Animals received aqueous extract of *Annona* leaf 0.05ml/100gm BW/ week rat orally.
Group IV (ME) – Animals injected methotrexate 1.0mg/100gm BW/ week intraperitoneally.
Group V (CYP+AMLE) – Animals received both cypermethrin and aqueous *Annona* leaf extract (0.05ml/100gm BW/week each) orally.
Group VI (CYP+ME) –Animals received both cypermethrin (0.05ml/100gm BW/week) orally along with methotrexate 1.0mg /100gm BW/day once a week injected intraperitoneally. All the treatments were given between 9.30 to 10.30 am in the morning. At the end of the treatment protocol, animals were anesthetized with ether and sacrificed by decapitation. Blood was collected in both EDTA coated and uncoated tubes and stored properly. All animals were dissected and their liver were rapidly excised, washed with the saline, blotted with a piece of filter paper and weighed. A bit of tissue from the region of liver were fixed in 10% formalin and used for histological studies.

Biochemical analysis

Estimation of serum transaminases - was done by Reitman and Frankel[11] method.
Estimation of alkaline phosphatase - by colorimetric method of Bowers and Mc Comb[12].

Histological studies

Anatomy of the liver tissues were to be studied by fixing a small portion of the tissue in 10% neutral buffered formalin as described by Luna[13]. Thin sections of 4-5µm were taken, stained with Haematotoxylin and Eosin and histological profiling undertaken.

STATISTICAL ANALYSIS

Results obtained were tabulated. Statistical analysis was carried out using Dunnetts "t" test. Any significant variation between the control and treated groups were recorded[14].

RESULTS AND DISCUSSION

Effect on body weight

In the present study, a significant increase in body weight can be seen in all the treatment groups except in methotrexate treated group, when compared to control, the rate of increase observed to be almost equal irrespective of treatment on comparison with cypermethrin treated control. Body weight changes serve as a sensitive indicator of general health status of animals. The body weight increase and weight of intraperitoneal tissue was significantly reduced by diets containing green tea, caffeine and theanine[15]. No significant changes on DEN treatment and cinnamon supplementation in body weight has been reported[16]. Similarly, no significant increase in weight of animals treated with 100mg of AMLE, but significant decrease in percentage body weight on treatment with 1000mg AMLE has been observed[17]. Studies have reported the significant lowering of body weight on cadmium treatment and reversal of these effects on curcumin supplementation has been reported[18]. A decrease in body weight on treatment with cinnamon extract has been reported.

Effect on liver weight

A significant increase in weight of liver on cadmium chloride treatment and subsequent reversal by curcumin supplementation has been reported[18].Also reported about significant increase in liver weight on cadmium treatment and a concurrent significant decrease in liver weight on green tea extract consumption along with cadmium. Similar increase in liver weight on cadmium treatment and subsequent decrease in co-administration with fenugreek seeds, rosemary and cinnamon as well as methotrexate has been reported[19]. But in the present study, a significant decrease in liver weight can be seen in all treatment groups except methotrexate supplementation group, on comparison with control. When compared to cypermethrin control, no significant changes can be observed on Annona leaf extract supplementation, while methotrexate supplementation seems to be more beneficial in nature.

Effect on liver enzymes

It is well known that elevation of transaminases is credited to hepatocellular damage and increase in ALP reflects a pathological alteration in biliary flow[20]. Serum AST, ALT, ALP levels are biomarkers for hepatic diseases and the increase in activities of these enzymes indicates liver damage and thus alteration in liver function[21]. The administration of cypermethrin has shown to produce oxidative stress by generating ROS and reducing the antioxidant defence systems. In the present study, the activity of SGOT, SGPT were observed to be singnificantly increased in all the treatment, groups except for methotrexate supplementation group, on comparison with control. When compared to cypermethrin treatment also, methotrexate supplementation seems to be more beneficial. The activity of ALP was also observed to be significantly increased in all the treatment groups when compared to control as well as to cypermethrin treated groups. Rats that are exposed to acute restraint stress showed a significant increase in the activities of liver enzymes AST, ALT and ALP. Administration of cinnamon decoction significantly reduced the activities of these enzymes. Co-administration of aqueous extracts of fenugreek seeds, rosemary and cinnamon with cadmium chloride reduced the enzyme activities towards the control values[22].

Effect on liver histology

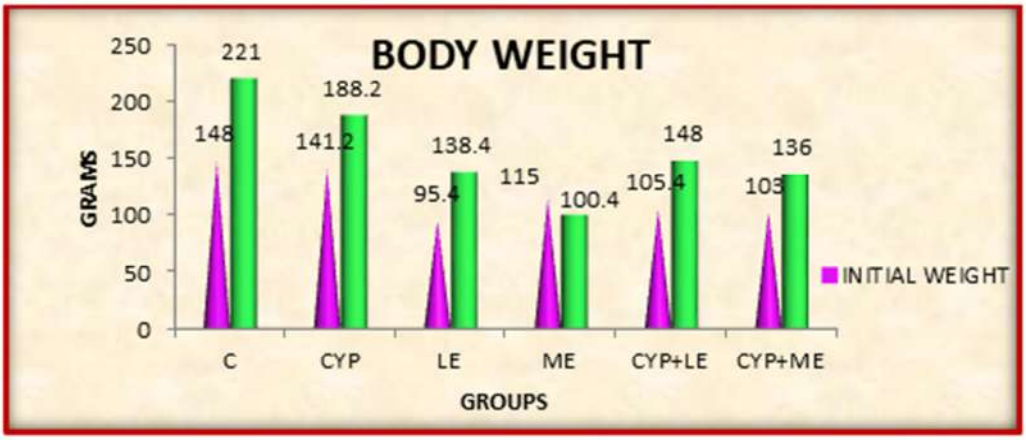
It is known that hepatocytes play a vital role in the proper functioning of the liver as they are the main functional cells of the liver. The hepatocytes contain glycogen, and also maintain a steady level of blood glucose[23]. A compromise in the integrity of the hepatocytes could lead to improper functioning of the liver. The degeneration and increased density of nuclear chromatin with very compact nuclear structure of hepatocytes in cadmium induced rats[24]. Histological changes observed in the photo micrograph of a section of the liver of rats in the hepatotoxic groups showed necrosis and regeneration in the group fed *Gentium africanum* supplemented diet[25]. The aqueous extract of *Psidium guajava* leaves on histological study did not show any adverse alteration in the morphological architecture of the liver tissues in both sexes of the animal

model[26]. Deranged architecture of hepatocyte with hydrophobic degeneration, multiple binucleated cells and deranged sinusoidal arrangement with dilation and vascular congestion have observed by on AME treatment[27]. Section of cypermethrin treated liver showed moderate enlargement of sinusoids. Vacuole formartion in hepatocytes and central vein congestion increase in cypermethrin dose was observed to bring about enlargement of sinusoidal degeneration of hepatic cord, congestion and haemorrhage in central vein[28]. In the present study, while no significant pathological changes were observed on AMLE treatment, cypermethrin as well as methotrexate brought about an alteration in lobular architecture. Concurrent supplementation was AMLE showed altered lobular architecture with vacuolations and necrosis in hepatocytes, while methotrexate supplementation showed normal lobular architecture with mild interface hepatitis.

Table 1: ***The effect of aqueous leaf extract of annona muricata on cypermethrin induced changes on body weight of albino rats.***

GROUPS	BODY WEIGHT (GRAMS)	
	INITIAL WEIGHT	FINAL WEIGHT
C	248±4.636	321±3.316
CYP	141.2±1.772*	188.2±0.860*
LEAF EXTRACT	95.4±1.720*	138.4±1.630*
METHOTREXATE	115±2.549*	100.4±2.315*
CYP+LE	105.4±1.720*a b	148±2.549*a b
METH+CYP	103±2.097 *a c	136±1.370* ac

Fig 1: ***The effect of aqueous leaf extract of annona muricata on cypermethrin induced changes on body weight of albino rats.***



Values are expressed as Mean ± S.E.M of five rats.

*Significance at 5% level, [a] Significance at 5% level of G2 Vs G5, G6. [b]Significance at 5% level of G3 Vs G5. [c]Significance at 5% level of G4 Vs G6.

C – Control, CYP- Cypermethrin, LE- Leaf extract, ME- Methotrexate, CYP+LE- Cypermethrin + Leaf extract, CYP+ME – Cypermethrin + Methotrexate.

Table 2: ***The effect of aqueous leaf extract of annona muricata on cypermethrin induced changes on liver weight of albino rats.***

GROUPS	LIVER WEIGHT (GRAMS)
C	8.52±0.123
CYP	5.901±0.052*
LE	5.226±0.325*
ME	6.754±0.194*
CYP+LE	5.656±0.227*a
CYP+ME	7.928±0.353

Fig 2: ***The effect of aqueous leaf extract of annona muricata on cypermethrin induced changes on liver weight of albino rats.***

Values are expressed as Mean ± S.E.M of five rats.

*Significance at 5% level, [a]Significance at 5% level of G2 Vs G5,G6. [b]Significance at 5% level of G3 Vs G5. [c] Significance at 5% level of G4 Vs G6.
C – Control, CYP- Cypermethrin, LE- Leaf extract, ME- Methotrexate, CYP+LE- Cypermethrin+Leaf extract, CYP+ME – Cypermethrin + Methotrexate.

Table 3: The effect of aqueous leaf extract of annona muricata on cypermethrin induced changes on liver enzymes in albino rats.

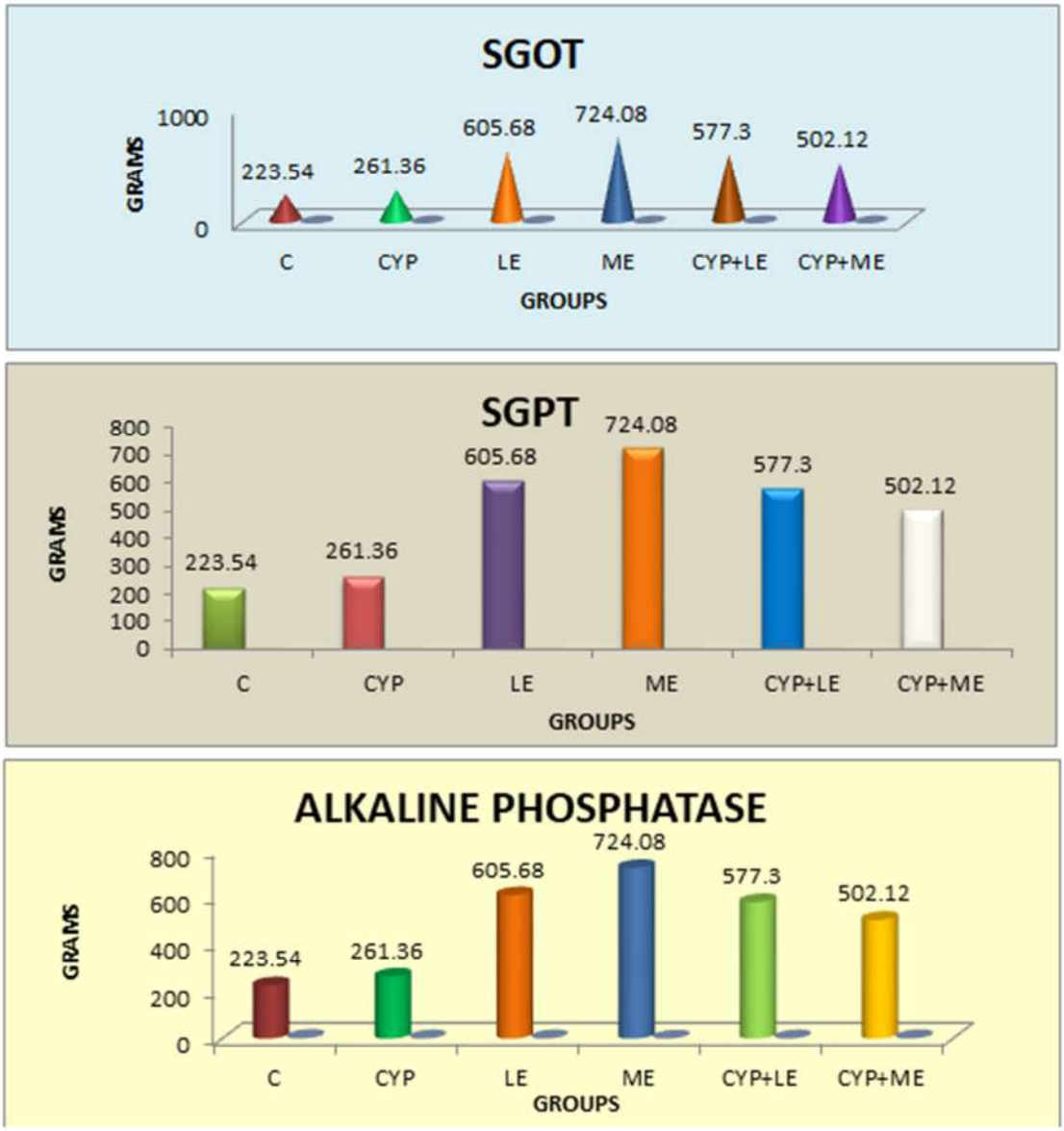
PARMETERS	SGOT (g/dl)	SGPT (g/dl)	ALP (U/I)
C	247.7 ± 0.769	136.40 ± 0.515	223.54 ± 3.664*
CYP	298.46 ± 4.612*	195.29 ±3.671*	261.36 ± 1.775
LE	321.9 ± 1.124*	777 ± 0.814*	605.68 ± 1.746*

ME	437.72 ± 0.849*	86.84 ± 0.527*	724.08 ± 0.609*
CYP+LE	583.6 ± 0.817*[a b]	184.6 ± 1.357*[a b]	577.3 ± 1.132*[a b]
CYP+ME	248.92 ± 4.164 [æ]	61.02 ± 0.93*[æ]	502.12± 2.566*[æ]

Values are expressed as Mean ± S.E.M of five rats.

*Significance at 5% level, [a]Significance at 5% level of G2 Vs G5,G6. [b]Significance at 5% level of G3 Vs G5. [c] Significance at 5% level of G4 Vs G6.
C – Control, CYP- Cypermethrin, LE- Leaf extract, ME- Methotrexate, CYP+LE- Cypermethrin+Leaf extract, CYP+ME – Cypermethrin + Methotrexate.

Fig 3: ***The effect of aqueous leaf extract of annona muricata on cypermethrin induced changes on liver enzymes in albino rat***

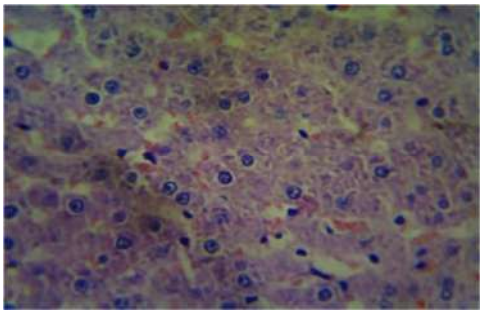


Values are expressed as mean ± S.E.M of five rats

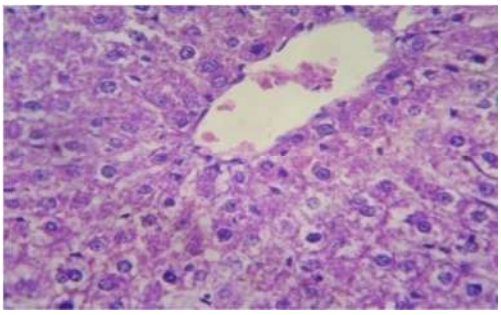
*Significance at 5% level, [a]Significance at 5% level of G2 VsG5,G6. [b]Significance at 5% level of G3 Vs G5. [c] Significance at 5% level of G4 Vs G6.
C-Control, CYP – Cypermethrin, LE – Leaf extract, ME-Methotrexate, CYP+LE- Cypermethrin +leaf extract, CYP+ME- Cypermethrin + Methotrexate.

HISTOLOGY OF LIVE

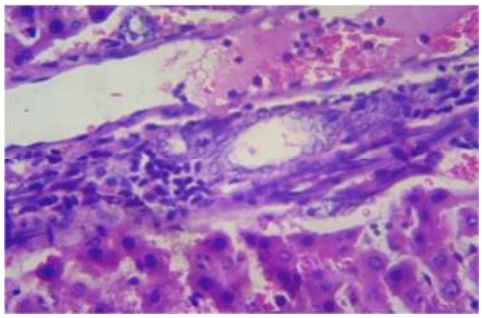
1. CONTROL – LIVER



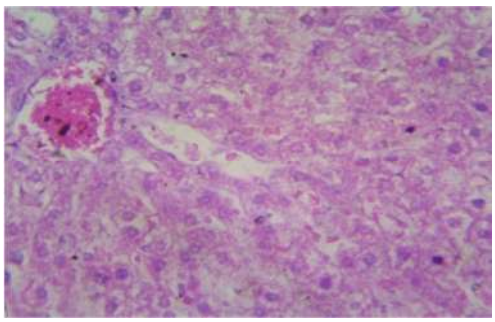
4. METHOTREXATE TREATED



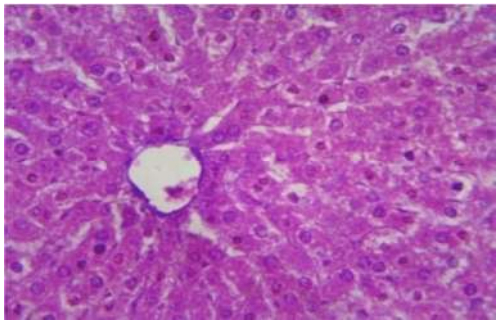
2.CYPERMETHRIN TREATED



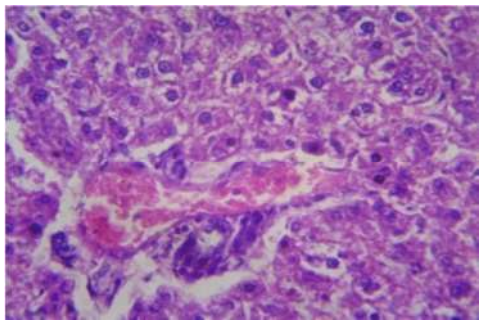
5.LEAF EXTRACT+ CYPERMETHRIN THEATED



3. LEAF EXTRACT TREATE



6. METHOTREXATE+ CYPERMETHRIN TREATED



CONCLUSION

Through this study the following objectives have been established. Cypermethrin bring about severe hepatotoxicity causing a reduction in liver weight and increasing the serum output of SGOT and SGPT as well as ALP, the markers enzymes of prompt liver function. Aqueous leaf extract *Annona muricata* does bring about certain changes in structure and function of liver due to the presence of acetogenins and other alkaloids. When the leaf extract is given along with cypermethrin, a partial revesral can be observed. Methotrexate, a known anticancer drug seems to have a more beneficial effect than aqueous leaf extract of *Annona muricata* at the present dosage and duration, A higher dosage with different duration may do the magic.

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SP-4

EFFECT OF TERATOGENICITY IN THE AYURVEDIC DRUG BHRINGARAJAADHI CHURNAM USING ZEBRA FISH AS MODEL ORGANISMS

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ABSTRACT

The term teratogenicity means the ability to cause congenital malformation or birth anomalies in the developing fetus or the embryo. The teratogens can cause damage to the DNA of the developing embryo or the fetus. They may cause observable and internal damages like limb abnormality, weight reduction and skeletal deformities. In this study the zebra fish embryo is being used as the model organism to test the teratogenic effect of the drug. Bhringarajaadhi Churnam is a Ayurvedic drug that helps mainly in the growth of hair and also plays a role in the liver disorders. This drug has a main component Bhringarajaadhi plant, sesame seeds, Indian gooseberry (Amla). In this case eight different concentrations of the drug was inoculated into the zebra fish embryo to study the effect of the drug. E3 medium was used as the negative control. , 1% and 2% ethanol was used as the positive control. The fish embryo was observed under light microscope for a period of 5 days to observe the deformities. The effect of the drug on the embryo was the drug did not lead to the proper hatchability of the larvae. The larvae have to hatch at 48 hours but the hatchability rate was low with a few notable deformities. Our results indicate that the zebrafish is a convenient system for studying the teratogenic potential of drugs. This approach can easily be adapted to test other chemicals and drugs for their effects on early vertebrate development.

KEYWORDS: ***Teratogenicity, malformation, zebra fish embryo.***

INTRODUCTION

The term teratology was derived from the Greek *teras* meaning 'marvel' *logos* 'the study of'. As early as the 17th century the teratology referred to a discourse on prodigies and things that are seen as extraordinary or as an abnormal (1). In the 19th century it acquired more meaningful growth that is related to the biological deformities. In the present it is the most widely used method in determining the biological and congenital deformity or an individual with significant malformations. A teratogen is an agent that can disturb the development of the embryo or the fetus. Teratogen may cause birth defect in the child or the teratogen can halt the pregnancy outright. The teratogens can be classified into radiation, maternal infections, chemicals and drugs such as alcohol, antibiotics, cocaine, lithium.etc., Bhringarajaadhi churna is a phyto-pharmaceutical formulation of *Eclipta Alba* with the goodness of Indian gooseberry and sesame seeds. Ancient medical treaties describe that *Eclipta Alba* is prescribed for perfect health as it is rejuvenator and it is free from aging, pain, degeneration and premature death. *Eclipta Alba* which is otherwise known as "False Daisy" it belongs to the family Asteraceae. The root of this plant is well developed, cylindrical, greyish. "Bhringaraj" in Sanskrit is kehraj and in Tamil it is called karisalankani. This plant improves mental cognition, enables the mind to understand inner deep-down traits. On the regular consumption of this plant leads to the improvement in the immune system, quick recovery from diseases, delays ageing effects like wrinkles and grey hair, clears vision abnormalities and improves clear audible range. The biological composition of the drug is Indian gooseberry and sesame seed powder. The chemical composition of the drug Ecliptic, Heptacosanol, Des-me thyl, Wedelolacetone, Stigamasterol, sixteen polyacetylenic thiophenes. Presently wide variety of model organisms are commonly used for studying the diseases and their mechanism, using *Drosophila* and *Caenorhabditis elegans* ,and mammals such as mice, rats and primates etc. There are a wide gap exists between the invertebrate and vertebrate model systems. Thus to compensate the gap between the invertebrate and vertebrate the zebra fish was used as the model organism. Since 1960s the zebra fish has become increasingly important to scientific research. It has many

characteristics that make it a valuable model for studying human genetics and disease. In this study the teratogenic effects of the drug Bhringarajaadhi Churnam in zebra fish embryo in eight different concentration (10, 50, 100,200,400,600,800, 1000 µg/mg). The fish embryo is being observed in different time intervals from 4hours-96hours. The mortality rate, deformity and the hatchability of the fish embryo has been observed and recorded.

MATERIALS AND METHODS

Drug preparation

10 ml of the drug Bhringarajaadhi Churnam was weighed and was dissolved in about 1ml of distilled water to prepare the stock.

E3 medium (for zebrafish embryos)

34.8 g NaCl, 1.6 g KCl, 5.8 g CaCl2·2H2O, 9.78 g $MgCl_2 \cdot 6H_2O$

To prepare a 60X stock, dissolve the ingredients in H_2O, to a final volume of 2 L. Adjust the pH to 7.2 with NaOH. Autoclave. To prepare 1X medium, dilute 16.5 mL of the 60X stock to 1 L. Add 100 µL of 1% methylene blue

Embryo Harvesting

Maintain zebrafish at 28.5 °C, pH 7, conductivity between 500-1,500 µS, and a light/dark cycle of 14 hr light and 10 hr dark(2) Set up the fish for mating the night before harvesting eggs by adding fish system water into a mating tank. Add a male and female fish into the tank and separate the two fish with a divider. Each fish pair will produce a range of 50-300 eggs. To ensure that enough eggs will be produced, set up 30 pairs of fish. Typically, about 50% of fish pairs at the prime mating age (6-9 months old) will produce eggs, resulting up to 200 eggs per pair and a maximum of up to 3,000 eggs for this experiment. The next morning after the light turns on, remove the divider to initiate mating. Check the mating tanks for eggs every 15 min. Once the fish lay eggs, harvest all embryos using a tea strainer and combine them into one large container with E3 buffer. At 1.5 hpf, remove and discard unfertilized eggs with a plastic transfer pipet under a dissecting microscope. Unfertilized eggs are opaque while fertilized eggs are transparent (3, 7). Transfer 50 embryos into a 100 x 15 mm glass Petri dish containing 50 ml E3 buffer for each treatment condition.

Inoculation of drug in the zebra fish egg

The E3 medium was poured in a sterile plate and two replicate eggs were inoculated and the teratogenic effect was observed.

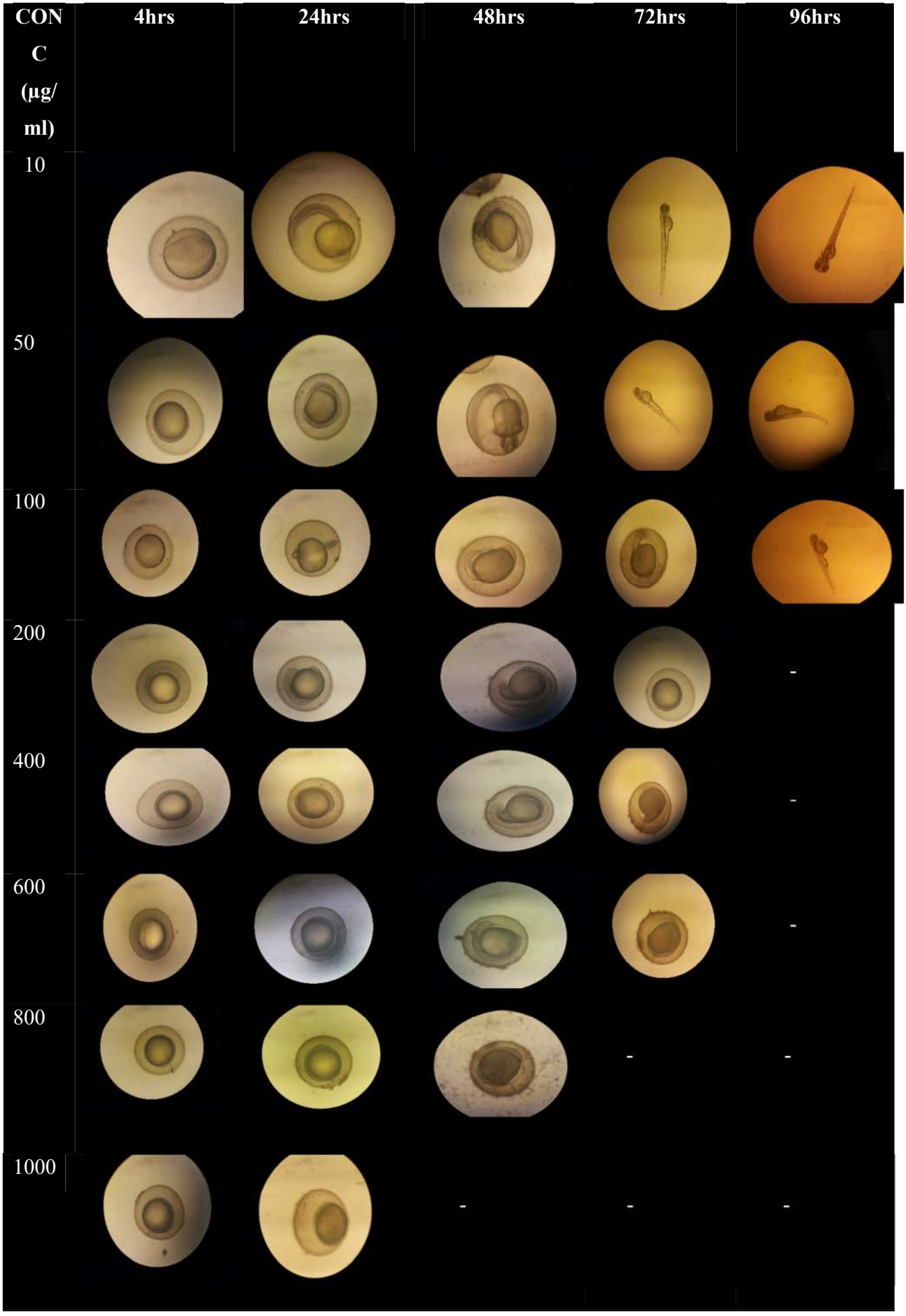
Treatment of the drug

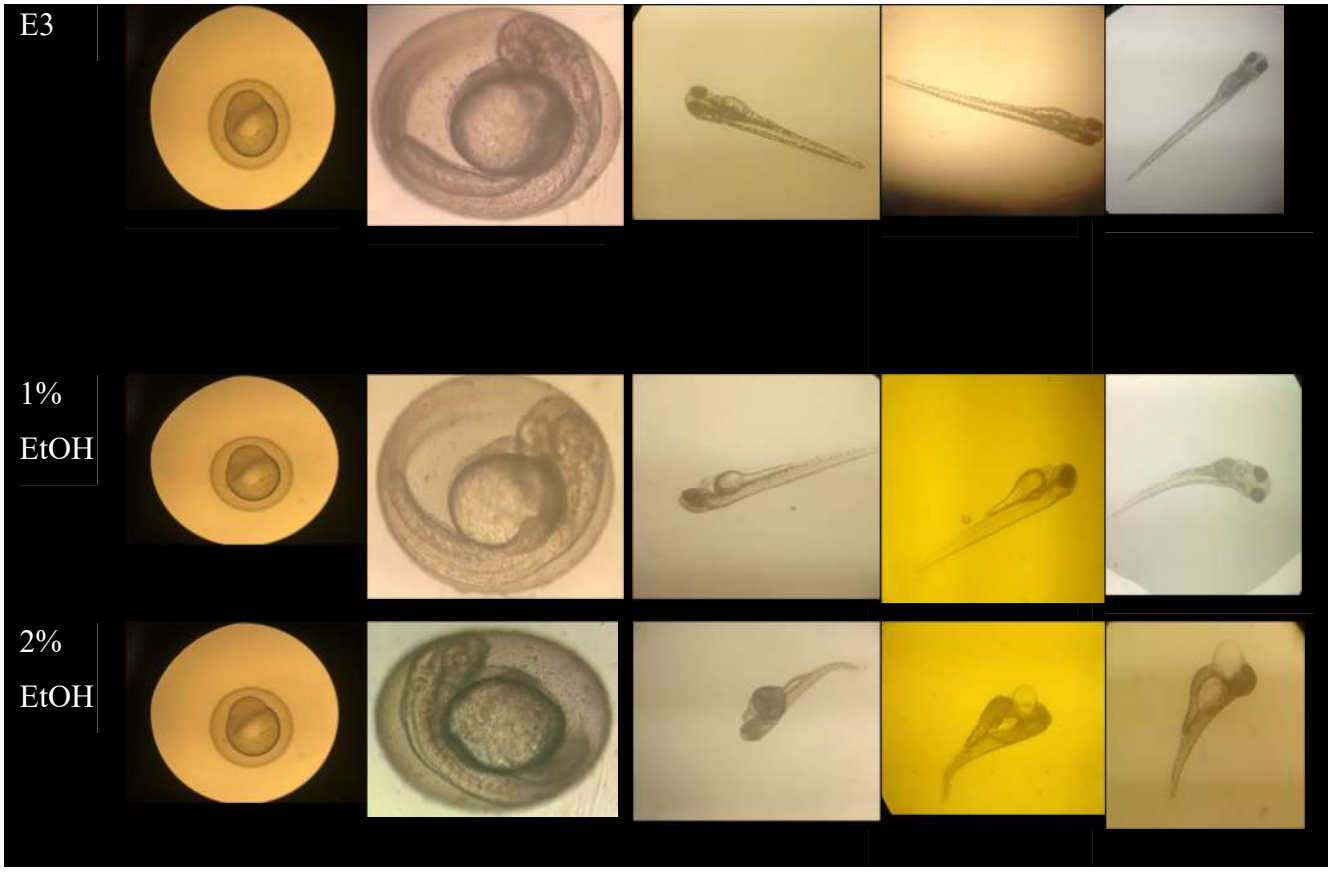
Various concentration of the Ayurvedic drug – Bhringarajaadhi Churnam (10, 50, 100, 200, 400, 600, 800, 1000 µg/ml) was prepared by the method of serial dilution using distilled water. E3 medium was used as the negative control. The positive control 1% and 2% ethanol was also prepared.

RESULT AND DISCUSSION

The teratogenic effect of the drug Bhringarajaadhi Churanam is being detected using eight different concentration on the zebra fish embryo. In this experiment the positive control is 1% ethanol and 2% ethanol. The negative control in this experiment is E3 medium. The study were done with different concentrations that is being used are 10µg, 50µg, 100µg, 200µg, 400µg,600µg,800µg and 1000µg. The zebra fish embryo observed in different intervals of time from 4hours-96hours. The deformity that was observed in the 50µg,100µg concentration of drug. In the drug concentration 200µg, 400µg, 600µg,800µg and 1000µg was the embryo did not hatch at 48 hours, pericardial edema and body curvature were observed . Positive control the development of the zebra fish embryo does not go normal, the embryo that is being inoculated with the drugs dies as the days goes by. In the negative control the developed of the zebra fish embryo takes place as it has to grow according to the regular cycle.

Figure (1) ***Embryos treated with ethanol(Positive Control), E3 medium (negative Control) and Drug (Bhringarajaadhi Churanam) showed***





CONCLUSION

The zebra fish embryo is an important asset in the teratogenicity. In this study the Bhringarajaadhi Churnam is a rich source of iron vitamins and other healthy supplement. When the drug is given in a very low concentration 10µg the zebra fish embryo grows properly, but as the concentration gets higher the growth of the embryo is affected. When the embryo is kept in the higher concentration on a longer period of time the embryo does not hatch resulting in the deformity of the fish larvae. In the higher concentration that is in 800 and 1000µg the larvae dies. In the positive control that is in 1% and 2% ethanol the fish embryo is totally deformed and dies as it is kept for a longer period of time. In the negative control E3 medium there is a proper development of the fish larvae. Compared to other vertebrates, zebrafish have advantages that include high fecundity, external fertilization, optical transparency, and rapid development. Available mutants that lack pigmentation (such as the casper zebrafish)(4) also help to enhance visibility of internal organs. It is also easy to generate transgenic zebrafish with reporter genes to facilitate analysis in live fish (5). Because the zebrafish genome is conserved with humans, information gained from their studies can lead to translational results in humans (6)

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SP-5

REVIEW ARTICLE

BENEFICIAL EFFECTS OF BACTERIAL SECONDARY METABOLITES

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ABSTRACT

Microorganisms are the potential source of unique low molecular weight bioactive secondary metabolites. Microbes are capable of synthesizing number of valuable secondary metabolites find diverse uses in human welfare. Secondary metabolites are species specific and can be produced only during certain period of their growth phase and compete with other microbes and involves in communication purpose. Some of the secondary metabolites have deleterious effect on animal, human and plant. Actinomycetes, fungi and bacteria species contributed varied types of precious secondary metabolites. Bacteria are proven to be a major source of secondary metabolites and plays important role in controlling the growth of infectious pathogens, also in the field of agricultural, pharmacological industrial and cosmetics industry. This review outlines about the benefic effects of bacterial secondary metabolites.

Keyword: ***Secondary metabolites, Pigments, Bacteria, Pheromones, Antibiotics.***

INTRODUCTION

Bacteria are microscopic organism, which may exist in its single-celled form or in a colony of cells and are extremely diverse in nature. Bacteria have been beneficial to us in many ways and some of them are pathogenic in nature. Bacteria are found in everywhere in earth includes soil, air, water, in human body, thermal vents, hot spring, highly acidic environment, extremely cold conditions and in marine water. Microbes like Bacteria, Fungi and Actinomycete have given thousands of valuable products to the health and well-being of people [1]. Metabolism is a set of chemical reactions which helps to maintain the organism's growth, reproduction and stimuli to environment. Anabolism and catabolism are the two types of metabolism in living organism. Primary metabolic reaction and secondary metabolic reactions involves biosynthesis of natural products with industrial applications called as metabolites from plants, animals and microbes. Metabolites are either macromolecules or micro molecules classified into primary and secondary metabolites. Primary metabolites are directly involved in growth, development and reproduction of organism synthesized in trophophase of the growth cycle, which includes amino acids, sugars, vitamins, nucleotide, ethanol, polyols acetone and lactic acid. In addition to synthesizing primary metabolites microbes also synthesize low molecular mass secondary metabolites [2].

IMPORTANCE OF SECONDARY METABOLITES

The term secondary metabolite was used in microbiology by Bu'lock in 1961[3]. Secondary metabolites are industrially important and unique bioactive substances produced by microbes and plants. In contrast to primary metabolites, secondary metabolites are not directly involved in the growth and regulation of the microbes. Plants produce a lot of secondary metabolite, such as alkaloids, flavanoids, terpenoids, steroids and carbohydrates .Microbes contributes 22500 types of biologically active secondary metabolites during idiophase of the growth cycle referred as idiolites[4].Among them 45% are synthesized by actinomycetes, 38 % by fungi and 17 % by bacteria which includes antibiotics, alkaloids, gibberellins and toxins. Bacterial species synthesize different kinds of secondary metabolites with unique activities, novel structures and properties, most of them are used in pharmacology and agriculture field. Secondary metabolites are not essential for the growth of bacteria but provide potent application in human health. Only specific strains of bacteria produce secondary metabolites. Bacterial secondary metabolites exhibit wider application such as antibiotics, antitumor compounds, immunosuppressant, antiparasitic agents, herbicides and enzyme inactivating agents[5]. Secondary metabolites widely used as an anti-infective agent and sold for 55 billion

dollars in 2000. Primary metabolites are act as a building block in making certain secondary metabolites. Shikimic acids are used to produce ergot alkaloid, candicidin and chloramphenicol. Antibiotics such as penicillin, cephalosporin and cephamycins, gramicidin are derived using amino acids. Acetyl-CoA used as building block to synthesize erythromycin (antibiotic), avermectin (antiparasitic compound) doxorubicin and taxol (antitumor agent). Streptomycin and kanamycin are derived from sugars. Fermentation technology has been used for the large scale production of secondary metabolites. Secondary metabolites can be industrially produced through both submerged fermentation and also by solid state fermentation. Solid state fermentation yield higher amount of required secondary metabolites than submerged fermentation[6].

BACTERIAL SECONDARY METABOLITES

Bacteria are prokaryotic microorganism are known to be a rich source of bioactive substance with diverse chemical structures and biological properties. It includes biologically active substances like antibiotics, antitumor, pigments and bacterial toxins.

ANTIBIOTICS

Antibiotics are wonder drugs act as a powerful medicine to treat against bacterial and fungal infection. 'Antibiotic' the term coined by Selman Waksman in 1941. Antibiotics are the secondary metabolites that antagonizes the growth other pathogenic microorganism[7]. Penicillin was the first antibiotic discovered by Alexander Fleming in 1928 from *Penicillium notatum*. Antibiotics like tetracycline, erythromycin, and kanamycin, were discovered during 1950-1960 considered as the Golden age of antibiotic production. Nearly 5000 types of antibiotics have been produced by fungi, Gram negative and Gram positive bacteria[8]. Most of the potent antibiotics are contributed by soil microorganism. Antibiotics are the chemical substances which retard or kill the growth of pathogenic microorganism. Antibiotics are low molecular weight compounds, applied as an antitumor agent, immunosuppressive agent and hypocholesterolemic agent. Microbial antibiotics like actinomycin D, mitomycin, bleomycins, anthracyclines, daunorubicin and doxorubicin are used to treat tumor acts as an antitumor agent. Bacterial species have greatly contributed distinct types of antibiotics. *Bacillus subtilis* produces two antibiotics iturine and surfactin. Iturine possess antifungal property. Surfactin shows surfactant and antibiotic activities. Marine bacteria are known to be the unique source of antibiotics. Marine *Vibrio* species produces the antibiotic andrimid. *Alteromonas* species contributes 2-n-pentyl-4-quinolinol[9]. The demand for developing new antibiotics with potent antipathogenic activity is an urgent need nowadays. Antibiotic resistance is the major issue in medical community. Superbugs are the certain strains of microorganism, which are resistant to antibiotics. So there is a need for search of new antibiotics with desirable efficient properties. The work on development of new antibiotics should be intensified to get new antibiotics to overcome the multidrug resistant bacteria.

BACTERIAL PIGMENTS

Pigments are organic compound acts as colorants. Natural pigments are much preferable than synthetic pigments due to their bioavailability, stability, effectiveness, biodegradability, non carcinogenic, non toxic and antimicrobial property against pathogens [10]. Pigments find applications in various industries which include textile, cosmetics, plastic, paint, paper and printing. Natural pigments have been obtained from microbes, animals, insects, plant and ores [11].Plant pigments play a pivotal role in photosynthesis process and also attract the insects for pollination. The root, bark, leaves and wood of the plant contribute different types of pigments it includes porphysin, carotenoid, anthocyanin and betalain. Microorganisms contribute a lot of pigments have been helpful in photosynthesis process in certain photoautotrophic organisms, antioxidant activities and UV radiation exposure. Pigments are the helpful tool in taxonomic studies. It provides a potential way to classify the algae, fungi and bacteria. Microbes contribute diverse types of pigments such as carotenoids, melanin, quinones, flavins, prodigiosins, monascins actinorhodin, zeaxanthin and violacein [12]. Environmental factors play a key role in controlling the pigment production. Factors such as oxygen availability, temperature range, nutritional condition, chemical composition, colony age and strain improvement determines the type of pigment to be produced. For example temperature plays a key role in blue pigment production from *Arthobacter atrocyaneus*. *Arthobacter atrocyaneus* synthesize blue pigment at 24° C, but there is no significant pigment production in 37°C [13].Bacteria such as *Rhodospirillum rubrum, Micrococcus lutens* and *Saraina aurantiaco* exhibit production in various temperatures. Carotenoids are the major class of pigments this is very important in photosynthetic process in Cyanobacteria also resistance to oxidative damage and photo damage. Only few species alone produces purple and blue pigment.

*Chromobacterium.*spp*, Janthinobacter.*spp*,* and *Iodobacter.*spp produces a versatile purple color violacein pigment possess antibacterial, antiviral, antioxidant, antifungal and antitumor properties. Indigoidine, a blue pigment has been produced by *Erwinia, Arthrobacter, Corynebacterium* and *Vogesella* genera shows antioxidant and antimicrobial activity[14]. Free radicals are the unstable molecule produced during oxygen metabolism it leads to Alzheimer's disease, dementias, cardiovascular disease, diabetes, rheumatoid arthritis, cancer, and cataracts. Aging is associates with free radicals. Antioxidants have the ability to prevent free radicals. Certain pigments have antioxidant property such as carotenoid, naphthaquione and violacein [15]. Xanthomonadin possess antioxidant property. *Serratia marcescens* synthesize a red color pigment prodigiosin with antibacterial activity against Gram positive and gram negative bacteria also possess antimalarial and antifungal property[16]. Prodigiosin also shows apoptotic activity against cervix carcinoma cells [17]. Prodigiosin is also produced from different strains like *Serratia marcescens, Pseudomonas magneslorubra, Vibrio psychroerythrous, Vibrio gazogenes* and *Rugamonas rubra.* Some of the pigments can be produce only by marine derived microorganism. Riboflavin pigment used to treat blood borne pathogen. *Pseudomonas aeruginosa* is the source of pyocyanin, an antifungal and antibacterial agent.

HYDROGEN CYANIDE

Hydrogen cyanide or Prussic acid can be biosynthesized from *Pseudomonas aeruginosa and pseudomonas fluorescens* referred as Hydrogen cyanide producing bacteria HPB [18]. HPB supports the growth of the plant by enhancing the phosphate availability [19].

PHEROMONES

Pheromones are the secondary metabolites of the bacteria involves in the microbe-microbe interaction. Majorly this is produced by Myxobacteria, Actinomycetes and Cyanobacteria. Pheromones are small diffusible molecule secreted into the environment to communicate with other bacteria. Pheromones secreted by bacteria triggers a specific response from other bacteria such as luminescence, virulence factor production and conjugation. Fruiting bodies are formed by aggregation of Myxobacteria. *Bacillus* species sporulate and synthesize antibiotics. Pheromones produced by *Vibrio fischeri* and *Vibrio harveyi* involves in bioluminescence activities. The virulent gene of the *Pseudomonas aeruginosa* is auto induced by pheromones.

GIBBERLLINS

Microbes have given valuable plant growth regulators which includes gibberellins, auxins, abscisic acid, ethylene, fusicoccin and jasmonic acid. Gibberellins are plant hormones helps in plant growth regulation. Gibberellins were first isolated from *Gibberella fujikuroi*. Gibberellins are also secreted by some of the bacteria which include *Acetobacter diazotropicus, Azospirillum lipoferum, Azospirillum brasilence, Bacillus licheniformis*, *Bacillus pumilus, Herbosporillum seropedicae* and *Rhizobium phaseoli* [20].

CONCLUSION

Bacteria are the potential source of secondary metabolites has wider application in human healthcare. Bacteria are known to produce distinct types of secondary metabolites. Bacterial secondary metabolites used for human need. Mainly Secondary metabolites are find applications as antimicrobial agents, antitumor agent, antiobesity agent and food colorant. But it produces only in less quantity. There is an urgent need to develop new distinct types of secondary metabolites in higher amount. Nowadays antibiotic resistance is the major problem. So there is a need of searching new antibiotics. Microorganism are present in different environment have distinct characters. Discovery of new microbes helps in identifying new biologically active secondary metabolites with new properties. Pharmaceutical industries are in need of higher quantity secondary metabolites. This can be achieved through gene manipulation techniques and through mutations. In genetic engineering microbial genes can be altered to produce maximum number of antimicrobial agents. Mutation through physical and chemical agents also helps in synthesis of new secondary metabolites.

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SP-6

BIOSYNTHESIS AND CHARACTERIZATION OF *Cassia Fistula* MEDIATED LITHIUM COATED IRON OXIDE NANOPARTICLE ($LiFe_2O_3$) AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

The present study focuses on biological evaluation of synthesised lithium coated iron oxide nanoparticles using *Cassia fistula* aqueous extract. Ultraviolet-visible spectroscopy, Fourier transform infrared spectrometer, energy dispersive- X-ray spectrometer, X-ray diffractometer and scanning electron microscopic studies were applied for the confirmation of synthesized lithium coated iron oxide nanoparticles. The characterization studies confirmed that synthesized nanoparticles existed high stability and crystalline configuration. It is observed that, the size of *Cassia fistula* mediated lithium coated iron oxide nanoparticle was about 24 - 170 nm by using Scherrer formula. The antimicrobial effects of lithium coated iron oxide nanoparticles showed efficient antibacterial activity against *E.coli* (12.50 ± 0.50mm), *Bacillus subtilis* (13.16 ± 0.28mm) at 50 mg ml^{-1} concentration. Thus, biologically synthesized *Cassia fistula* mediated lithium-coated iron oxide nanoparticles used as antibacterial agent in pharmaceutical application.

Keywords: ***Antibacterial activity, Biological synthesis, Cassia fistula, Lithium coated iron oxide nanoparticles***

INTRODUCTION

There are a large variety of methods are used in the production of nanoparticles, making use of techniques from both physics and chemistry. Among the first ones, arc-discharge, high-energy ball milling, laser pyrolysis and laser ablation are the most commonly used. Electrochemical and chemical vapor deposition, sonochemistry and different wet chemistry routes (e.g. sol-gel, co-precipitation, inverse micelles, etc.) are widely employed[1]. With the development of new chemical or physical methods, the concern for environmental contaminations is heightened as the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous byproducts. Thus, there is a need for 'green chemistry' that includes a clean, nontoxic and environment-friendly method of nanoparticle synthesis[2,3]. As an alternative to conventional methods, biological methods are considered safe and ecologically sound for the nanomaterial fabrication[4]. Bacterial resistant of different antibiotic is a severe clinical problem in public health. Therefore, researcher are focus to find out best antibiotic from different sources. Nanomaterial is emerging technology for synthesizing different antimicrobial agent[16]. *Cassia fistula* know as golden tree and belonging to the family fabaceae. It is consider as purgative, self-medication and strongly advised against Ayurveda. It contain phytochemicals like phenol, terpenoid, flavone and sterol compound. Herein, we report biological synthesis of lithium coated iron oxide nanoparticles ($LiFe_2O_3$) and evaluating it antibacterial activity by well diffusion methods (Fig 1).

MATERIALS AND METHODS

Materials

Cassia Fistula were collected at Mettupalayam region Coimbatore, Tamil Nadu, India. Bacterial pathogens (*E. coli* MTCC 912, *Bacillus subtilis* MTCC 121 and *Streptococcus pyrogenes* MTCC 1925) obtained from IMTech, Chandigarh, India. The bacterial pathogens are subculture and maintained in nutrient agar media for future use. All the chemicals used in the experiments were purchased from Sigma Aldrich, India.

Methods

Preparation of Cassia Fistula leaf extract

10 grams of fresh *Cassia fistula* leaves rinsed thoroughly in tap water and future wash with distilled water to remove debris. Samples were crushed well using mortar and pestle with 100ml of distilled water for 15min. Then the extract was filtered using filter paper and stored in refrigerator for further investigation[14].

Synthesis of lithium ferrous oxide nanoparticles

Lithium nitrate and Ferric chloride (0.1 N) was used as precursor. Lithium nitrate and Ferric chloride solution was prepared in deionized water. 50% of leaf extract was prepared and made up to 250ml with deionized water. The plant extract (50 ml) and 250 ml of Lithium Ferrous solution were mixed. The mixture was stirred by using magnetic starrier for 2 hours at 80 ^{o}C. The mixture was centrifuged and supernatant was discarded. The pellet was washed with deionized water followed by ethanol for three times and dried using hot air oven[20].

Characterization of Lithium Ferrous oxide nanoparticles

The optical property of $LiFe_2O_3$ nanoparticles were examined by UV absorption spectroscopy (Shimadzu). The phase purity of the synthesized *Cassia Fistula* $LiFe_2O_3$ nanoparticles were analysed using an X-ray diffractometer (XRD). Fourier transform infrared (FT-IR) spectroscopy was done to categorize the functional groups present in synthesized $LiFe_2O_3$nanoparticles. Morphological and size distributions of the particles were analysed using scanning electron microscope (SEM) images. Elemental analysis of particles were observed using Energy Dispersive X-Ray spectrometer (EDX)[21].

Antibacterial activity of Lithium Ferrous oxide nanoparticles

Antibacterial activity of *Cassia fistula* mediated $LiFe_2O_3$ nanoparticles were determined by well diffusion method. Bacterial culture swapped on nutrient agar plate and 5mm size of well punched on agar surface with help of sterile gel puncher. Various concentration of $LiFe_2O_3$ nanoparticles such as 25, 50 mg/L and streptomycin (positive control – 10 µg/ml) added in appropriate well. The plates were incubated at room temperature for 24 hours. The zone of inhibition were measured (millimetre in diameter) and mean values are recorded[17].

STATISTICAL ANALYSIS

All results are presented as mean ± standard deviation (SD). Using SPSS statistical tool, all data were analysed at the significant level ≤ 0.05 by T-test to test difference between St-MgO, Che-MgO and Control group. One way analysis of variance (ANOVA) were also perform to test effect of magnesium oxide nanoparticles dose on the test parameters. *P*-values of ≤ 0.05 were considered statistically significant.

RESULTS

Synthesis and characterization of lithium coated iron oxide nanoparticles

Figure 2 shows the UV-Visible absorption spectrum of bio-based Lithium coated iron oxide nanoparticles. It has an optical absorption range around 342 nm. UV-Visible absorption spectra reveal that Lithium coated iron oxide nanoparticles are mono dispersed. FTIR spectral analysis was used to illustrate and identify the biomolecules that bound on the synthesized Lithium coated iron oxide nanoparticles (Fig 3). The FTIR spectrum of *Cassia fistula* mediated lithium coated iron oxide show peak at 3446 cm^{-1} are corresponding to N-H symmetric represent that Amines. 1595 and 1383 cm^{-1} are responsible to N-H bend, CH_3C-H bend. It indicate the functional group of Amines, Alkanes and alkalys. 609 cm^{-1} are responsible for C-Br stretch and it indicates presence ofAlkyl halides. From this result, indicate most of amine functional group are involved in synthesis of Lithium coated iron oxide nanoparticles. The element confirmation of $Li_2Fe_2O_3$ by EDX analysis. The Ferrous oxide 72.46 % and Lithium 27.54 % strong signal is formed which indicate Lithium Ferrous Oxide NPs formation. (Fig 5). The XRD analysis of bio-based LiF_e2O_3 nanoparticles shown in Fig. 4. The XRD peaks were developed at (111), (200), (220), (311), (222), (400), (331), (420), (422), (333) and (531) Bragg's reflection based on crystal phase of $LiFe_2O_3$ NPs. These peak areas could be mentioning as spherical phase and assessed with JCPDS card data No. 4026. The particles sizes in the range of 24 - 170 nm

was determine by using Scherrer formula. Fig 6 showed SEM analysis of NPs. From the result, it is apparent that the NPs were irregular spherical shape and well distributed without any aggregation.

Antibacterial activity of LiF_e2O_3 nanoparticles

Cassia fistula mediated lithium coated iron oxide nanoparticles shows an excellent antibacterial activity against bacterial pathogenic. Highest zone of inhibition observed in $LiFe_2O_3$ against *Bacillus subtilis* with diameter of 13.16 ± 0.28 mm at a concentration 50 mg/ml and lowest inhibition obtained in *Streptococcus pyrogenes* with zone diameter 10.33 ± 0.76 mm at same concentration (Table 1 & 2). It is clearly shows that lithium coated iron oxide nanoparticles have bactericidal activity due to cell membrane disruption, which lead to cell death. Rajiv et al. explained the important of antibacterial activity of Eichhornia mediated Fe_2O_3 nanoparticles against *E. coli*, *P. fluorescens* and *S. aureus*[16].

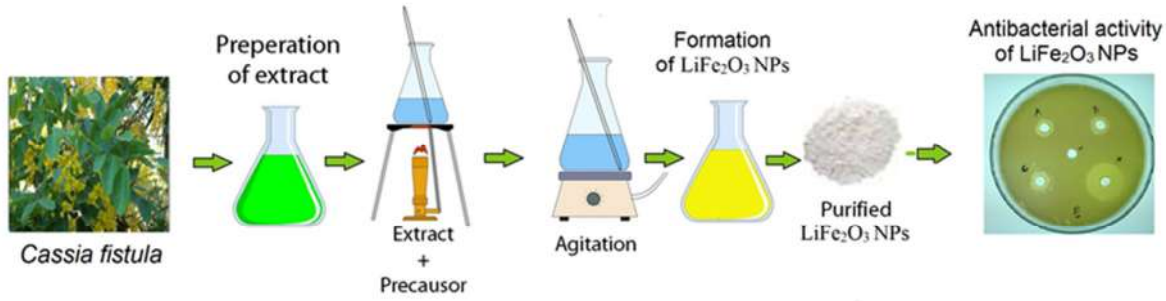

Fig 1. ***Graphical representation of cassia fistula mediated lithium coated iron oxide nanoparticles and its antibacterial activity***

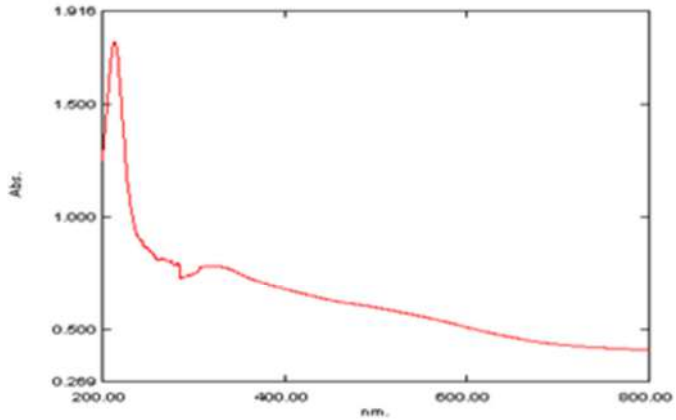

Fig 2. ***UV- visible spectrum of biosynthesized Lithium coated iron oxide using aqueous extract of Cassia fistula leaf extract***

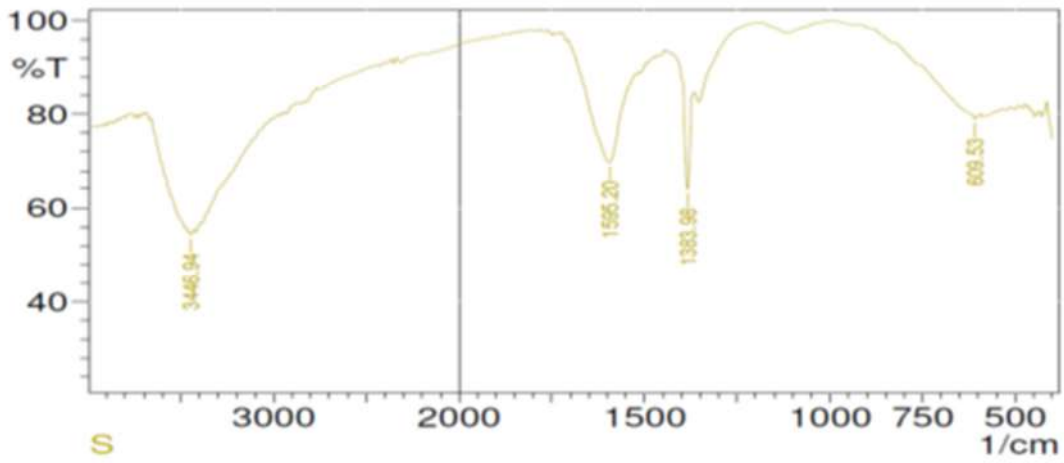

Fig 3. ***FTIR spectrum of Cassia fistula extract mediated lithium coated iron oxide nanoparticles***

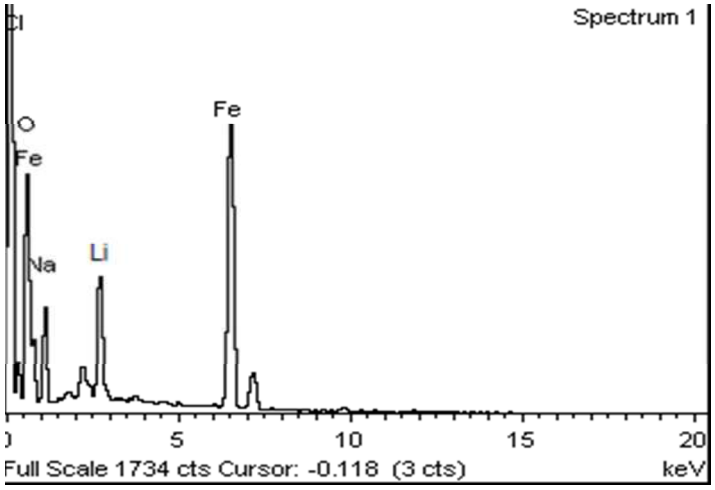

Fig 4. ***EDX of biosynthesized lithium coated iron oxide nanoparticles using Cassia fistula extract***

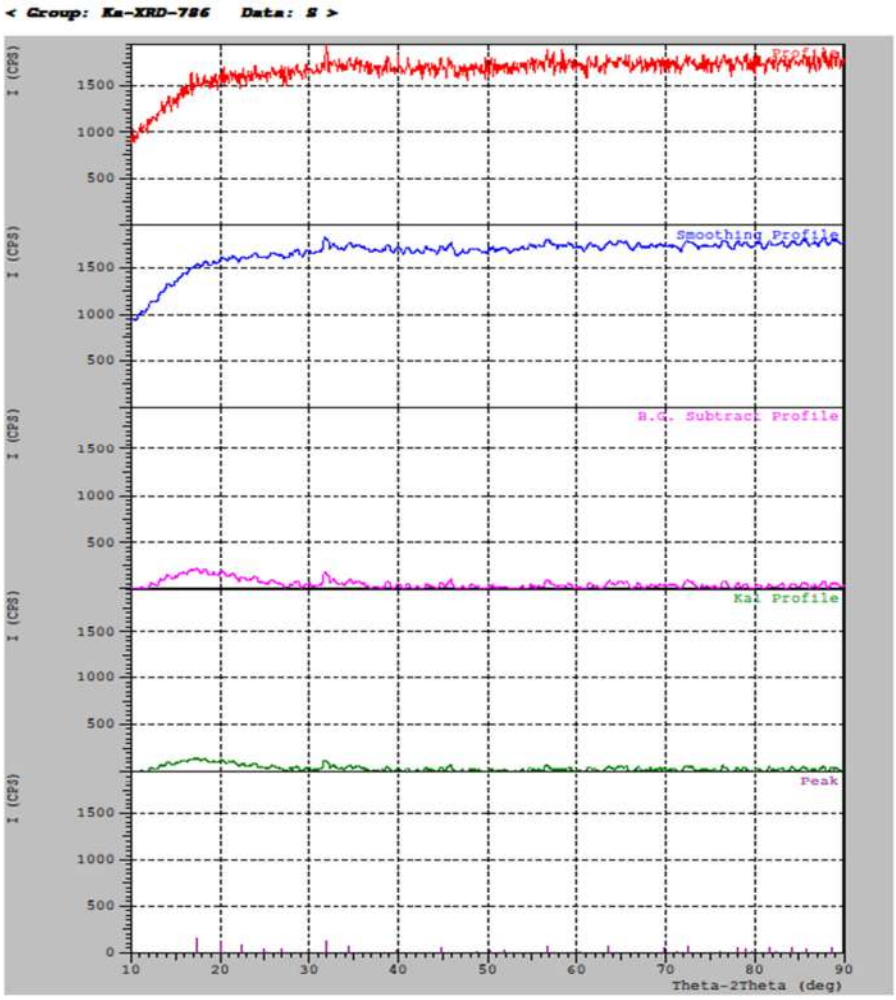

Fig 5. ***XRD image of lithium coated iron oxide nanoparticles***

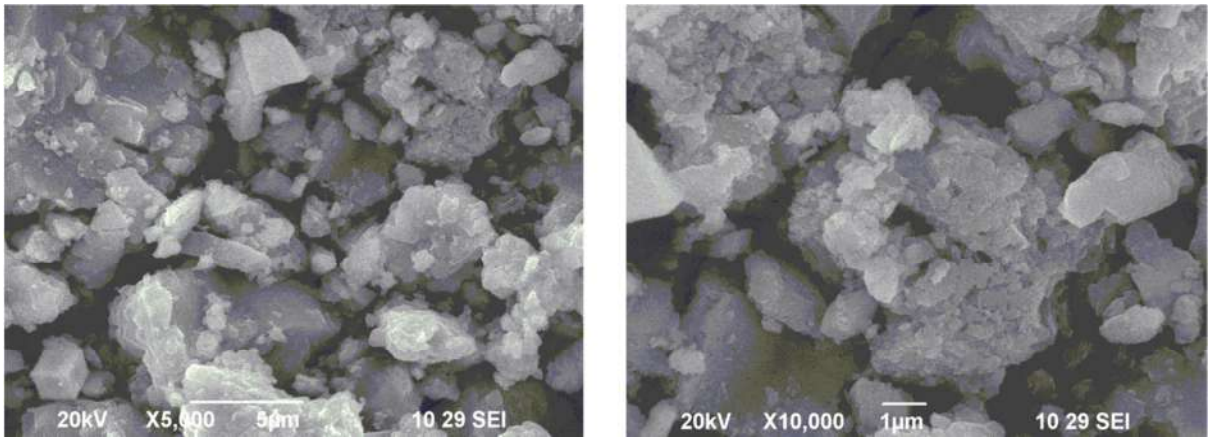

Fig 6. ***Scanning Electron Microscope image of synthesized lithium coated iron oxide nanoparticles at different resolution***

Table 1. ***Antibacterial activity of $LiFe_2O_3$ nanoparticles against bacteria pathogen. The resulted zone of inhibition (mm) are expressed in Mean ± SD***

Pathogens	Different concentration of $LiFe_2O_3$ Nanoparticles		Control
	25 mg/L	50 mg/L	(Streptomycin 10 µg/ml)
E.coli	10.83 ± 0.76	12.50 ± 0.50	10.83 ± 1.76
Bacillus subtilis	12.16 ± 0.28	13.16 ± 0.28	12.33 ± 0.28
Streptococcus pyrogenes	8.33 ± 0.28	10.33 ± 0.76	11.58 ± 0.97

Table 2. ***Anova analysis of variance for the data on inhibition zone of $LiFe_2O_3$nanoparticles against bacterial pathogen. All the results are statistically significant (P value ≤ 0.05)***

Pathogens		Sum of Square	Mean Square	F	Sig.
E.coli	Between group	4.167	4.167	10.00	0.034*
	Within group	1.667	0.417		
	Total	5.834			
Bacillus subtilis	Between group	1.500	1.500	18.00	0.013*
	Within group	0.333	0.083		
	Total	1.833			
Streptococcus pyrogenes	Between group	5.042	5.042	30.25	0.005*
	Within group	0.667	0.167		
	Total	5.708			
Control	Between group	3.375	3.375	10.12	0.033*
	Within group	1.333	0.333		
	Total	4.708			

Sig: Significant, *P<0.05

DISCUSSION

In the present study, biosynthesis of $LiFe_2O_3$ was carried out using plant extract of *cassia fistula* as there are no records of research work done in $LiFe_2O_3$ synthesis using this plant. It is cultivated in tropical countries including Sri Lanka, India and Malaysia. The *cassia fistula* are used for medicinal purposes[6]. Extracts of *cassia fistula* have been found to show antibacterial[11], antifungal[12, 16] and anti-helminthic activity[15]. In the chemically synthesized metal nanoparticles, the toxic chemicals and strong reducing agents like sodium hydroxide are involved in the synthesis process and by products formed during the synthesis play a major role in producing toxic effect. Capping agents or the stabilizing agents are reducing agents as sodium hydroxide gives more negative surface charge to the nanoparticles. This negative surface charge also plays a pivotal role in the toxic effect of the chemically synthesized nanoparticles[18,19]. When the biologically synthesized nanoparticles are considered when compared with that of chemically synthesized nanoparticles, the toxic effect is more for chemically synthesized nanoparticles[22, 28]. The least toxicity was observed for biologically synthesized lithium coated ferrous oxide nanoparticles as it is a pure green synthesis method which does not involve the use of any other toxic chemicals except corresponding metal halides like lithium nitrate[28]. Phytochemical play a major role in the biological synthesis process. It also act as capping or stabilizing agents to the nanoparticles instead of the toxic chemicals or reducing agents as in the case of chemically synthesized metal nanoparticles[23, 24, 25]. This might have contributed to the lesser toxicity levels of biologically synthesized nanoparticles. Synthesis of $LiFe_2O_3$ using plant extracts is getting more popular[28]. Curtis et al. synthesized $LiFe_2O_3$ by using the *cassia fistula* extract at 24 h of incubation[5]. Interestingly, $LiFe_2O_3$ were synthesized rapidly within 30 min of incubation period. The aqueous lithium ferrous solution was turned to brown color within 30 min, with the addition of leaf extract. Intensity of brown color increased in direct proportion to the incubation period[10]. Although such systems were not repeated in plant mediated synthesis nanoparticles, the phytochemical constituents are attributed to the formation of nanoparticles. Caffeine and theophylline present in tea extracts were also reported to catalyze the synthesis of nanoparticles [7]. Phyllanthin from *Phyllanthus amarus* was also reported as the capping ligands in the synthesis of ferros oxide[8]. Quercetin and polysaccharides have been used for ferrous oxide nanoparticle synthesis [9].

CONCLUSION

Lithium coated iron oxide nanoparticles synthesized by *Cassia fistula* extract act as a capping and reducing agent with lithium nitrate and ferric chloride precursor. Synthesized *Cassia fistula* mediated $LiFe_2O_3$ nanoparticles are spherical in shape 24 - 170 nm size. $LiFe_2O_3$ shows promising antibacterial activity against *E.coli* and *Bacillus subtilis* at concentration of 100 µg/ml. *Cassia fistula* mediated $LiFe_2O_3$ nanoparticles can be used as antibacterial agent in the field of pharmaceutical application.

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SP-7

CYTOTOXIC ACTIVITY OF GREEN AND CHEMICALLY SYNTHESIZED MAGNESIUM OXIDE NANOPARTICLES AGAINST SiHa CELL LINE

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ABSTRACT

Magnesium oxide nanoparticles are synthesis using *Solanum Trilobatum* aqueous extract (St-MgO) and sodium hydroxide (Che-MgO) by co-precipitation method. The size and shape of St-MgO, Che-MgO nanoparticles are determined by characterization analysis like UV-Visible spectrophotometer, Fourier transform infrared spectroscopy, X-ray diffraction, Energy dispersion X-ray, Scanning electron microscope and particles size analysis. The structure and morphological analysis reveals that nanoparticles are spherical shape with 30 nm (St-MgO) and 42 nm (Che-MgO). The Cytotoxic activity of *Solanum trilobatum* and sodium hydroxide mediate magnesium oxide nanoparticles determine by MTT assay against SiHa cell line. The present studies explained that chemically synthesized nanoparticles (Che-MgO) are more toxic when compared to green synthesis (St-MgO). Therefore, *Solanum Trilobatum* mediated magnesium oxide nanoparticles can used for drug delivery system.

Keywords: ***Cytotoxic assay, Solanum Trilobatum, Biological synthesis, Chemical synthesis, Magnesium oxide nanoparticles, SiHa Cell Line, Cervical cancer.***

INTRODUCTION

Cancer is one of major health problem in our country. Cancerous cells normally gain ability of uncontrolled growth, invasion and metastasis [1, 2]. Several researcher are trying to keep cancer cell in controlled growth by radiation and chemotherapy treatment. Unfortunately, those treatment methods have several limitation. Hence, it required alternative method for control growth of cancerous cell[3,4]. In previous study explained, that nanomaterial have ability to control the proliferation of cancer cell lines. Nanomaterial have different application like molecular diagnosis, drug delivery and cancer therapy that attract the attention of recent investigation [5]. Magnesium oxide NPs have extremely high surface areas and unusual crystal morphologies. Nanoscale MgO possesses unique optical, electronic, magnetic, thermal, mechanical and chemical properties due to its characteristic structures [6]. Magnesium oxide is an important functional metal oxide that has been widely used in various fields, such as catalysis, refractory materials, paints, and superconductors [7]. In the literature, there are several methods declared for synthesis of nano-sized MgO particles, including the sol–gel method, chemical gas phase deposition, laser vaporization, hydrothermal synthesis, and combustion aerosol synthesis [8, 9]. Biological methods for the synthesis of MgO NPs with the use of plant materials have not been widely exploited. The ability of magnesium oxide nanoparticles to kill various concentration of cancer cell like HeLa, AGS, HepG2 and SNU-16. Promising result was obtained from HepG2 cell line, MgO NPs have ability to activate the pathway of apoptotic mechanism[3,4]. In previous study, Magnesium oxide nanoparticles synthesized using *Solanum trilobatum* having excellent antioxidant and antibacterial properties when compared to chemical synthesis [11]. In this present study, we investigate green (St-MgO) and chemically synthesized (Che-MgO) nanoparticles used for cytotoxic activity against cervical cancer SiHa cell line by MTT assay.

MATERIALS AND METHODS

Materials

St-MgO and Che-MgO nanoparticles are spherical shape with 30 nm and 42 nm [11]. Chemical used in this experiment are analytical grade, purchase from sigma Aldrich. SiHa cell line are purchase from National

Centre for Cell Science, Pune, India. The cells are cultures in EMEM added with 10% FBS at 37°C in a CO_2 incubator.

Methods

Synthesis of St-MgO nanoparticles

Solanum trilobatum leaf washed with distilled water; shade dried for 5 days; make powder with help of mortar and pestle. One gram of powdered leaf sample dissolved in 100 ml of distilled water and heat at 100 °C for 5 mins. After heating, filter the sample with Whatman filter paper and stored the crude extract for further uses. 50 ml of crude plant extract was added (dropwise) to a beaker, which contain 50 ml magnesium nitrate (0.5 M) solution under magnetic stirring for 4 hours until the formation of precipitate settled in bottom of conical flask. This precipitate was centrifuged at 5000 rpm for 5 mins and collect the pellet. This precipitate further purified by washing with Millipore water and ethanol for several times to remove impurities. After purification step, precipitate is annealed at 400 °C for 8 hours and make it fine powder by using mortal pestle. Final product was stored in screw cap bottle for further use [10].

Synthesis of Che-MgO nanoparticles

To synthesize Che-MgO NPs, a 0.5 M magnesium nitrate ($MgNO_3$) and 0.1 M sodium hydroxide (NaOH) was prepared in double distilled water. A 100 ml of MgNO3 solution added dropwise to a beaker contain 100 ml of NaOH solution placed over magnetic stirrer with high-speed stirring. After 20 minutes of stirring, beaker kept for 2 hour as undisturbed for settlement of precipitate. The precipitated Che-MgO NPs washed with Millipore water followed by ethanol until removal of impurities and then vacuum dried at 80 °C in hot air oven. Then Che-MgO NPs transferred to airtight screw cap bottle for further analysis [12].

Characterization of St-MgO and Che-MgO nanoparticles

UV-Visible spectroscopy analysis use to determine optical density (UV-2450, Shimadzu). FTIR analysis is used to analysis the functional group is responsible for synthesis of magnesium oxide nanoparticles. XRD (Perkin-Elmer spectrum) and EDX (Energy Diffraction X-ray, Model QuanTax 200, Germany) analysis for crystal nature with element composition of nanoparticles. The size and shape of magnesium oxide nanoparticles are determine by SEM (Scanning Electron Microscope, Model JSM 6390LV) and particles size analysis[11].

Determination of Cytotoxic studies of St-MgO and Che-MgO nanoparticles

Cervical cancer SiHa Cell line (Cell culture) were obtained from National Centre for Cell Science, Pune, India. The cells were cultured in Eagles Minimum Essential Medium (EMEM) added with FBS (10%, v/v) at 37 °C in a CO2 incubator (95% air, 5% CO2 and 100% relative humidity). To evaluate the cytotoxic activity of the green and chemically synthesized magnesium oxide nanoparticles against SiHa cells, the cells were collected in the exponential stage of growth, seeded into 96-well plates (15,000 per well) and permitted to adhere for 48 h. Then, Different concentrations (6.5, 12.5, 25, 50, 100 µg/ml) of St-MgO, Che-MgO nanoparticles were added to the desired wells and incubated for 48 h. A 20 µL of EMEM medium having MTT (5 mg/mL) was added to each well and incubated for 4 h at 37 °C. Later, the medium was altered with 100 µL of DMSO, and optical densities were measured at 570 nm. All studies were performed in triplicates and expressed as mean ± standard error. The percentage of viability is calculated using this formula

$$\% \text{ viability} = (\text{OD of Test} / \text{OD of Control}) \times 100$$

RESULTS AND DISCUSSION

Synthesis and Characterization of St-MgO and Che-MgO nanoparticles

St-MgO nanoparticles are synthesized by using *Solanum Trilobatum* aqueous extract by co-precipitation method. For Che-MgO nanoparticles, sodium hydroxide are used for synthesis. Magnesium nitrate ($MgNO_3$) precursor used for synthesis of both nanoparticles (fig 1). The characterization analysis of St-MgO and Che-MgO nanoparticles are described in the table 1[11].

Cytotoxic assay

Cytotoxic activity of St-MgO and Che-MgO nanoparticles was evaluate by MTT assay against SiHa cervical cancer cells. IC_{50} value for St-MgO, Che-MgO nanoparticles was found to be 37.5 µg/ml and 47.2 µg/ml

(Fig 2 & 3). Maximum percentage of inhibition by magnesium oxide nanoparticles observed at concentration (100 µg/ml) more than 50 %. Nasser et al. [13] synthesis mesoporous magnesium oxide nanoparticles showed promising anticancer activity against HepG2 liver cancer cell line with IC_{50} of 27.5 µg/ml and conclude that magnesium nanoparticle have ability for micro carrier.

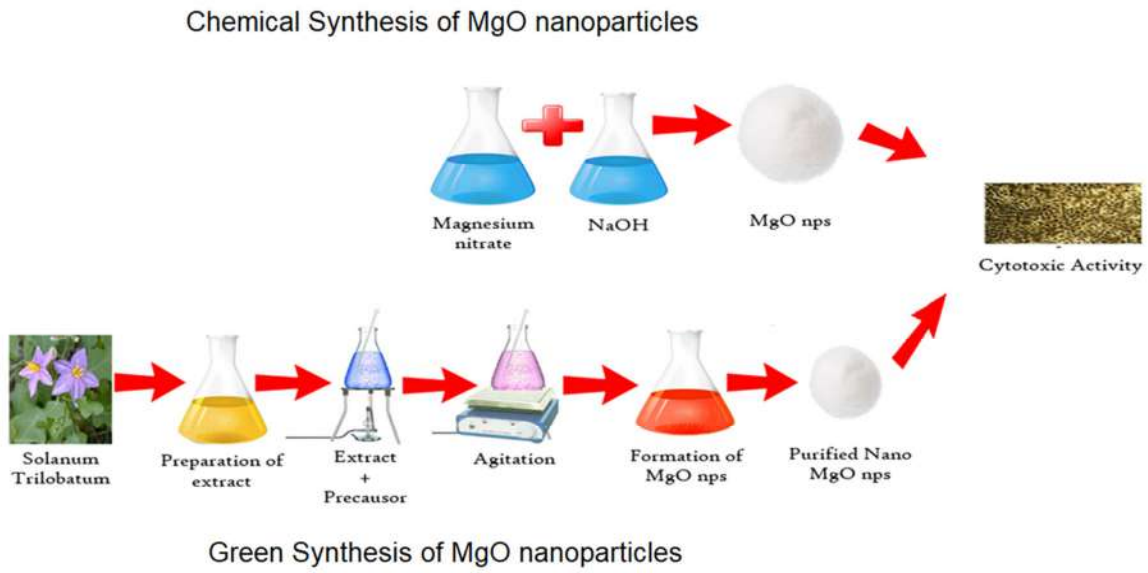

Fig 1. Graphical representation of green and chemical synthesis of magnesium oxide nanoparticles against SiHa cell line

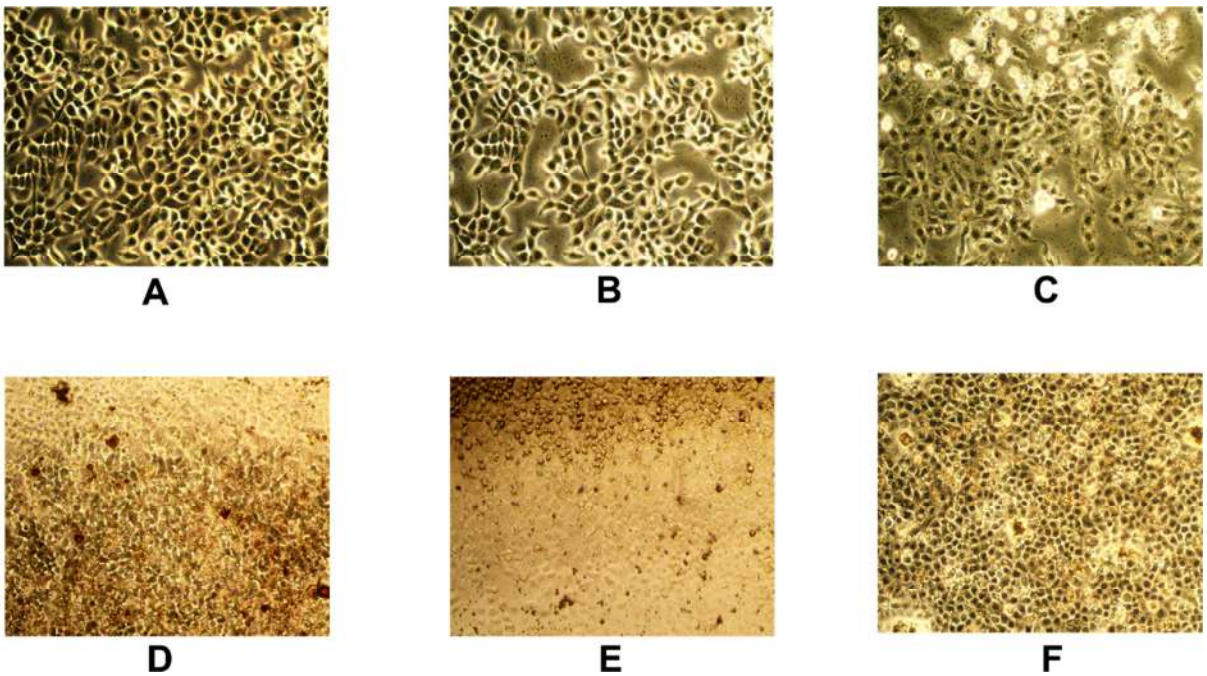

Fig 2. Cytotoxic activity of St-MgO nanoparticles on SiHa cell lines. The cells were cultured in 10 % FBS medium and treated with 6.5–100 µg/ml St-MgO nanoparticles, morphological changes were observed at 40X magnification. (A) Control; (B) 6.5 µg/mL (C) 12.5 µg/mL (D) 25 µg/mL (E) 50 µg/mL (F) 100 µg/mL of St-MgO nanoparticles treated cells.

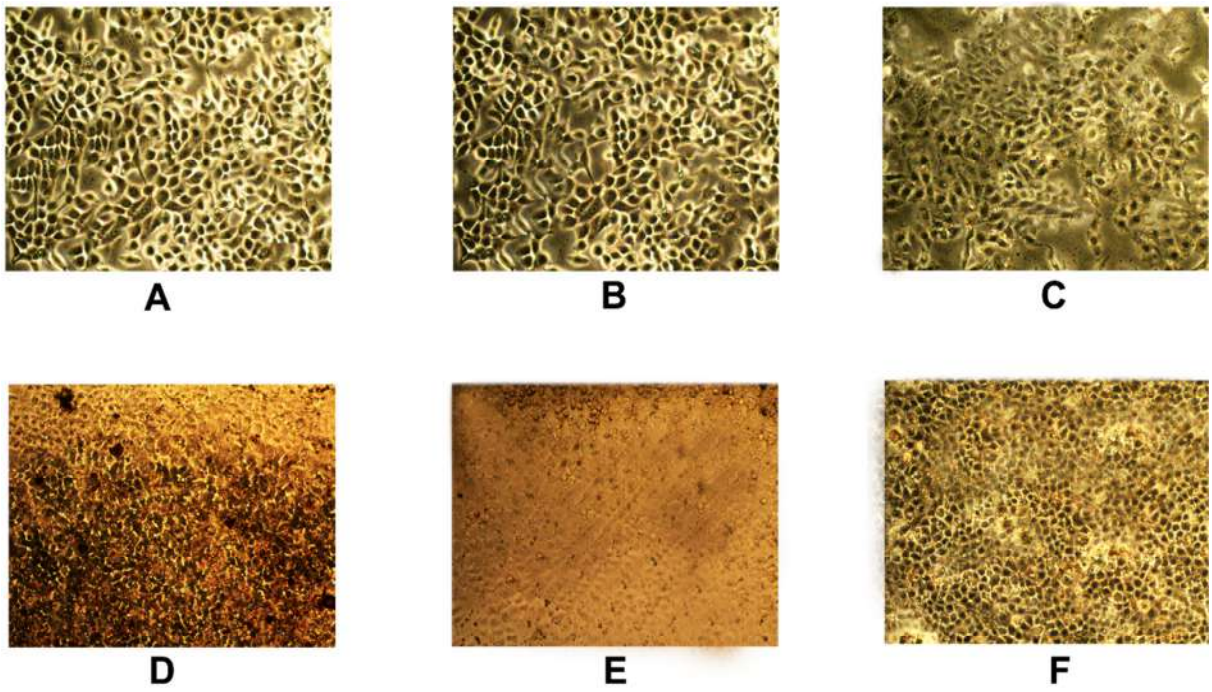

Fig 3. Cytotoxic activity of Che-MgO nanoparticles on SiHa cell lines. The cells were cultured in 10 % FBS medium and treated with 6.5–100 µg/ml Che-MgO nanoparticles, morphological changes were observed at 40X magnification. (A) Control; (B) 6.5 µg/mL (C) 12.5 µg/mL (D) 25 µg/mL (E) 50 µg/mL (F) 100 µg/mL of Che-MgO nanoparticles treated cells.

Table 1: Structural and morphological analysis of St-MgO and Che-MgO nanoparticles

Characterization analysis	St-MgO	Che-MgO
UV-Visible (Absorbance)	362 nm	374 nm
Fourier transfer infrared spectroscopy	1219.01, 1365.60, 1635.64, 1728.22, 2144.84, 2322.29, 2576.90, 2723.49, 2962.66, 3379.29 and 3950.22 cm-1	440.75, 1633.78, 3462.37 and 3699.63 cm-1
X-ray diffraction	crystalline nature (JCPDS-89-4248)	crystalline nature (JCPDS-45-0946)
Energy dispersion X-ray	Mg – 53.76 %, O – 46.23 %	Mg – 83.26 %, O – 16.74 %
Scanning Electron Microscope	Spherical shape	Spherical shape
Particle size analysis	30 nm in size	42 nm in size

Table 2. Viability percentage of SiHa Cell line treated with various concentration of St-MgO and Che-MgO nanoparticles.

Nanoparticles	Concentration of nanoparticles (µg/ml)				
	6.25	12.5	25	50	100
St-MgO	92.45	85.76	73.63	68.04	57.51
Che-MgO	81.45	69.76	65.63	61.06	53.51

CONCLUSION

The green and chemically synthesized MgO nanoparticles were characterized by different techniques for size, shape and morphology. The different concentration of St-Mgo and Che-MgO effect on cytotoxic activity of SiHa Cell line by MTT assay. St-MgO nanoparticles show minimum inhibition against SiHa cell

line related to Che-MgO nanoparticles. Therefore, *Solanum Trilobatum* mediated magnesium oxide nanoparticles can be effectively used as a drug delivery in medical field.

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SP-8

ANTI-INFLAMMATORY AND ANTIBACTERIAL ACTIVITY OF *NERIUM OLEANDER*

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ABSTRACT

The protective response made by body using the immune cells, vessels and molecular mediators this is eliminating the initial cause of cell injury, clear out necrotic cells and tissues damaged and this is called inflammation. The anti-inflammatory property of a substance or treatment that reduces inflammation or swelling. Plants have a potential to heal and may have its own antimicrobial characteristics. *Nerium oleander* leaf extracts is been used for gall ailments and to prevent miscarriage. Its phyto-constituents are alkaloids, tannin, saponins, glycosides, anthraquinones, terpenoids and sterol. The results which suggest that *N. oleander* has considerable potency in anti-inflammatory action and has prominent effects by *in-vitro* anti-arthritic assay protein denaturation method.

Keywords: ***Nerium oleander, inflammation, anti-inflammatory activity, Protein denaturation, anti-bacterial.***

INTRODUCTION

Health is functional level or metabolic efficiency of a living organism. In humans they develop their ability of individuals or communities which make them to manage towards the mental or social challenges and to adapt[1]. The pathogens, damaged cells or irritants can stimulate the body system which leads to develop the inflammation[2]. The difference between the inflammation and infection mainly dealt with the presence and absence of the microbial invasion in the system, atherosclerosis, hypersensitivity and trauma. The microbial invasion does not result in classic inflammatory response where in the pathological situations such as parasitosis and eosinophilia[3]. Trails of anti-inflammatory treatment for existing Alzheimer's disease have shown a minimal effect on the diseases. The macular degeneration may develop in the case of regular use of aspirin for over ten years[4]. Some herbs, health supplements and drugs also possess the anti-inflammatory activities. Such as include devil's claw (*Harpagophytum procumbens*), hyssop (*Hyssopus officinalis*), ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), *Arnica montana* (containing helenalin) and willow bark (containing salicylic acid). Other unproven, potentially anti-inflammatory dietary sources such as pomegranate (*Punica granatum*), green tea (*Camellia sinensis*), cat's claw (*Uncaria tomentosa* and *Uncaria guianensis*), Indian olibanum (*Boswellia serrata*), or blue cheese like Roquefort and brome lain from pineapples (*Ananas comosus*)[5]. In the developing and non-developing countries bacterial diseases rapidly multiplicate leads to the health problems. The emergence of hitherto unknown disease-causing microbes and the drug resistant microorganisms pose immense clinical problem in the treatment of public health concerns[6]. Rational drug design does not always yields' effective antimicrobials. The limited action of the synthesised drugs has also the enzyme inhibitors activity too which also has the complex issue of drug uptake by the body cells[7]. Plants have a potential to heal and may have antimicrobial characteristics which accepted a long before. The phytoconstituents of have a variety of biological activity such as antimicrobial, antioxidant, anticancer and diabetic etc[8]. From the old civilization period itself plants as a drug is been used in Ayurveda, Homeopathic, Unani and Chinese system for the healthcare[9]. Nearly 1700 natural antibiotics are identified and there detailed function doesn't know yet. Many reports on antibacterial properties, but they are mainly on proven medicinal plants[10], essential oils[11], or on single plant[12].

MATERIALS AND METHODS

The fresh plant *Nerium oleander* was collected from the local areas in and around the Perambalur and Ariyalur districts of Tamilnadu (11°16.612'N and 78°53.933'E). From the collected plant samples, the

leaves were separated and shadow dried for 15 – 20 days under the room temperature. After the leaf drying process, the leaves were powdered using the electric blender and stored in sealed container for the further studies

Preparation of plant extract

To 500g of *Nerium oleander* leaf powder, 1500 ml of chloroform was added for the extraction as the solvent for 24h at room temperature, after which the supernatant of each solvent was recovered by filtering through Whatman filter paper. This process was repeated thrice and the respective solvent from the supernatant was evaporated in a rotary vacuum evaporator to obtain the crude extract. These extracts were stored at 4°C until used for the evaluation of anti-microbial activity.

Phytochemical Screening:

Phytochemical test was carried out on Chloroform extracts of *N. oleander*, using standard procedures to identify the constituents such as alkaloids, fatty acids, flavonoids, glycosides, phenols, resins, saponins, steroids, tannins, terpenoids, Xanthoproteins, reducing sugars and anthraquinones[13].

In vitro anti-inflammatory activity of plant extracts

Inhibition of albumin denaturation

The anti-inflammatory activity of plant extract was studied by using inhibition of albumin denaturation technique which was studied according to Sakat *et al.* (2010)[14] followed with minor modifications. The reaction mixture (0.5 ml; pH 6.3) consisted of 0.45 ml of bovine serum albumin (5% aqueous solution) and 0.05 ml of distilled water. pH was adjusted at 6.3 using a small amount of 1 N HCl. Different concentrations of plant extract were added to the reaction mixture and were incubated at 37ºC for 20 min and then heated at 57ºC for 5 min after cooling the samples, 2.5 mL of phosphate buffer saline was added. Turbidity was measured spectrophotometrically at 600 nm. The percentage inhibition of protein denaturation was calculated as follows:

$$\text{Percentages Inhibition (\%)} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}} \times 100$$

HRBC membrane stabilization method

Blood was collected (2 mL) from healthy volunteers and was mixed with equal volume of sterilized Alsevers solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl in distilled water) and centrifuged at 3000 rpm. The packed cells were washed with isosaline solution and a 10 % v/v suspension was prepared with normal saline and kept at 4ºC undisturbed before use. Different concentrations of plant extract (50, 100, 200, 500 and 1000 µg /0.5 ml) in normal saline, Aspirin as standard (50, 100, 200, 500 and 1000 µg / 0.5 ml) and control (distilled water instead of hypo saline to produce 100 % hemolysis) were separately mixed with 1 ml of phosphate buffer, 2 ml of hyposaline and 0.5 ml of 10% HRBC suspension was added to prepared. All the assay mixtures were incubated at 37º C for 30 min and centrifuged at 3000 rpm for 20 min and hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. The percentage of HRBC membrane stabilization[15] or protection was calculated by using the following formula:

$$\text{Percentages stabilization (\%)} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}} \times 100$$

STATISTICAL ANALYSIS

Data obtained from this study were expressed as mean ± SD. Statistical analysis was performed using SPSS version -17, were considered statistically significant.

Antibacterial activity

Chloroform leaf extracts *Nerium oleander* were tested by the well diffusion method. Different concentration of the extracts (100µg/ml) was prepared by reconstituting with Chloroform. The test microorganisms (*Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli* and *Klebsiella pnemoniae*) were seeded into respective medium by spread plate method 10µl (10 cells/ml) with the 24hr cultures of bacteria growth

in nutrient broth. After solidification the filter paper wells (5mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. Gentamycin (10µg) used as standard for antibacterial test. The antibacterial assay plates were incubated at 37°C for 24hrs. The diameters of the inhibition zones were measured in mm.

RESULTS AND DISCUSSION

Anti-inflammatory activity

Inflammation is a common phenomenon which reacts towards the injury in response to living tissues. The results of preliminary phytochemical analysis of the leaf extracts shown in Table-1 which showed the presence of alkaloids, tannin, saponins, glycosides, anthraquinones, terpenoids and sterol etc.

Table-1: ***Qualitative analysis of Chloroform leaf extract of Nerium oleander***

Phytochemicals constituents	Qualitative analysis
Alkaloids	+
Glycosides	++
Phenol	+
Flavonoids	+
Steroids	++
Tannins	+
Terpenoids	++
Saponins	+

The anti-inflammatory effects of the medicinal plants possessing several flavonoids[16]. The total phenolics and antioxidant activity has a high positive relationship between them in the plant system[17]. The anti-inflammatory activity a property of many flavonoids, tannins and sterols[19] and these phyto constituents also possess antioxidant property[17]. The *N. oleander* extracts possess different constituents and this was observed in the present study. In summary, the results showed that *Nerium oleander* has considerable potency in anti-inflammatory action and has prominent effects by *in-vitro* anti-arthritic assay protein denaturation method. In the treatment of rheumatism *N. oleander* extracts can be used.

Protein Denaturation method

In the arthritic disease the production of auto antigen due to denaturation of protein. Percentage inhibition of chloroform extract was found to be 62.80% at 500µg/ml and this was compared with Aspirin, a standard drug (Table-2 and Figure-1). The results emphasize that the controlling the production of auto antigen by the chloroform extract and also inhibits the protein denaturation. The traditional medicine is been act as a complementary and alternative systems of medicine which was reported more than 70% of the developing world's population. The popularity and its importance has become an efficient and also possess a cost effective.

Table 2. Albumin denaturation assay

Concentration (µg/ml)	Chloroform extract	Aspirin
50	8.25±0.25	10.11±0.31
100	12.58±0.48	14.25±0.68
200	13.20±0.54	16.96±0.11
300	15.75±0.71	18.26±0.22
400	17.82±0.22	21.55±0.39
500	19.54±0.39	23.53±0.43

Values are expressed as mean ± SD (n=5)

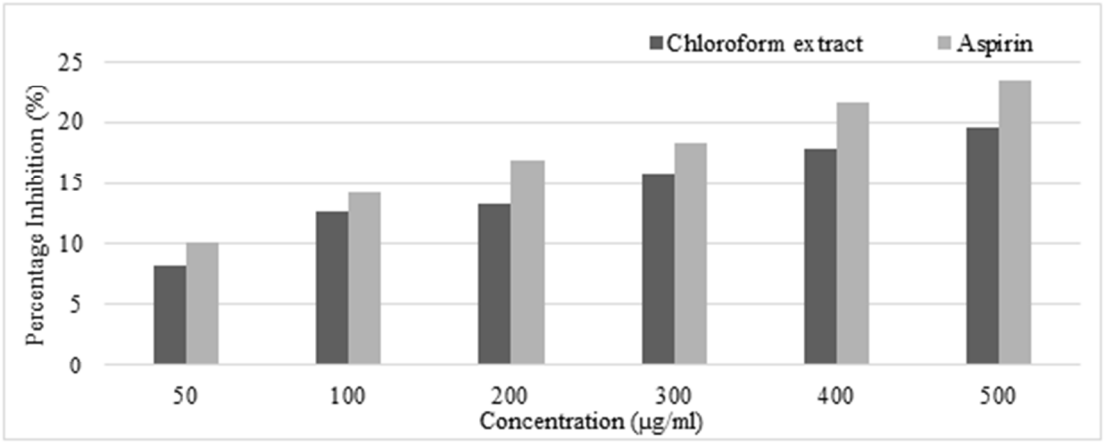

Figure 1. ***Albumin denaturation assay by the Chloroform extracts of Nerium oleander leaves***

By using protease enzyme inhibition method *in vitro* anti-inflammatory activity was evaluated[19]. In a study, two Bangladeshi medicinal plants namely *Mesua nagassarium* and *Kigelia pinnata* researchers[20] has revealed the significant *in vitro* membrane stabilizing effect and so it reveals the medicinal plants contain the anti-inflammatory activity. Researchers have isolated the anti-inflammatory compounds from *Persicaria stagnina*[21], *Scoparia dulcis*[12] and *Sidacordi folia*[22] by using the standard protocols. The anti-inflammatory study using the crude extracts of medicinal plants showed a significant activity. The albumin denaturation assay and membrane stabilization assay were evaluated in *in-vitro* anti-inflammatory activity the chloroform extracts of *N. oleander* leaves showed significant results. The results of *in-vitro* models showed that *N. oleander* has potential anti-inflammatory activity and acts through multiple mechanisms. *Mimusops elengi* leaf alcoholic extract also showed a similar result in *in vitro* anti-inflammatory activity[23]. The maximum activity of membrane stabilization in *N. oleander* was found at 73.85±0.80% at a dose of 1000µg/0.5ml and 86.23% of protein denaturation was found at a dosage of 250µg/ml in reference to standards in anti-inflammatory activity[24].

***In vitro* anti-inflammatory activity**

In this current research work, HRBC membrane stabilization and protein denaturation methods the *in vitro* anti-inflammatory activity was performed and shown in the Table-3 and Figure-3. HRBC is similar to lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membrane.

Table 3. ***Membrane Stabilization Assay***

Concentration (µg/ml)	Chloroform extract	Aspirin
50	16.25±0.21	17.56±0.54
100	19.36±0.56	20.21±0.98
200	21.63±0.69	23.79±0.23
300	24.66±0.12	27.86±0.33
400	26.88±0.11	28.55±0.20
500	29.96±0.09	30.29±0.12

Values are expressed as mean ± SD (n=5)

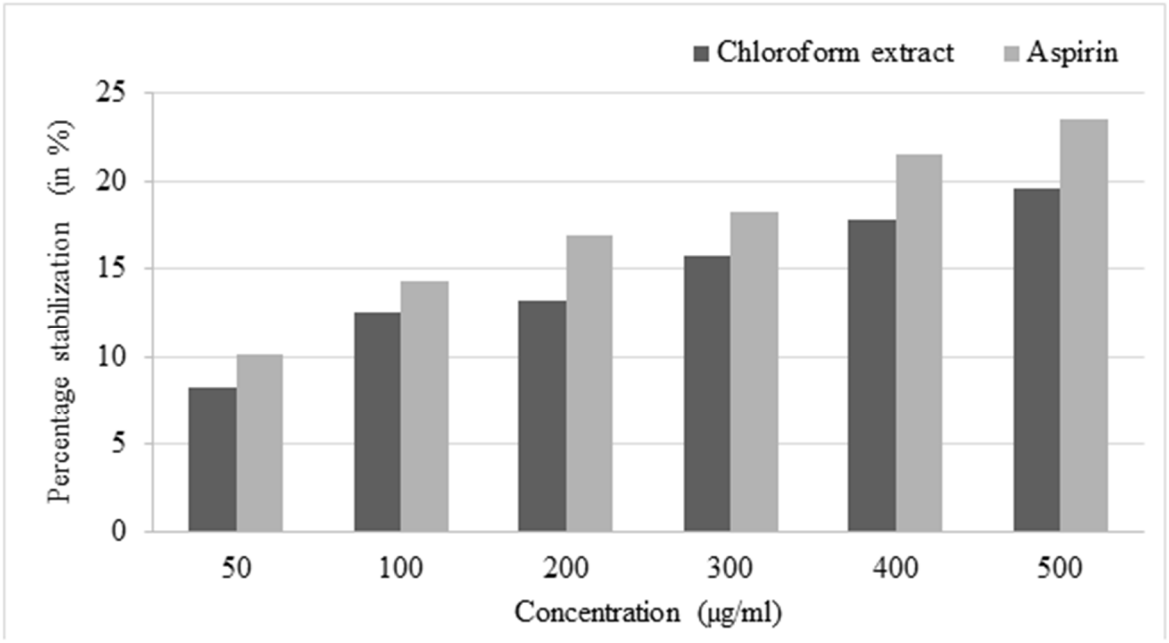

Figure 2. ***HRBC stabilization assay by the Chloroform extracts of Nerium oleander leaves***

HRBC membrane stabilization method

The *in vitro* anti-inflammatory method involves the stabilization involves stabilization of hypotonicity induced membrane lysis in the HRBC membrane. About 59.25% protection of Chloroform extracts was observed at 500 µg/ml and this effect was compared with Aspirin (standard drug). The leaf extracts of *Basella alba*. Linn[15] also showed a similar studies on *in-vitro* anti-inflammatory activity which exhibited the membrane stabilization effect. The methanolic extracts of methanolic extract of *Murraya koenigi*[25] leaves showed a significant anti-inflammatory activity in dose dependent manner[26]. The crude extracts of the various parts or the whole plants of the medicinal plants and isolated compounds from the medicinal plants showed statistically significant anti-inflammatory activity both in *in vivo* and *in vitro* assay[19]. The *Nerium oleander* chloroform extracts of leaves was subjected to erythrocyte (RBC) membrane stabilization induced haemolysis by hypotonic solution[27]. The vitality of cells depends on the integrity of their membranes, exposure of RBC's to injurious substances such as hypotonic medium results in lysis of its membrane accompanied by the oxidation and hemolysis of hemoglobin[28]. The synergistic action of the phytochemicals in *N. oleander* extracts should have the protection of the cell membrane against injurious substances which will have the membrane stabilizing properties[29].

Antibacterial Activity

Nerium oleander chloroform leaf extracts showed their antibacterial activity against the gram-negative (*E. coli* and *K. pneumoniae*) and gram-positive organisms (*S. pyrogenes* and *S. aureus*). (Table 4). Based on the dosage level the extracts showed a significant value in the antibacterial activity. The results were compared with Ampicillin, a known antibiotic or antimicrobial agent. The antimicrobial activity against all the isolates showed a similar result. The extracts of the aqueous showed a lesser activity than the chloroform extracts in the medicinal plant samples[30].

Table 4: ***Antibacterial activity Nerium oleander leaf extracts.***

S. No	Pathogenic bacteria	Chloroform extract Zone of inhibition (mm)			Standard (Ampicilin) Zone of inhibition (mm)
		20 µl	40 µl	60 µl	
1.	*Escherichia coli*	14	15	18	11
2.	*Klebsiella pneumoniae*	16	18	19	10
3.	*Staphylococcus aureus*	15	17	19	13
4.	*Streptococcus pyogenes*	16	17	20	12

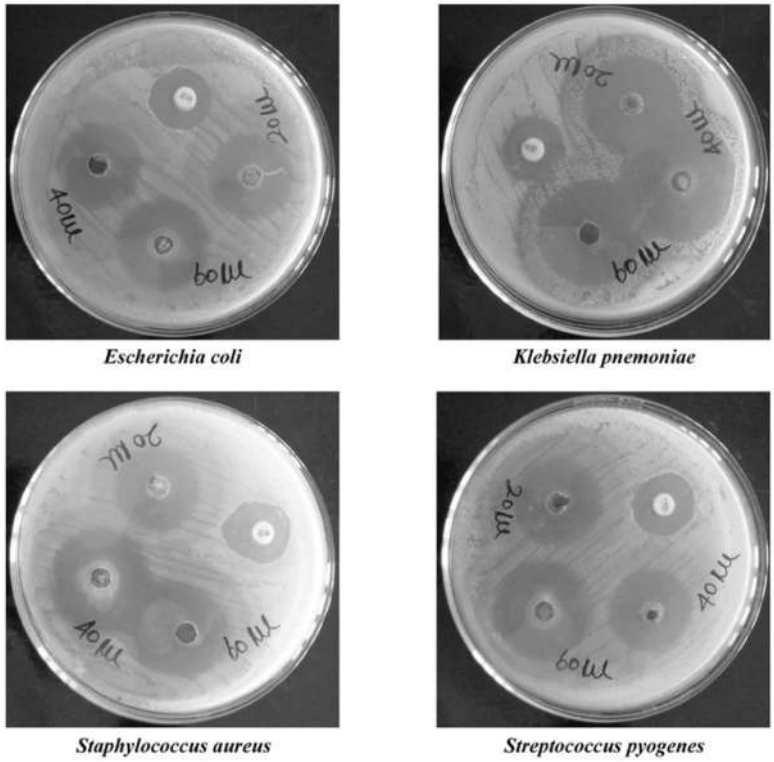

Figure 3. ***Antibacterial activity of Nerium oleander leaf extracts***

In this study, chloroform extract of the *N. oleander* showed minimum inhibitory concentration (MIC) at 20µl concentration itself showing a range of 14mm for *E. coli*, 16mm for *K. pneumoniae*, 15mm for *S. aureus* and 16mm for *S. pyogenes.* From there findings the leaf extract at a minimum concentration exhibits a maximum level of antibacterial activity against these microbial samples than the chemo drug ampicillin. The minimum quantity of the *N. oleander* leaf extracts can be used for the curing of microbial diseases in faster way. Briefly, the higher level of antibacterial activity was observed against *K. pneumonia* and the low level of antibacterial activity was observed against *S. pyrogenes* and *E. coli*. In addition, *K. pneumonia* is more liable than other test microorganisms to chloroform extract of *N. oleander*. The plants extract showed significant antimicrobial activity against the endodontic pathogens[31].

CONCLUSION

Inflammation is a common spectacle and it is a reaction of living issue towards damage. The existing study involved a primary phytochemical investigation of the leaf extracts showed the occurrence of alkaloids, tannin, saponins, glycosides, anthraquinones, terpenoids and sterol etc. In summary, our results advocate that *N. oleander* has considerable potency in anti-inflammatory action and has prominent effects on *in vitro* anti-arthritic assay protein denaturation method. Chloroform extracts of *N. oleander* leaves showed significant results in *in-vitro* anti-inflammatory model and in addition, the maximum antimicrobial activity was also observed.

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SP-9

SCREENING FOR PROBIOTIC EFFICIENCY AND ANTIMICROBIAL ACTIVITY OF THE ISOLATES OBTAINED FROM CURD AND COW DUNG

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ABSTRACT

Probiotics are beneficial live microbial feed supplements which enhance the hosts immune response. The potential attributable role of probiotics accounted to colonization, immune stimulation& Antimicrobial Activity. Probiotic organisms were isolated from the probiotic product of curd & cow dung using selective medium [MRSA – De Man, Rogsa and Sharpe Agar]. Microscopic, macroscopic, biochemical & molecular characterization were done to confirm the probiotic isolates. Based on the result, it was confirmed to be *Bacillus cereus & Bacillus sp*. Probiotic isolates evaluated for temperature tolerance and evaluated for lactose utilization according to the color change. Both the isolates documented the lactic acid production which has been evidenced by the color change which is very much required for lactose intolerance effect. Antimicrobial susceptibility testing of various antibiotic disc against the chosen clinical isolates exhibit significant sensitivity. Antimicrobial activity of probiotic isolates against chosen clinical isolates witness the presence of antimicrobial activity. Comparative analysis was done between probiotic isolates and probiotic with commercial probiotics against chosen clinical isolates stated a promising alternative therapeutic target which could be achieved with the help of probiotics with minimum side effects over commercially available antibiotics.

Keywords: ***Probiotic organism, Lactose tolerance, Antimicrobial activity***

INTRODUCTION

'Probiotic' is a word which has been derived from Greek language 'pro bios' which means 'for life'. In 1989, the meaning use today was improved by Fuller. Thus, probiotics are live microbial supplements which increases the microbial load in the intestine and improves the host's health. Havenaar and Huis in't Veld broadened this definition in 1992 including mono or mixed culture of live microorganisms which applied for animal and man [1,2,3]. Probiotic strains of bacteria are added in the food system which traverse to the lower intestinal tract through the mouth. Probiotic bacteria should be resistant to the enzymes like lysozyme in the oral cavity. Then the flow will be moving into the stomach and reaches the upper intestinal tract which consists of bile. In this stage the strains should be highly resistant towards the digestion processes. It is observed that time for the first entrance to release from the stomach takes about three hours. The strains has to be more resistant towards the stressful conditions created by the gastric juice in stomach (pH 1.5-3.0) and bile which is present in the upper respiratory tract [4,1]. Survival in low pH and in gastrointestinal environment can shows that it can be used as a promising probiotic strain. The most important criteria for selecting a probiotic is its antimicrobial activity. Antimicrobial activity targets the enteric undesirables and pathogens [5]. Organic acids, carbon dioxide, hydrogen peroxide, diacetyl, low molecular weight antimicrobials and bacteriocins are the substances which are produced as a result of the antimicrobial activity by the lactic acid producing bacteria[6, 1]. Till today some researches shows that different species produce different antimicrobial substances. This study focus on the isolation of Probiotic isolates from different sources and determining its antimicrobial efficiency in comparison to commercially existing antibiotics.

MATERIAL AND METHODS

Sample collection

Two different samples were collected. Samples consisted of two groups: group I, fresh dairy product of curd; group II, Cow dung. 10g and 10ml (for curd) of Group I sample has been weighed. Sample group II

were taken and weighed to 1 gram in 10ml of sterile distilled water and it was finely chopped using a sterile cutter and take 10g of it, then smashed it to isolate the product.

Isolation and purification of lactic acid bacteria from sample

Isolation of LAB, serial dilution agar technique was used. 10g of each sample (except curd taken 10 ml) was dissolved in 90 ml of De Man, Rogosa and Sharpe broth. After added into the MRS broth it has been shaken homogeneously and it was incubated at 37°C for 24 hours in an aerobic condition. In the serial dilution agar plate technique, 10ml of stock solution was suspended and dissolved in 90 ml of water blanks to form a microbial suspension. Serial dilution of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were made by adding 10ml into 90ml water blanks. 10 ml has been taken from each dilution and it was inoculated in the MRS agar plates (it was prepared by pouring 15ml of sterile and cooled molten media in the petri dishes) and it was subjected for incubation at 37^0C for 24 hours for bacterial growth. The plates were observed to detect the morphology of colonies and number of colonies were counted on each plate of different dilution [7]. Bacteria were isolated by spread plate method on MRS agar and it was incubated for 24hrs at 37°C and it was transferred into the sterile MRS agar slants and then it was maintained in refrigerator at 40^0C for further analysis.

Temperature sensitivity

The selected bacterial cultures were grown at varying temperatures, i.e. 25, 30, 37 and 40^0C for 48-72 hrs. Then 0.1ml of bacterial culture was transferred to the MRS agar plates by using pour plate method and it was incubated for 48hrs at 37^0C. The growth of lactic acid bacteria on MRS agar plates were used to designate isolates as temperature resistant [8].

Lactose utilization

The color change of the medium indicates the acid production by selected bacterial isolates. Sterilized fermentation medium containing 10g peptone, NaCl 15g, phenol red 0.018g, lactose 5g, for 1L distilled water and final pH 7.0 was inoculated with different cultures and it was subjected for incubation at 35^0C for 24-48 hrs. Color change from red to yellow shows acid production [9].

Molecular Characterization

DNA Isolation and Quantification

DNA has been isolated from the samples by modified salting-out procedure. The isolated DNA was quantified by reading the absorbance rate at 260 & 280 nm. The quality of the extracted DNA was checked on 0.8% agarose gel, by staining it with Ethidium bromide. The electrophoresis was performed at 70V for 20 min. Quantity of the extracted DNA was checked in UV spectrophotometer (SHIMADZHU, JAPAN) by reading the optical density (OD) at 260 nm and 280 nm. DNA quality was also determined and amplified. The sequence was analysed using 16s rRNA sequencing.

Gene	**Direction**	**Sequence (5′ – 3′)**
16S rRNA	Forward	**AGAGTTTGATCMTGGCTCAG**
	Reverse	**TACGGYTACCTTGTTACGACTT**

Screening for antibacterial activity

For screening of isolated bacterial cultures, the cultures were inoculated in MRS broth and it was incubated at 37°C for 24-48 hours in shaker to carry out the fermentation process. After incubation, 2 ml of each fermented culture and supernatant was taken to check the antimicrobial activity by using Well diffusion method against the six test microorganisms. Each culture was tested against every test microorganism. pathogens grown in their respective medium at 37 °C was diluted to a turbidity equivalent to that of a 0.5 McFarland standard [10].

Screening for antibiotic susceptibility

The antibiotic susceptibility of isolated LAB was assessed using antibiotic discs diffusion method on MRS agar plates. Broth cultures of LAB was prepared by using MRS and changed to 0.5 McFarland standards. A

100µl suspension of freshly grown bacterial cultures was spread on MRS agar plates. The antibiotic discs were placed on the agar surface and the plates were incubated at 37°C for 48 hours

RESULTS

Table 1. ***Isolation of Probiotic Organism***

S.No	Source	Media	Organism
1.	Curd	MRS Agar	*Bacillus sp.*
2.	Cow dung	MRS Agar	*Bacillus cereus.*

Table 2. ***Macroscopic Characterization of Probiotic Organism***

S.No	Organisms	Selective Media	Appearance
1.	*Bacillus sp.*	MRS Agar	Small,Creamy,Whitish, Convex
2.	*Bacillus cereus.*	MRS Agar	White, Mucoid

Table 3. ***Temperature tolerance & Lactic acid production ability of Isolates***

S. No	Organism	Lactic Acid Production	Temperature Range			
			25^0	30^0	37^0	40^0
1.	*Bacillus sp*	+	+	+	+	+
2.	*Bacillus cereus*	+	+	+	+	+

Inference
+→Present

Figure 1. ***PCR amplification of 16S rRNA gene***

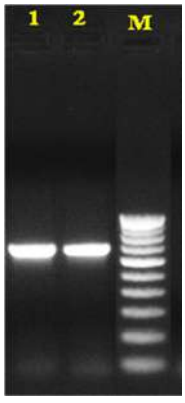


Table 4. ***Sequence and Phylogenetic tree for Probiotic Organisms***

Analysis

Analysis	Phylogeny Reconstruction
Scope	All Selected Taxa
Statistical method	Neighbor-joining

Phylogeny Test

Test of Phylogeny	Bootstrap method
No. of Bootstrap Replication	1000

Substitution Model

Substitutions Type	Nucleotide
Model/Method	Tamura 3-parameter model
Substitutions to Include	d: Transitions + Transversions

Rates and Patterns

Rates among sites	Uniform rate
Pattern among lineages	Same (Homogeneous)

Data Subset to Use

Gaps/Missing Data Treatment	Complete deletion

Bacillus sp.

>GTAAGACTGGGATAACTCCGGGAAACCGGAGCTAATACCGGATAGTTCCTTGAACCGCATGG
TTCAAGGATGAAAGACGGTTTCGGCTGTCACTTACAGATGGACCCGCGGCGCATTAGCTAGTT
GGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACA
CTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGA
CGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTG
TTAGGGAAGAACAAGTGCAAGAGTAACTGCTTGCACCTTGACGGTACCTAACCAGAAAGCCAC
GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGG
GCGTAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGG
GTCATTGGAAACTGGGAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGA
AATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACT

Tree

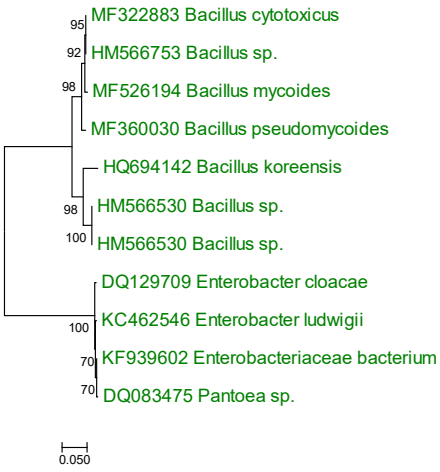


Figure 2: ***Evolutionary relationships of taxa***

By using Neighbor-Joining method, the evolutionary history was inferred (Saitou N. and Nei M. 1987). The optimal tree with the sum of branch length = 0.44069154 is shown. The percentage of replicate trees in which he associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. To infer the phylogenetic tree, the tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used. The evolutionary distances were calculated using the Tamura 3-parameter method and units of the number of base substitutions per site. The analysis involved sequences of 11 nucleotide. All the positions which contains gaps and missing data were eliminated.A total of 537 positions in the final dataset were observed. Evolutionary analyses were done using MEGA7.

Bacillus cereus

>TGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACT
CCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGG
CTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACC
AAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCC
CAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCA
ACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAAAACAAGTGC
TAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAATCCACGGCTAACTACGTGCCAC
CAGCCGCGGT

Tree

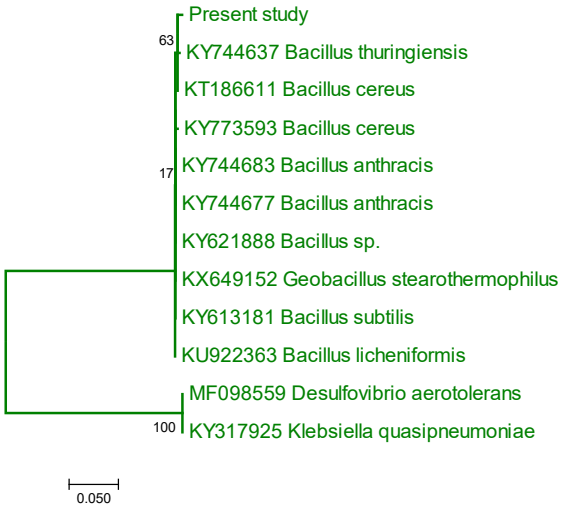
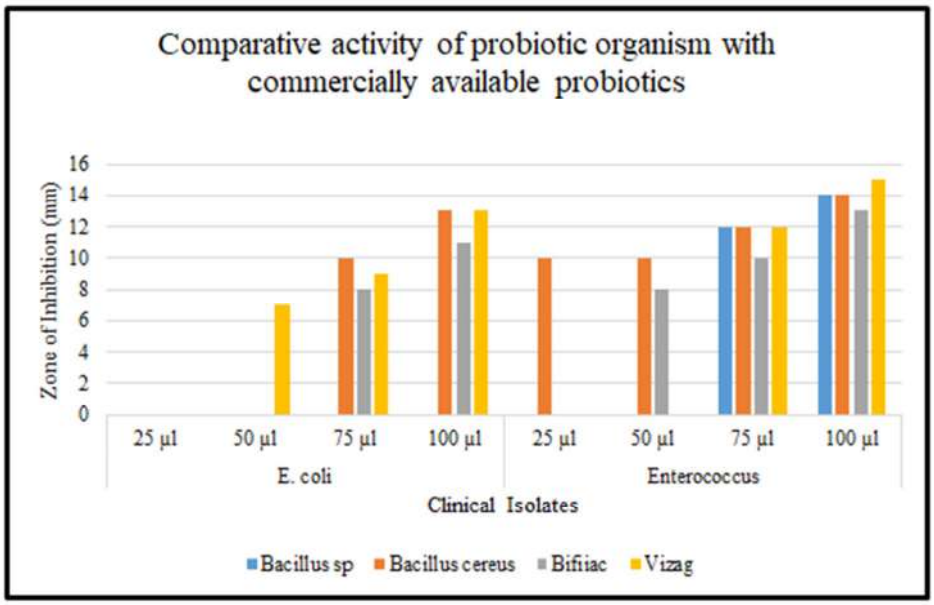


Figure 3: ***Evolutionary relationships of taxa***

The evolutionary history was evaluated using the Neighbor-Joining method. The ideal tree with the sum of branch length = 0.36229143 is shown. The percentage of replicate trees in which the closely related taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The phylogenetic tree is inferred using the tree drawn to scale, with branch lengths in the same units as those of the evolutionary distances. The evolutionary distances were calculated using the Tamura 3-parameter method and are in the units of the number of base substitutions per site. The 12 nucleotide sequences were analyzed. Gaps and missing data were eliminated from all the positions. There were a total of 437 positions in the final dataset. Evolutionary analyses were computed in MEGA7.

Figure 4. ***Comparative activity of probiotic organism with commercially available probiotic***



DISCUSSION

In human studies, shown that probiotic bacteria can have some positive effects on the immune system in their hosts. The medical world has long been interested in finding out an alternative choice of medicine with minimal side effects and for better harmonies human health. Probiotic organisms are found to be an alternative choice with demonstrable health benefits and have generally regarded as safe and proven low risk of inducing or being associates with etiology of diseases. In Present study, we focused on characterizing the potential benefits of probiotic organism of dairy and animal source. In Dairy source: Curd is a regular and common food component of India with special reference to South India. Curd is a part of milk which is formed by the action of enzymes. The curd bacteria especially lactic acid bacteria have been widely accepted as GRAS (Generally Recognized As Safe) for human consumption [11]. Apart from dairy source it has been discovered that cow dung possess a good probiotic niche. In this study we have selected curd (Dairy product), cow dung as probiotic source. Probiotic product consists of various enzymes, vitamins, capsules, and some fermented products contain microorganisms which play a beneficial role on the health of host. The oral consumption of probiotic microorganism exhibits a protective effect on the gut microflora. Lots of studies suggest that probiotic have beneficial role on microbial disorder of the gut, but it is really difficult to prove the clinical effect of such products. The preparation of probiotic used for traveller's diarrhoea, antibiotic associated diarrhea and acute diarrhea which shown that they have positive therapeutic effect [12, 1, 6]. Lots of factors may change the balance away from potentially beneficial or health promoting bacteria like lactobacilli and bifidobacteria to potentially harmful or pathogenic microorganisms like clostridia, sulphate reducers and *Bacteroides* species. It makes the host more susceptible to the illnesses. In present study, isolated probiotic organisms were able to survive at temperature 25, 30, 37 and 40^{0}C. Temperature is a most important factor which can dramatically affect the growth of bacteria. The reason for choosing this temperature range was to detect whether the isolated cultures were able to grow within range of normal body temperature or not. The results obtained were positive for growth at chosen temperature range. Lactose utilization of probiotic organisms isolated from Curd (Dairy product). Lactose intolerant people cannot metabolize lactose due to the lack of β-galactosidase enzyme. symptoms such as abdominal pain, cramping and diarrhoea were occur when they consume milk or lactose-containing products. If lactose passes via the small intestine, it is converted into gas and acid in the large intestine by the action of colonic microflora. Also the presence of breath hydrogen is a signal for lactose indigestion. The studies shows that the addition of certain starter cultures with milk products, allows the lactose intolerant people to consume those products without the usual rise of breath hydrogen or associated symptoms[13,14,15]. Antibiotic susceptibility pattern against selected clinical isolate was observed by using Kirby-Bauer disc diffusion method. The organisms are sensitive to certain drugs. Here *E.coli* is sensitive to streptomycin, ampicillin, norfloxacin and resistant to tetracyclin, penicillin G, amphotericin B and in *Enterococcus* are sensitive to streptomycin, ampicillin, norfloxacin and resistant to amphotericin B, penicillin G, tetracyclin. Study

document the existence of sensitivity but there is a chance of developing multi drug resistance over the period of time with adverse side effects. The issue of multi drug resistant and side effects could be addressed with the presence of probiotic organism which act as an alternative choice of the therapeutic causes with minimum side effects. Antimicrobial effects of LAB are formed by excreting some substances such as organic acids (lactic, acetic, propionic acids), carbon dioxide, hydrogen peroxide, diacetyl, low molecular weight antimicrobial substances and bacteriocins [1].

CONCLUSION

Till today some researches shows that different species of microorganisms produce different antimicrobial substances in which gut associated microbiota play a crucial role as a successful therapeutic tool.

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SP-10

SCREENING OF OCULAR CANDIDIASIS AMONG THE LEPROSY PATIENTS

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ABSTRACT

Mycobacterium leprae is the causative agent of Leprosy. Leprosy is the cause of Blindness in 3.2% of those affected by the disease. Blindness can have drastic consequence for those who probably already have sensory loss of the hand and feet. This disease is caused by Candida which is a normal flora that turns to a pathogenic one because of the Immunocompromised state. In a compromised individual serious sight threatening and life threatening infections may intervene. Present study aim to screen for ocular candidiasis among leprosy patients. The study group involves 40 male and 40 female. Ocular swabs were collected and screened for the presence of Candidiasis using Hi-Chrome candida agar. Study documents prevalence of ocular candidiasis in age-wise and treatment-wise manner among leprosy patients. Study concludes the risk of ocular candidiasis among the leprosy patients due to immunocompramised condition of the host and improper co-ordination of optic nerves which provides sustainable platform for the establishment of ocular candidiasis. Proper and an effective therapeutic care need to emphasis/reduce the burden of blindness due to ocular candidiasis.

Keywords: ***Ocular candidiasis, Leprosy, Immunocompromised***

INTRODUCTION

Mycobacterium leprae is the causative agent of leprosy. Historically leprosy has affected mankind since over 6000 years ago. India represents nearly 76% of the global burden of the disease[1]. An intracellular acid fast bacterium *M.leprae* is aerobic rod shaped and is surrounded by waxy cell membrane coating. *Mycobacterium leprae* was discovered by G.H. Armaeuer Hansen in Norway in 1873, the incubation period for the bacteria can last anywhere from two years to ten years or a maximum of thirty years. Leprosy is the cause of blindness in 3.2% of those affected by the disease. Blindness can have drastic consequence for those who probably already have sensory loss of the hand and feet. The disease comprises the eye through nerve damage and by direct bacillary invasion of the skin or eye itself. Bacterial, Fungal, Viral and parasitic pathogen all can cause systemic infection and can spread to eye. Some infections cause intra ocular damage by indirect mechanism during immunosuppression leading to opportunistic infections. Fungi are opportunistic in the eye since they rarely infect healthy, intact ocular tissue. In a compromised or Immunosuppressive individual, serious sight threatening and life threatening infections may intervene. Fungi may infect the cornea and other ocular structures. Species of *Fusarium, Aspergillus and Candida* are predominant. Ocular fungal infections or ophthalmic mycoses are being increasingly recognized as the predominant cause of morbidity and blindness. Dissemination of pathogens via the blood stream can lead to the direct involvement of the eye [2]. Candida endogenous interaction with the eye occurs as a direct result of the modern medical practice that sustains patients lives with broad spectrum antibiotics that eliminate micro biota of the host [3] and also in immunocompromised. Present study aim to screen for ocular candidiasis among the leprosy patients.

MATERIALS AND METHODS

Selection of study group

Total number of forty nine leprosy patients undergoing treatment in the leprosy Rehabilitation Centre, Madurai was subjected to the present. The study population involves 40 males and 9 females.

Ethical clearance
Biological samples were collected from 49 volunteers with informed concern.

Collection of Sample
Sterile moistened cotton swabs were used for culture collection on the eye lid. Previously the cotton swabs were dipped in sterile water. Patients were requested to look up and the moistened swab were rubbed gently on to the lower eyelid and transferred in to peptone broth to maintain the viability of the culture during isolation.

Screening for Ocular Candidiasis
The swab was taken from the ocular region and transferred into a peptone broth, kept for overnight incubation. Ocular culture swabbed over the HI-Chrome candida agar and plates were incubated for further characterization.

Germ Tube Test
This is a rapid test for identifying *C.albicans* and is known as Reynolds Braude Phenomenon. A light inoculum emulsified in 0.5ml of Horse or Human serum in a small test tube with a loose cotton plug. This was incubated at 37°c for 2-3 hrs. Prolonged incubation is not recommended as it may obscure the germ tube.

RESULT

Age-wise prevalence of Ocular Candida among the leprosy patients represented in Table 1 and Figure 1. Highest percentage of occurrence was observed in the age group 30-39, 50-59, above 70 (100%) Treatment-wise prevalence of Ocular Candida among the leprosy patients represented in Table 2 and Figure 2. Highest percentage of occurrence was observed in the treatment year 20-29, 30-39, 40-49 (100%)

Table 1. ***Age wise prevalence of Candida among leprosy patients***

Age	**Candida Prevalence (%)**
30-39	78
40-49	84
50-59	100
60-69	100
Above 70	100

Table 2. ***Treatment stage wise prevalence of Candida among Leprosy patients***

Years	**Candida Prevalence (%)**
1-9	77.7
10-19	93.7
20-29	100
30-39	100
40-49	100

Figure 1. Age wise prevalence of Candida among Leprosy patients

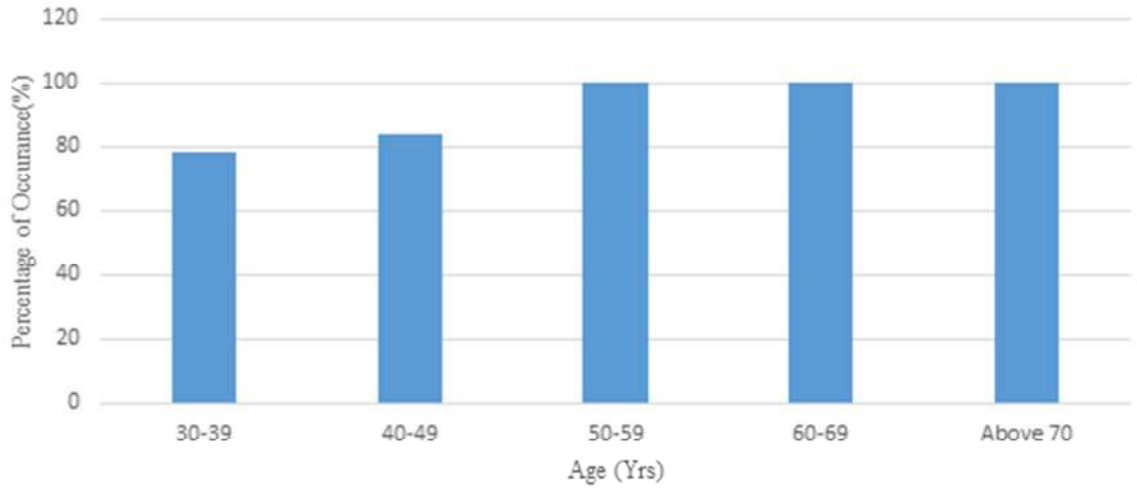
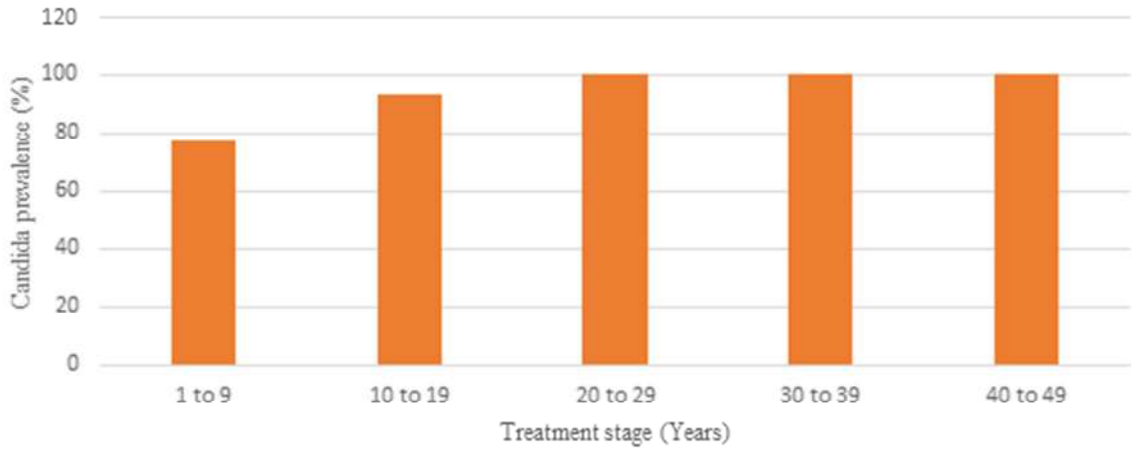


Figure 2. Treatment stage wise prevalence of Candida among Leprosy patients



DISCUSSION

Leprosy is chronic granulomatous disease caused by the acid fast bacterium Mycobacterium leprae. The incubation peroid for the bacteria can last anywhere from two to ten years. India reperesents 76% of the global burden of the disease [1]. Eye is one of the highly protected organs. The surface of the eye is armed with mechanical and immunological functions to defend itself against a hostile environment. The defense mechanisms are native and acquired both generalized and specific. The vascular supply to the surface of the eye is a major conduit of the immune defenses. The ocular inflammatory response invloves vascular dilation and exudation of immunologically active substances and cell, including macrophages, polymorphonuclear leukocytes and lymphocytes [3,4,5,6]. Present study documents the prevalence of ocular candidiasis among agewise and reflux that all the age catogory are volunarable to ocular candidiasis infection and also observed increased evidence of ocular candidiasis among all the treatment stage which effectively symbolize the immunocompramised nature of the host. Due to immunocompramised condition the immune mechanisms of the eye becomes deprived leading to opportunistic infections. Candida is a opportunistic pathogen of the eye. The spread of these organisms to the eye occurs through blood. Prevalence of Candida among leprosy patients is observed to be high due to their immunocompramised state due to which the normal role of Candida turns to be a potent pathogen and cause deleterious impact to the eye if left untreated leading to blindness. These factors can occur in combination and result in four main causes of visual loss: Corneal Ulceration, acute and chronic irridocylitis and Secondary cataract.

CONCLUSION

Study concludes that early diagnosis and proper therapeutic care to reduce the incidence of Ocular candidiasis and the proper nutritional supplementation need to be regulated to improve the immunocompramised nature of the host. Thereby we can reduce the burden of blindness due to *Candida albicans* in the leprosy victims.

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SP-11

BIOSYNTHESIS OF SILVER NANOPARTICLES OF *Stoechospermum marginatum* & EVALUATING ITS ANTIBACTERIAL & ANTIOXIDANT ACTIVITY

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ABSTRACT

Approximately 70% of the earth's surface is covered by oceans. Seaweeds, amongst the marine plants are reliable potent resources of the marine environment (1). The present study involves synthesis of silver nanoparticles from *Stoechospermum marginatum*, a brown seaweed, and evaluation of its antimicrobial, antioxidant and antitumor activity. Ninety ml of 0.1N silver nitrate was kept for incubation at room temperature along with 10ml of isolated bioactive compounds for the synthesis of silver nanoparticles. Fourier Transform Infrared (FT-IR) was used to characterize the synthesized silver nanoparticles. Further, antibacterial activity was determined by agar well diffusion method using different volume (25, 50, 75 &100µL) *Escherichia coli*, *Enterococcus* sp, *Klebsiella* sp and *Staphylococcus* sp. The stable DPPH's potential for hydrogen donation was used to measure antioxidant activity, by incubating, 1mL of 0.1mM DPPH with 1mL of extracts in ethanol at different concentrations (25, 50, 75, 100 µg/mL), at room temperature for 30min.

Keywords: ***Seaweeds, Stoechospermum marginatum, antioxidant and antibacterial activity.***

INTRODUCTION

Approximately 70% of the earth's surface is covered by oceans. Seaweeds, amongst the marine plants are reliable potent resources of the marine environment (1). To overcome the emerging problems of microbial resistance to antibiotics, new natural sources of drugs have been investigated. Marine organisms have caught the attention of researchers because they are wealthy sources of structurally new and biologically dynamic metabolites (2). Extensive studies of seaweeds have proven its potential as a primary and secondary metabolites source (3). Seaweeds are the important marine resources and thus used as potential sources of food and medicine (4). Seaweeds are one of the large and diverse ecosystems; it plays an essential function in marine environment. It is mainly involved in global primary production and providing food and shelter for variety of organisms. Seaweeds surface provisions protected and nutrient rich environment for the bacteria (5). Seaweeds are a source of bioactive compounds with properties like cytostatic, antihelminthic, antiviral, antifungal and antibacterial. They have also been exploited to treat diseases like cancer, arthritis etc. in many parts of the world, seaweeds have been used as food, feed and fertilizer, being the renewable living sources. Antioxidant and antibacterial properties are the most widely studied amongst all the other properties (6). Besides, some seaweed has proven to have also significant antitumoral, antileukemic, antiprotozoan and hypolipidemic activities (7). In places like China, Korea and Japan, there is evidence that the incidence of some cancers is lower due to regular consumption of seaweeds (8,9). *Stoechospermum marginatum* is a brown marine algae found in Indian Ocean to Australian ocean. It is potentially used as human food in salads, drugs, and raw material for production of high percentage of alginic acid and mannitol. Nanotechnology refers to an promising field of Science that includes synthesis and development of various nanomaterials. Nanoparticles (NPs) can be defined as a small object that behaves as a whole unit in terms of its transport and properties. Nanoparticle are being widely exploited for diverse purpose including, medical treatments, industry production of fuel batteries to large inclusion into various materials of daily use such as cosmetics or clothes (10). Silver nanoparticles, in particular, have been broadly used in the last few years in diverse applications due to their renowned success in biomedical (11), electronic (12), catalysis (13) and optical applications (14). In particular, a wide variety of nanosilver- coated wound dressings, surgical instruments, implants and contraceptive devices have been developed due to the exceptional antimicrobial properties of Ag-NPs (15).

MATERIALS AND METHODS

Sample collection and processing

Commonly available marine seaweeds such as *Stoechospermum marginatum* (Brown algae) was chosen for the study. Dried *Stoechospermum marginatum* and fresh *Stoechospermum marginatum* was from the intertidal regions of the Mandapam coastal regions (9˚ 17.417 N; 79˚ 08.558 E) of Gulf of Mannar. Dried *Stoechospermum marginatum* was then cut into small pieces and rinsed with sterile distilled water. The cleaned macro-algae was then left for shade drying for a week at room temperature. The shade-dried seaweed was powdered and extractions were prepared.

Preparation of Solvent Extract by Percolation method

This method is used most commonly for the extraction of active ingredients in the preparation of tinctures and fluid extracts. A percolator is usually used. About 50gm of dried fine powder of *Stoechospermum marginatum* was reflexed successfully with ethanol using percolation apparatus for 40 hours separately. After decanting, ethanol extract of *Stoechospermum marginatum* given residue stored in air tight container was subjected to qualitative test for identification of various constituents.

Synthesis of Silver Nanoparticles (Ag-NPs)

Ninety ml of 0.1N Silver nitrate was added to 10 ml of isolated bioactive compound and kept at room temperature for 5 hours for the reduction into Ag+ ions. The synthesis of silver nanoparticles was indicated by the change of color from green to brown (17).

Characterization of Biosynthesized Silver Nanoparticles

Fourier Transform Infrared (FTIR) Analysis Of Silver Nanoparticles

FT-IR spectral analysis was carried out to categorize the probable biomolecules involved in the reduction of the Ag+ ions and the capping of the AgNPs synthesized by *Stoechospermum marginatum* extract. The chemical composition of silver nanoparticles was analyzed using FTIR spectrometer after bio reduction with silver nitrate. 2 ml of bio synthesized liquid silver nanoparticles were scanned using infrared in the range of 400-4000 cm^{-1}.

Determination of Antimicrobial Activity

Antibacterial Activity - Agar Well Diffusion Method

Agar well diffusion method is extensively used to estimate the antimicrobial activity of plants or microbial extracts (18,19). A specified volume of the microbial inoculums was spread on a Muller- Hinton agar plate and hoes of diameter of 6 to 8 mm were punched using a sterile cork borer and a volume (25, 50, 75 &100 µL) of the *Stoechospermum marginatum* crude extract solution and AgNPs synthesized extract solution at preferred concentration was introduced into the well. The agar plates were then incubated under appropriate conditions depending upon the test microorganism. The growth of microbial strain tested is inhibited as the antimicrobial agent diffuses in the agar.

Quantification Of Antioxidant Activity

DPPH (2, 2- Diphenyl-1-Picrylhydrazyl) Screening Assay

0.1mM solution of DPPH in ethanol was prepared. This solution (1ml) was added to 1ml of extracts in ethanol at different concentrations (25, 50, 75, 100 µg/ml). Extracts solubilized in ethanol were only used in this test and dilution method was used to prepare various concentrations of the extracts. The mixture was shaken vigorously and was permitted to stand at room temperature for 30 min. Then, absorbance was measured at 510nm by using UV- spectrophotometer. Reference standard compound being used was ascorbic acid and the experiment was done in triplicate. The percent DPPH scavenging effect was calculated by using the following equation,

$$\text{Inhibition (\%)} = Ac - As / Ac \times 100$$

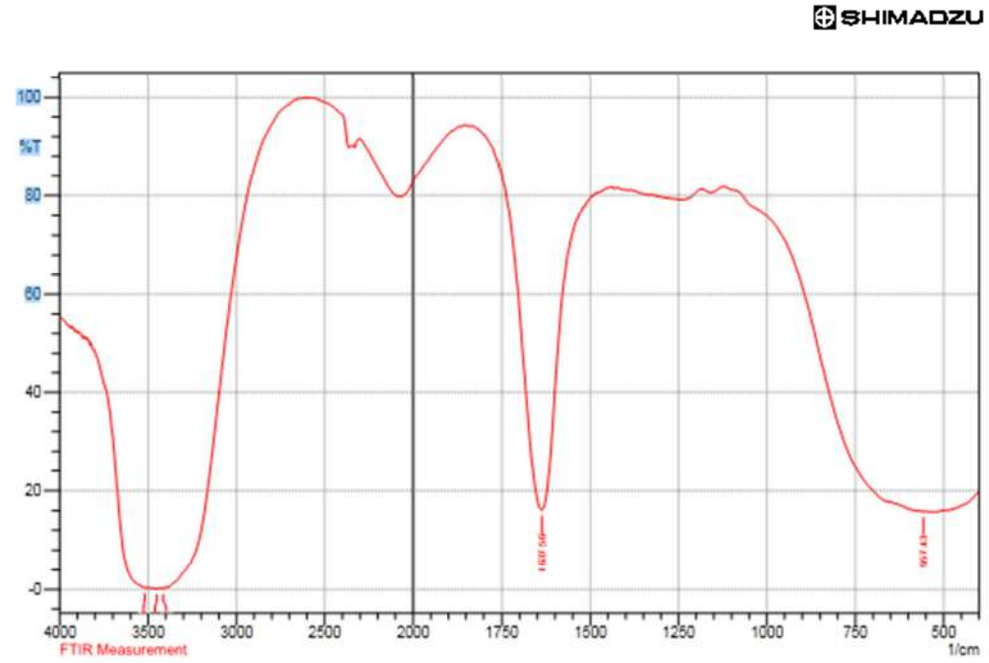
Where,

As – Absorbance of the sample

Ac – Absorbance of the control (Ethanol & DPPH radical solution).

RESULTS

Figure 1: ***Fourier transform infrared spectroscopy***



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	557.43	15.805	0.689	1120.64	551.64	234.501	13.124
2	1637.56	16.08	67.962	1815.02	1519.91	82.517	62.029
3	3415.93	0.255	0.241	3417.88	2609.69	458.327	0.048
4	3450.65	0.151	0.027	3454.51	3435.22	51.882	0.238
5	3520.09	0.324	2.666	3749.62	3504.66	319.687	3.71

Figure 2: ***Antimicrobial activity of crude extract of Stoechospermum marginatum***

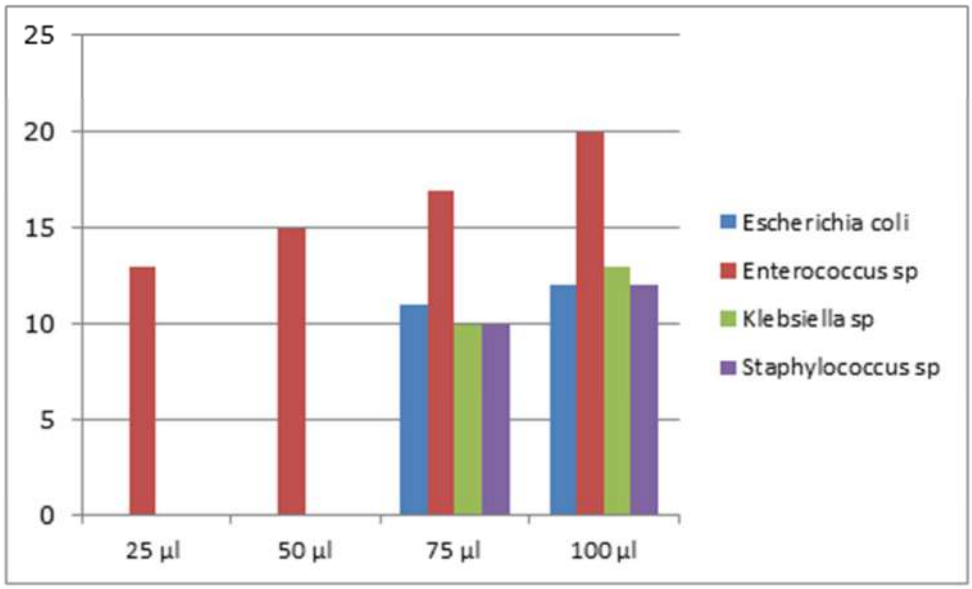


Figure 3: Antimicrobial activity of silver nanoparticles of *Stoechospermum marginatum*

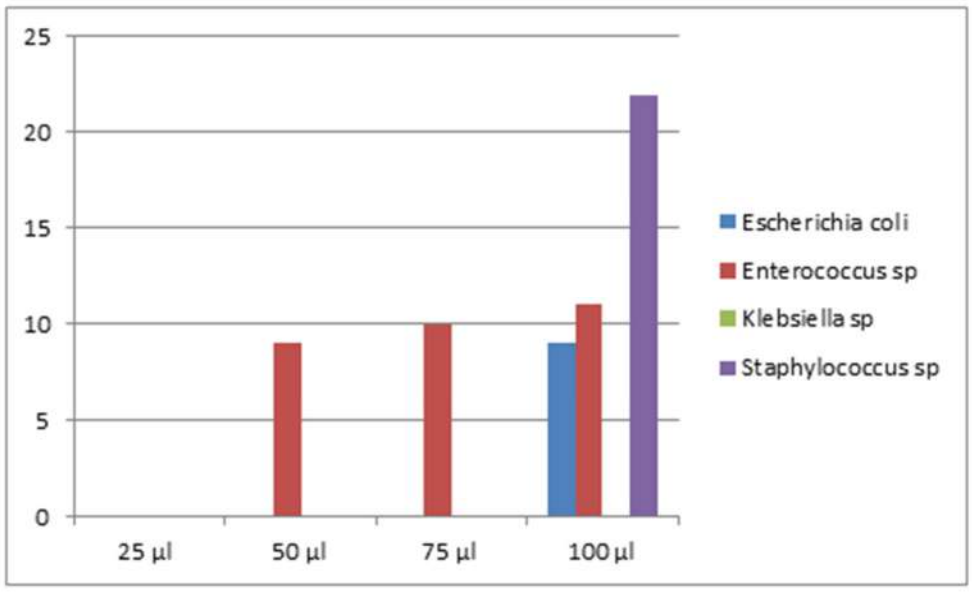
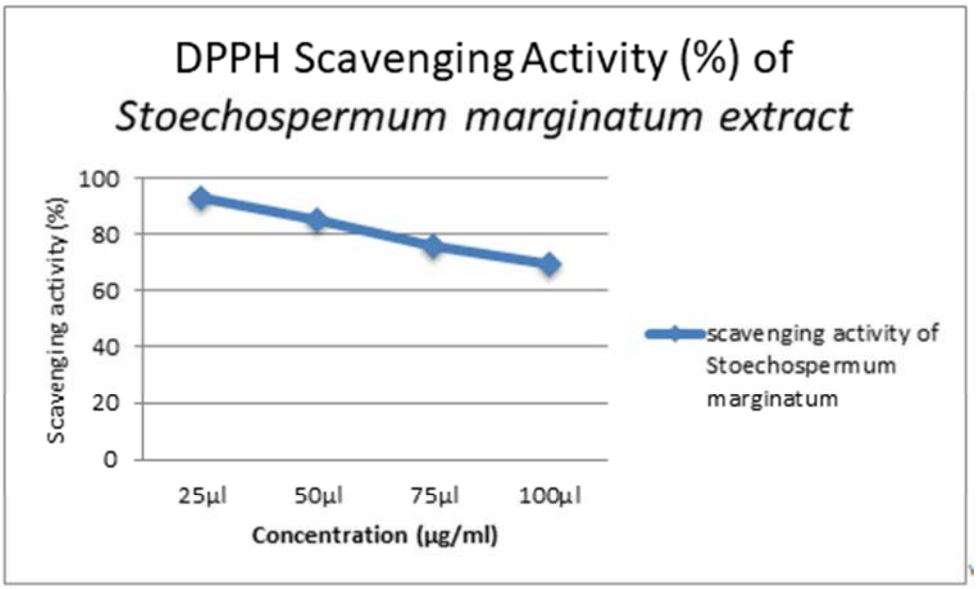


Figure 4: *DPPH scavenging activity of ethanol extract of Stoechospermum marginatum*



DISCUSSION

Investigations of natural models can provide a more efficient way of discovering novel chemicals with distinctive pharmacological properties or biomedical uses (20). Phycologists may be amazed to learn how repeatedly seaweed natural products are discussed in medicine. Several bioactive substances ought to have evolved due to ecological pressures acting on seaweeds. The chemical means that are employed by algae to overcome these problems can be potentially useful to humans and may result in new technologies such as natural antifoulants and novel UV-sunscreens. The brown seaweed *Stoechospermum marginatum* was collected from the intertidal regions of the Mandapam coastal region of Gulf of Mannar. Currently, there have been stupendous efforts to develop clean, nontoxic, reliable and eco-benign procedures for the synthesis and assembly of nanoparticles with desired sizes and morphologies to increase their biomedical applications. Nano- biotechnology dealing with metal nanoparticles has gained rising interest due to its cutting-edge nature and extensive application range in nearly every field of science and technology including biomedical sciences. Presently, metal nanoparticles are of much importance because of their catalytic activity, optical properties, electronic properties, antimicrobial activity and magnetic activity. Nanomaterials are used in medicine, with explicit applications in tissue engineered scaffolds and devices, drug delivery systems, cancer therapy and bio analytical diagnostics and therapeutics. Many chemical, physical, biological and other hybrid techniques are available for the synthesis of metal nanoparticles.

Usually, physical and chemical methods are not preferred due to their non eco-friendly and toxic nature. A large number of therapeutically important biodynamic compounds are present in seaweeds, which provide important ideas for developing novel drugs against cancer, microbial infections and inflammations. The formation of silver nanoparticles is indicated by the change of color of the reaction mixture to brown after the addition of silver nitrate solution. FTIR measurements were obtained to determine the presence of different functional groups in biomolecules responsible for the bioreduction of Ag^{+} and capping/stabilization of silver nanoparticles. The bands at 3415.93 & 3450.65 cm−1 in the spectra correspond to O–H stretching vibration indicating the presence of alcohol and phenol. The components which are confirmed by use of the FTIR influence their role in antimicrobial activity The Antibacterial activity was investigated by agar well diffusion test using *Escherichia coli, Enterococcus sp., Klebsiella sp., Staphylococcus sp.,* against different concentration of crude ethanolic extract and the AgNP of *S.marginatum* and it shows better activity against the bacterial strains. The *in vitro* anti-oxidant activity of *Stoechospermum marginatum* was determined using DPPH hydrogen peroxide scavenging assay method. It was observed that the algae exhibited significant antioxidant properties by scavenging the free radicals. The hydrogen donating ability of the antioxidants is thought to be responsible for their effect on DPPH radical scavenging. DPPH is a stable free radical and it accepts an electron or hydrogen radical to become a stable diamagnetic molecule. A stable non-radical form of DPPH is attained when it is mixed with a hydrogen atom donor, along with the concurrent change in the color of the solution to pale yellow from violet. Hence, DPPH has been broadly used as a free radical to estimate reducing substances and is thought to be a practical reagent for determining the free radical-scavenging activities of compounds . The anti-oxidant activity of the ethanolic extract was found between 65-95% with the maximum scavenging activity of 92.5% in 25µl concentration and minimum scavenging activity was 69.6% in 100 µl. Therefore the activity increases with the decrease in concentration and the extract showed better activity at low concentration itself.

CONCLUSION

The antibacterial and antioxidant activities of the nanoparticles synthesized from the crude extract of *Stoechospermum marginatum* was less efficient than that of the crude extract but was still in the acceptable range. This leads to a conclusion that if the nanoparticles are further purified and characterized, it may prove to be a better therapeutic agent against many infections.

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SP-12

COMPARATIVE STUDY ON PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITIES OF RAISINS (*VITIS VINIFERA L.*)

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ABSTRACT

The study was investigated to evaluate the phytochemical analysis and Antibacterial activities of *Vitis vinifera*. *Vitis vinifera* belongs to the family *Vitaceae* and seen in tropical, subtropical and warm temperate regions. The quantitative phytochemical analysis of *Vitis vinifera* exhibited the presence of secondary metabolites such as terpenoids, steroids, phenolic compounds, flavonoids, glycosides and saponins. Antibacterial activity was performed against Gram Positive (*Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*) and Gram Negative Microbes (*Proteus vulgaris* and *Shigella flexneri*) using Agar-well diffusion methods. It was found that *Bacillus subtilis* showed highest sensitivity of 13mm and 20mm at 625µg of concentrations in both black and green raisins respectively among Gram-positive bacteria. In Gram-negative *Shigella flexneri* showed 12mm and 20mm zone of inhibition at 625µg of concentration in both black and green raisins respectively where as *Proteus vulgaris* showed no activity in green raisin. Thus, from the study it can be concluded that *Vitis vinifera* produce various bioactive compounds and have an upstanding Antibacterial activity.

Keywords: ***Phytochemical analysis, Agar well diffusion method, Steroids, Bacillus subtilis, Proteus vulgaris, Vitis vinifera.***

INTRODUCTION

Vitis vinifera, the common grape vine is native to Mediterranean region, central Europe and south-western Asia, from Morocco and Portugal north to southern Germany and east to northern Iran.[1] They grow up to 32m in length with flaky bark. The fruit is a berry, known as grape that grow in cluster of 15 to 300 and are found in black, purple, green and pink. These plant species are found in humid forest and streamside. Dry grapes also known as Raisins are commercially produced by drying harvested grape berries. The dried grapes are used to treat heart diseases, blood pressure, diabetes, acidosis, anaemia, allergies and constipation.

TAXONOMIC CLASSIFICATION OF *Vitis vinifera*

Kingdom: Plantae
Division: Tracheophyta
Class: Magnoliopsida
Sub class: Rosidae
Order: Vitales
Family: Vitaceae
Genus: *Vitis L.- grape*
Species: *vinifera*

Fig.1.Raisins (*Vitisvinifera*)

MATERIALS AND METHODS

Collection of Plant Material and Preparation of Extract

The green and black raisins were collected from the market, Nanganallur, Chennai, Tamilnadu, India and soaked in ethanol for 72 hrs. The supernatant was filtered and condensed in a rotor evaporator at 50°C to yield gummy extract.

Qualitative Phytochemical Analysis

The green and black raisin extracts were subjected to preliminary Phytochemical screening for identification of phytoconstituents using specific standard procedures [2,3,4].

Estimation of Total Phenolic Content

To determine the total phenolic compounds, Folin-Ciocalteau reagent method was followed with slight modifications [5,6]. One hundred µL of ethanolic extracts (1mg/mL) of black and green raisins were mixed with 900 µL of ethanol and 1 mL of Folin-Ciocalteau reagent (1:10 diluted with distilled water). After 5 min, 1 mL of 20% (w/v) Na_2CO_3 solution was added. The mixture is then incubates for 30 minutes in dark at room temperature. The absorbance was measured spectrophotometrically at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (µg/mg of extract), which is a common reference compound.

Estimation of Total Flavonoids

The total flavonoid content of ethanolic extract of Raisin of *Vitis vinifera* was determined using aluminium chloride reagent method[7]. Five hundred µL of extract (1mg/mL) was mixed with 500 µL of methanol and 0.5 mL of 5% (w/v) sodium nitrite solution and incubate for 5 minutes at room temperature. Then, 0.3 mL 10% (w/v) aluminium chloride solution was added and incubate for further 5 min at room temperature followed by addition of 1 mL of 1 M NaOH solution. The total volume was made up to 5 mL with distilled water. Absorbance was measured at 510 nm using spectrophotometer. The result was expressed as (µg/mg of extract) quercetin equivalent.

Antibacterial Activity

Microbial Strains

The microorganisms of Gram negative strains such as *Proteus vulgaris* and *Shigella flexneri* as well as Gram positive strains such as *Staphylococcus aureus*, *Bacillus subtilis* and *Micrococcus luteus* were used for evaluation of antibacterial activity.

Nutrient Broth Agar Medium Preparation

Nutrient broth agar medium was prepared according to the standard methods (peptone-5 g, yeast-3 g, NaCl-5 g, distilled water- 1000 mL, agar-20 g). Depending upon the availability of strains, the medium was prepared, stirred, boiled to dissolve and then autoclaved at 15 lbs or at 121 ºC for 15 min[8]. The medium was then poured into sterile petriplates and allowed to solidify for 15 min.

Agar Well Diffusion Method

Antibacterial activity of ethanolic extract of black and green raisins was carried out using agar well diffusion method[8]. The solidified nutrient agar in the petriplates was inoculated by dispensing the inoculum using

sterilized cotton swabs which is previously immersed in the inoculum containing test tube and spread evenly onto the medium. Five wells were created by using a sterile cork - borer of 8 mm diameter. Different concentrations of 250, 375, 500 and 625µg/mL were poured into each well. All the plates were incubated at 37ºC for 24 h. The antibacterial activity was assessed by measuring the diameter of zone of inhibition formed around the well[9]. Tetracycline (25 µg) was used as positive control.

RESULTS AND DISCUSSIONS

Preparation of Extract

The black and green raisins were soaked separately in ethanol for 72 hrs to produce gummy extract. The supernatant was filtered and condensed in a rotor evaporator at 50˚C to yield the gummy extract

Phytochemical Analysis

The phytochemical analysis of extracts of *Vitis vinifera* showed the presence of terpenoids, steroids, phenolic compounds, flavonoids and glycosides whereas alkaloids, saponin and tannin where absent. A similar work was carried out [10] using ethanolic extract of grape seeds which expressed the presence of saponins, tannin, alkaloids, flavonoids and terpenoids where as flavonoids and terpenoids where only present in black and green raisin compared to the work carried out in the study[10].

Table 1*: Qualitative analysis of ethanol extracts of black and green raisins*

S. No	Phytochemicals	Tests	Results	
			Black	Green
1	Alkaloids	Mayer's test Hager's test	- -	- -
2.	Terpenoids	$CHCl_3$ + conc. H_2SO_4	+	+
3.	Steroids	Liebermann – Burchard test (acetic anhydride + Con. H_2SO_4)	+	+
4.	Flavonoids	NaOH solution	+	+
5.	Phenols	$FeCl_3$ solution	+	+
6.	Glycosides	Sodium nitroprusside solution + Con. H_2SO_4	+	+
7.	Saponins	Foam test	-	-
8.	Tannin	Dis H_2O + $Fecl_3$	-	-

Total Phenol and Flavonoid Content

Flavonoids and phenolic compounds are known to play major roles in the antioxidant and prooxidant capacities exhibited by plant extracts. The antioxidant effect conferred by these compounds are due to the phenolic hydroxyl groups attached to their respective ring structures that can act as reducing agents, hydrogen donors, singlet oxygen quenchers, super oxide radical scavengers and as metal chelators. They are also said to reduce α–tocopherol radicals or tocopheroxyls, activate antioxidant enzymes, mitigate nitrosative stress, and inhibit oxidases[11]. After proton donation, these compounds are oxidized to resonance-stabilized radicals that can further act as prooxidants at high concentrations, high pH, and in the presence of metal ions[12]. Flavonoids and phenolic acids present in food such as quercetin, myricetin, caffeic acid, gallic acid, chlorogenic acid, coumaric acid, ferulic acid, and ellagic acid have been proven to exhibit dual *in vitro* behaviour[13]. The total phenolic and flavonoid content of black and green raisin of ethanolic extract were found to be 358.4 ± 25.08 and 238.6 ± 16.70; 216.2 ± 15.13 and 203.0 ± 14.21 µg/mL respectively. A similar study [14] was carried out for the estimation of phenol using two varieties of grape berry pulp (Öküzgözü and Boğazkere) for three years (2011, 2012, 2013) and their average was taken and the amount of phenols present were 757.93 and 523.43µg GAE/mg respectively which were high when compared to the amount present in black and green raisins. The same study [14] showed that the amount of average flavonoids present in Öküzgözü variety and Boğazkere variety for three years were 17.32 and 29.65 µg GAE/mg respectively which was low than the amount present in black and green raisins

Table 2. Total Phenolic and Flavonoid Content Present in Ethanolic Extract of Raisins

S. No	Tests	Amount µg/mL	
		Black	Green
1	**Phenols**	358.4 ± 25.08	238.6 ± 16.70
2	**Flavonoids**	216.2 ± 15.13	203.0 ± 14.21

Antibacterial Activity

Antibacterial activity of ethanolic extracts of black and green raisin was determined by agar well diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) [15,16]. The ethanolic extract was investigated for *in vitro* antibacterial activity against microorganisms including Gram-positive (*Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus*) and Gram-negative (*Shigella flexneri, Protease vulgaris*). The antibacterial sensitivity of both the extracts and their potency were assessed by measuring the diameter of clear zone in cultures in petriplates. It was found that Gram-negative *shigella flexneri* showed highest sensitivity in green raisin at 375, 500, 625 µg/mL concentrations when compared to black raisin among Gram-negative bacterium. *Proteus vulgaris* showed least sensitivity in black raisin where as green raisins showed no sensitivity. Among Gram-positive bacteria *Bacillus subtilis* showed highest sensitivity in green raisin in 375, 500, 625 µg/mL concentrations when compared to black raisin. These results were identical to the previous reports that showed Bacillus subtilis (17+2.1mm)[17] with maximum activity using grape seed extract.

Table 2: ***Antibacterial activities of ethanolic extract of black raisin on various pathogenic organisms.***

S.No	Organisms	Zone of Inhibition, (mm)				
		250µg	375 µg	500µg	625µg	Standard (Tetracycline)
1	*Bacillus subtilis*	-	11	12	13	26
2	*Micrococcus luteus*	-	-	-	13	28
3	*Staphylococcus aureus*	-	-	-	12	22
4	*Proteus vulgaris*	-	-	11	12	16
5	*Shigella flexneri*	-	-	-	12	24

Table 3: Antibacterial activities of ethanolic extract of green raisin on various pathogenic organisms.

S.No	Organisms	Zone of Inhibition, (mm)				
		250µg	375 µg	500µg	625µg	Standard (Tetracycline)
1	*Bacillus subtilis*	-	14	16	20	28
2	*Micrococcus luteus*	-	11	12	13	16
3	*Staphylococcus aureus*	-	12	14	16	26
4	*Proteus vulgaris*	-	-	-	-	-
5	*Shigella flexneri*	-	15	19	20	28

Figure 2*: Antibacterial activity of ethanol extract of black raisinson various pathogens*

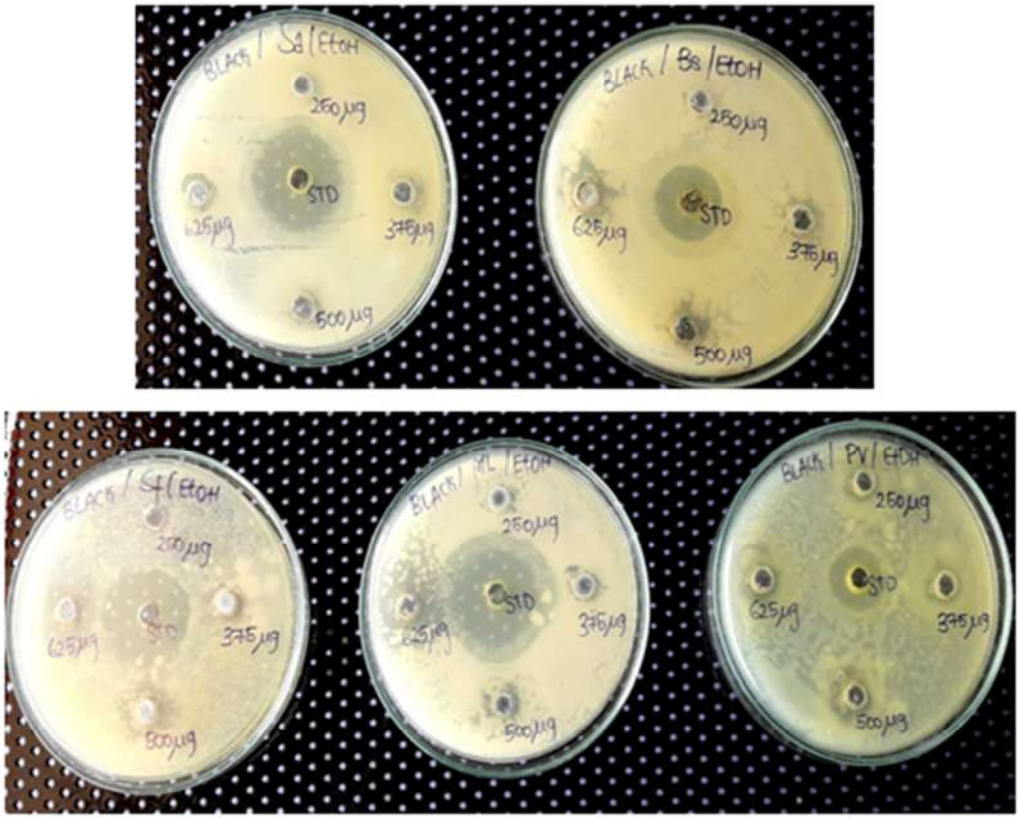
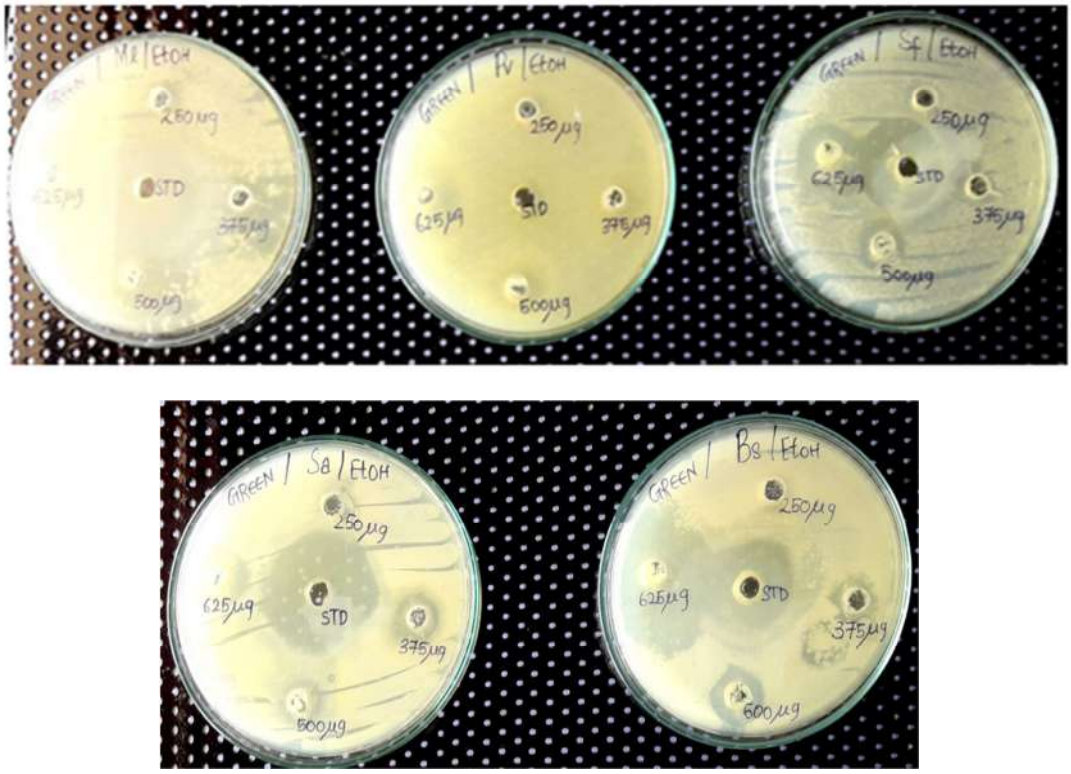


Figure 3*: Antibacterial activity of ethanol extract of green raisinson various pathogens.*



CONCLUSION

Thus it has been concluded that when compared to black raisin, green raisin were found to have an upstanding amount of secondary metabolites and consumption of raisins can be used for medicinal uses against diseases such as cancer, diabetics, inflammation etc.. The plant extracts act as source of great potential antibacterial compounds against the microorganism. Thus, it can be used in the treatment of infectious diseases caused by resistant microbes. So further research has been extended in the study of *In-*

vivo activities (Anti-oxidant, Anti diabetic and Anti-inflammatory) and to identify potential bio active compounds using GC-MS analysis.

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SP-13

COMPARATIVE GENE ANALYSIS OF DRK GENES IN FRUITFLY (*Drosophilamelanogaster)*

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ABSTRACT

The JAK-STAT signaling pathway transmits information from extracellular chemical signals to the nucleus resulting in DNA transcription and expression of genes involved in immunity, proliferation, differentiation, apoptosisand oncogenesis in insects. Herein, comprehensively identified 6 genes families involved in STAT pathway in the silkworm, *Bombyx mori* as well as 7 in *Drosophila melanogaster*, 3 in *Anopheles gambiae*, 6 in *Apis mellifera*, 5 *in Triboliumcastaneum*. Comparative analysis showed that *Drosophila melanogaster* have higher number of STAT pathway related gene families are present and it have a unique gene family called as upd which is not present in other insects. In conclusion, phylogenitic tree provide useful clues about the evolutionary relationship between the surveyed sequences of STAT-related genes in silkworm and other insects. Further, this study will be helpful to identify immuno related diseases that affects in silkworm and their effective prevention and paves way to improve the quality of silk and its production rate.

INTRODUCTION

It has become increasingly clear that changes in gene regulation have played an essential role in adaptive evolution both between and within species. Over the past five years, comparative studies have moved beyond simple characterizations of differences in gene expression levels within and between species to studying variation in regulatory mechanisms. We still know relatively little about the precise chain of events that lead to most regulatory adaptations, but we have taken significant steps towards understanding the relative importance of changes in different mechanisms of gene regulatory evolution (Athma*et al.*,2014).Cell-cell signaling represents an essential hallmark of multicellular organisms, which necessarily require a means of communicating between different cell populations, particularly immune cells. Cytokine receptor signaling through the Janus kinase/Signal Transducer and Activator of Transcription/Suppressor Of Cytokine Signaling (CytoR /JAK /STAT / SOCS) pathway embodies one important paradigm by which this is achieved. This pathway has been extensively studied in vertebrates and protostomes and shown to play fundamental roles in development and function of the immune and other cells. However, our understanding of the origins of the individual pathway components and their assembly into a functional pathway has remained limited.

Stat pathway

The JAK-STAT signaling pathway transmits information from extracellular chemical signals to the nucleus resulting in DNA transcription and expression of genes involved in immunity, proliferation, differentiation, apoptosisand oncogenesis. The JAK-STAT signaling cascade consists of three main components: a cell surface receptor, a Janus kinase(JAK) and two Signal Transducer and Activator of Transcription (STAT) proteins. Disrupted or dysregulated JAK-STAT functionality can result in immune deficiency syndromes and cancers (*Aaronson et al., 2002).*

Stat pathway associated gene in silkworm

*Innate immunity was critical in insects defensive system and able to be induced by Janus kinase/signal transducer and activator of transcription cascade transduction (JAK/STAT) signaling pathway. Currently, it had been identified many JAK/STAT signaling pathway-related genes in silkworm, but little function was known on insect innate immunity. T*he silkworm JAK/STAT pathway related major genes including BmSTAT, BmHOP, BmSOCS, BmDRK, Bmken, BmPIAS respectively **(**PENG et al., 2012). Due to this importance, we tried to explore the significant genes that associated with Signal Transducer and Activator of Transcription (STAT) pathway of silkworm, *Bombyx mori. Also, comparative analysis of STAT pathway genes of silkworm with other related species namely Drosophila melanogaster (fruit fly), Anopheles*

*gambiae (mosquito), Apis mellifera (honey bee), Tribolium castanenum (red flour bettel)*and examined similarity of sequences and evolutionary relationship between this organism were studied computationally.

Highlights

- Insilico identification of drk genes in the fruitfly (*Drosophila melanogaster)*
- Comparative analysis of *Drosophila melanogaster's*gene drk with other organisms.
- To understand the evolutionary relationship of*Drosophila melanogaster*gene with the other organisms such as*Bombyx mori* (silkworm*), Anopheles gambiae (mosquito), Apis mellifera (honey bee), Tribolium castanenum (red flour bettel).*

MATERIALS AND METHOD

The *Drosophila melanogasters* genome and protein sequences were downloaded from the flybase database (Jing et al., 2004) and utilized for this study. The predicted protein sequences associated with drk genes of *Drosophila melanogaster*, *Anopheles gambiae*, *Apis mellifera*, *Tribolium castaneum*, were downloaded from the NCBI database (Senator Claude Pepper et al., 1987), Ensembl Metazoan database (Kerseyet al., 2012), or species-specific databases such as bombyx mori (Drysdale et al., 2008), BeeBase for *A. mellifera* (Honeybee Genome Sequencing Consortium, 2006). The drk gene sequences that collected from the various database of each organism are merged. This set of sequences were blasted using standalone blast against *Drosophila melanogaster*sequence which are downloaded from flybase database (Jing et al., 2004) Based on the multiple sequence alignment results, neighbor-joining phylogenetic trees (Naruya et al.,1988) forstat-related genes from all surveyed insects wereconstructed using MEGA4.0 (Masatoshi,1993).

Standalone blast

In addition to providing BLAST sequence alignment services on the web, NCBI also makes these sequence alignment utilities available for download through FTP (Tao.,2010).This allows BLAST searches to be performed on local platforms against databases downloaded from NCBI or created locally. These utilities run through DOS-like command windows and accept input through text-based command line switches. There is no graphic user interface.

Steps Involving In Standalone Blast

- Download standalone blast from the website https://blast.ncbi.nlm.nih.gov/Blast.cgi according to the version of the computer
- Use the command prompt to instruct standalone blast
- Download all the protein sequences associated withstat pathway fromspecializeddatabases and keep separately under different gene group for the selectedorganism.
- Make a database of gene drk by merging sequence that collected from four different organisms (*bombyx mori, Apis mellifera, Tribolium castaneum, Anophelesgambiae*)

Download the whole protein sequences of*Drosophilamelanogaster*fromhttps://flybase.org/.

- Selected the drive in the command prompt that you keep your file and standalone blast programs (remember to keep the databases that you created, the protein sequence that downloaded from silkdb and stand-alone blast program in a single folder)
- The input command for the execution of blastpis given below,
  1. G:\>cd G:\blast-2.2.30+ (copy the address of the folder that contains standalone programs, sequencedatabase of protein that from a different organism and protein sequence of *Bombyx mori* that download from Silkdb database)
  2. G:\blast-2.2.30+>makeblastdb –in silkpep.fa (bombyxmori protein sequence) –dbtypeprot –out database (database name that you going to creat)
  3. G:\blast-2.2.30+>blastp.exe –query dbofhop.tx(data base gene sequence that we make) -db database -evalue 0.001 -outfmt 7 -num_alignments 5 –out outfile
  4. Then output file is created in the name of outfile
  5. Analyse the results

NCBI CD (Conserved Domains) search

1. The collected sequences of various organisms related to stat pathway and their similarity sequences available in the silkworm protein sequences were further analyzed for domain information using CD search.
2. Open the home page of NCBI CD search by using the URL https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi
3. Paste the predicted protein sequences of silkworm on the search box and click on submit button.
4. Similarly, the silkworm sequence that matches with gene sequence also paste on the query box and press on submit button
5. Compare the domains of protein sequences with *Bombyx mori* sequence based on comparison *Bombyx mori* sequence with similar domainswas selected for further analysis.

Clustalw

ClustalW (Thompsonet al., 1994) is one of the standard programs implementing one variant of the progressive method in wide use today for multiple sequence alignment. The W denotes a specific version that has been developed from the original Clustal program.

The basic steps of the algorithm implemented in ClustalW are:

- Compute the pairwise alignments for all against all sequences. The similarities are stored in a matrix (sequences versus sequences).
- Convert the sequence similarity matrix values to distance measures, reflecting the evolutionary distance between each pair of sequences.
- Construct a tree (the so-called guide tree) for the order in which pairs of sequences are to be aligned and combined with previous alignments. This is done using a neighbor-joining clustering algorithm. In the case of ClustalW, a method by Saitou & Nei is used.
- Progressively align the sequences/alignments together into each branch point of the guide tree, starting with the least distant pairs of sequences. At each branch point, one must do either a sequence-sequence, sequence-profile, or profile-profile alignment.

Steps involved

1. Make a database of the protein sequence that from a different organism and silkworm sequence that shares similar domains
2. Open the home page of clustalw by the URL http://www.genome.jp/tools-bin/clustalw
3. Enter the sequence from the database that containssimilardomain click on submit button after appearing the result of MSA create a rooted phylogenetic tree by click on execution
4. A rooted phylogenetic tree is createdsave it as a tree format

MEGA,(Molecular Evolutionary Genetics Analysis)

Molecular Evolutionary Genetics Analysis (MEGA) is computer software for conducting statistical analysis of molecular evolution and for constructing phylogenetic trees.

MEGA program (Molecular Evolutionary Genetics Analysis)

1. Download the MEGA software from *www.megasoftware.net/ and install it*
2. Open mega → data and select the phylogenetic tree in tree format after opening the phylogenetic tree click on view →option →labels and sequence can be a label with a differentcolour based on the organism.

RESULTS & DISCUSSION

Inventory of drk genes involved in stat pathway inDrosophila melanogaster and other insects

The protein sequences related to stat pathway were retrived from the database Ensmbl metazova ,NCBI and also with organism specific databases such as flybase for *Drosophila melanogaster*, Beebase for *Apis mellifera* and the number of protein sequence were retrived information is represent in Table 1.

Table 1 Stat -related genes in insects.

Genes	*Drosophila melanogaster*	*Tribolium castaneum*	*anopheles gambiae*	*Apis mellifera*
drk	5	1	-	2

The amino acid sequences of known insects of stat pathway-related genes to search against the whole proteome *silkworm* using the standalone blast program.the blast is performed with an evalue of 0.001 the resultant sequence that from both silkworm and known insects sequence numbers are represented in table 2 after avoiding repeating sequence . The JAK-STAT signalling pathway transmits information from extracellular chemical signals to the nucleus resulting in DNA transcription and expression of genes involved in immunity, proliferation, differentiation, apoptosis and oncogenesis. Previous studies have reported the silkworm JAK/STAT pathway related major genes including BmSTAT, BmHOP, BmSOCS2, BmSOCS5A, BmSOCS5B, BmSOCS6, BmDRK, Bmken, BmPIAS1, BmPIAS2 and BmPI3K were cloned, respectively, but the homologues of Drosophila Upd1, Upd2 and Upd3 were not cloned(PENG et al., 2012) ,similar results is occur in this work also upd gene family is not able to identify in *Bombyx mori.*

Genes	*Bombyx mori*		*Tribolium castaneum*		*anopheles gambiae*		*Apis mellifera*		Total sequence		
	No of seq from gene	Silk seq	No of seq from gene	Silk seq	No of seq from gene	Silk seq	No of seq from gene	Silk seq	Gene sequences	Gene seq match with silk	After avoiding repetation
drk	5	5	1	5			2	5	8	15	6

Drk gene related domain in stat pathway

Initially the Drk gene related protein domains of all known insects that collected for comparative analysis are find using cd search software and it represent on table , then the matching domain of silkwormwith insects were find out using CD search software which is represented in Table . By comparing the domains on Tables 13 and 14,select the *B. mori* sequence domain that present commonly with the insects domain that we select for comparative analysis .from the above table we select the *B.mori* sequences BGIBMGA002069-PA,BGIBMGA000600-PA,BGIBMGA003789- PA,BGIBMGA012094-PA, and the other sequences does not contain any domain common with the insects sequence

Number of insects and silkworm sequence found out after domain search

Sequence found after domain search using ncbi cd search is represented in Table

Table Domain search resultant sequences

Genes	Sequence from the insects	Silkworm sequence
drk	8	4

Multiple sequence alignment based phylogenitic tree construction

Based on the domain search result the sequence that collected from the insects and silkworm protien sequences are compained and make a single file then these sequences are selected for multiple sequence alignment using the online software called clustalw based on the results construct a phylogenitic tree in tree format further its visualiesd by software called as mega and figure 1repersent phylogenitic analysis of drk gene

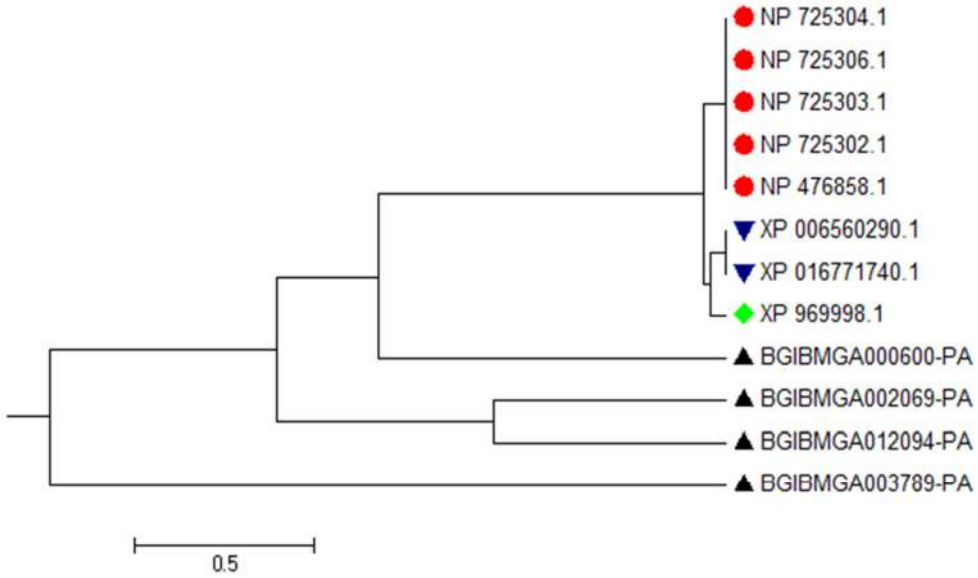

Figure 1- Phylogenetic tree of the Drk genes in *B. mori* and other insects.The amino acid sequences encoding by Drkgenes from seven insect species,including *Bombyx mori*, *Drosophila melanogaster*, *Apis mellifera*, *Tribolium castaneum*, *Anopheles gambiae* were used to build a phylogenetic tree.

The phylogenetic tree of Drk gene mainly contains four cladistic group with amino acids sequence per site is taken as 0.5 and the distance between the branches represent the rate of evolution,the drosophila sequence NP725304.1 closely related with first clade and which share similar domain of SH3_GRB2_like_C and in the third clade *B.mori* sequences BGIBMGA012094-PA,BGIBMGA002069-PA is distantly related with clade group one and two which shares similar domain of SH2_Grb2_like and the *B.mori* sequence BGIBMGA003789-PA in the fourth clade shows evolutionary distance from other clades.

Summary

In summary, The common fruit fly, *Drosophila melanogaster*, is a well studied and highly tractable genetic model organism for understanding molecular mechanisms of human diseases. Many basic biological, physiological, and neurological properties are conserved between mammals and *D. melanogaster*, and nearly 75% of human disease-causing genes are believed to have a functional homolog in the fly,by the identification drk related gene function in other organisms beneficial in human immune related development .further experiments needs to supports the facts

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Internet Resources

1. Blast alone online tutorial https://www.ncbi.nlm.nih.gov/books/NBK52637/
2. For download BLAST (standalone) version https://blast.ncbi.nlm.nih.gov/Blast.cgi
3. *Ncbi cd search online tools address* https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi
4. Clustal w online tool address www.genome.jp/tools/clustalw/
5. *Molecular Evolutionary Genetics Analysis (mega) online tutorial* www.megasoftware.net/
6. JAK-STAT_signaling_pathway information from wikipedia https://en.wikipedia.org/wiki/JAK-STAT_signaling_pathway

SP-14

A STUDY ON ANTIDIABETIC POTENTIAL OF SPIRULINA ON ORAL GLUCOSE TOLERANCE TEST AND ALLOXAN INDUCED DIABETIC MICE

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ABSTRACT

The study was undertaken to investigate the effects of Spirulina platensis on body weight, blood glucose on alloxan induced diabetic rats. Twenty four male rats at six weeks of age were used for the experiment. Diabetes was induced by intraperitoneal injection of alloxan@ 120 mg/kg and experiment was carried out for a period of 6 weeks. The Rats were divided into five groups and each group contains 6 rats. The groups were i) healthy control ii)diabetic control iii) Metformin(350mg/kg) iv) Spirulina platensis @ 200 mg/kg v) Spirulina platensis @ 400 mg/kg Results from the study showed that Spirulina platensis reduced blood glucose significantly ($P<0.01$) and increased body weight significantly ($P<0.05$) in contrast to diabetic control group after 6 weeks. Lipid profile analysis with Spirulina platensis @ 400 mg/kg showed that total choleste. Our findings showed that oral administration of Spirulina platensis could reduce the adverse effect of alloxan induced diabetes in rats. Based on this research it can be concluded that Spirulina platensis has antihyperglycemic effects in alloxan induced diabetic

Keywords : ***Spirulina platensis, Alloxan, Metformin, Intreperitoneal, Antihyperglycemic.***

INTRODUCTION

Diabetes mellitus is a metabolic disorder which cause deleterious effects on a patient's physical and psychological state (Halpern et al., 2000)[6]. Alloxan is a toxic glucose analogue , that has been noted to exert its diabetogenic action when administered parenterally to the animals (Federiuk IF et al.,2004)[4]which selectively destroys the Langerhans islet ß-cells (Bhattacharya SK .,1995)[2] and use to produce experimental diabetes to animals (Etuk.EU .,2010)[3] Spirulina are multicellular and filamentous blue-green microalgae (Huntington et al.,2008)[5].It contains phycocyanin, β-carotene and xanthophyll pigments,α-tocopherol and phenolic compounds(Mishra S., 2006)[9].The spirulina has the ability of potent anti-viral (Hayashi.T and Hayashi.K,1996)[7], anti–cancer (Lisheng,1991), hypocholesterolemic (Kato et al.,1984) and health improvement (Annapurna et al.,1991)[1]agent .It has been used as a dietary supplement by the people which is collected from natural water , dried and eaten and it is also been used as dietary ingredient of feed for fish,shrimp and poultry(Huntington et al.,2008)[5].

MATERIALS AND METHODS

Experimental Animals

The studies were conducted in compliance with guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA no. 971/bc/06/CPCSEA),Government of India and approved by the Institute of Animal Ethics commission All the studies were conducted as per the norms of the committee for the purpose of supervision of experiments on animals. Male mice weighing 140-170g were purchased from Biogen Bangalore, India. All animals were housed 2/cage and kept in the animal house for one week for proper acclimatization before starting the experiment under controlled conditions of illumination (12 h light/12 h darkness) and temperature ranging 20-25°C. They were housed under the above laboratory conditions, maintained on standard pellet diet and water.

Powder Preparation

Powder product of Spirulina platensis were donated by S.K organic farms, Ambattur, Chennai. It is a spray dried product in powder form, standard in quality and a part of bulk production by the industry

.

Drugs and Chemicals

The chemical and drugs used in the study were Test drug, Alloxan, Glucose, metformin, isoflurane (anaesthetic agent) was purchased from Sigma–Aldrich, St. Louis, MO, USA. Animal restrainer (e.g., Broom restraint, Plas Labs), glucometer (Accu-Check, Roche, Germany) and Advia (Hematology analyzer).

Oral glucose tolerance test (OGTT) in mice

Experimental animals were kept on fast for 16-18 hours (overnight) prior to induction of diabetes.Animals were randomized according to their baseline blood glucose level.The animals were dosed as specified, a glucose load of 2g/kg and either vehicle (water) or were simultaneously administered orally.Blood glucose concentrations were measured at 15,30, 60, 90 and 120 min (T15–T120) post treatment by tail cut method using glucometer (Accu-Check, Roche, Germany).The reduction in blood glucose produced by the compounds metformin and testing drugs were calculated using the area under the curve method with basal value as the zero. (AUC 0–120 min).

Alloxan induced diabetic mice model

Alloxan Preparation

Alloxan was weighed and dissolved in 4% saline solution to make a concentration of 120mg/kg

Metformin

350mg of metformin was dissolved in carboxymethyl cellulose.

Alloxan induced Diabetes mellitus

After one week of the acclimatization, rats were injected once with low-dose of Alloxan (80mg/kg,i.p.) to induce partial insulin deficiency. The glucose value was noted using glucometer before Alloxan injection.This is considered as Basal value.After 48-96 hrs of Alloxan injection, the rat's fasting blood glucose value (glucometer) were noted using tail flick method. The animals would display hyperglycemia and glucose intolerance.Animals with similar degrees of hyperglycemia (mostly above 95mg/dl) were considered and according to their glucose value, animals were randomized and divided to groups as follows

Group 1 – Normal Group
Group 2 – Diabetic control (Untreated)
Group 3 – Diabetic group + Metformin (300 mg/kg, p.o),
Group 4 - Diabetic group + spirulina (200mg/kg) and
Group 5 – Diabetic group + spirulina (400mg/kg)

Animal blood collection

Animals were kept fasting for 12 hours on the day before glucose estimation. Then the animals tail were flicked, fasting blood glucose levels were noted on the Day 0 and every alternate days of the entire observation period.

Body weight

Body weight of individual animals will be recorded from the Day 0 and every day 1 of the entire observation period

Feed intake

Cage-wise feed intake will be recorded on from the Day 0 and every day 1 of the entire observation period

RESULTS AND DISCUSSION

The oral treatment of *Spirulina* decreased the blood glucose levels in diabetic mice. Spirulina (200 and 400 mg/kg) showed significant ($P < 0.001$) decrease in Blood glucose level at 2, 4 and 6 h. Continuous treatment with SPIRULINA (200 and 400 mg/kg) one hour prior to glucose dose oral dose showed a significant (P <

0.001) decrease in the blood glucose level in diabetic mice. Maximum reduction of blood glucose level in acute and subacute occurred at the dose of 400 mg/kg. The Spirulina showed short onset and short duration of anti hyper glycaemic action. The ability of Spirulina to prevent body weight loss seems to be due to its ability to reduced hyperglycaemia. Spirulina significantly enhanced glucose utilization in OGTT in both nondiabetic mice. From the data obtained OGTT, it is clear that administration of Spirulina effectively prevented the increase in blood glucose level without causing a hypoglycaemic state.

Fig 1. ***OGTT values***

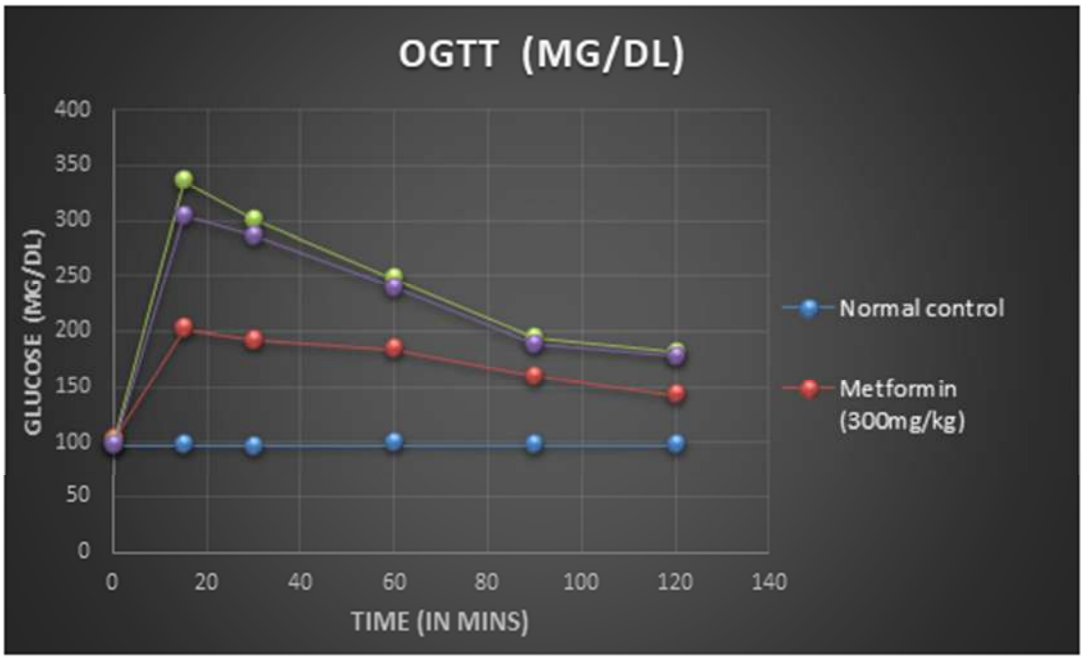

Table 1: ***OGTT- glucose values in mg/dl***

Time (in hrs)	Normal control	Metformin (300mg/kg)	Spirulina	
			200 mg/kg	400 mg/kg
0	98.5	103.2	100.5	98
15	98.1	203.2	336.4	305.4
30	97.2	192.5	300.5	285.6
60	99.5	184.7	247.8	239.8
90	98.6	160.3	194.3	188.6
120	99.3	143.2	181.3	178.6

Fig 2. ***Body Weight Chart***

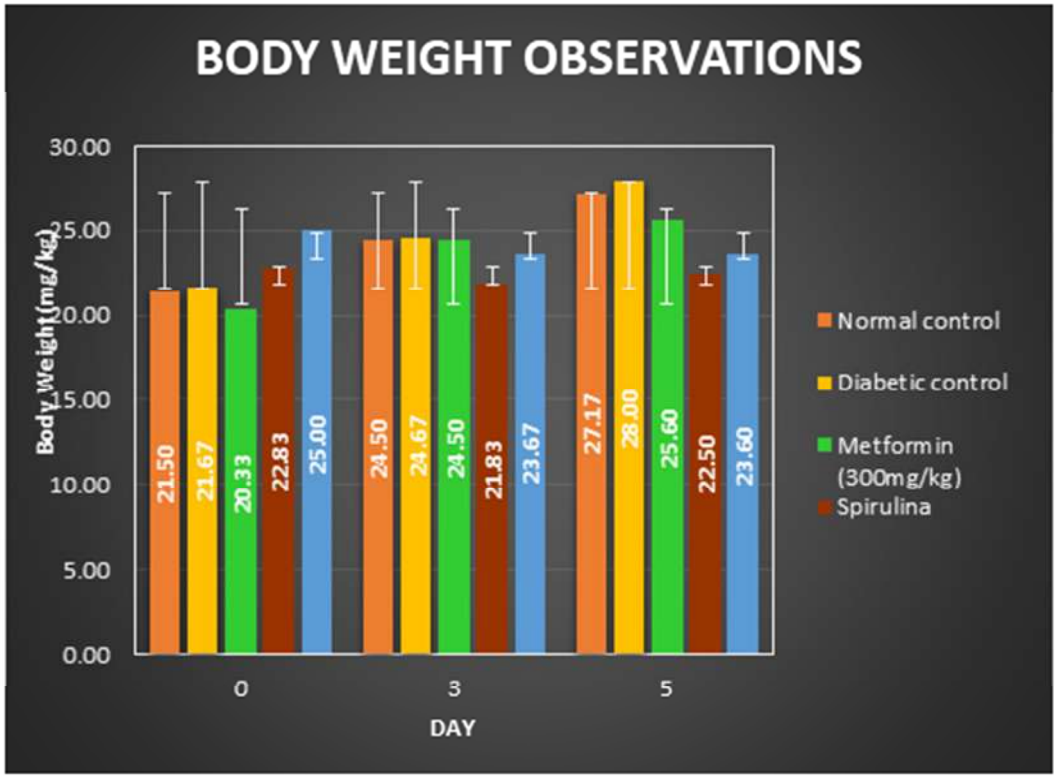

Table 2. ***Body weight chart (weight in grams)***

Days	Normal control	Diabetic control	Metformin (300mg/kg)	Spirulina	
				200 mg/kg	400 mg/kg
0	21.5±2.59	21.67	20.33	22.83	25
3	24.5	24.67	24.5	21.83	23.67
5	27.17	28	25.6	22.5	23.6

Fig 3. ***Feed intake chart***

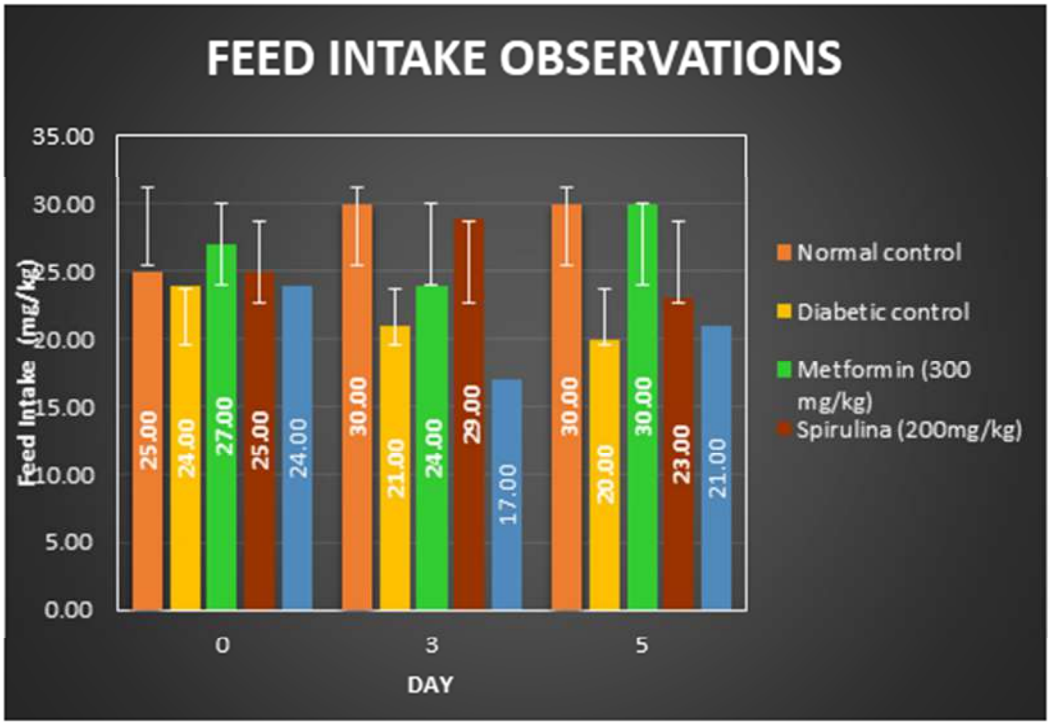

Table 3. ***Feed intake chart***

Days	Normal control	Diabetic control	Metformin 250 mg/kg	Spirulina 200mg/kg	Spirulina 400 mg/kg
0	25	24	27	25	24
3	30	21	24	29	17
5	30	20	30	23	21

CONCLUSION

Spirulina was found to be very active against alloxan induced diabetic rats by reducing the insulin levels and glucose parameters. This indicates the algal extarct is effective against type 1 diabetes.
In future, further chronic studies and antioxidant studies evaluation will add on to the advantage of the plant in becoming a lead molecule in type 1 diabetes

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SP-15

THE EXAMINATION OF PHYTOCHEMICAL CHARACTERIZATION OF *Melia dubia* LEAF EXTRACTS, BIOSYNTHESIS OF SILVERNANOPARTICLES AND ITS ANTICANCER ACTIVITY AGAINST HEP-G2 CELL LINE.

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ABSTRACT

Cancer is a multi-step, dangerous and widely distributed disease. Cancer of the liver is one of the prevalent cancers that occur in the world, in which hepatocellular carcinoma [HCC] is commonest. *Melia dubia* belonging to *Meliaceae*, also called as forest neem is thought-out to be a medically significant plant with a deluge of medicinal properties, especially like antibacterial activity, anti-inflammatory activity, anti-cancer activity,hepatoprotective activity and furthermore. Nanotechnology is one of the fastest growing fields and it is bring into being to be one of the promising technology for science, research and development. As nanoparticles serve as the base for nanotechnology applications, silver nanoparticles [AgNPs] synthesized by the plant extracts has focused huge consideration as a result of its physical, chemical and biological properties that attributed to anti-angiogenic activity, catalytic activity, bactericidal effects and found application in nanobiotechnology research. This examination targets on the in vitro anticancer property of silver nanoparticle of *Melia dubia* leaf extract against Hep-G2 cell line. Leaf of *Melia dubia* was collected, ethanol extracts of leaves were prepared using percolation method. Then, the phytochemical analysis of leaf extracts was performed. Further silver nanoparticles were synthesised and characterization of these nanoparticles was performed. Followed by the screening of anticancer activity of crude extract and silver nanoparticles synthesised extract was done using Hep-G2 cells by MTT assay. The results concluded that the silver nanoparticles synthesized from *Melia dubia* leaf exhibited important anticancer activity against Hep-G2 cells. Thus the nano synthesis of AgNPs can be exploited in effective therapeutics target against different forms of cancer.

KEYWORDS: ***Silver nanoparticles, hepatocellular carcinoma, Melia dubia***

INTRODUCTION

Traditional herbal medicine and their preparations have been broadly utilized for the thousands of years in progressing and progressed countries owing to its natural origin and insignificant side effects.[1] The traditional knowledge system required to be examined, documented, conserved and used for the welfare of humankind, before it is lost forever. In India, medicinal plants have played a crucial role in the development of our ancient material medica.[2]*Melia dubia* belonging to the family *Meliaceae* is also well-known as Mahaneem or forest neem which is rapidly growing tree species of India, Sri Lanka, Malaysia to Australia and Angola.[3] A survey of literature revealed that *Melia dubia* has lot of pharmacological activities like Anti-Bacterial activity &AntiFungal activity ,[4]Anti-Inflammatory activity,[5] Anti-oxidant activity, Hepatoprotective activity,[6] Anti-Diabetic activity,[7]AntiUrolithiatic activity [8]and AntiCancer activity. [9] The rising price of modern medicine and predominance of diseases have resulted in claim for uncovering of inexpensive and effective drugs. [10] The medicinal value of these plants lies in the bioactive phytochemical constituents that bring out specific physiological effects on human body. These natural compounds formed the base of modem drugs that we use today .[11]Phytoconstituents are the natural bioactive compounds found in plants .Nanoparticles serves as the base for various nanotechnology applications. Nanotechnology, and apace with nanostructured materials, play an magnificent role in science, research and development as furthermore better products found on nanostructured materials are brought in to the market. [12]They are also seen as a solution to various challenges in environmental, technological and medical fields. Silver is a white, smooth, lustrous transition metal which possesses high electrical and thermal conductivity. It has been known longer than the recorded history as a result of its medical and therapeutic advantages before realizing that the microbes were the causative agents for infections. It is the chief therapeutic agent in medicine for

surgical infections and infectious diseases. The silver nanoparticles have more beneficial properties as than that of the risk factors. [13] Synthesis of metallic nanoparticles utilizing plant extracts is inexpensive, easily scaled-up, and environmentally benign. Different characterization methods are used in evaluating its potential applications .[14]Silver nanoparticles synthesized by the plant extracts lead a role in biological activities such as antimicrobial, antioxidant and anticancer. Cancers of the liver are one of the prevalent cancers that occur in the world, the commonest of which is the hepatocellular carcinoma (HCC), which is thought out to be the 5th commonest cancer in the world. Eighty per cent of all HCCs take place in India arise with cirrhosis of liver in the background and 60% of all these cases are hepatitis B positive carriers. Symptoms can be seen with advanced stage of the disease. Surgery, exclusive curative modulus available, unfortunately which is not possible in case of 95% of HCC patients.[15]Liver cancer is considered as one of the most widespread malignancies across the globe. According to a recent evaluation, about 782,000 people were diagnosed for liver cancer; out of which 746,000 people died .[16] Various reports disclosed that silver nanoparticles have significant anti-angiogenic properties. Compounds that hold anti-angiogenic properties may have antitumor activity as they have the capability to block the activity of abnormally expressed signaling protein. [17] Silver nanoparticles synthesized by the plant extracts lead to a remarkable role in biological activities such as antimicrobial, antioxidant and anticancer. [9] In the present investigation *Melia dubia* has been chosen due to its medical significance.Thus, this study targets on the examination of photochemical characterization of *Melia dubia* leaf extracts, biosynthesis of silvernanoparticles and its anticancer activity against Hep-g2 cell line.

METHODOLOGY

Collection of plant

The leaves of the plant *Melia dubia (Malaivembu)* was collected from the available area near Singampunari, Sivagangai District and their leaves were collected and washed with tap water then rinsed with distilled water, dried, cut into fine pieces and were crushed into fine powder and stored at 37^{o} C.

Preparation of solvent extract by percolation method

This is the procedure used most commonly to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator (a narrow, cone-shaped vessel open at both ends) is generally used. The solid ingredients are moistened with an suitable amount of the specified menstruum and allowed to stand for approximately 4 h in a well closed container, after which the mass is packed and the top of the percolator is closed. Additional menstruum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 h. The outlet of the percolator then is opened and the liquid contained in that respect is allowed to drip moderately. Additional menstruum is added as necessary, until the percolate measures about three-quarters of the required volume of the finished product. The marc is then pressed and the expressed liquid is added to the percolate. Sufficient menstruum is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting .[18] About 50gm of dried fine powder of *Melia dubia* were reflexed successfully with ethanol using percolation apparatus for 40 hours separately. After decanting, ethanol extract of *Melia dubia* given residue stored in air tight container were subjected to qualitative test for identification of various plant constituents.

Screening of phytochemical components

The leaf extracts of *Melia dubia* were examined for the presence of alkaloids, carbohydrates, saponins, phenolic compounds, flavonoids, proteins and aminoacids as stated in the common phytochemical methods such as Mayer's test, Wagner's test, Molisch's test, Benedict's test, Fehling's test, Froth test, Foam test, Ferric chloride test, Alkaline reagent test, Lead acetate test, Xanthoproteic test, Ninhydrin test.

Detection of alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of yellow colored precipitate signifies the presence of alkaloids.

Wagner' Test
Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown or reddish precipitates the presence of alkaloids.

Detection of carbohydrates
Extracts were dissolved separately in 5ml distilled water and filtered. The filtrates were utilized to test for the presence of carbohydrates.

Molisch's Test
Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of violet ring at the junction signifies the presence of carbohydrates.

Benedict's Test
Filtrates were treated with Benedict's reagent and heated mildly. Orange red color precipitate signifies the presence of reducing sugars.

Fehling's Test
Filtrates were hydrolyzed with diluted Hydrochloric acid, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red color precipitate signifies the presence of reducing sugars.

Detection of saponins
Froth Test
Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam signifies the presence of saponins.

Foam Test
0.5gm of extract was shaken with 2ml of distilled water. If the produced foam remained for ten minutes it signifies the presence of saponins.

Detection of phenols
Ferric Chloride Test
Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color signifies the presence of phenols.

Detection of tannins
Gelatin Test
1% gelatin solution containing sodium chloride was added to the extract. Formation of white color precipitate signifies the presence of tannins.

Detection of flavonoids
Alkaline Reagent Test
Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

Lead Acetate Test
Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

Detection of proteins and aminoacids
Xanthoproteic Test
The Extracts were treated with few drops of concentrated Nitric acid. Formation of yellow color signifies the presence of proteins.

Ninhydrin Test
To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of aminoacid.

Detection of glycosides
Extracts were hydrolyzed with diluted Hydrochloric acid, and then assigned to test for glycosides.

Legal's Test
Extracts were treated with Sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red indicates the presence of cardiac glycosides.

Detection of steroids and triterpenoids
Detection of Steroids
To 1ml of the test solution, equal volume of chloroform and 3 drops of concentrated sulphuric acid was added. Formation of brown color ring signifies the presence of steroids.

Detection of Triterpenoids
To the extract solution, chloroform and concentrated H_2SO_4 was added. Formation of red color indicates the presence of triterpenoids.

Characterization of biosynthesized silver Nanoparticles
UV-Visible Spectrophotometer Analysis
The formation and completion of AgNPs was characterized by UV-Visible spectroscopy by using Electro UV-Visible spectrophotometer. The bio-reduction of the Ag+ ions in solution was monitored by periodical sampling of aliquots and the UV-Visible spectra of these aliquots were examined as a function at time of reaction in 200-600 nm range operated at a resolution of 1nm. Distilled water was utilized as a blank. [19]

Fourier Transform Infrared (FTIR) Analysis of Silver Nanoparticles
FT-IR spectral analysis was carried out to determine the possible biomolecules accountable for the reduction of the Ag+ ions and the capping of the AgNPs synthesized by *Melia dubia* extract. The chemical compositions of silver nanoparticles were analyzed using FTIR spectrometer after bio reduction with silver nitrate. 2 ml of bio synthesized liquid silver nanoparticles were scanned using infrared in the range of 400-4000 cm^{-1}.

Anticancer activity of crude extract and agnps synthesized extract
Cell culture
Hep-G2 (Human liver carcinoma cells)cell line were cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 u/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37°C.

MTT Assay
The crude extract and AgNPs synthesized extract of *Melia dubia* leaves was tested for *in vitro* cytotoxicity, using Hep-G2 cells by 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) assay.Concisely, the cultured Hep-G2 cells were harvested by trypsinization, pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200 µL) into 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS and treated with various concentrations of crude and AgNPs synthesized extract of *Melia dubia* leaves in a serum free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO2 incubator for 24 h. After the incubation period, MTT (20 µL of 5 mg/ml) was added into each well and the cells incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope. Eventually, the medium together with MTT (220 µL) were aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a micro plate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC50 value was calculated using GraphPad Prism 6.0 software (USA).

RESULTS

Table 1: Phytochemical screening of *Melia dubia* extract

S.No	Phytochemicals	Presence of Phytochemical in ethanolic extract of *Melia dubia*
1.	Flavonoids	+
2.	Saponin	+
3.	Polyphenols	+
4.	Carbohydrates	+
5.	Alkaloids	+
6.	Proteins	-

Inference

+ - Presence - - Absence

Figure 1: *Silver Nanoparticles Synthesized from Melia dubia leaf extract*

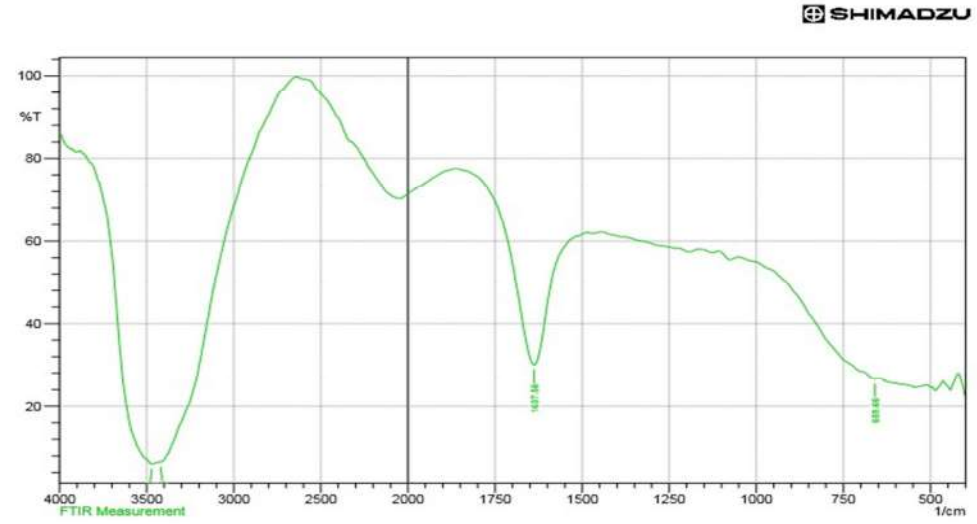
Before Synthesis After Synthesis

Table 2: *Visible Spectroscopy of AgNPs synthesized Melia dubia leaf extract*

S.No	UV Readings	Absorbance Value
1.	430 nm	2.44
2.	435 nm	2.48

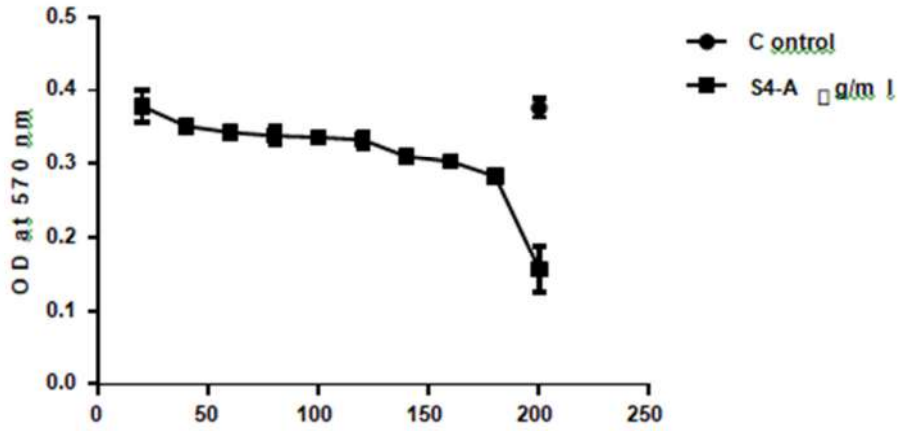
Fourier Transform Infrared Spectroscopy of AgNPs synthesized Melia dubia leaf extract

Graph 1:

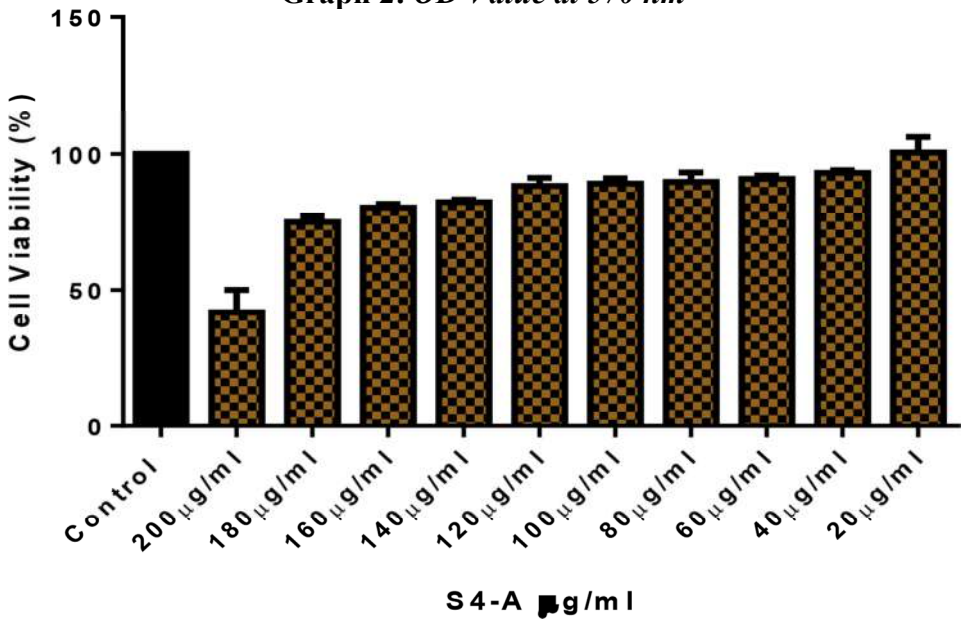


	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	659.66	26.556	0.762	1008.77	651.94	144.948	1.772
2	1637.56	30.028	38.584	1836.23	1485.19	90.891	34.708
3	3417.86	6.493	0.743	3423.65	2648.26	271.472	0.175
4	3473.8	6.01	6.679	3822.91	3441.01	239.59	17.251

MTT assay for crude Melia dubia leaf extract



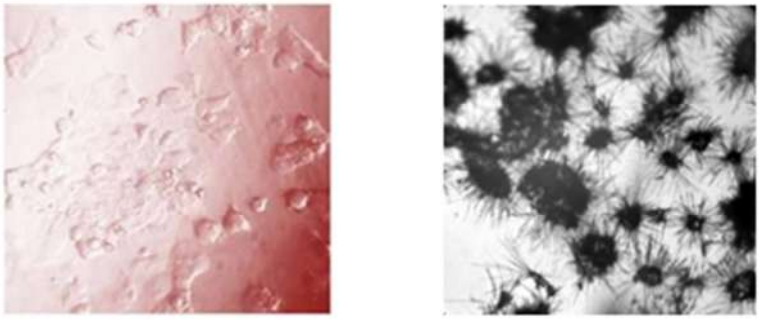
Graph 2: *OD Value at 570 nm*



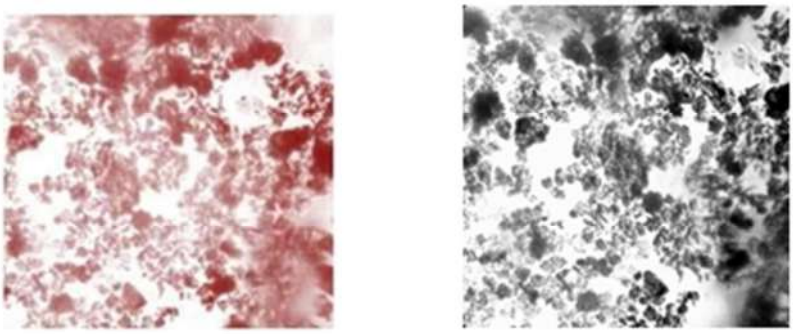
Graph 3 : *Cell Viability (%) of crude Melia dubia leaf extract*

Figure 2

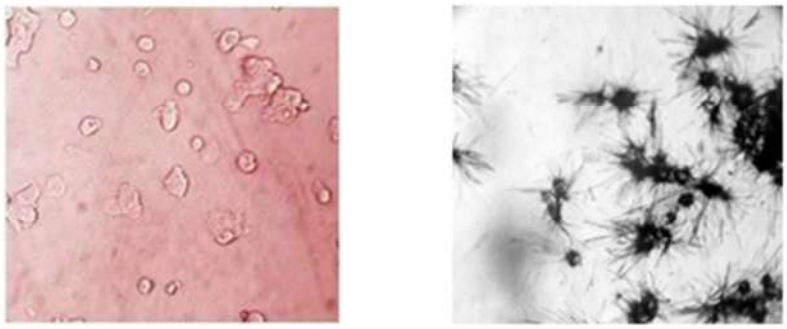
Formation of formazan crystals in control cells and Crude extract treated cells.



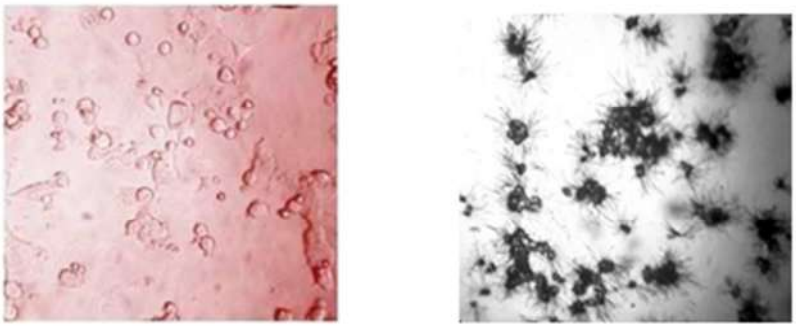
Control cells



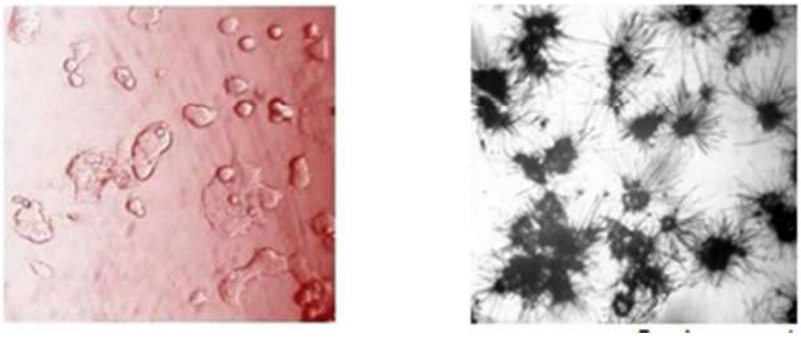
S4-A 200 µg/ml



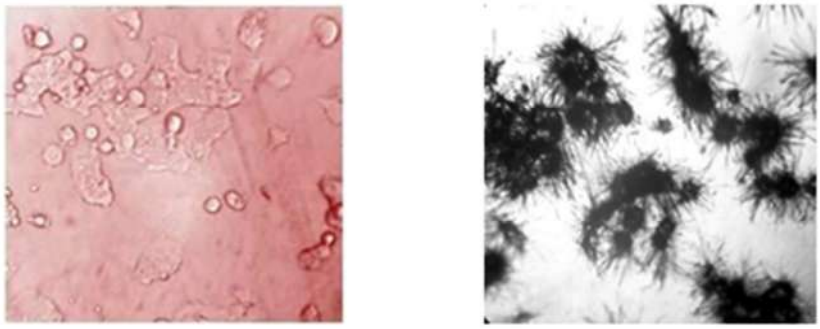
S4-A140µg/ml



S4-A 100 µg/ml

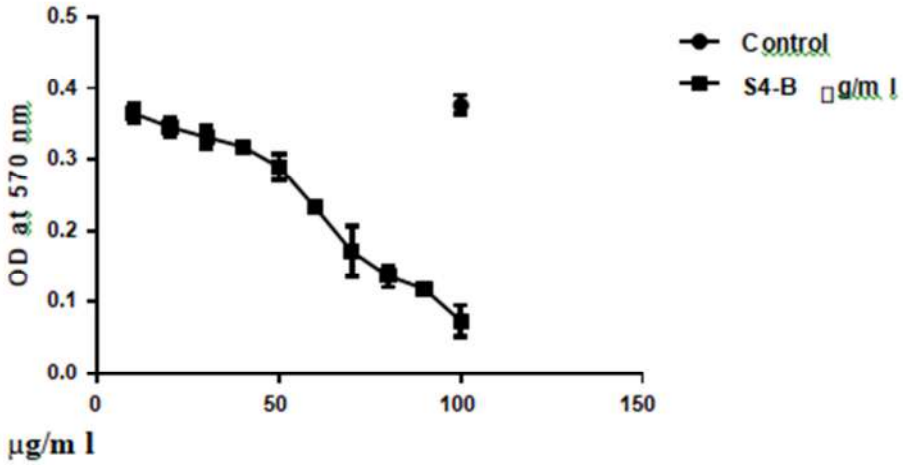


S4-A 40 µg/ml

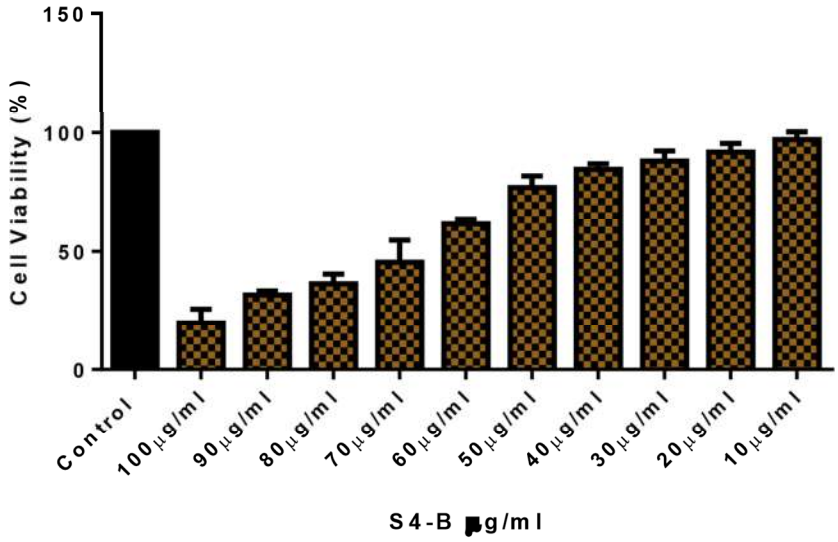


S4-A 20 µg/ml

MTT assay for agnps synthesized melia dubia leaf extract

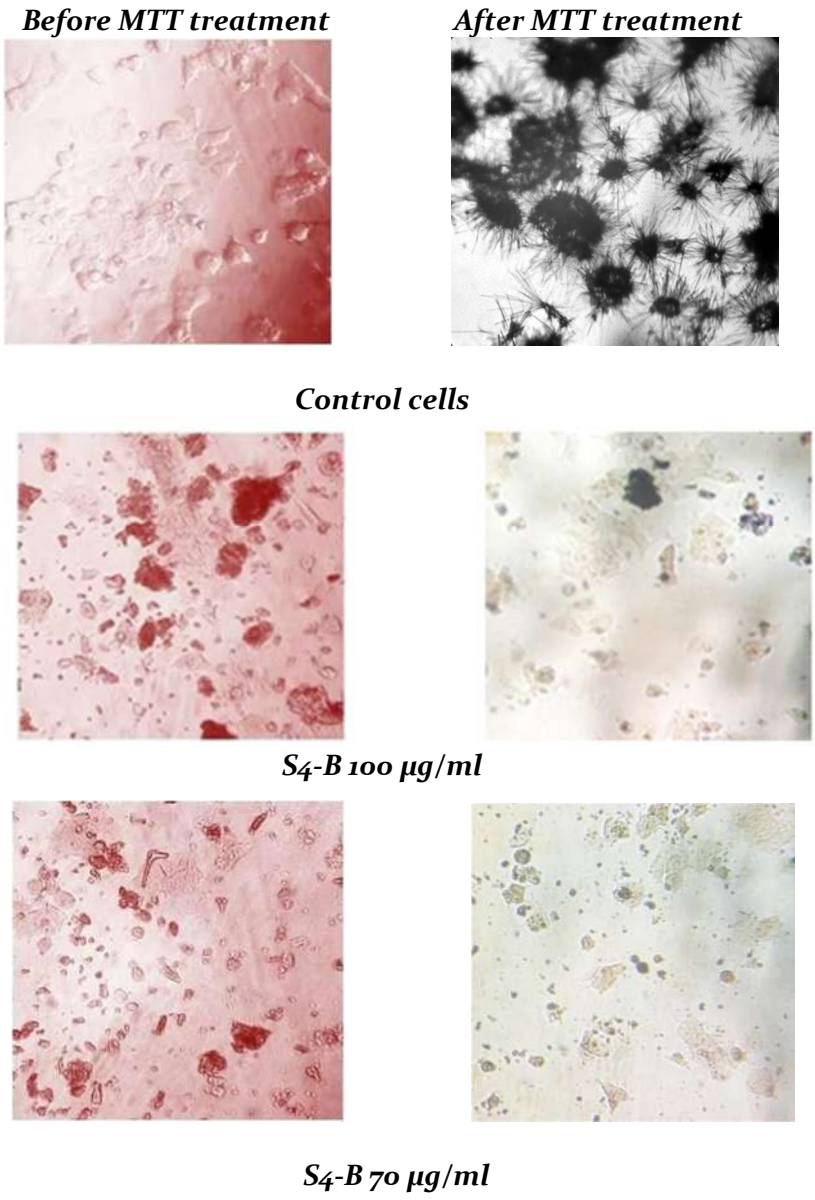


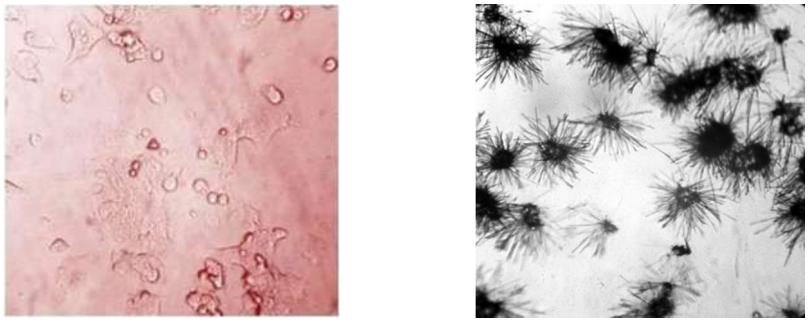
Graph 4: ***OD Value at 570 nm***



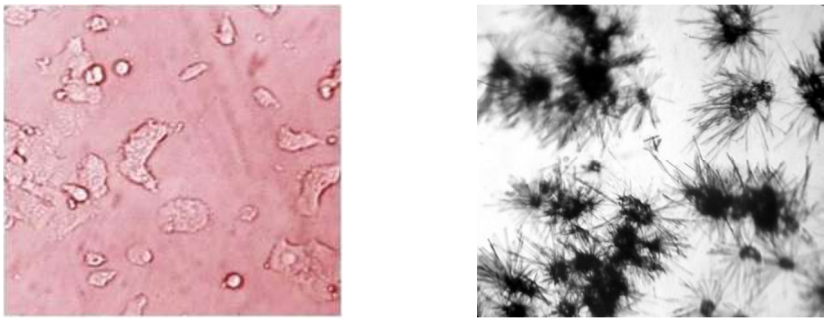
Graph 5: ***Cell Viability (%) of AgNPs synthesized Melia dubia leaf extract (Ethanol)***

Figure 3:
Formation of formazan crystals in control cells and AgNPs treated

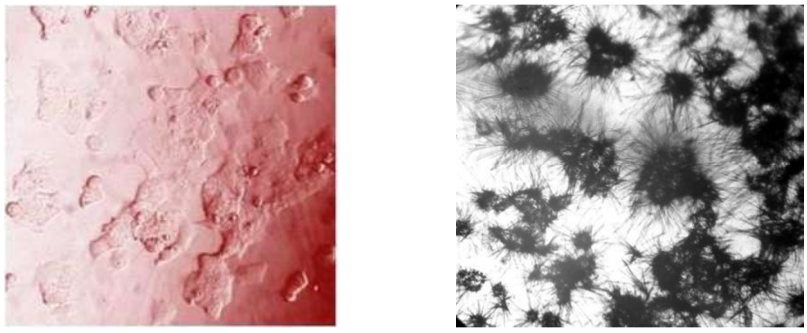




S4-B 50 µg/ml



S4-B 30 µg/ml



S4-B 10 µg/ml

DISCUSSION

Phytochemical screening of Melia dubia

Melia dubia also called as mahaneem or forest neem is an immensely valuable medicinal plant of India, Srilanka, Malaysia, Australia and Angola.[15] The phytochemical screening disclosed that the presence of alkaloids, carbohydrates, proteins, phenolic compounds and flavonoids in all the fractions and the results listed in the table-1. The result of phytochemical screening shows the phytoconstituents present in ethanol extract. The main secondary metabolites identified in the leaf extract of *Melia dubia* were polyphenols such as flavonoids and tannins (hydrolysed and condensed). Flavonoids were well known phytoconstituents that strive a wide range of biological activities including antimicrobial, antiinflammatory, antioxidant, and cancer preventive, antiarthritic and anti-coronary. The phenolic compounds are one of the biggest and most prevasive groups of plant metabolites that hold biological properties such as anti-apoptosis, anti-ageing, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and enhancement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities. With the rise in antibiotic resistance around the world, it remains important to look for new antimicrobial molecules and active extracts containing polyphenols can be of interest in this regard. Saponins were strongly confirmed to be as antimicrobial and in addition, they inhibit moulds thereby protecting the plant from attacks. Glycosides are well-known to reduce the blood pressure.

Synthesis of AgNPs

The first indication of nanoparticles formation is colour change. A clear yellowish brown colour was formed as a result of the reduction of silver ion within 30 min when 1mM AgNO3 was added into the leaf

extract of *Melia dubia*, which specify the synthesis of AgNPs (Figure 1).The colour of synthesised AgNPs clearly changes to reddish brown within 24 h of incubation at room temperature. This change of colour apparently signifies the bioreduction of $AgNO_3$ into AgNPs. The employment of plants as the production assembly of silver nanoparticles has focused huge consideration as a result of its environmentally friendly, rapid, non-pathogenic, economical protocol and providing a single step method for the biosynthetic processes .[8]

UV-Visible Spectroscopy

The absorption spectra of the prepared nanosized silver samples were characterized by UV-Visible spectroscopy. The surface plasmon resonance (SPR) peak of nanoparticles are reported within 400-460nm. Similarly in *Melia dubia,* it was observed that the peak value 2.44 at 430nm and 2.48 at 435nm, which signifies the formation of silver nanoparticles and this wide peak, is due to surface property (SPR) of silver nanoparticles. An absorption peak between 430-435 nm confirms the presence of silver nanoparticles (Table 2).

Fourier Transform Infrared Spectroscopy

FTIR measurement was carried out to identify the possible biomolecules in *Melia dubia* leaf extract responsible for capping leading to efficient stabilization of AgNPs. The IR spectrum of AgNPs manifests prominent absorption bands located at 3473.8 cm−1, 3417.86 cm−1, 1637.56 cm−1, and 659.66 cm−1 (Graph 1). The absorption band at 3473.8 cm−1and 3473.86 cm−1 signifies the presence of medium, N-H stretching primary amine. The band at 1637.56 cm−1 signifies the presence of strong, C=C stretching alkene. The band at 659.66 cm−1 signifies the presence of strong C-I stretching halo compound. The results of the FTIR studies further confirm that the compounds present in the *Melia dubia* leaf extract reduced $AgNO_3$ into AgNPs. (Antibacterial, antioxidant & anticancer activites etc).

Determination of cell viability by MTT assay

The AgNPs synthesized by using *Melia dubia* against induced cell damage in HEP G2 cell line was assessed by estimating the percentage cell viability Tryphan blue dye uptake and measuring viable cells in the medium. The average percentage cell growth inhibition in HEP G2 cell was turned out to be dose dependent in nature. The cytotoxic nature of *Melia dubia* synthesized AgNPs was assessed using MTT assay, the various concentration (20, 40, 60, 80, 100, 120, 140, 160, 180 & 200 µL) of crude *Melia dubia* leaf extract. The OD value for crude *Melia dubia* leaf extract concentration in triplicates at 570 nm ranges from 0.359 nm, 0.402 nm, 0.375 nm at 20 µg/mL, 0.331 nm, 0.333 nm, 0.345 nm at 100 µg/mL and 0.121 nm, 0.179, 0.171nm at 200 µg/mL and the Control mean OD value is 0.392 nm, 0.371 nm and 0.367 nm (& Figure 12). The cell viability for crude *Melia dubia* leaf extract, the mean value 100.57 % was reported at 20 µg/mL and gradually decreased in accordance with the concentration and reached the mean value 89.30% at 100 µg/mL and 41.68% at 200 µg/ml. The IC50 value for crude *Melia dubia* leaf extract is 164.2 µg/mL . Figure 2, shows the formation of formazan crystals in control cells and crude *Melia dubia* leaf extract. The various concentration (10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µL) of AgNPs synthesized *Melia dubia* leaf extract were used. Increasing of AgNPs increased the percentage of cell growth inhibition. The OD value for AgNPs synthesized *Melia dubia* leaf extract concentration in triplicates at 570 nm ranges from 0.375 nm, 0.351 nm, 0.370 nm at 10 µg/mL, 0.269 nm, 0.295 nm, 0.305 nm at 50 µg/mL and 0.091 nm, 0.049, 0.082nm at 100 µg/mL and the Control mean OD value is 0.392 nm, 0.371 nm and 0.367 nm . The cell viability for crude *Melia dubia* leaf extract, the mean value 97.00 % was reported at 10 µg/mL and gradually decreased according to the concentration and reached the mean value 76.91% at 50 µg/mL and 19.64% at 100 µg/mL . The IC50 value for AgNPs synthesized *Melia dubia* leaf extract is 60.70 µg/mL .The half maximal inhibitory concentration (IC50) is a measure of the potency of a substance in inhibiting a specific biological or biochemical function. It is generally used as a measure of antagonist drug potency in pharmacological research. According to the FDA, IC50 represents the concentration of a drug that is necessary for 50% inhibition in vitro. Figure (3), shows the formation of formazan crystals in control cells and AgNPs synthesized *Melia dubia* leaf extract. The anticancer property was effective due to the presence of phytoconstituents present in *Melia dubia* leaf extract. The phytoconstituents like flavonoids and polyphenols acts as an antiproliferative mechanism for cancer cells.[20] Alkaloids aids in the inhibition of cancer cell growth. Terpenoids aids in MCF-7 cell apoptosis.[21]Since natural flavonoids have minimum toxicity associated with them, they are being considered as potential anticancer agents. Our results are data

with previous studies wherein flavonoids have been stated to generate reactive oxygen intermediates in cancer cells. The accumulation of intercellular ROS leads to the disruption of the mitochondrial membrane potential, the release of cyctochrome c into the cytosol with subsequent activation of the caspase cascade, and eventually leading to apoptosis. [22, 21, 23]

CONCLUSION

The choice of the plant in this study was *Melia dubia* (Malaivembu). *Melia dubia* leaves has a countless of benefits. *Melia dubia* leaves rich in flavonoids, polyphenols which acts as excellent antimicrobial agent, a good antioxidant, anticancer agent. The ethanolic extracts of *Melia dubia* leaves were prepared through percolation method. About 50gm of dried fine powder of *Melia dubia* were reflexed successfully with ethanol using percolation apparatus for 40 hours separately. After decanting, ethanol extract of *Melia dubia* given residue was stored in air tight container and were further subjected to qualitative test for identification of various plant constituents. Preliminary phytochemical screening was performed and the bio active compounds were identified. *Melia dubia* leaf extracts showed positive results for Alkaloids, Carbohydrates, Saponins, Phenols, Flavonoids, Tannins, glycosides, and proteins The synthesis of nanoparticles using aqueous extracts of *Melia dubia* leaves, which can be advantageous over other biological synthesis processes which involve eco-friendly and low cost approach with less toxicity. The synthesized silver nanoparticles (AgNPs) of *Melia dubia* leaf extract were characterized by UV-Visible spectrophotometer and Fourier Transform Infrared Spectroscopy (FTIR). Human Liver carcinoma cells (HepG2) were used for this study, Crude extract and silver nanoparticles synthesized from *Melia dubia* leaves were tested for *in vitro* cytotoxicity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. *Melia dubia* crude extract showed positive results for cytotoxicity with an IC50 value of 164.2µg/ml. Silver nanoparticles synthesized from *Melia dubia* leaves showed the significant cytotoxicity with an IC50 value of 60.70µg/ml. Compare than crude extract, silver nanoparticle synthesized from *Melia dubia* seed exhibit significant antitumor activity against Human Liver Carcinoma cells (HepG2). Our study evidenced that *Melia dubia* leaf extract showed an effective therapeutic property against in vitro anticancer activity of liver cancer (HepG2) cell line. Further chacterization will enable us to explore the potential possibility of *Melia dubia* as a successful therapeutic tool to combat hepatocellular carcinoma.

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SP-16

STUDY ON PHYTOCHEMICAL ANALYSIS AND ANTICANCER ACTIVITY (AGAINST COLON CANCER) OF SILVER NANOPARTICLES SYNTHESIZED FROM *Carica papaya* LEAF EXTRACT

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ABSTRACT

Cancer is the second chief reason of death worldwide, with colon cancer as third most common cancer. As bowel dysfunction is a repercussion of chemotherapy, natural therapeutic is needed. . Nano biotechnology is one of the most significant areas of research in biomedical sciences. Nanomaterials are undoubtedly a efficient mode of drug delivery in modern science. *Carica papaya,* usually called as papaya has surfeit of medicinal values including antiplasmodic, antioxidant, anti cancer, anticoagulant etc. Phytochemical components of papaya leaf increases apoptosis and inhibits proliferation of cells. It also acts against enzymes responsible for cancer cell growth especially colon cancer inducing toxins In this evaluation, *Carica papaya* leaf extract was prepared by percolation method and phytochemical analysis was performed. Silver nanoparticles (AgNPs) were biosynthesized from papaya leaf and characterized by UV-visible spectrophotometry and Fourier Transmission Infrared spectroscopy. MTT assay was performedto examine the invitro cytotoxicity of AgNPs using HT-29 cell lines. From the results, we could observe that AgNPs synthesized from papaya leaf showed significant cytotoxicity. Compared with crude extract, AgNPs from leaf extract exhibit significant anti tumor activity against human carcinoma cell line (HT-29 cells). Thus we can draw the conclusion that the biosynthesized AgNPs can be thrived into a promising anticancer agent for colon cancer. This serves as an eco-friendly, cost effective therapeutics with more bioavailability against colon cancer.

Keywords: Colon cancer, silver nanoparticle, *Carica papaya*, Phytochemical analysis.

INTRODUCTION

Cancer is a predominant cause of morbidity and mortality in developing and developed countries[1]. In terms of prevalence, Breast cancer (17.9%), colorectal cancer (11.5%), and Prostate cancer (9.6%) are highly prevalent worldwide. Colon cancer is a class of disease in which a group of cells which depending on their three malignant properties which results an uncontrolled growth, invasion and sometimes metastasis in colon. Reasons for Colon cancer include physical inactivity, a miniscule diet of fruits and vegetables, obesity and smoking. Although Colonoscopy considered as first rate for Colon cancer screening, and it is expensive and it requires skilled examiner and is less convenient to the patient[2]. The treatment for cancers of rectum and colon varies by the location of tumor and stage at diagnosis[3]. Maximum cancer patients have a mixed therapies, including Surgery with chemotherapy or Radiation therapy, Targeted therapy, Hormonal therapy and Stem cell transplant. The major side effects of chemotherapy and radiation therapy are Bowel dysfunction, permanent ostomy and risk of bladder problems. Approximately one-half of patients treated with surgery will result in the reoccurrence of cancer within 3 years[4]. *Carica papaya* familiar as papaya, a perennial herb belongs to *Caricaceae* family. Young *Carica papaya* leaves are naturally loaded with alkaloids (pseudocarpaine, carpaine, dehydrocarpaine 1 and 2), flavonoids (myricetin and kaempferol), cyanogenetic compounds (benzylglucosinolate), and phenolic compounds (caffeic acid, ferulic acid and chlorogenic acid)[5]. Nanobiotchnology is one of the most hopeful areas in modern nanoscience and technology. Applications of AgNPs is broad, such as in drug delivery, food industries, biomedical engineering, agriculture, textile industries, water treatment, as an antioxidant, antimicrobial, anticancer agents[6]. The biosynthesis of nanoparticles can be achieved using enzymes, microbial strains , metabolites, biodegradable products and plant extracts. Biosynthesis of silver nanoparticles using *Carica papaya* leaves increases Bio availability and the synthesize silver nanoparticles found highly cytotoxic against cancer cells.

So for synthesized AgNPs for medical applications, *Carica papaya* plant leaf extracts can be utilized as reducing and stabilizing agent. *Carica papaya* a plant that is known for anticancer therapy. Proliferation is restrained and apoptosis is increased by this plant. DNA Topoisomerase 2 enzyme has an significant role in replication, transcription and DNA recombination processes, and cancer cell proliferation will increase . Methanolic extract of papaya has suppression activity against DNA Topoisomerase 2. Thus by the inhibition of this enzyme activity there is a longer lasting bond between the enzyme and DNA and Protein-Linked DNA Breaks (PDBs) occurred and ended with apoptotic death[7.] Papaya is powerful cancer fighter that is highly efficacious against hormone related to colon cancer as well as other cancer. Papaya can prevent the growth of cancer cell by cease metastasis and normalized cell cycle. Bioactive compounds like Alkaloids, Phenolics, Caratinoids, and Glucosinolate act through various mechanisms such as cancer cell signaling, apoptosis, proliferation, migration, invasion, as well as angiogenesis and carcinogen elimination to exhibit in vitro and in vivo anticancer activities[8,9]. The fiber of papaya binds to cancer causing toxins in the colon thus keeping them away from the healthy colon cells. Hence the current study was aimed at screening for phytochemical constituents and evaluating anticancer activity of AgNPs synthesized from *Carica papaya* leaves on HT-29 colon cancer cell line in comparison with effects of crude extract.

MATERIALS AND METHODS:

Period of study
June 2017 – April 2018.

Sample collection
From the Cumbum Valley, Theni District leaves of *Carica papaya* plant were collected. Collected leaves were cleaned with tap water then rinsed with distilled water, dried, cut into fine pieces and were crushed into fine powder and stored at 37^{o} C.

Percolation method for preparing Carica papaya solvent extract
This is the procedure used mist frequently to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator (a narrow, cone shaped vessel open at both ends) is generally used. The solid ingredients are moistened with an appropriate amount of the stated menstruum and allowed to stand for about 4 hours in a well closed container, after which the mass is packed and the top of the percolator is closed. Additional menstruum is added to form a shallow layer above the mass, and the condition is allowed to macerate in the closed percolator for 24 hours. The outlet of the percolator is then opened and the liquid contained therein is allowed to drip slowly. Additional menstruum is added as required, until the percolate measures about three-quarters of the needed volume of the required finished product. The marc is then pressed and the expelled liquid is added to the percolate. About 50gm of dried fine powder of *Carica papaya* were reflexed successfully with ethanol using percolation apparatus for 40 hours separately. After decanting, ethanol extract of *Carica papaya* given residue stored in air tight container were subjected to qualitative test for identification of various plant constituents.

Screening of phytochemical components
The leaf extracts of *Carica papaya* were analyzed for the presence of carbohydrates, alkaloids, saponins, phenolic compounds, flavonoids, amino acids and proteins on the report of common phytochemical methods such as Mayer‘s test, Wagner‘s test, Molisch‘s test, Xanthoproteic test, Benedict‘s test, Fehling‘s test, Froth test, Foam test, Alkaline reagent test,Ferric chloride test, Lead acetate test, Ninhydrin test.

Detectionof alkaloids
Extracts were dissolved separately in dil. Hydrochloric acid and filtered.

Mayer’s Test
Filtrates were treated with Mayer‘s reagent (Potassium Mercuric Iodide). Yellow colored precipitate formation indicates the presence of alkaloids.

Wagner' Test
Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). The presence of alkaloids is indicated by formation of brown/reddish precipitate.

Detection of carbohydrates
Extracts were dissolved separately in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test
Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

Benedict's Test
Filtrates were treated with Benedict's reagent and heated gently. Presence of reducing sugars is confirmed by formation of orange red precipitate.

Fehling's Test
Filtrates were hydrolyzed with diluted Hydrochloric acid, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate shows the presence of reducing sugars.

Detection of saponins

Froth Test
Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicates the presence of saponins.

Foam Test
0.5gm of extract was shaken with 2ml of distilled water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of phenols

Ferric Chloride Test
Extracts were treated with 3-4 drops of ferric chloride solution. Presence of phenols is indicated by formation of bluish black color

Detection of tannins

Gelatin Test
To the extract, 1% gelatin solution having sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of flavonoids

Alkaline Reagent Test
Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

Lead Acetate Test
Extracts were treated with few drops of lead acetate solution. Presence of flavonoids is confirmed by formation of yellow color precipitate.

Detection of proteins and aminoacids

Xanthoproteic Test
The Extracts were treated with few drops of conc. Nitric acid. Presence of proteins is indicated by formation of yellow color.

Ninhydrin Test
To the extract, 0.25% w/v ninhydrin reagent was added and heated for few minutes. Presence of amino acid is confirmed by formation of blue color .

Detection of glycosides
Extracts were hydrolyzed with diluted Hydrochloric acid, and then tested for glycosides.

Legal's Test
Extracts were treated with Sodium nitropruside in pyridine and sodium hydroxide. Presence of cardiac glycosides is indicated by formation of pink to blood red.

Detection of steroids and triterpenoids

Detection of Steroids
To 1ml of the test solution, equal volume of chloroform and 3 drops of conc.sulphuric acid was added. Formation of brown color ring shows the presence of steroids.

Detection of Triterpenoids
To the test solution, chloroform and concentrated H_2SO_4 was added. Appearance of red color indicates the presence of triterpenoids.

Biosynthesis of silver nanoparticles
10 ml of aqueous extract of *C. papaya* leaves were added to 90 ml of 1mM aqueous $AgNO_3$ solution in 250 ml conical flask and incubated with continuous stirring process at 28^0 C for 24 hours. The bio-reduction of silver nitrate ($AgNO_3$) into silver nanoparticles can be confirmed visually by the change in color from colorless to dark brown. The color change indicated the formation of silver nanoparticles by *Carica papaya* leaf extract. The bio reduction of silver ions in the solution was monitored by sampling the aqueous component after incubation period and the absorption maxima was scanned at different wavelengths (420-500 nm) using a UV-Visible Spectrophotometer.

Charecterization of biosynthesized silver nanoparticls

Uv-visible spectrophotometric analysis of silver nanoparticles
For the spectrometric analysis of AgNPs biosynthesized, an ELICO SL-159 UV- visible spectrophotometer was utilized. The bio-reduction of the Ag+ ions in solution was monitored by periodical sampling of aliquots and the UV-Visible spectra of these aliquots were monitored as a function at time of reaction in 430 & 435 nm range operated at a resolution of 1nm. Distilled water was taken as a blank .A spectrum of silver nanoparticles was plotted with wave length on x-axis and absorbance on y-axis.

Fourier transform infrared (ftir) analysis of silver nanoparticles
FT-IR spectral analysis was done to detect the possible bio molecules responsible for the Ag+ ions reduction and the capping of the AgNPs synthesized by *C. papaya* extract. The chemical composition of Ag nanoparticles was analyzed using FTIR spectrometer after bio reduction with silver nitrate. 2 ml of bio synthesized liquid silver nanoparticles were scanned using infrared in the range of 400-4000 cm^{-1}.

Determination of anticancer activity

MTT assay for cell cytotoxicity

Principle

MTT (3-4, 5 dimethylthiazol-2yl-2, 5-diphenyl tetrazolium bromide) assay, is used to test the cell cytotoxicity. It is based on the ability of a mitochondrial dehydrogenase enzyme of viable cells to cleave the tertrazolium rings of MTT which is pale yellow and form dark blue colored formazan crystals which is largely impermeable to cell membranes. Because of its impermeablity, it accumulates within healthy cells. Crystals which are solubilized are liberated by solubilization of cells by the addition of detergents (DMSO). The number of surviving cells is directly proportional to the level of formazan crystals created. The color can be quantified using a multi-well plate reader.

Materials required

MTT (3-4,5 dimethylthiazol-2yl-2,5-diphenyl tetrazoliumbromide)(5 mg/ml) and DMSO (Dimethyl sulfoxide) were from Sigma, (USA), Fetal Bovine Serum (FBS), DMEM medium and antibiotic solution were from Gibco (USA),1X PBS was from Himedia, (India). wash beaker and 96 well tissue culture plate were from Tarson (India).

procedure

Cell culture

HT-29 cells (Human colon carcinoma cells)cell line were cultured in liquid medium (DMEM) augmented 10% Fetal Bovine Serum (FBS), 100 u/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37^{o}C.

MTT Assay

The Crude extract and Silver nanoparticles of *Carica papaya* Leaves Samples were tested for *invitro* cytotoxicity, using HT - 29 cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay. Briefly, by trypsinization, the cultured cells were harvested pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200 µL) into 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS and treated with various concentrations of the Crude extract and Silver nanoparticles of *Cariaca papaya* Leaves samples in a serum free DMEM medium. Both the Crude extract and Ag nanoparticles of *C. papaya* Leaves samples were replicated three times and the cells were incubated at 37°C in a humidified 5% CO2 incubator for 24 hours. After the incubation period, MTT (20 µL of 5 mg/ml) was added into the well and the cells incubated for another 2-4 hours until purple precipitates were clearly visible under an inverted microscope. Finally, the medium along with MTT (220 µL) were aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, DMSO (100 µL) was added to dissolve formazan crystals and the plate was shaken for 5 min. Using a micro plate reader (Thermo Fisher Scientific, USA), the absorbance for the well was measured at 570 nm and using Graph Pad Prism 6.0 software (USA) the percentage cell viability and IC50 value were calculated .

RESULTS

Table 1: ***Phytochemical screening of Carica papaya leaf extract***

S.No	Phytochemicals	Presence of Phytochemical in ethanolic extract of *Carica papaya*
1.	Alkaloids	+
2.	Carbohydrates	+

3.	Saponins	+
4.	Phenols	+
5.	Tannins	+
6.	Flavonoids	+
7.	Proteins and Aminoacids	+
8.	Glycosides (Cardiac glycosides)	+
9.	Steroids	-
10.	Triterpenoids	-

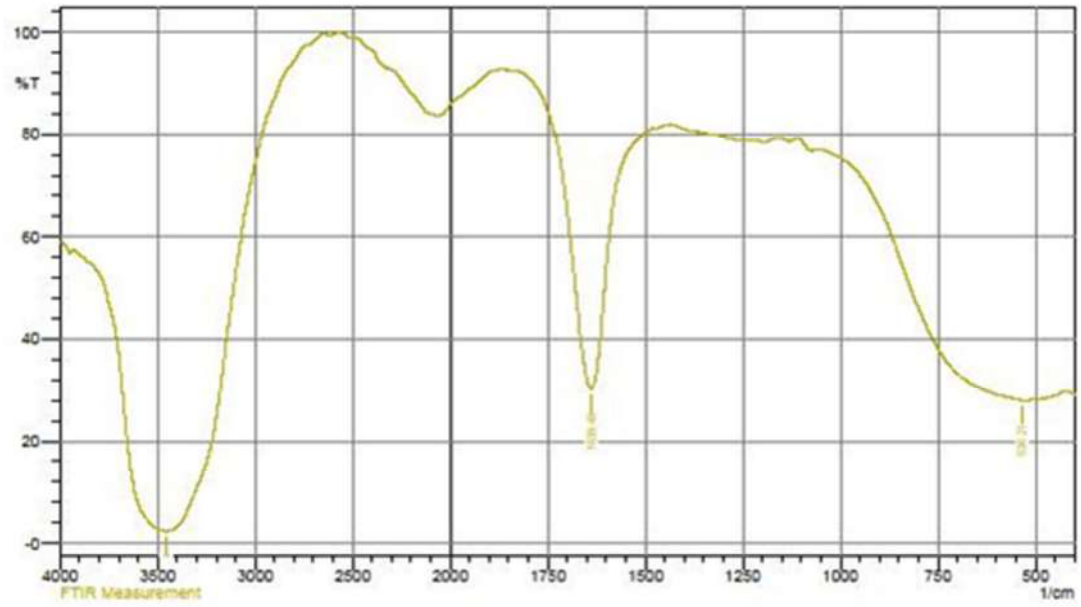
Inference:

+ **Present**

- **Absent**

Figure 1: ***Bio synthesis of Carica papaya silver nanoparticles***

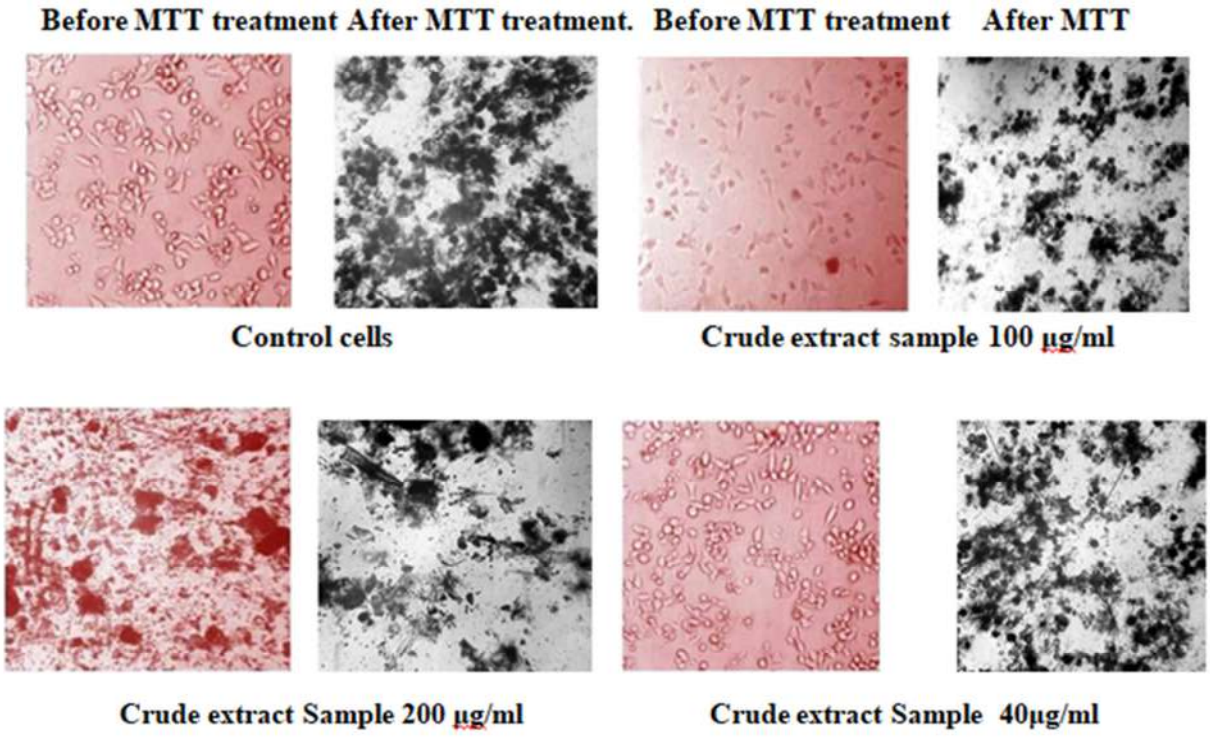
Figure 2: FTIR spectra of Silver nano particles synthesized from *Carica papaya* leaf extract

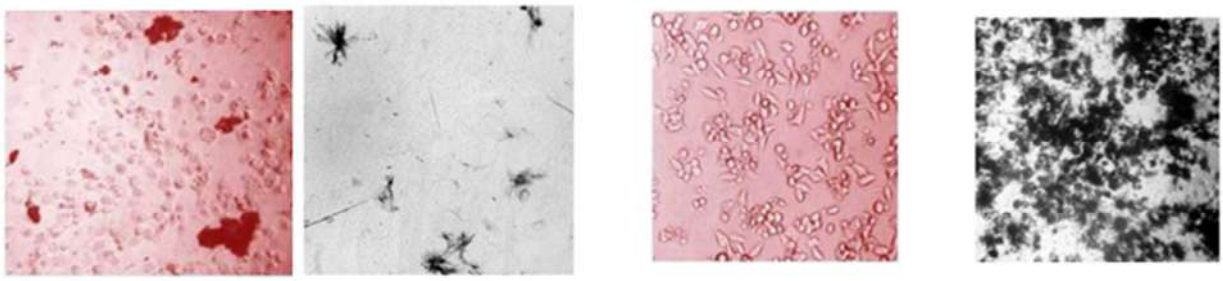


	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	536.21	27.977	0.554	1055.06	530.42	177.257	11.973
2	1639.49	30.274	55.293	1826.59	1498.69	62.067	40.868
3	3456.44	2.356	66.565	3832.56	2650.19	686.641	532.249

Figure 3

Formation of formazan crystals in control cells and Crude extract Sample treated cells

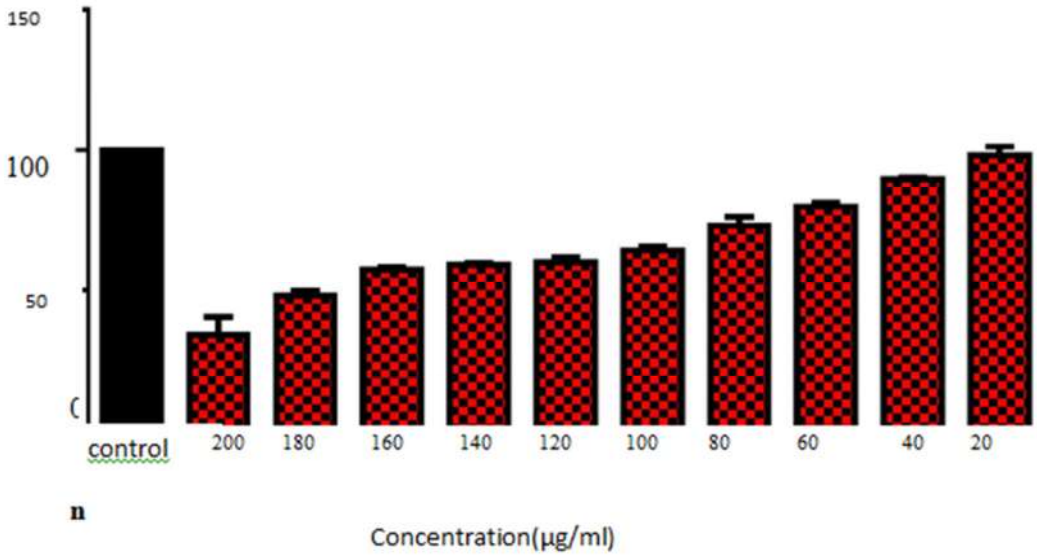


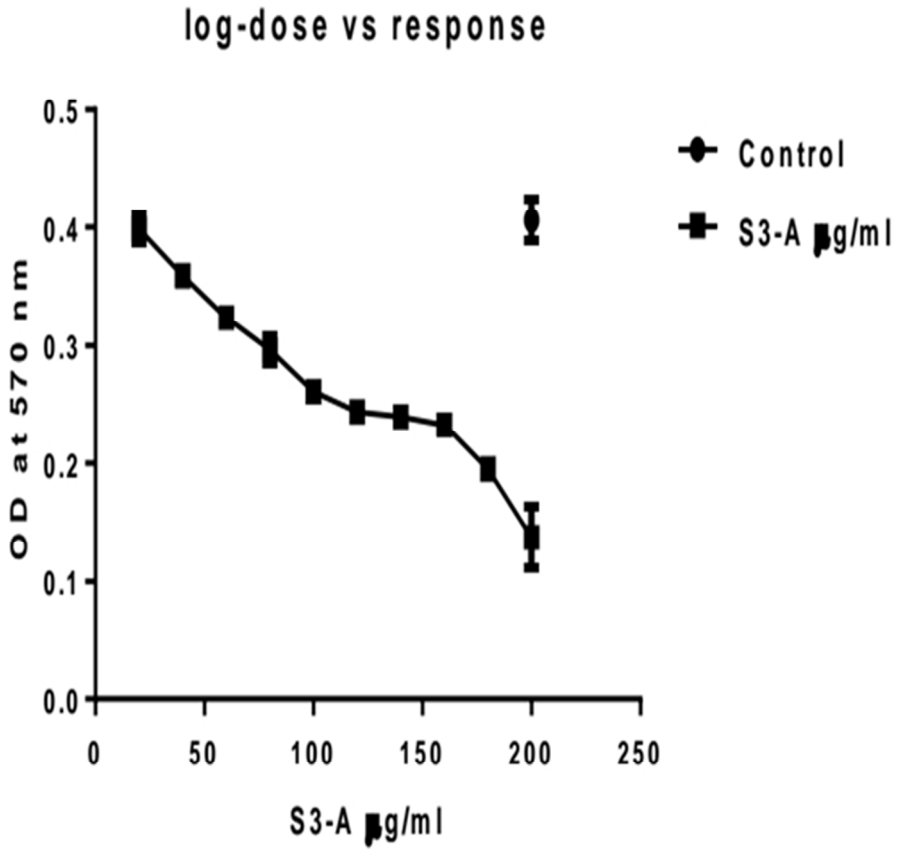


Crude extract Sample 140 µg/ml

Crude extract sample 20 µg/ml

Graph 1: ***Cell viability (%)***



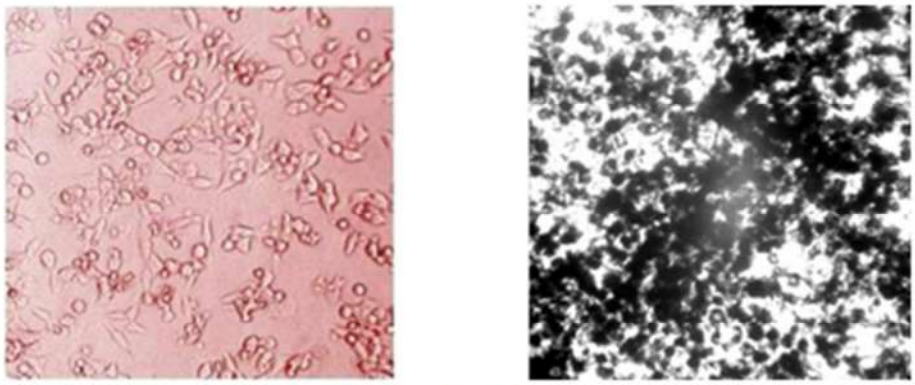


Graph 2: OD Value at 570nm.

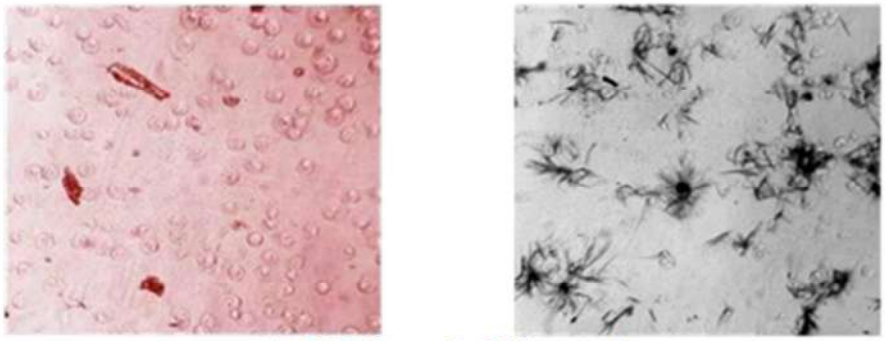
Figure 4

Formation of formazan crystals in control cells and AgNPs Sample treated cells

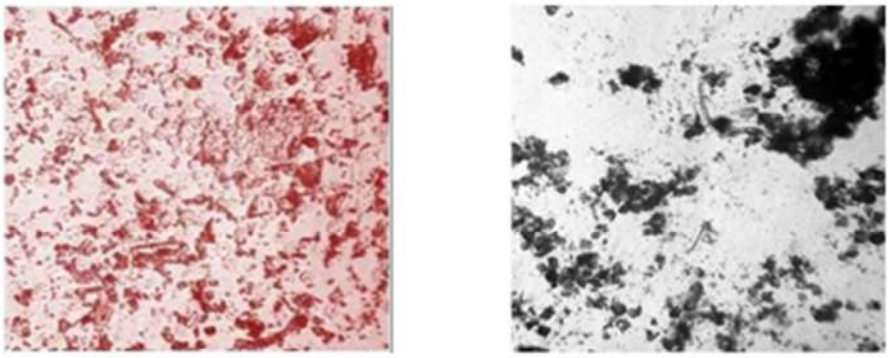
Before MTT treatment ***After MTT treatment***



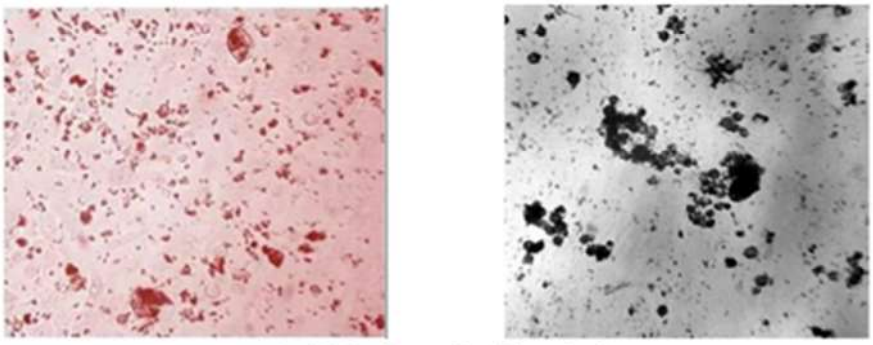
Control cells



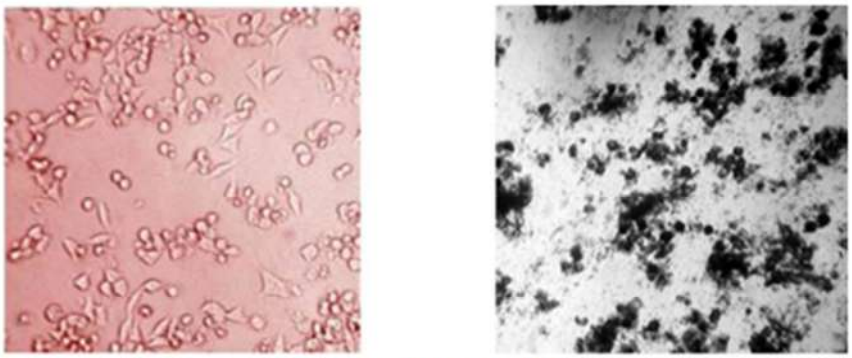
AgNPs Sample 100 µg/ml



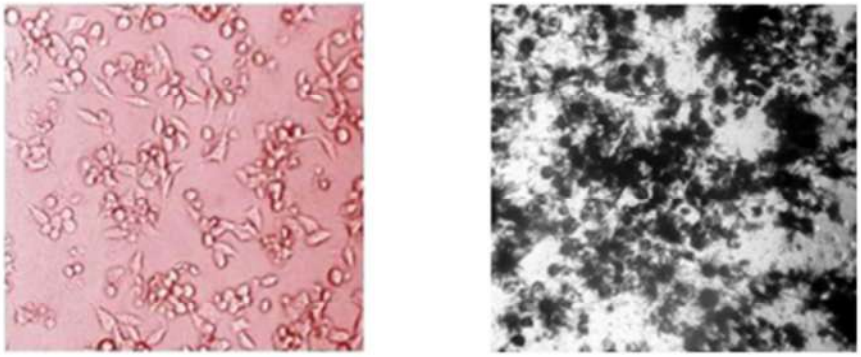
AgNPs Sample 70 µg/ml



AgNPs Sample 50 µg/ml

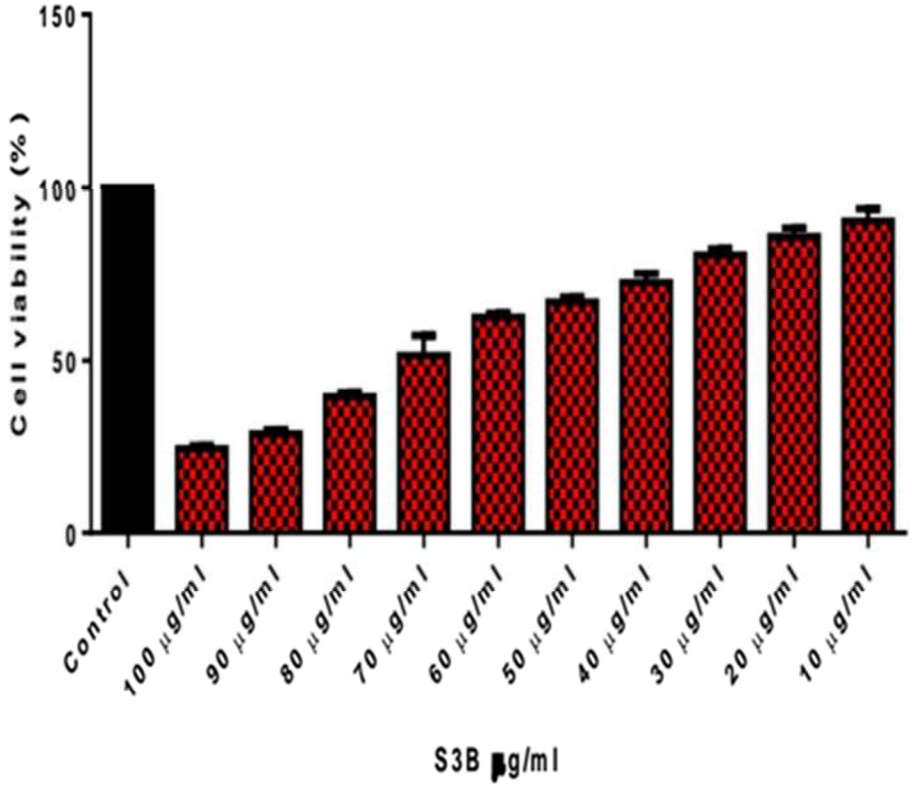


AgNPs Sample 30 µg/ml

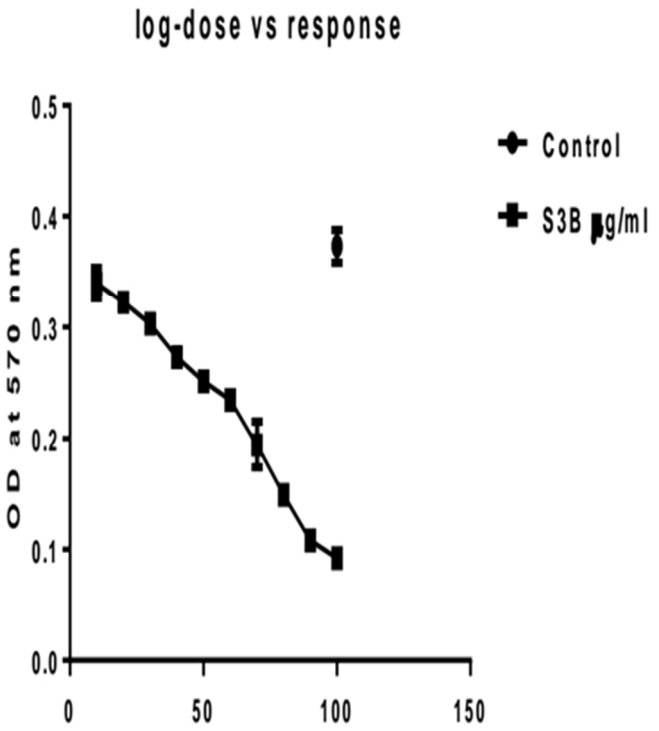


AgNPs Sample 10 µg/ml

Graph 3: ***Cell viability % of AgNPs***



Graph 4: ***OD value at 570nm***



DISCUSSION

Phytochemical examinations of plants are important to study about its effect on human disorders and diseases. Papaya leaf extracts and their bioactive compounds found in them are responsible for antimicrobial, antioxidant, anticancer activity, which have to be screened for their valuable information. These bioactive compounds act as pharmacological agents and are used mainly because these compounds obtained from natural plant sources. Phytochemical screening done in the first phase, revealed the *Carica papaya* leaf extracts showed positive results for metabolites of Alkaloids, Carbohydrates, Phenols, Tannins, Cardiac glycosides, Saponins, Proteins, Flavonoids and Amino acids (Table 1). Nano materials is unquestionably a efficient mode of drug delivery in modern Medicine. The exploitation of microbes, medicinal plant materials and enzymes for the synthesis of nanoparticles has been transmogrified in recent years and could serve as replacement for anticancer agent.[10] In the present study, synthesis of Silver nanoparticles (AgNPs) was performed using *Carica papaya* leaves extract. The advancement of green synthesis nanoparticles than physical method is economical, eco-friendly, and easily scaled up for huge scale synthesis of nanoparticles. Owing to the small size of nanoparticles, they may confer the ability to invade the cells and accomplish the anticancer property. Silver ions are bioreduced into silver nanoparticles with the help of the *Carica papaya* extracts and is followed by color change to dark brown (Figure 1). The reaction mixture turning into dark brown color after silver nitrate solution is added and certain time period of incubation is a clear indication of the formation of silver nanoparticles. The change of colour was seen due to the Surface Plasmon Resonance (SPR). SPR is the resonant oscillation of conduction electrons at the interface between a negative and positive permittivity materials stimulated by incident light. Sometimes the color changed by metallic nanoparticles is due to the coherent excitation of all free electrons which are released by phenolic compounds present in the extract[11]. The characterization of nanoparticles are usually done based on their shape, size, surface area and dispersion. The *Carica papaya* nanoparticles characterized using various techniques such as UV- Visible spectrophotometer, Fourier Transform Infrared (FTIR). UV-Visible spectrophotometer used to confirm the formation, size, stability of silver nanoparticles in the aqueous colloidal solution. The UV-Vis spectrum showed an SPR peak of silver nanoparticles at 430 nm. The size and shape of the silver nanoparticles reflects the absorbance peak. The absorption spectrum produced showed a strong surface plasmon resonance band maximum at 435 nm a characteristic peak of silver nanoparticles. The absorption maxima were obtained around between 405 nm to 450 nm indicates that particles are poly dispersed. FTIR measurements were preformed to identify the bio molecules for capping and effective stabilization of the metal nanoparticles synthesized. The FTIR spectrum of silver nanoparticles synthesized by *carica papaya* showed sharp absorbance between 400 and 4000 cm^{-1}. (Figure 3) The FTIR

spectrum of the *Carica papaya* leaf extracts shows the presence of AgNPs with transmission peaks at 536.21, 1639.49 and 3456.44 cm^{-1} respectively. The absorption peak at 3456.44 cm^{-1}corresponded to OH groups present in alcohols and phenolics; band appearing at 1639.49 cm^{-1} indicates amide bond coming from carbonyl group of a protein, absorption peak at 536.21cm^{-1} appear from C-H stretch and aromatics. The proteins could be most possibly from a coat enveloping the metal nanoparticles to inhibit agglomeration of the nanoparticles and biological stabilizing them in the medium. This shows that the biological molecules such as phenolics, proteins, may perform the role of the formation and stabilization of the silver nanoparticles in aqueous medium. The crude extract and silver nanoparticles synthesized by *carica papaya* leaves were tested for *invitro* cytotoxicity, using HT–29 cells (Human colon carcinoma cells) by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. MTT assay, is based on the ability of a mitochondrial dehydrogenase enzyme of viable cells to cleave the tertrazolium rings of the pale yellow MTT and form dark blue colored formazan crystals which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. Solubilization of cells by the addition of detergents (DMSO) results in the liberation of crystals which are solubilized. The number of surviving cells is directly proportional to the level of formazan product created. The *carica papaya* crude extract decreased cell viability in longer time exposure in a dose dependent manner. Crude extract subjected to cytotoxicity assay exhibited potential cytotoxicity against HT – 29 cell lines with IC50 value of 98.43 µg/ml . From these results, *Carica papaya* crude extract, it was observed that concentration is the minimum lethal dose that kills approximately 50% of HT – 29 cells. During the high dose fixation 200 µg/ml, at this high concentration the viability of cells exhibited as 33.90 %. Silver nanoparticles synthesized from *carica papaya* leaves subjected to cytotoxicity assay exhibited significant cytotoxicity against HT – 29 cell lines with IC50 value of 58.25µg/ml . It was noted that 58.25µg/ml concentration of silver nanoparticles are the minimum lethal dose that kills approximately 50% of HT – 29 cells. The cell viability assessed using different concentration (µg/ml), the maximum concentration (100 µg/ml) exhibited the 24.55% of cells were viable . Due to the small size of nanoparticles, they extend the ability to penetrate the cells and increase the Bio-availability. From this study, Silver nanoparticles synthesized from *Carica papaya* exhibit the significant antitumor activity over crude extract. Formation of ROS was responsible for this cytotoxic effect. Reactive oxygen species (ROS) commonly include the superoxide radical, hydrogen peroxide and the hydroxyl radical, which cause damage to cellular components such as lipids, DNA and proteins and eventually lead to death. Further research need to be emphasized on the extraction of pure components from *Carica papaya* leaves for better therapeutic strategy to combat against colon cancer.

CONCLUSION

The choice of the plant in this study was *Carica papaya* (Papaya). Papaya leaf has a uncountable benefits. *Carica papaya* leaves rich in proteolytic enzymes, phenols and vitamins which serves as excellent antimicrobial agent, a good antioxidant, anticancer agent. By percolation method, the ethanolic extract of *Carica papaya* leaves was prepared. About 50gm of dried fine powder of *Carica papaya* were reflexed successfully with ethanol using percolation apparatus for 40 hours separately. After decanting, ethanol extract of *Carica papaya* given residue stored in air tight container were subjected to qualitative test for identification of various plant constituents. The bio active compounds were identified by preliminary phytochemical screening, and *Carica papaya* leaf extracts showed positive results for Alkaloids, Carbohydrates, Phenols, Tannins, Cardiac glycosides, Saponins, Amino acids, Flavonoids and Proteins. The synthesis of nanoparticles using aqueous extracts of *Carica papaya* leaves, which can be advantageous over other biological synthesis processes which involve eco-friendly and low cost approach with less toxicity. The synthesized silver nanoparticles (AgNPs) of *Carica papaya* were characterized by UV-Visible spectrophotometer and Fourier Transform Infrared (FTIR). Human colon carcinoma cells (HT – 29 cell lines) used for this study, Crude extract and silver nanoparticles synthesized from *Carica papaya* leaves were tested for *in vitro* cytotoxicity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. *Carica papaya* crude extract showed positive results for cytotoxicity with an IC50 value of 98.43µg/ml. Silver nanoparticles synthesized from *Carica papaya* leaves showed the significant cytotoxicity with an IC50 value of 58.25µg/ml. Compare than crude extract, silver nanoparticle synthesized from *Carica papaya* leaves exhibit significant antitumor activity against Human colon carcinoma cell lines (HT-29 cells).Hence, from this evaluation, it is very clear that biological production of nanoparticles will have huge impact in the nanoparticle based medicinal applications. Biosynthesis of the silver nanoparticles lead to

produce novel and cost effective nano based anticancer agents. The results of this study, implies that the biosynthesized silver nanoparticles from *Carica papaya* leaves can be developed in to a promising drug candidate for biomedical applications. Further research into its bioactivity has to be explored and the use of silver nanoparticles might be the future thrust in the area of medicine due to the ease carrier in drug delivery system with minimized toxicity and promising biocompatibility.

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SP-17

DYE DEGRADATION THROUGH *INVITRO* SCREENING OF PAH BACTERIA

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ABSTRACT

Polycylic Aromatic Hydrocarbons (PAH) are by-products of the incomplete combustion of organic materials. PAH contains mutagenic agents and hence are considered to be the priority pollutants in the environment. Bioremediation is one of the adoptable and affordable methods for degrading PAH. Microbes such as Bacteria, Yeast, Fungi are capable of degrading PAH. These studies mainly focus on biodegradation of dyes using microbes isolated from the soil sample polluted with PAH. The result was obtained by performing plate assay, tube assay and Microtiter plate assay. Degradation of dyes was performed by TLC. From this study, it is concluded that the potent isolates were identified as *Bacillus sp and Pseudomonas aeruginosa* which are capable of undergoing dye degradation.

Keywords: ***PAH, TLC, Bioremediation, Microtiter plate assay, Tube assay, Plate assay***

INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are a large class of organic compounds containing two or more fused aromatic rings made up of carbon and hydrogen only. They are ubiquitous in nature. Over 100 PAHs have been identified in atmospheric particulate matter and about 200 in tobacco smoke. PAHs have very low water solubility, and are soluble in many organic solvents and highly lipophilic (tending to combine with or dissolve in lipids or fats). Polycyclic Aromatic Hydrocarbons (PAHs) are organic compounds that are mostly colorless, white, or pale yellow solids, Portella *et.al,* (2005)[1]. Some PAHs are manufactured in the industry. It has been proved that PAHs can cause carcinogenic and mutagenic effects and are potent immune-suppressants. Effects have been documented on immune system development, humoral immunity and on host resistance. The major source of PAHs is the incomplete combustion of organic material such as coal, oil and wood and the minor sources of PAH are volcanic eruption. The dye is generally applied in an aqueous solution, and may require a mordant to improve the fastness of the dye on the fibre. Dye has become common industrial environmental pollutants. The best alternative method for the dye treatment is the biological treatment method. Microorganisms that have the ability to decolorize dyes have been reported by several researchers. Many microorganisms are capable of decolorizing the azo dyes including bacteria and fungi. The dye degrading bacterial strains include *Bacillus sp*, *Enterobacter sp*, *Pseudomonas sp*. Abdul and Hussain, (2016)[2].

MATERIALS AND METHODS

Sample Collection

Soil samples were collected from the sites polluted with petroleum near the petrol bunk of Ernakulam district, Cochin. The samples were carefully sealed and stored at room temperature.

Isolation of Bacteria

The isolation of dye degrading bacteria was done by standard serial dilution method. Aliquots (0.1 ml) of 10^{-2}, 10^{-4} and 10^{-6} were spread plated on Nutrient agar medium. Inoculated plates were incubated at 30°C for 24 hours. After the incubation, morphologically distinct bacteria from nutrient agar medium were selected for further analysis.

Screening of Dye Degrading Bacteria

Qualitative Analysis using Congo Red Plate Assay and Tube Assay: Gupta and Suhas, (2009)[3]

The bacterial isolates were spotted on the plate containing nutrient agar containing Congo Red dye. This is done to identify and isolate the dye degrading bacteria. The isolated bacteria that showed degradation which was confirmed by the presence of clear zone around the bacterial colonies in the Congo Red dye was chosen for plate assay. The Congo Red dye was prepared at a concentration of 0.1gm per 100 ml of distilled water. Later the inoculated plate is kept in the incubator for overnight and the result is observed. The same procedure is also performed with Malachite Green dye. The plate in kept in the incubator and the result is observed. The same procedure is again carried out with the consortium (two or more microbial group living symbiotically). The potent isolates were chosen for making consortium and the plate was then inoculated with consortium and incubated at 37^0C for 24 hours and the results were observed.

Quantitative assay using Microtiter Plate Assay: Powers (2015)[4]

The method of **Powers (2015)[4]**, with slightly modified and the following procedure is performed. The isolated bacteria that showed degradation which was confirmed by the presence of clear zone around the bacterial colonies in the Congo red dye and Malachite Green dye were chosen for Plate Assay. As a result of microtiter plate assay, OD values are calculated using photoelectric colorimeter.

$$\% \text{ of decolorization} = [(Ai - Af)/Ai] * 100$$

Whereas, Ai = absorbance of the control

Af = absorbance of the test solution.

Thin Layer Chromatography for Dye Degrading Bacteria Saranraj (2013)[5]

Thin layer chromatography was performed to monitor the degradation of dyes on the precoated TLC silica gel plates. A 10µL of the sample was spotted on the TLC plates using a microsyringe. The solvent system used was methanol. The dye chromatogram was observed in day light and in UV light (254nm).

Identification of Bacteria

The morphological identification of the potent isolates was characterized by several methods such as Colony characterization, Gram staining, Motility and followed by Biochemical Analysis using Bergey's Manual as Reference

Cross Streak Method Wakeham et.al (1980)[6]

Consortium was checked with cross streak method. Nutrient agar plates were prepared and inoculated with a test organism in a single streak in the center of the petridish. After incubation at 37°C the plates were seeded with indicator bacteria by a single streak at a 90° angle to that of test organism. The microbial interactions were analyzed by the observation of the inhibition zone Madigan *et.al.* (1997)[7]. The antagonism was checked for the preparation of microbial consortia capable of efficient dye degradation.

RESULT AND DISCUSSION

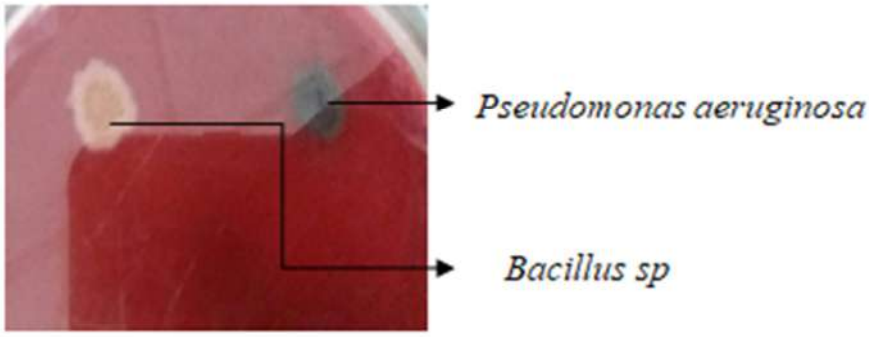
Isolation of Bacteria

Five isolates were obtained, out of those five isolates two organisms were selected and pure culture of *Pseudomonas aeruginosa* and *Bacillus sp* were stored at 4^0C and are subcultured for every 15days to prevent contamination.

Plate assay

Plate assay confirms the degradation of dye by two organisms (*Pseudomonas* sp and *Bacillus* sp), showed strongest degradation. Clear zones were observed. According to the work of Peeters, (2008)[8], Microtitre plates are essential tool for biofilm research since it allows high through put screening of biofilm forming strains or in the assay of anti-biofilm drugs. *Staphylococcus aureus* V329 biofilm was formed on pre-sterilized 96 well flat bottom 8 polystyrene micro-titre plates in triplicates.

Figure 1.*Congo Red Plate Assay*



Clear zones indicate the degradation of dye undergone by *Pseudomonas aeruginosa* and *Bacillus sp.*

Tube assay

Tube assay was conducted with two dyes including Congo Red and Malachite Green. The degree of decolorization of the tested dye was measured at its respective maximum absorbance at 640 nm wavelength using supernatent by using UV - Visible Spectrophotometer.

Figure 2.1. ***Tube Assay performed with Congo Red dye***

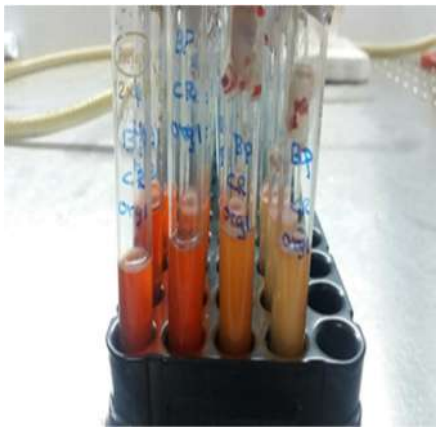


Figure 2.2. ***Tube Assay performed with Malachite Green dye***

Quantitative Assay by Microtiter plate assay

Microtiter plate assay was done as quantitative analysis of dye degradation by 2 potent isolates (*Pseudomonas sp* and *Bacillus sp)* in different dilutions. According to Kuo-Cheng Chan,(2003)[9], Decolorization activity was expressed in terms of percentage decolorization and was determined by observing the decrease in absorbance at 500 nm of respective dyes.

Figure 3. ***Degradation shown for Congo Red dye***

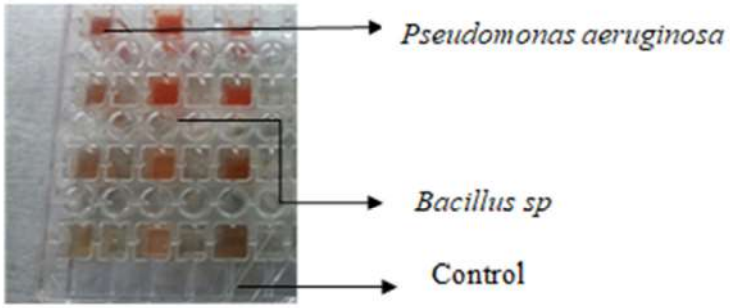
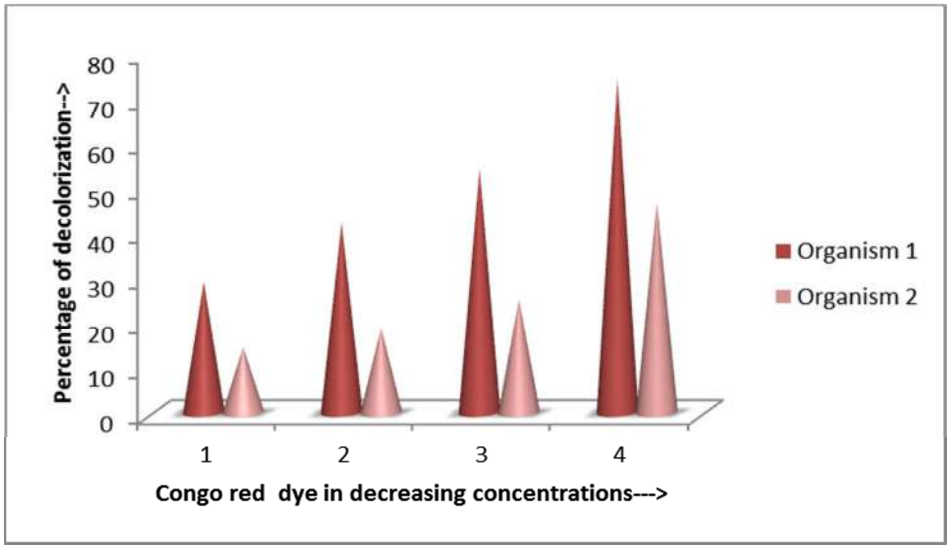


Figure 4. ***Showing the percentage of decolorization of Congo Red by 2 potent organisms in decreasing concentrations (10^{-1} – 10^{-4}).***



Org 1 – Pseudomonas aeruginosa,
Org 2 – Bacillus sp

Pseudomonas aeruginosa (74%) gave high percentage of decolorizaton for Congo Red compared to *Bacillus sp* (46.6%) in higher dilutions. This is because *Pseudomonas sp* has the higher intensity and capability of undergoing dye degradation than the *Bacillus sp.*

Figure 5. ***Degradation shown for Malachite Green dye***

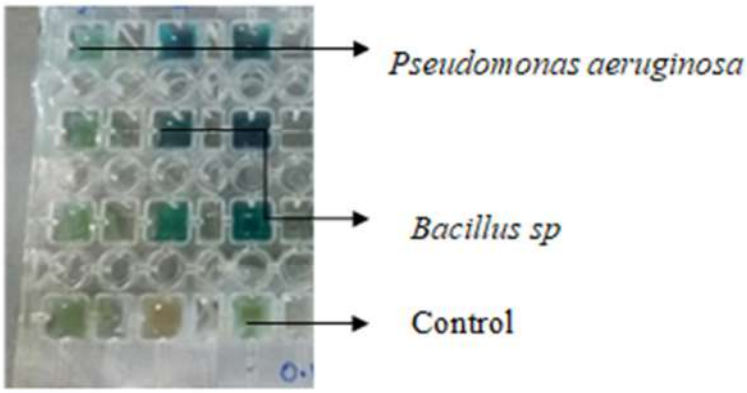
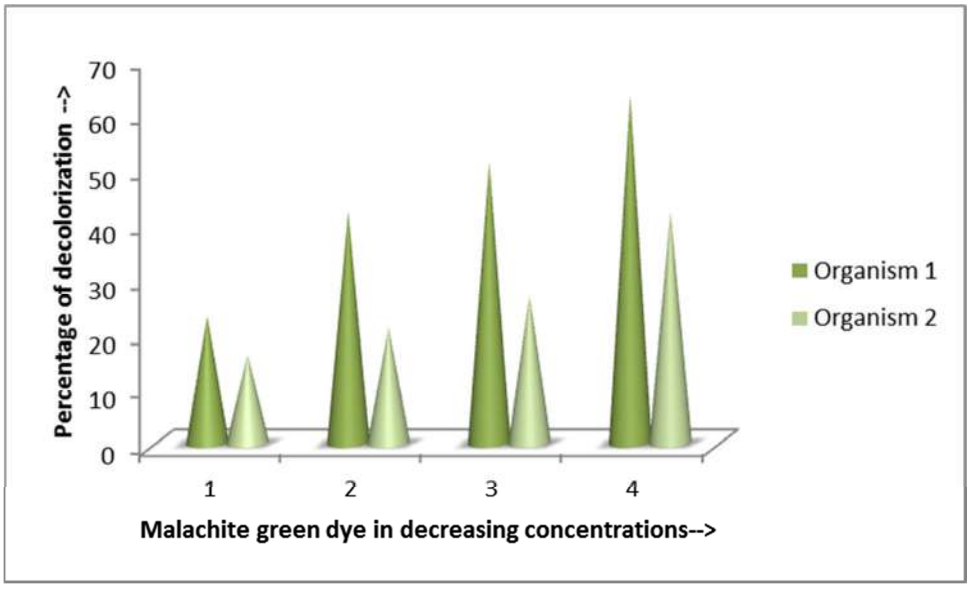


Figure 6. ***Showing the percentage of decolorization of Congo Red by 2 potent organisms in decreasing concentrations (10^{-1} – 10^{-4}).***



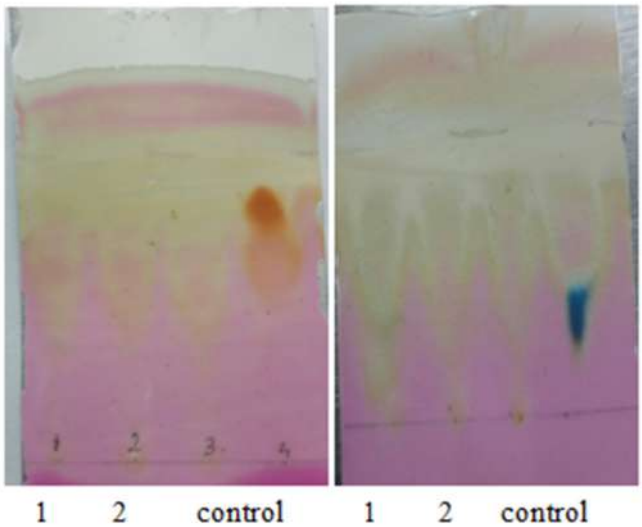
Org 1 – Pseudomonas aeruginosa,
Org 2 – Bacillus sp

Pseudomonas aerugenosis (63 %) gave high percentage of decolorizaton for malachite green compared to *Bacillus sp* (42 %) in higher dilutions. This is because *Pseudomonas sp* has the higher intensity and capability of undergoing dye degradation than the *Bacillus sp.*

Thin Layer Chromatography for Dye Degrading Bacteria

Transformation of dyes was further confirmed by thin layer chromatography. This clearly indicates that decolorization was due to degradation of dyes into intermediate products. The initial step in bacterial degradation of dye is reductive cleavage of N=N (azo) bond leading to formation of colorless aromatic amines. These amines are further oxidized into simpler forms. Nilanjana and Preethy, (2011)[10] conveys the spot observed in the initial dye solution (control of Reactive Black 5 dye) was different from the spot observed in supernatant obtained after decolorization of Reactive Black 5 dye by *Lysinibacillus fusiform.*

Figure 7. ***TLC results showing degradation of dye.***



1. represents the dye degradation shown by *Pseudomonas aerugenosis*
2. represents the dye degradation shown by *Bacillus sp*

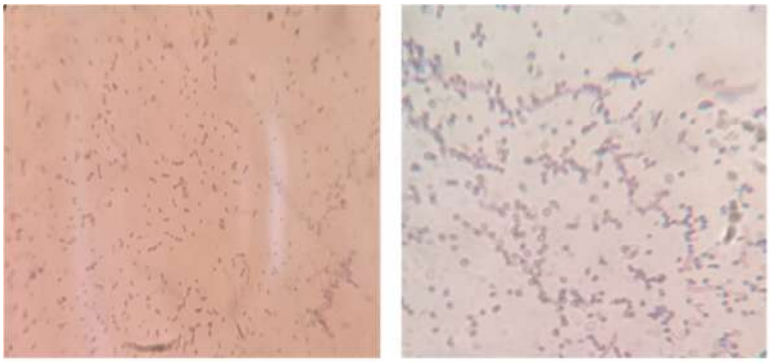
Identification of Bacteria

On nutrient agar plates, pure cultures were obtained. Colony morphological characteristics, Gram staining, motility and Biochemical test are listed in table 1.

Table 1. ***Characterization of the Organisms***

Serial.no	Biochemical Tests	*Pseudomonas aeruginosa*	*Bacillus sp*
1.	Indole test	Negative	Negative
2.	Methyl Red test	Negative	Negative
3.	Voges-Proskeur test	Negative	Negative
4.	Citrate Utilization test	Positive	Positive
5.	Urease test	Positive	Negative
6.	Sugar fermentation test Maltose	Positive	Negative
7.	Lactose	Positive	Negative
8.	Sucrose	Positive	Negative
9.	Glucose	Positive	Negative
10.	Triple Sugar Iron Agar test	Alkaline slant and Alkaline butt	Alkaline butt and AlkalineSlant
11.	Catalase test	Positive	Positive
12.	Oxidase test	Positive	Positive
13.	Gram staining	Gram negative	Gram positive
14.	Motility	Motile	Motile

Figure 8. ***Gram Staining***



According to the morphological characterization, it was found that the organism 1 was *Pseudomonas aeruginosa* and organism 2 was *Bacillus sp*.

CONCLUSION

From the performed biochemical test it is found that the organism 1 is *Pseudomonas aeruginosa* and organism 2 is *Bacillus sp.* was capable of undergoing dye degradation even in higher concentrations. Through this work, it is evident that bacteria can be employed as a vital biological tool for developing

decentralized wastewater treatment systems for decolourization of dye effluents through biodegradation. *Pseudomonas aeruginosa* and *Bacillus sp* are used for degrading dyes such as congo red and malachite green with a large intensity. But *Bacillus sp* fails to show high amount of degradation in crystal violet dye. Therefore for industrial purposes, it is very much important to select a potent isolate that is highly capable of undergoing dye degradation.

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SP-18

EFFECT OF *SARACA INDICA* ON LIPOPOLYSACCHARIDE FROM *ESCHERICHIA COLI* SEROTYPE ($0111:B_{4)}$ INDUCED CYTOKINE RESPONSE TO INFLAMMATORY STIMULI IN HUMAN PBMC AND EFFECT OF TUMOR NECROSIS FACTOR-A IN RODENT

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ABSTRACT

Sepsis remains a common, serious and heterogeneous clinical entity that is difficult to define adequately. Despite its importance as a public health problem, efforts to develop and gain regulatory approval for a specific therapeutic agent for the adjuvant treatment of sepsis have been remarkably unsuccessful one step in the critical pathway for the development of a new agent for adjuvant treatment of sepsis is evaluation in an appropriate animal model of the human condition. Additionally, many species including mice and baboons, are remarkably resistant to the toxic effects of bacterial lipopolysaccharide, whereas humans are exquisitely sensitive. Therefore a definite diagnosis is only possible by histological analysis. The use of tumor necrosis factor, constitute a new, interesting tool for the treatment of sepsis, but larger, adequately designed studies are needed to confirm existing data**.**

KEYWORDS: ***lipopolysaccharides, lipoxygenase, cycloxygenase, endotoxin, tumor necrosis factor.***

INTRODUCTION

The word inflammation is derived from the Latin "inflammare" (to burn). It is one of the most important processes involved in the defense of an organism against local injury and infections. However, it often progresses to painful or chronically harmful diseases requiring pharmacological treatment (Vane *et al*., 1994; Gao *et al*., 1996). Typical inflammatory disease such as psoriasis, rheumatoid arthritis and colitis are among the leading causes of death and disability in the world (Amenta *et al.* 2000). The normal immune response contains a series of feedback loops intended to restore homeostasis in response to an infection or injury. Raised levels of anti-inflammatory cytokines, persistent inflammation, and immunosuppression can lead to sepsis and possibly multiorgan dysfunction if the body is unable to return to homeostasis (Lord,*et al.,* 2014) Sepsis is a devastating condition characterized by the systemic activation of inflammatory and coagulation pathways in response to microbial infection of normally sterile parts of the body. Severe sepsis, defined as sepsis with at least one dysfunctional organ, is the leading cause of death in non-coronary intensive care units and is associated with mortality rates of 30-50%. The development of experimental sepsis models to elucidate the progression and pathophysiology of clinical sepsis spans the past eight decades. Studies utilizing models of intra-abdominal sepsis began in the 1930's with the isolation of endotoxin and the intravenous or peritoneal infusion of live organisms, a model which dominated sepsis research for over 30 years. In the 1960's, a transition was made from endotoxemia models to a focus on bacteremia. Such models include the injection of live bacteria (Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus), inoculation of feces, and intramuscular, intraperitoneal and subdermal implantation of feces-containing capsules and sponges. Following the use of endotoxemia and bacteremia models, various models involving ischemia and bowel perforation were developed. These models led to the development of the most frequently used sepsis model today Limitations in both the use of animal models and design of experimental studies contribute to the poor translation of preclinical animal studies to clinical sepsis. Some limitations are inherent to the use of animals to model any clinical condition. It is commonly the case that young animals of a specific gender, species, genetic background, and nutritional status housed in a pathogen-free, sterile facility unexposed to the natural environment are used (5). Many of these elements are tightly controlled to maintain consistency at the expense of clinical relevance. However, an attempt at balancing both consistency and clinical relevance can be made if the investigator designs treatment groups for different animals (e.g. separate treatment groups for females and males, groups for

young and aged mice, etc.) given the heterogeneity of the sepsis patient population. Other limitations may be appropriately addressed by establishing clear research questions and implementing an experimental protocol which would adequately investigate these questions. For instance, if the objective of an animal study is to test the clinical applicability of a therapeutic agent, it would be more clinically relevant if therapies currently used for the management of sepsis including the adequate administration of resuscitation fluids, antibiotics, and supportive therapies (14) were incorporated into the experimental protocol of the animal study. Additionally, the experimental protocol of animal studies can be modified to include clinically relevant management procedures such as constant monitoring and assessment for hemodynamic parameters, tissue perfusion, or dehydration as would occur in clinical sepsis. Other factors which may contribute to the gap between findings in experimental and clinical studies include the time at which therapeutic agents are administered, the lack of staging of sepsis to reflect disease progression at different severities on the sepsis continuum or different patient populations, and the risk of experimenter bias in animal studies . Traditional medicine is as old as human kind and is practiced by virtually all cultures, each one with its own indigenous knowledge, health practices and benefits. Scientific and non-scientific knowledge generally has been transmitted by oral tradition from generation to generation since antiquity until it finally became a significant part of the foundation of today's school of medicine. However, Knowledge about the medical benefits of plants still persisted in the population. The inflammatory response is a dynamic continuum, initiated by the host in response to tissue injury or infection, induces the arachidonic acid. The most abundant polyunsaturated fatty acid in the phospholipid bilayer of cell membranes which may be metabolized by two different pathways to form eicosanoid (Wolfe, 1982). The cyclooxygenase pathway generates prostaglandins and thromboxanes. The second pathway leads to formation of leukotrienes by 5-lipoxygenase enzyme. The potential of blood to release eicosanoids e.g. COX, LOX and cytokines TNF-α, in response to an inflammatory stimulus, e.g. LPS and calcium ionophore, has proven as a useful measure to evaluate immune status of patients with psoriasis, IBD, rheumatoid arthritis (Zangerle *et al*., 1992) and sepsis (Ertel *et al*., 1993). There is much experimental evidence suggesting that metabolites of arachidonic acid metabolism, whether produced via the lipoxygenase or the cyclooxygenase pathway, are important mediators in the pathogenesis of inflammatory and/or allergic diseases Samuelsson (1983). Shirumalla *et al*., (2006) reported eicosanoids and cytokines are key mediators of the immune response with central roles in the development, differentiation and regulation of immune cells. Change in inflammatory mediator response results predictive clinical outcome in selected disease states (Mira *et al.,* 1999). Therefore, quantification of cytokine production can provide a valuable biomarker tool for the identification of various diseases and for monitoring response to treatment. Accurate and reproducible methods for measuring eicosanoids and cytokines, such as PGE_2, LTB_4 and TNF-α, are critical for the application as clinical biomarkers for diseases. Kirchner *et al*., (1980) first described *ex vivo* stimulation of cytokine release in whole blood in the early 1980s. Whole blood (WB) *ex vivo* stimulation is a useful tool to investigate eicosanoids and cytokine response to a variety of stimuli, including bacterial endotoxin (LPS), antigen, allergen and antibiotics. Basic principle of the e*x vivo* assays from patient blood, have proven to be a powerful tool for assessing cellular responses which determine the effects that potential inhibitors (e.g., pharmacological agents) may have on inflammatory processes. The culture of WB approximates the state of circulating cells *in vivo* and contains physiological concentration of factors that influence immune cell function more closely than does the culture of peripheral blood mononuclear cells (PBMC). WB culture may be the most appropriate milieu in which to study *ex vivo* cell activation and cytokine production *in vitro* by a simple procedure which eicosanoids such as prostaglandin E_2 (PGE_2), leukotriene B_4 (LTB_4), thromboxane B_2 (TXB_2) and Tumor necrosis factor-alpha (TNF-α) can be measured using commercial enzyme immunoassays (EIAs) in the supernatant of whole blood stimulated with inflammatory stimuli. Recent research has focused on finding anti-inflammatory agents with selective pharmacology and less toxicity. The current work aims at demonstrating the anti-inflammatory property. Based on the earlier reports and was taken to evaluate the preliminary inflammatory response. The whole blood assay was used to evaluate the involvement of eicosanoids and cytokine pathway, which is responsible for inflammation.

METHODOLOGY

Chemicals

All solvents and chemicals employed were of analytical grade

Healthy Volunteers

Normal patients were selected for this study from Best Diagno lab Chennai. Each patient consented to the use of residual blood from a routine laboratory draw to measure PGE_2, and TNF-α assay.

Reagents, Chemicals and EIA kits

Saraca indica L, leaves, distilled water, Whatman filter paper, dimethyl sulfoxide (DMSO) (Lancaster, Cat. No: 4699), lipopolysaccharide (*Escherichiacoli* serotype (0111:B_4) sigma, cat. no: L3012, Phosphate Buffer Saline (PBS) (Sigma, Cat. No: P7059), CO_2 incubator (Heraus), Micropipettes (Eppendorf), Centrifuge (eppendorf Model: 5810R), Micro centrifuge tubes (Axygen), Deep freezer -80^oC (Thermo Forma), ELISA reader (Molecular Device), RPMI-1640 from Sigma Aldrich, CAT. No. R4130, Prostaglandin E_2 enzyme immunoassay EIA Cat No: 514010, and TNF-α (human) EIA Kit from cayman chemicals, CAT. No.589201, Heparin (Heparin sodium injection I.P) 5000 I.U. /ml, 96-well micro titer plate from Greiner bio-one, CAT. No.655 180, rofecoxib (unichem Pharma (Mumbai), Rolipam (Cat No. 0905) TOCRIS bioscience.

Preparation of Plant extract

The leaves materials were washed, air-dried at room temperature (26°C) for 2 weeks, after which it was ground to a uniform powder. The dry powder was extracted by reflexed in 100 mL aqueous solution for 24 h, using a Soxhlet apparatus (Khan *et al*., 1988). The extract was filtered using Whatman filter paper, No. 1. The filtrate was then evaporated using rotatory evaporator (Super fit-ROTAVAP, India). And dried at 37°C. Dried extract was stored at 20°C in labeled, sterile capped bottles.

Phytochemical Tests

Test for carbohydrates

To 2ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish color indicates the presence of carbohydrates.

Test for tannins

To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for saponins

To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.

Test for flavonoids

To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

Test for alkaloids

To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

Test for quinones

To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones.

Test for glycosides

To 2ml of plant extract, 3ml of choloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

Test for cardiac glycosides

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

Test for terpenoids
To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown color at the interface indicates presence of terpenoids.

Test for triterpenoids
To 1.5ml of extract, 1ml of Libemann–Buchard Reagent (acetic anhydride + concentrated sulphuric acid) was added. Formation of blue green color indicates presence of triterpenoids.

Test for phenols
To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green color indicates presence of phenols.

Test for coumarins
To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow color indicates presence of coumarins.

Steroids and phytosteroids
To 1ml of plant extract equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids.

Phlobatannins
To 1ml of plant extract few drops of 2% HCL was added appearance of red color precipitate indicates the presence of phlobatannins.

Anthraquinones
To 1ml of plant extract few drops of 10% ammonia solution was added, appearance pink color precipitate indicates the presence of anthraquinones.

Drug Preparation
Stock solutions of 100mM of the*Saraca indica L,* were prepared in 100 % DMSO. The stock solution was serially diluted thereafter with DMSO to get < 1 % concentration in drugs (0.1, 0.3, 1, 3, 10, 30 and 100 µM) stock solutions. It was then labeled properly and the preparation was stored in cool dry place.

Analysis of Human PBMC -PGE_2 Assay
Stimulation and Incubation of Human PBMC
Blood was drawn from healthy volunteers into heparin coated vacutainer tubes. 5ml of heparinized blood was added to 20ml of RPMI 1640 (supplemented with 100 units/ml penicillin, 100µg/ml streptomycin and 40µg/ml acetylsalicylic acid). According to Syahida *et al*. (2006) procedure RPMI 1640 diluted blood was transferred into 96 well plates, 170µl/well was added, and then 20µl of different drug concentrations of drug solutions each were transferred to the respective wells and one well without drug served as control. The plates were incubated at 37^{o}C in a thermo mixer at 400 rpm for 60 minutes. After an hour of incubation, 10µl of the LPS (from *E.coli* B_4) was added to each well to obtain a final concentration of 1µg/well. The plates were incubated at 37^{o}C in the thermo mixer at 400 rpm for 120 minutes and then kept at 37^{o}C in a CO_2 incubator for two more hours (total stimulation time is four hours). Finally, the supernatant was collected carefully without disturbing the pellet at the bottom and then stored at -20^{o}C for further analysis by PGE_2 assay.

Reagent Preparation for EIA

EIA Buffer
This vial contains 10 ml of concentrated EIA Buffer 1M phosphate, containing 1% BSA, 4 M NaCl, 10 mM EDTA and 0.1% sodium azide.

Wash Buffer

This vial contains concentrated wash buffer (4M phosphate, pH.7.4). To prepare for use, dilute 5 ml vial wash buffer provided in the kit was diluted to 2 L of ultrapure water and 1 ml of Tween 20.

Tracer

The provided dye should be added to Cayman's AChE tracers. Add the dye to the reconstituted tracer at a final dilution of 1:100 (add 60 µl of dye to 6 ml tracer).

Antibody

PGE_2

The Vial contains 100dtn peptide affinity–purified IgG rabbit containing 50% glycerol, 0.5mg/ml BSA, and 0.02% sodium azide TNF-α This vial contains lyophilized TNF-α (human) Acetyl cholinesterase (AChE)-FAb' EIA Conjugate (a covalent conjugate of monoclonal anti-TNF- αFAb' and electric eel AChE.Reconstitute 100 dtn vials with 10 ml EIA Buffer

Ellman's Reagent

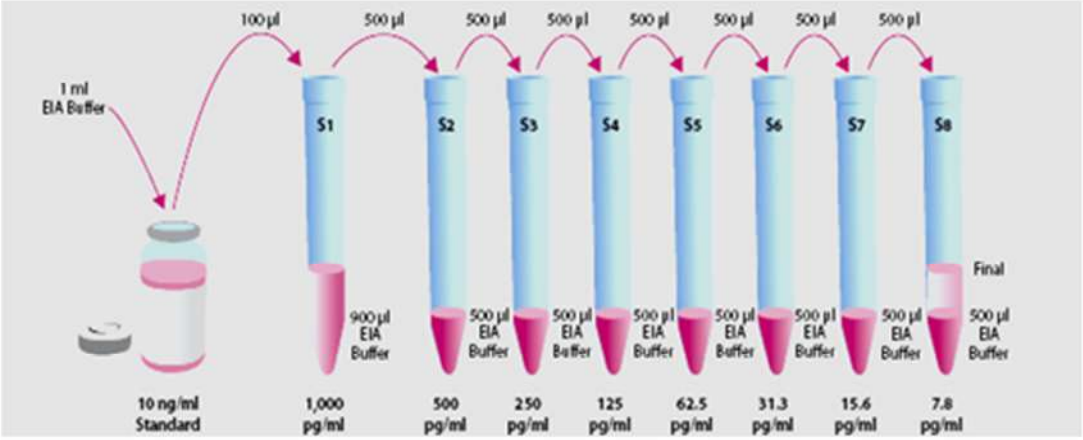
This reagent has been tested and formulated to work exclusively with these kits for development of plates. The vial contain 100 dtn provided in the kit was reconstituted with 20ml of ultra pure water.

Effect of Saraca indicaon PGE_2 byEIA Method (Using Cayman kit) (Cat No: 514010)

All buffer preparation as well sample preparation was followed as per the kit instructions.

Standard

Prepared as described in the figure below.



Procedure

Steps	Reagent	Blank	TA	NSB	B_0	Std/Sample
1. Add reagents	EIA	-	-	**100µl**	**50µl**	-
	Std/sample	-	-	-	-	**50µl**
	Tracer	-	-	**50µl**	**50µl**	**50µl**
	Antibody	-	-		**50µl**	**50µl**
2. Incubation	Cover plate and incubate overnight at 4°C					
3. Wash	Wash all wells five times					
4. Add Reagents	-	-	**5µl**	-	-	-
	200µl	**200µl**	**200µl**	**200µl**	**200µl**	**200µl**
5. Incubation	Cover plate and incubate 60-90 minutes RT with gentle shaking					
6. Read	Read plate at a wavelength between 405-420 nm					

The microplate was set up with sufficient wells for running of all blanks, standards and samples in duplicate. EIA assay buffer (100µl) was transferred into the non-specific binding (NSB) wells and 50µl of EIA assay buffer was transferred into the zero standard (B_0) wells.50µl of sample was transferred into the appropriate

wells. Diluted antibody of 50µl was also transferred into all wells except the blank (B) and NSB. The plate was covered with the lid provided and was incubated at 4^0C overnight. The wells were aspirated and washed 5 times with wash buffer ensuring that all wells are completely filled and emptied at each wash. 200µl of Ellman's reagent was added to each well and 5µl tracer added to TA wells. The plate was covered with plastic film. The plates were kept for optimum development by using an orbital shaker for 60-90 minutes. Readings were taken at 405-420nm.

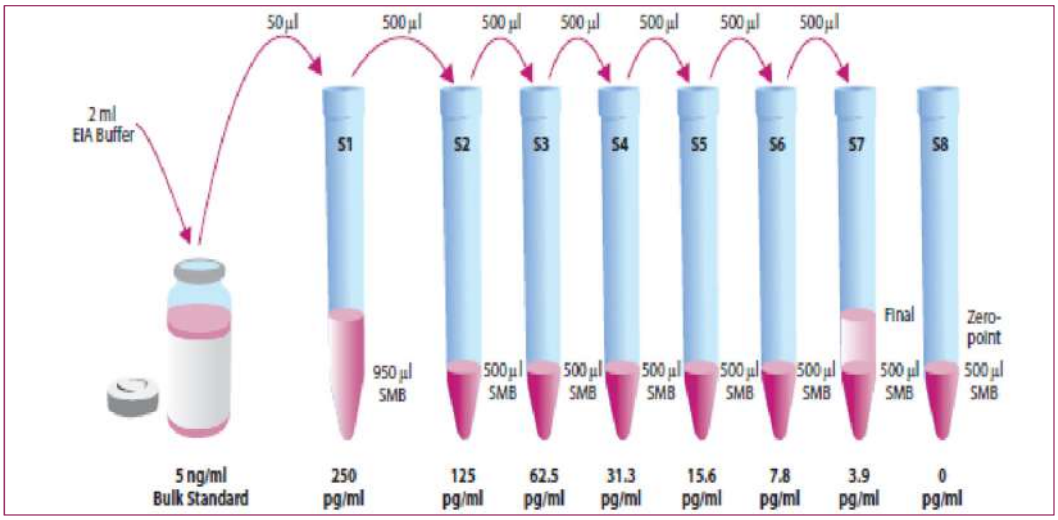
Analysis of Human PBMC in TNF-α Assay

Stimulation and Incubation of Human PBMC Assay

The assay procedure remains same as mentioned in PGE_2; the supernatant was collected carefully and stored at -20°C for TNF-α assay.

Standard

Prepared as described in the figure below



The micro plate was set up with sufficient wells for running of all blanks, standards and samples in duplicate. 100µl standard sample were added in each respective well. The TNF-α Fab conjugate 100µl were added to all well except blank. The plate was covered with plastic film and incubated overnight at 4^0C. After incubation the plate was washed and 200µl of Ellman's reagent was added to each well and kept in orbital shaker for 90 minutes. The plate was read at a wavelength between 405-420 nm**.**

Procedure

Steps	Reagent	Blank	Std/Sample
1. Add reagents	Std/sample	-	100µl
	Conjugate	-	100µl
2. Incubation	Cover plate and incubate overnight at 4°C		
3. Wash	Wash all wells five times		
4. Add Reagents	Ellman's	200µl	200µl
5. Incubation	Cover plate and incubate 30-90 minutes RT with gentle shaking		
6. Read	Read plate at a wavelength between 405-420 nm		

Formulations of Saraca indica Leaves

Oral dose formulation was made dried leaves and mixed with aqueous suspension containing 2% polysorbate 80 and 0.5% carboxymethylcellulose. The dose volume for ***Saraca indica*** was 2ml/kg in rats for

Intra peritoneal administration. LPS (Lipopolysaccharide) was formulated using PBS and stored at 4°C. All doses of LPS and ***Saraca indica*** were freshly prepared on the day of the study.

Experimental Design (Dose Response Activity of Saraca indica In LPS Induced SepsisModel In Rat)
LPS-Induced TNF-α Production in Rats
Female wistar rats (7 weeks old) were fasted overnight and intraperitoneally injected with lipopolysaccharide (LPS, *Escherichia coli* serotype 055:B5, Sigma-aldrich) dissolved in saline (300µg/kg). ***Saraca indica*** (350 and 500 mg/kg) and Dexamethasone (1mg/kg) was administered orally 60 min prior to LPS injection. Serum was collected 1 h after LPS injection. The amounts of TNF-α in serum were measured by Enzyme Linked Immunosorbent Assay (ELISA).

Tumour necrosis factor alpha TNFα rat, Biotrak ELISA system (RPN2744)
Materials Provided
Anti-Rat TNF-α precoated 96 well strip plate, Pretreatment buffer (1 x phosphate buffer saline), Lyophilized recombinant mouse TNF-α standards, 2 vials, Biotinylated antibody reagent, 1 vial, 8 ml, containing 0.1% sodium azide. Standard diluent 1 vial, 20 ml, wash buffer, 1 vial, 50 ml Streptavidin-Horse Reddish Peroxidase (HRP) concentrate, 1 vial, 12ml, stop solution, 1 vial, 13-15 ml, <1% sulfuric acid and adhesive plate covers.

Sample Dilution
Collected serum is diluted with standard diluent (1:10).

Wash Buffer
The entire contents of the 30 x wash buffer bottle were diluted to a final volume of 1.5 liters with distilled water.

Preparation of standard curve
Six tubes were labeled, one for each standard curve point: 2500 pg/ml, 833 pg/ml, 278 pg/ml, 93 pg/ml, 31 pg/ml, and 0 pg/ml. Initially 1:6 dilution followed by 1:3 serial dilutions for the standard curve were prepared as follows: 600µl of appropriate diluents was pipetted into each tube.120µl of the reconstituted standard was transferred into the first tube (i.e., 2500 pg/ml) and mixed. 300µl of this dilution was transferred into the second tube (i.e.,833 pg/ml). The serial dilutions (using 300µl) three more times to complete the standard curve points.

Procedure
a. Pretreatment buffer 50µl was added followed by 50µl-diluted samples and standards were added to each wells and incubated for 1 hour.
b. Plates were washed for 3 times using the wash buffer
c. Biotinylated antibody reagents 50µl were added to all wells, in duplicate.
d. Plates were incubated for another 1 hour at room temperature.
e. The aspiration/wash step was repeated as in step b.
f. Streptavidin-HRP 100µl conjugate were added to each well and incubated for 30 minutes at room temperature.
g. The aspiration/wash step was repeated as in step b.
h. Tetramethylbenzidine (TMB) 100µl substrate solution were added to each well and incubated for 10 minutes at room temperature in the dark.
i. Stop solution 100µl were added to each well.
j. Optical density of each well was noted using a spectrophotometer set to 450 nm.

DATA ANALYSIS

ED_{50} &IC_{50}determination of the log dose of *Saraca indica* / standard drug was plotted vs. the percentage of inhibition and regression analysis of log dose–response curve was used to calculate ED_{50} &IC_{50} by using Graph Pad Prism 3.0 (Graph Pad Software).

RESULTS AND DISCUSSION

TESTS	SARACA INDICA
1. CARBOHYDRATE TEST	POSITIVE
2. TANNIN	POSITIVE
3. SAPONIN	NEGATIVE
4. FLAVONOID	POSITIVE
5. ALKALOID	POSITIVE
6. QUINONES	POSITIVE
7. CARDIAC GLYCOSIDES	NEGATIVE
8. TERPENOIDS	POSITIVE
9. PHENOL	POSITIVE
10. COUMARINS	POSITIVE
11. STEROIDS AND PHYTOSTEROIDS	POSITIVE(STEROIDS)
12. PHLOBATANNINS	NEGATIVE
13. ANTHRAQUINONES	NEGATIVE

In the present study, an assessment was carried out for the eicosanoid response of human PBMC, which determined the eicosanoid release in response to the inflammatory stimulus. Initially, the estimation of blood for PGE_2 and TNF-α.

Effect of Saraca indica on PGE_2 (Prostaglandin) in Normal Human PBMC

PGE2 levels after PBMC stimulation with LPS was 3068.37 pg/ml in normal volunteer Saraca indica inhibited PGE2 production in a dose-dependent manner in both normal volunteer (IC50= 22.63 µM) (Fig. 1; Table.1) (Brenneis et al., 2006) reported rofecoxib, effective concentration of 120nM, which was used as a drug control in this study. In present study, Rofecoxib shows IC50 of 120nM in normal patients in Human PBMC.

Effect on TNF-α Production Stimulated by LPS in Human PBMC:

Normal PBMC provides a protein and cell rich milieu appropriate for the study of biochemical efficacy of compounds. LPS stimulated TNF- α levels in normal volunteer was 10144.14 pg/ml (Fig.2) (Fig. 2.) was 10144.14 pg/ml. Saraca indica significantly inhibited TNF-α production IC50 9.96µM in normal PBMC. Swaroop et al., (2007) reported Rolipram, effective concentration of 1.1 µM which was used as a drug control in this study. In our experiment, rolipram showed IC50 of 3.24µM in normal Human PBMC.

Effects of Saraca indica on LPS-induced TNF-α production in rats

Amount of plasma TNF-α were measured 1hour after LPS injection since TNF-α level were maximal at this time. The average baseline TNFα levels were 9572 pg/ml in rats. The TNFα level then underwent rapid decline to baseline by about3 to 4 hours post LPS administration. As displayed in (Table.3) ***Saraca indica*** (350 and 500mg/kg, per oral resulted in dose-dependent inhibition of LPS-induced TNF-α production, *Saraca indica* were significantly inhibited 67.35 % at 500mg/kg.

Table : 1 Effect of *Saraca indica* on PGE_2 (Prostaglandin) in normal Human PBMC

Conc. (µM)	Normal patient Whole blood assay					
	Rofecoxib			*Saraca indica*		
	Level of PGE_2 (pg/ml)	SEM	% Inhibition	Level of PGE_2 (pg/ml)	SEM	% Inhibition
0.001	3115.78	108.79	-1.51	ND	ND	ND

0.003	2945.23	68.86	4.02	ND	ND	ND
0.01	2606.73	179.91	15.05	ND	ND	ND
0.03	2312.14	85.09	24.66	3079.56	175.82	-0.34
0.1	1827.14	81.20	40.45	2979.56	254.76	2.92
0.3	870.09	56.38	71.63	2825.71	159.46	7.93
1.0	392.57	10.38	87.22	2736.59	190.99	10.82
3.0	24.36	2.72	99.19	2434.12	158.94	20.67
10	ND	ND	ND	1887.34	115.20	38.49
30	ND	ND	ND	1456.45	63.25	52.54
100	ND	ND	ND	898.79	49.34	70.72
300	ND	ND	ND	326.00	26.30	89.35
LPS	**3068.37**					
IC_{50}	**120nM**			**22.63µM**		

****ND-Not Done***

Fig.1. ***Effect of Saraca indica on PGE_2 (Prostaglandin) in normal Human PBMC.***

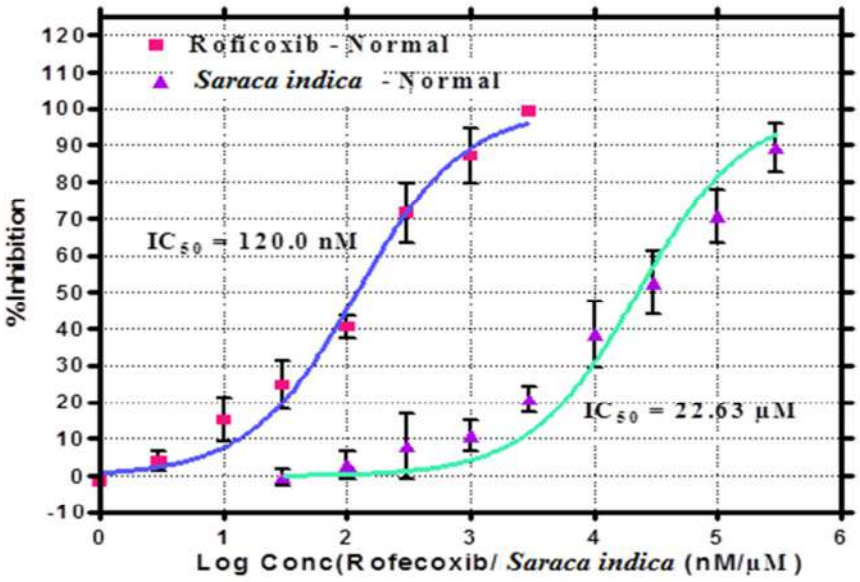


Table-2 ***TNF-α inhibition of rolipram and Saraca indica in Human PBMC***

CONC. (µM)	Normal Human PBMC					
	Rolipram			***Saraca indica***		
	Level of TNF-alpha	**SEM**	**% Inhibition**	**Level of TNF-alpha**	**SEM**	**% Inhibition**

0.03	10159.01	1024.11	-0.15	10376.98	1003.03	-2.30
0.1	9928.57	924.23	2.13	10025.47	1018.36	1.17
0.3	9135.25	922.13	9.95	9309.12	922.33	8.23
1	7837.14	715.22	22.74	8984.56	841.22	11.43
3	4567.16	456.32	54.98	6920.36	678.12	31.78
10	3145.32	324	68.99	5187.15	511.33	48.87
30	1567.14	196.33	84.55	3403.24	342.23	66.45
100	230.25	23.22	97.73	864.45	80.36	91.48
LPS	**10144.14**					
IC_{50}	**3.24µM**			**9.96µM**		

Figure-2 ***TNF-α inhibition of Rolipram and Saraca indica in Human PBMC***

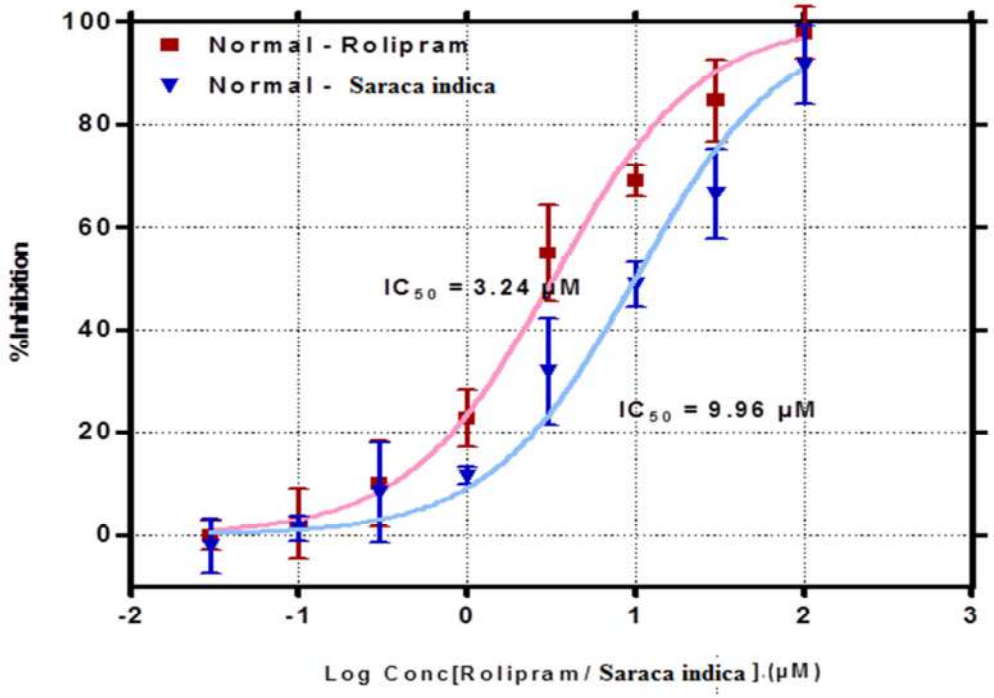


Table -3 ***Significant activity of Saraca indica in LPS induced sepsis model***

Dose	**Rat TNFα (pg/ml)**	
	Levels(pg/ml)	**% Inhibition**
***Saraca indica*350mg/kg**	3456	63.89
***Saraca indica*500mg/kg**	3125	67.35
Dexamethasone (1mg/kg)	859	91.03
LPS	**9572**	

CONCLUSION AND DISCUSSION

Eicosanoids and cytokines are pleiotropic immunological messengers that play an important role in the progression of many diseases including Psoriasis and rheumatoid arthritis (Calder, 2006). Both the mediators have been implicated as markers of subclinical inflammation and later risk of disease in healthy subjects (Danesh *et al*., 2000).Kirchner *et al*., (1982) first described ex vivo stimulation of cytokine release in whole blood in the early 1980s. This technique has proven useful in identification of sepsis patients, Heagy *et al.* (2000) determined the high and low eicosanoids and cytokines producers. Linden *et al*., (1998) first provided pharmacological assessments of compounds inwhole blood and human PBMC assay. Some studies have used for eicosanoids and cytokine production in whole blood or PBMC cultures as a proxy for monocytic cytokine production (Wilson *et al*., 1991; Abbate *et al*., 1996). Two earlier papers (De Groote *et al.,* 1992; Yaqoob *et al.,* 1999) evaluated the association between inflammatory mediators from whole blood cultures and PBMC, but neither of them investigated the correlation with monocytic cultures. However, the group observed that for most cytokines whole blood and PBMC cultures showed different production kinetics and that the inter-individual variation was higher in PBMC than in whole-blood cultures (De Groote *et al.,* 1992). They also found a larger relative loss of monocytes compared to lymphocytes (T- and B-cells) in the PBMC isolation procedure. A lower relative content of monocytes in PBMC cultures may explain some of the discrepancy between PBMC cytokine production on one hand and the production from whole blood and monocytes on the other. However, compared to culture medium whole-blood represents a physiologically much more relevant environment for the cells. Furthermore, isolation, centrifugation and washing PBMC at 4°C are unphysiological processes, which may affect the membrane properties and activation state of the cells. Subsequent isolation of monocytes is most commonly done by positive selection and the use of antibodies that bind the surface marker CD14, which acts as a co-receptor for LPS in toll-like receptor (TLR) 4 signaling (Guha and Mackman, 2001). This procedure may pre-activate the cells before stimulation. Overall, whole-blood culture is well suited for large trials where a number of other measurements are performed and in trials among infants where blood volumes are limited. According to (Camilla *et al.,* (2009) summarized cytokine production from whole blood cultures is a valid, low-cost and convenient.The method seems to be better suited than PBMC culture and is recommended. Moreover, it was found a good reproducibility in whole-blood cytokine production, even with measurements 8 weeks apart. Human whole blood offers many advantages, for one it is based on primary human cells stemming from different donors as opposed to assays making use of transformed cell lines. This makes it more relevant to the human situation than assays based on preparations from the first source (Martel-Pelletier *et al.*, 2003). Furthermore, because whole blood is used, large supplies of assay material are readily available, thus giving it a clear advantage. So we chose human whole blood instead of animal enzymes, cells or cell lines (Martel-Pelletier *et al.,* 2003). As was mentioned previously, corticosteroids are not used long term due to the side effects of chronic use. NSAIDS have proven to be helpful in reducing the severity of certain inflammatory diseases. However, these drugs only provide partial relief and simply slow the progression of the disease. It is well-known from herbalist literature that certain plants possess medicinal qualities that are beneficial in reducing inflammation, and many recent studies have begun to assess the anti-inflammatory properties of plant derived compounds. One herb known for its anti-inflammatory properties is embelin. Current treatment for inflammatory diseases is NSAIDs (Bley *et al*., 1998). (Stefano *et al*.,2001) which are classified and tested into two main groups, which range from compounds that inhibits both COX-1 and COX-2 are classified under (group one). The compounds that selectively inhibit COX-2 with weak activity against COX-1 (group two) (Warner *et al.,*1999). Comparing these groups of NSAIDs to the epidemiological studies, NSAID showed severe gastrointestinal toxicity. In this regard, the development of COX/5-LOX inhibitors may represent a new promising alternative therapy (Syahida *et al*., 2006). Based on our results embelin lies in Group 1 category. Indeed, by inhibiting both pathways embelin may lead to a better anti-inflammatory effect accompanied by reduced gastric side-effects. In addition, dual inhibitors of the COX/LOX pathways may exhibit anti-inflammatory activity with a wider spectrum than that of classical NSAIDs (Liwen *et al.,* 2009). In the present study, Cyclooxygenase-2 activity was assessed by quantifying the amounts of prostaglandin E_2 released by white blood cells following incubation of whole blood with stimulus lipopolysaccharide in normal patient. ***Saraca indica*** upon prostanoid generation was very significant in both PGE_2 and TXB_2, which reduced levels in a dose-dependent fashion. The inhibition of prostanoid synthesis is the major desirable effect of NSAID's, which leads to reduction of inflammation by *Saraca indica* Tumour Necrosis Factor alpha (TNF-α) is a ubiquitous, multifunctional cytokine produced

primarily by activated monocytes, macrophages and T-cells, and plays a key role in fighting infection, eradicating tumours, and mediating the acute and chronic inflammatory effects of the immune system.Excessive production of TNF-α, however, has been directly implicated in a wide variety of diseases that differ considerably in their etiology and clinical manifestations. TNF-α is another significant proinflammatory mediator which is involved in the pathogenesis of several inflammatory diseases via induction of secretion of IL-1, IL-6 and IL-10 and activation of T cells (Brideau *et al.,*1996; Claveau *et al.,*2004) and therefore suppression of TNF-α would be beneficial in the management of inflammatory-related diseases. The significant inhibition of TNF-α production by embelin may be due to the fact that inhibition of NF-κB nuclear translocation causes reduced expression of TNF-α gene (Marriot *et al.,* 1997). *Saraca indica* showed significant inhibition of TNF-α production in human PBMS assay in normal Based on these results, *Saraca indica* showed potent inhibition on PGE2 and TNF α, thus proving as a broad anti-inflammatory and immunomodulatory activity. Inhibiting TNF-α at 9.96μM made our interest to select TNF α as a target. TNF-α is important in the pathogenesis of many disease because it induces synthesis of IL-1 and IL-8, which cause the inflammation. TNF-α also promotes proliferation and angiogenesis, and thus inhibiting this cytokine should halt the development of the disease at multiple loci (Lynne, 2005). Preliminary screening for eicosanoids and cytokine inhibition by *Saraca indica* has led us to believe that the compound has anti-inflammatory activity. Current study has concluded that *Saraca indica* has significant inhibitory effects upon proinflammatory mediator release in the cellular systems employed. It is therefore highly possible that *Saraca indica* is the active principle when used in folkloric medicine. Our results strengthen the findings of several reports regarding the anti-inflammatory activity of *Saraca indica*. Although a direct comparison cannot be made with other compounds reported in the literature due to differences in experimental procedures, it is evident that *Saraca indica* shows similar effects as described by Kim *et al.* (2001) upon prostanoid generation which was very significant in both the production of PGE_2 and TNF- α. The discovery of new compounds that can be administered orally and whose mechanism of action differs from that of the biological TNF-α inhibitors would significantly add yet another feather in the armamentarium available for treating psoriasis and other immunological disorders (Concetta et al., 2009). One molecule that fits such a profile from this study is embelin, a potent, selective and orally available compound that inhibits and decreases circulating TNF-α levels (Duan et al., 2003). LPS induced sepsis in rodent were used as a preliminary model in the present study to evaluate the pharmacodynamic activity of embelin. LPS-induced TNF-α production and release can be simplified as a series of events. LPS binding to LPS binding protein (LPB), and that complex then binds to CD14 which initiates a signaling cascade that culminates in expression of the TNF α gene. The TNFα precursor in the cell membrane, cleaves the precursor, and finally, release of the active form of TNF-α, a 17 kDa soluble fragment, into the extra-cellular spaces (Anderson et al., 2004; Gracie et al, 2002). Alterations in the concentration of TNF-α in fresh blood can be expected if the synthesis or release of TNFα is disrupted in animals during the response to LPS challenge by choosing an appropriate Pharmacodynamic model, one can establish an in vitro–in vivo correlation for drug candidates that are intended to modulate this cascade (Tinsley et al., 2004). Instead of directly initiating the specific animal models in psoriasis and chronic inflammatory models study, this sepsis model has served as a proof of concept for target validation and a quick assessment of in vitro– in vivo correlation. *Saraca indica* clearly reduced the serum levels of the proinflammatory cytokines TNF-α. From the study it was found that *Saraca indica*Significant reduction in the TNF-α exposure as 63% in rats. Decrease in the levels of TNF-α after *Saraca indica*administration contributes to some extent in understanding the mechanism of action. The detailed procedures of pharmacodynamics of TNF-α determination of TACE inhibitors in rat and mice were described by Zhang et al (2004). In Conclusion, our data shows that *Saraca indica* has inhibited both eicosanoid and cytokines which is the key for many inflammatory diseases. Based on these results further studies will be conducted to find out the impact of *Saraca indica* on other inflammatory disease related inflammatory disease and its ultimate effect. The present study revealed that both, *in vitro* and *in vivo*, seem to support the hypothesis that the *Saraca indica* is a potent inhibitor of TNF-α. These mechanistic insights, coupled with a positive sign to provide a novel and effective therapy in treatment of various chronic inflammatory disease. Based on these results further advances in realizing the potential of *Saraca indica* using other animal model has to been focused in near future in research.

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SCREENING FOR ANTIOXIDANT PROPERTIES OF SECONDARY METABOLITES OBTAINED FROM ACTINOMYCETES

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ABSTRACT

Actinomycetes have been intensively studied in several underexplored environments and extreme habitats in various parts of the world (including India) in the last few years. Yet, few reports were available regarding the isolation of actinomycetes from terrestrial medicinal plants. Therefore, an attempt has been made to isolate the actinomycetes from this lesser explored region in order to find novel species. Totally 13 actinomycetes strains were isolated from soil samples of different culture based on the gram staining and colony morphology. All the isolates were found to be positive in gram staining; biochemical screening had different morphological structures. The isolates were screened for their inhibitory activity against the human pathogenic bacteria. Primary screening was used to select the antibacterial isolates and determine the range of microorganisms that were sensitive to the antibiotics. Similarly secondary screening method was crucial to select the isolates for further studies. The qualitative approach is used to determine the range of the microorganisms that are sensitive to a potential antibiotic. The quantitative approach provides information about the yield of antibiotic that can be expected when the organism is grown in ISP2 broth. All the experimental measurements were carried out in triplicates and were expressed as an average of three analyses. Isolates I5, MS1005Fand MS1005E were highly active, while MS100E showed less activity against pathogenic bacterial strains. Isolate I5 exhibited the highest activity against MS1005F.

Keywords*: Actinomyctes, Medicinal plants, Biochemical tests, Morphology. Biochemical Screening.*

INTRODUCTION

Soil is a mixture of organic matter, mineral, gas, liquid and organisms as a ready reckoner for life in planet earth and its existence. It is commonly referred as a pedosphere, which has four defined functions such as a medium for plant growth, water storage, supply and purification. It also serves as a modifier of earth's atmosphere as a habitat for organisms, which in turn, modify the soil and its quality too. Soil shall be classed as, Alluvial, Black, Red, Yellow, Laterite, Arid, Desert, Saline, Alkaline, Peaty, marshy soils commonly available as forest and mountain soils. The soil samples were collected during the study from Chennai consists of clay, sedimentary rocks and sandstone. Red and yellow soil is available throughout Chennai and the soil available in Guindy, is literally known as terrestrial soil. Medicinal soils were obtained from the root nodules of medicinal herbs, have been discovered and used in traditional medicine practices, since prehistoric times. Medicinal plants are well known for its applications to the commonly encountered diseases and pathogens. The phytochemical as well as its secondary metabolites and beneficial organism were produced in plants assist those to survive in the soil environment against lot of potential damage causing organisms (Berdy, 2005). Plants can synthesize an array of chemical compounds which plays a vital role in defense against insects, fungi, diseases and herbivorous mammals. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine (Goodfellow, and Odonnell, 1989). Actinomycetes, (order Actinomycetales), any member of a heterogeneous group of gram-positive, generally anaerobic or facultative anaerobic bacteria noted for a filamentous and branching growth pattern that result in most forms such as an extensive colony or mycelium. The mycelium in some species may break apart to form rod or coccid shaped forms. There are number of genera also form spores; the sporangia, or spore cases, may be found on aerial hyphae, colony surface, or free within the environment.

Motility is positively conferred by flagella if it is available. Many species of actinomycetes available in soil are harmless to animals and higher plants, while some are important pathogens and many others are beneficial sources of antibiotics (Ventura, et al., 2007). Actinomycetes are one among the most important members of bacteria that produces a wide variety of useful secondary metabolites, many of which have potent biological activities, including commercially potentially available antibiotics and immunosuppressive compounds. In addition, few members are used for industrial bioconversion processes to manufacture commodity chemical, health products and agrochemicals. Several methods with basic research for improving the ability of actinomycetes have been developed, however, practical tools are very limited (Yuan-Gao, et al.2017). The notable characteristics of the actinomycetes are their ability to produce wide range antibiotics. Streptomycin, Neomycin, Erythromycin and Tetracycline are the commonly advised antibiotics against pathogens. Streptomycin is used to treat tuberculosis and infections caused by certain bacteria and Neomycin is used to reduce the risk of bacterial infection during surgery. Erythromycin is used to treat certain infections caused by bacteria, such as bronchitis, pertussis (whooping cough), pneumonia, ear, intestine, lung, urinary tract and skin infections (Wang. Y,et.al, 1999).Improved soil structure through bacteria increases water infiltration and holding capacity of the soil (Ingham, 2009). Downstream processing and analytical bio separation both refer to the separation or purification of biological products, but at different scales of operation for different purposes. Downstream processing implies manufacture of a purified product fit for a specific use, generally in marketable quantities, while analytical bio separation refers to purification for the sole purpose of measuring a component or components of a mixture, and may deal with sample sizes as small as a single cell (*Krishna Prasad and Nooralabettu, 2010). The secondary metabolites were isolated from actinomycetes and used for further screening in vivo.* To validate and purify the crude active compounds, were spotted on the baseline of silica gel plate (Stationary Phase), allowed to dry and run in the presaturated mobile phase inside the TLC chamber, the Rf value is calculated and the compound with bioactive has to be checked for its availability and purity with antioxidant property. The silica gel is the stationary phase, it is measured by the growth of microbes and it is referred by graph (Singhal N., et.al, 2009). Due, to the drastic increase of ESBL (Extended Spectrum Beta Lactamase) activity and MDR (Multi Drug Resistant) bacterial pathogen, the alternative agents which inhibits the growth has to be obtained from actinomycetes. The growth of pathogens was inhibited either through antibiotics or secondary metabolites of medicinal plants. In this study, an attempt made to identify the possible cure mediated through Actinomycetes *spp.* in other words bacteria against a bacterium in invitro condition. Therefore, this study aimed at isolating actinomycetes with antibacterial properties and to evaluate their secondary metabolites against selected multidrug resistant bacteria isolates (Ishida N, et al.1965). According to Hayakawa *et al*. (2008), Actinomycetes are distributed widely in various habitats but soil remains the most important with largest population found in surface layer. In natural soil habitat, streptomycetes exist as a major component of Actinomycetes population. There are several studies on isolation of Actinomycetes from soil. To date, there is interest in assessing antimicrobial potentials among soil Actinomycetes. The existence of marine Actinomycetes came from the description of the first marine Actinomycete *Rhodococcusmarinonascene* (Taechowisan*et al*. 2003). Actinomycetes have been detected in a unique marine environment such as marine organic aggregates and deep - sea gas hydrates reservoirs, where they were found to be the major components of the microbial communities (Madrid *et al*. 2001). The findings confirm the presence of indigenous marine Actinomycetes in the oceans and indicate that marine Actinomycetes are widely distributed in different marine environments and habitats. Both culture dependent and independent methods demonstrate that novel Actinomycetes found everywhere in the oceans from deep floor to coral reefs. The extensive variation among the different marine environments, the presence of Actinomycetes that are distantly related to known taxa in larger amount (Asano and Kawamoto, 1986). Vercesi*et al*. (1992) isolated Streptomyces *spp*. from other parts of grapevine berries, which exhibit widespread antagonistic activity against yeasts and fungi inhabiting the same environment. Besides Streptomyces *spp*., Matsumoto *et al*. (1998) isolated rare Actinomycetes like Microbispora*spp*. from surface-sterilized fallen leaves using different methods used healthy plant tissues from leaves, stems and roots to isolate eight endophytic actinomycetes which have antifungal potential, particularly Streptomyces *spp*.

MATERIALS AND METHODS

Soil sample collection

The plants from which the soil samples were collected are *Cissus quadranguenris* and *Alpinia officinarum*. The rhizosphere region and inner depth regions of guindy, chennaiwere considered further in this study. About 500 gms of soil samples were collected from the depth of 15 cm below the surface in the sterilized plastic bags during June 2018. The samples were air died in the laboratory at room temperature for a period of five to six days and further processed with standard microbiological methods

Extraction of Bioactive Compounds from selected Actinomycetes

After the period of incubation the filtrate was filtered by Whatman No-1 filter paper, centrifuged at 8,000 rpm for 15 minutes. Equal volume of ethyl acetate in the ratio of 1:1 is added and kept undisturbed overnight. The dried specific solvent is subjected further for the other analytical studies.

Spectrometric test

A lamp provides the source of light. The beam of light strikes the diffraction grating, which works like a prism and separates the light into its component wavelengths. The grating is rotated so that only a specific wavelength of light reaches the exit slit (Allen *et al*. 2010).

For flavonoids and phenol we using spectrometric test to find the presence of both compound in the given sample Isolate-5.

In-vitro Antioxidant activity for Secondary Metabolites

DPPH assay

The Antioxidant activity was determined by DPPH scavenging assay. Various concentrations of crude extract were pipetted out in clean test tubes. Freshly prepared DPPH (1, 1-Diphenyl-2-picryl hydroxyl) solution (1 ml) was added to each tube and the samples were incubated in dark at 37°C for 30 min and read at 517 nm. Ascorbic acid was used as reference compound (Allen *et al*. 2010). The percentage inhibition of radical scavenging activity was calculated as:

$$\%\text{DPPH potential} = \frac{(\text{control OD-sample OD})}{\text{Control OD}} * 100$$

Phosphomolybdenum assay

Various concentration from the crude extract were pipetted out and 1 mL of the reagent solution containing 4 mM Ammonium molybdate, 28 mM Sodium phosphate and 0.6 M Sulphuric acid was added, followed by incubation in boiling water bath at 95°C for 90mins. After cooling the sample to room temperature, the absorbance of the solution was measured at 695 nm in UV spectrophotometer. Ascorbic acid served as standard (Allen *et al*. 2010). The percentage of reduction was calculated as:

$$\%\ \text{Phosphomolybdenum reducing potential} = \frac{(\text{control OD-sample OD})}{\text{Control OD}} * 100$$

Ferric (Fe^{3+}) reducing power assay

The crude extract was taken in various concentration and was mixed with 2.5 ml of phosphate buffer (0.2 M, pH-6.6) and 2.5 ml of potassium ferricyanide (1% w/v), and incubated at 50°C for 30 minutes. Then, 2.5 ml of trichloroacetic acid (10% w/v) was added to the mixture and then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of upper layer solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (0.01% w/v) and the absorbance was measured at 700 nm. Ascorbic acid served as standard (Allen *et al*. 2010). The percentage of reduction was calculated as:

$$\%\ \text{Ferric reducing potential} = \frac{(\text{control OD-sample OD})}{\text{Control OD.}} * 100$$

TLC

Thin layer chromatography (TLC) is used to identify the mixture of compounds by using a mobile and stationary phase, silica gel is commonly used as the stationary material and coated o the slide (250 mg/10 ml). The mobile phase is a solvent chosen according to the properties of the components in the mixture. The principle of TLC is the distribution of a compound between a solid fixed phases (the thin layer) applied to a glass or plastic plate and a liquid mobile phase (eluting solvent) that is moving over the solid phase. The solvent is drawn up through the particles on the plate through the capillary action, and as the solvent moves

over the mixture each compound will either remain with the solid phase or dissolve in the solvent and move up the plate (Quach *et al*. 2004).

Rf values

An individual compound in TLC is characterized by a quantity Known as R*f* and is expressed as a decimal fraction. The R*f* is calculated by dividing the distance the compound travelled from the original position by the distance the solvent travelled from the original position, (Skoog *et al.* 2006).

$$Rf(\text{retention factor}) = \frac{\text{Distance travelled by solute(x)}}{\text{Distance travelled by solvent(y)}}$$

Estimation of phenol

Khare, (2007).Take two conical flask, A and B, fitted with reflux water condensers. Weigh accurately about 1 g of phenol and transfer in flask A. Connect the flasks to the reflux condensers and heat both flasks on boiling water-baths for 30 minutes. Then remove both the flasks from water-bath add pour 20 cm^3 of distilled water down each condenser, shaking the contents of each flask gently to ensure complete hydrolysis of the unreacted acetic anhydride (Nayar and Chopra, 1956). Then titrate the contents of each flask with NaOH solution, using phenolphthalein or an indicator. 100 µl of plant extraction+900 µl of methanol+1 ml of folin-clocalteau agent 1 ml reagent 9 ml water+1 ml of 20% sodium carbonate solution+20 g in 100 ml,1 g in 5 ml Incubation at RT for 30 mins in dark condition. If the precipitate appear centrifuge at 2000 rpm for 5 mins, Collect the supernatant and OD at 765 nm.

Estimation of flavonoid

Bin yang*et al*. (2001). A simple electrochemical method for estimating the antioxidant activity of flavonoids has been developed. They are also believed to be antithrombotic, antibacterial, antifungal, antiviral, and cancer protective, and also to protect against cardiovascular disease (Balant*et al*. 1984). 500 µl of plant extract+500 µl of methanol+500 µl of 5% sodium nitrate 0.25 g in 5ml+distilled water, 500µl of 10% sodium chloride 0.5 g in 5ml+distilled water. Incubate in room temperature for 5 mins. Add 1ml of 1M sodium hydroxide 0.2 g sodium+5 ml H_2O solution, Incubation in room temperature. Precipitation appear centrifuge at 3000 rpm for 5 mins. OD at 510 nm.

RESULT AND DISCUSSION

Solvent extraction

Figure 1. Extraction of Secondary Metabolites by Solvent Extraction Method

Culture I5 separated by ethyl acetate. Again to get the supernant by using separating funnel. Liquid–liquid extraction, also known as solvent extraction and partitioning, is a method to separate compounds or metal complexes, based on their relative solubility in two different immiscible liquids, usually water and an organic solvent.

Spectrometric test

In this process Spectrometric test involves in antioxidant activities of three type of assays, DPPH assay, phosphomolybdenum and ferric reducing power assay. The Actinomycetes species isolated from the

terrestrial sample collected from Phytosphere region were found to be effective in scavenging the free radicals when compared to the isolate-5.

In-vitro Antioxidant activity for Secondary Metabolites

DPPH assay

Antioxidant react with DPPH, which is a stable free radical and is reduced to the DPPH-H (hydrogen) form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds in terms of hydrogen donating ability. The isolate-5 at the concentration of 400 µg/mL

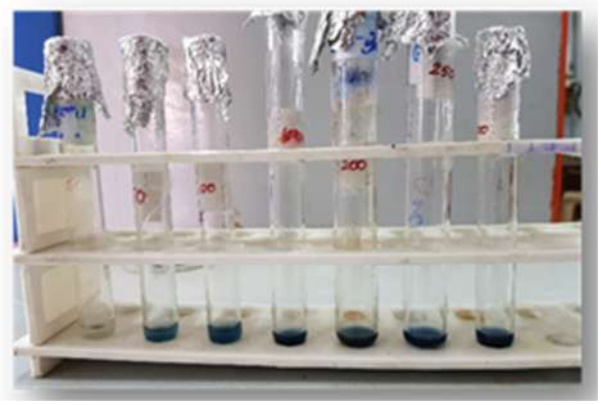

Figure 2. DPPH assay of Medicinal Soil Plants

S.No	Volume (µl)	MeoH (µl)	DPPH		Od value at 517nm
1	Control	1ml	1ml		0.193
2	20	980	1ml		0.138
3	40	960	1ml	RT/dark/	0.132
4	60	940	1ml	30mins	0.103
5	80	920	1ml		0.093
6	100	900	1ml		0.079
7	120	880	1ml		0.067

Table 1. ***The values of DPPH assay against Medicinal Plants Soil***

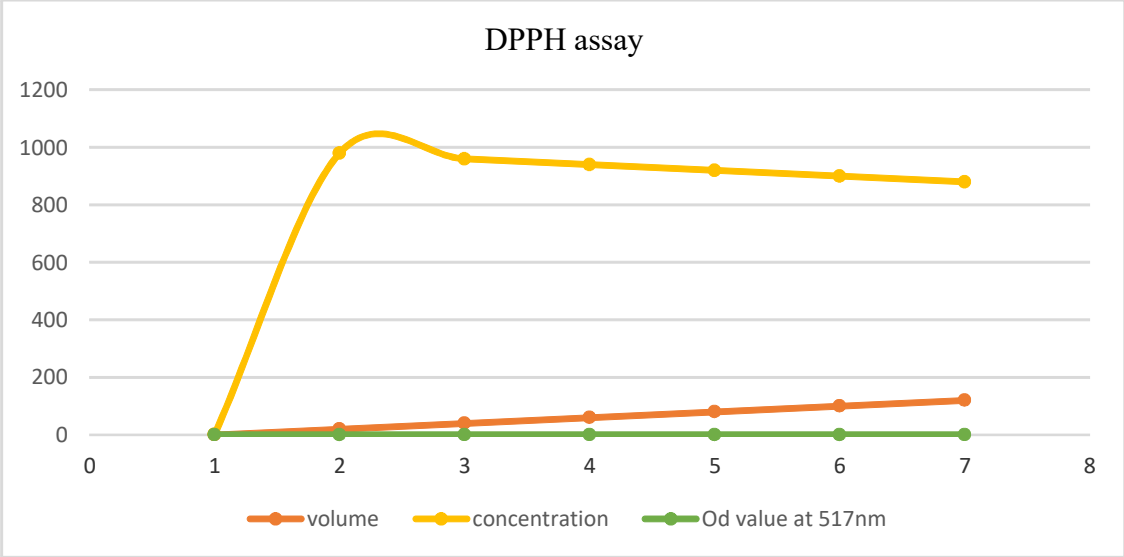

Figure 3. **OD values were plotted against Volume**

Phosphomolybdenum assay

The total antioxidant activity based on the reduction of pH increase in the absorbance value was well observed for the crude compound. The Phosphomolybdenum reducing potential was found to be 0.979

µg/ml at the concentration of 450 µg/ml for the isolate TS 1003 and comparatively the reducing potential was found to be 0.528 for TS 1006 at the same concentration.

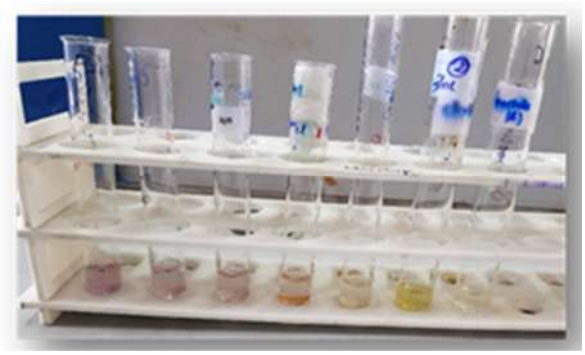

Figure 4. ***Phosphomolybdenum assay of Medicinal Soil Plants***

S.No	Volume (µl)	MeoH (µl)	Reagent solution		OD at 695 nm
1	Control	1ml	1ml		0.049
2	50	950	1ml	Incubation	0.307
3	100	900	1ml	In	0.628
4	150	850	1ml	water bath	0.976
5	200	800	1ml	(1/2hr)	2.480
6	250	750	1ml		1.869
7	300	700	1ml		2.098

Table 2. ***The values of phosphomolybdenum assay against Medicinal Plants Soil***

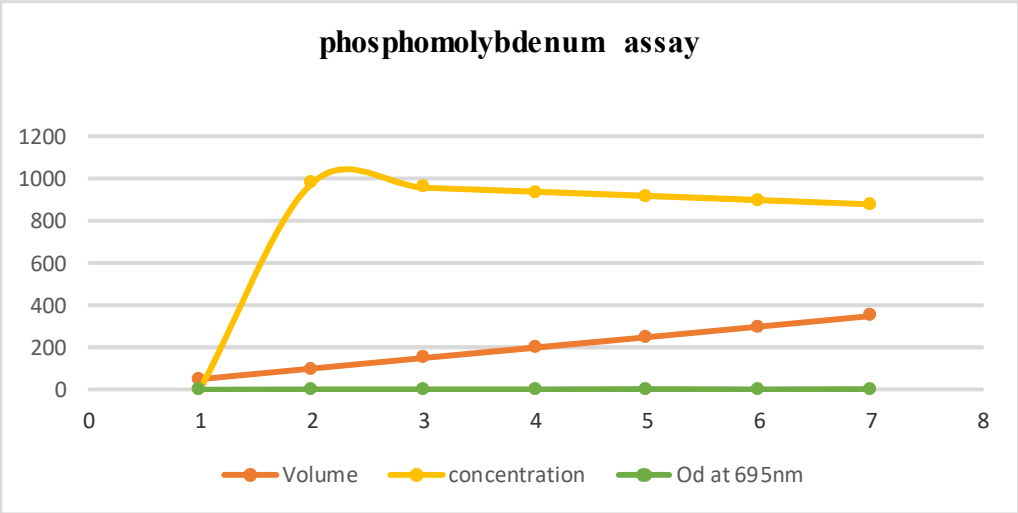

Figure 5. OD values were plotted against Volume

Ferric reducing power assay

An antioxidant capacity assay that uses Trolox as a standard. Antioxidant activity is a complex procedure usually happening through several mechanisms and is influenced by many factors, which cannot be fully described with one single method. Therefore, it is essential to perform more than one type of antioxidant capacity measurement to take into account the various mechanisms of antioxidant action (Wong *et al*. 2006).

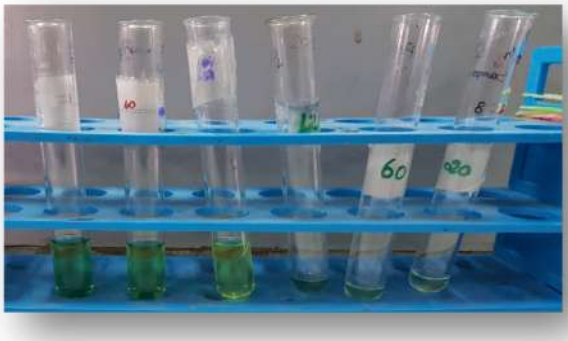

Figure 6. Ferric reducing power assay of Medicinal Plants Soil

S.No	Sample (µl)	MeoH (µl)	Po_4 buffer (µl)	Potassium ferocyanide	10%TCA	0.01% $fecl_3$		OD at 700nm
1	Control	1ml	1ml	1ml	500µl	100µl		0.174
2	20	980	1ml	1ml	500µl	100µl		0.194
3	40	960	1ml	1ml	500µl	100µl	If precipitate appearance	0.291
4	60	940	1ml	1ml	500µl	100µl	centrifuge	0.359
5	80	920	1ml	1ml	500µl	100µl	3000rpm/5mins	0.374
6	100	900	1ml	1ml	500µl	100µl		0.402
7	120	880	1ml	1ml	500µl	100µl		0.467

Table 3. The values of ferric reducing power assay against Medicinal Plant Soil

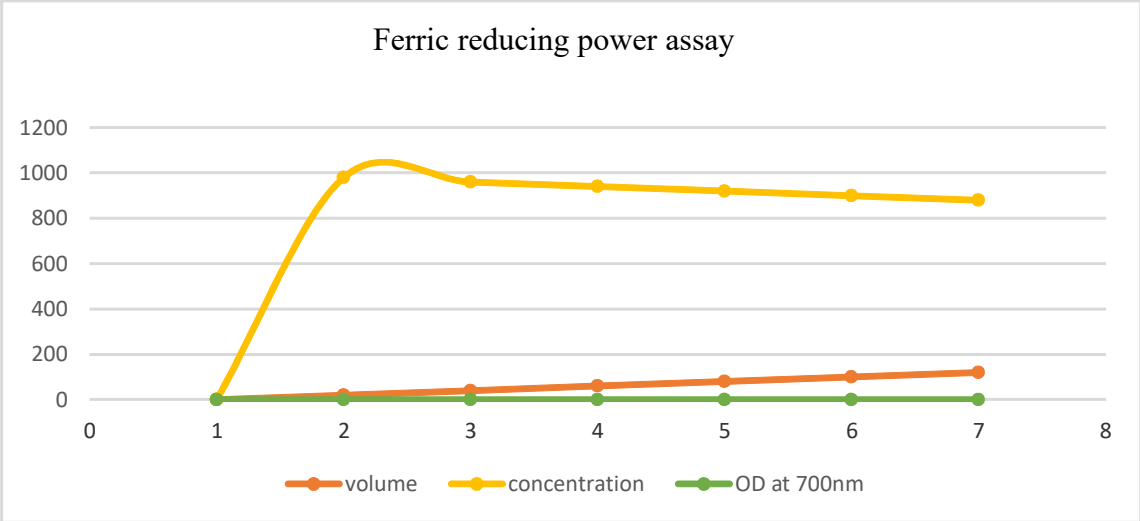

Figure 7. OD values were plotted against Volume

TLC

Sno.	Chemicals used	Amount	Result
1	Methanol	1ml	-ve
2	Ethyl acetate	1ml	-ve
3	Methanol:toluene	1ml:1ml	-ve
4	Methanol:ethyl acetate	1.5ml:0.5ml	+ve

Table 4. indicative of results obtained with different solvents

+ indicates positive result
- indicates negative result

The $R_f$value of I5 was 0.75 and the findings of this study is in mere agreement with similar findings of Boulder, (2015). If the values travels 2.1 cm and the solvent front travels 2.8 cm. Likewise the $R_f$was 1.7cm as reported by *Harry and Christopher (1989) was not in agreement with the present study. If the red component travelled 1.7 cm from the base line while the solvent had travelled 5.0 cm, then the* R_f *value is 0.34, hence this value is not agree with the resulted value.* UV image of I5 band and adding iodine. The secondary metabolites of isolates produced by small scale were extracted and tested for the presence of active compounds and their radical scavenging activity by TLC and from Dot plot assay it was observed that MS1005E, MS1005F and isolate-5 showed better radical scavenging activity.

x=2.7
y=3.5
X/y=0.77

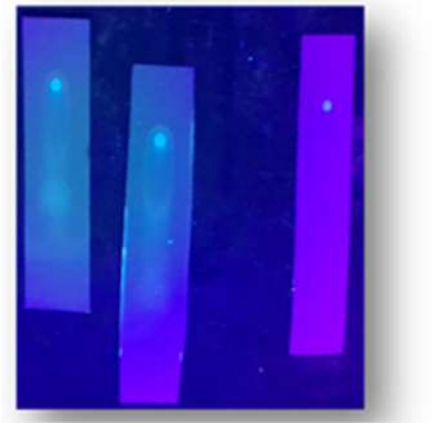

Figure:8(a)

Figure: 8 (b)

Under theUV light formation of the bands, tested in Ms1005E, MS1005F and I5. Finally the resulted paper was mixed with iodine to measure the solvent travelled.

ISP2 (International Streptomyces Project) for I5: Isolation of Actinomycetes

Actinomycetes have been intensively studied in several underexplored environments, niche and extreme habitats in various parts of the world in the past few years. Based on the colony morphology and stability in sub culturing, 13 suspected Actinomycetes cultures were purified on ISP-2 slants. Among 13 isolates 5, MS1005F and MS1005E were gray, white, green, blue, orange, and black pigmented, respectively. Interestingly, gray and white mycelial pigmented actinomycete*s* were prominent in the soil. For long time storage, the strains were grown in ISP-2 broth for 7 days. Out of this 13, I5 was selected based on its culture growth and further compound tests were done in this sample.

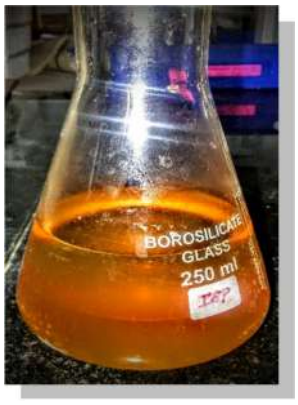

Figure 9. International Streptomyces Project-2

Estimation of phenol

In I5 sample the presence of phenol were observed. Phenolic compounds are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals (Shahidi*et al*. 1992).The final value =0.169 µg/ml

Estimation of flavonoids

The compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effect (Das and Pereira, 1990). It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health beneficial effects. The final value=1.710 µg/ml

CONCLUSION

An attempt has been made to isolate the actinomycetes from this lesser explored region in order to find novel species. Totally 13 actinomycetes strains were isolated from soil samples of different culture based on the gram staining and colony morphology. All the isolates were found to be positive in gram staining; biochemical screening had different morphological structures. The isolates were screened for their inhibitory activity against the human pathogenic bacteria. Primary screening was used to select the antibacterial isolates and determine the range of microorganisms that were sensitive to the antibiotics. Similarly secondary screening method was crucial to select the isolates for further studies. The qualitative approach is used to determine the range of the microorganisms that are sensitive to a potential antibiotic. The quantitative approach provides information about the yield of antibiotic that can be expected when the organism is grown in ISP2 broth. All the experimental measurements were carried out in triplicates and were expressed as an average of three analyses. Isolates I5, MS1005Fand MS1005E were highly active, while MS100E showed less activity against pathogenic bacterial strains. Isolate I5 exhibited the highest activity against MS1005F.

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SP-20

A STUDY ON CHEMINFORMATICS INTERPRETATION OF BIOACTIVE SUBSTANCES

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ABSTRACT

Bioinformatics and cheminformatics tools are of immense value to biologist in the extraction of information from vast repository of biological and chemical data. Computational tools are employed here to discover specific biological properties of newly generated chemical compounds. The objective of this work is to these molecules based on the structure and biological role they play and to elucidate the physical and chemical features of those molecules which contribute to their said biological function. This would help to maximize the pharmacodynamic and pharmacokinetic properties of those compounds. A commercial tool name by the TSAR which performs Quantitative Structure Activity Relationship Analysis(QSAR) is used to cluster the molecules on their similarity in sterric, lipophilic and electronic parameters. Using regression analysis the correlation between their predicted biological property and their structural parameters are studied. These could be after further refinement can be used as drug against bacterial infection and other specific targets.

Keywords: ***Computational tools, QSAR, Pharmacodynamics, Regression Analysis.***

INTRODUCTION

Cheminformatics was defined by F.K.Brown in 1998. Cheminformatics is transforming the data into information and information into knowledge for the intended purpose of making better decisions faster in the area of drug lead identification and optimization. Chemical information systems are concerned with storing, retrieving, and searching information about chemical compounds, and with storing relationships between bits of chemical data. These *In silico* techniques are used in pharmaceutical companies in the process of drug design. Chemical structures are represented *In silico* using formats such as XML based chemical Markup language or SMILES. These representations are often used for storage in large chemical databases. WLN was the first line notation to feature a canonical form, that is rules for WLN meant there was only one " correct'' WLN were able to write molecular structure in a line format , communicate molecular structure to one another and to computer programs. The best known and most widely used similarity metrics compare the two-dimensional topology that is; they only use the molecule's atoms and bonds without considering the shape. 3D Similarity Searches compare the Configuration of a molecule to other molecules. The "electronic surface'' of the molecule is the important bit – the part that can interact with other molecules, 3D searches compare the surfaces of two molecules, and how polarized or polarizable each bit of the surface is. 3D Similarity Searches are uncommon, for two reasons: It is difficult and it is slow. The difficulty comes from the complexity of molecular interactions. The properties are compared, which are computed or measured or both and declares that molecules with many properties in common are likely to have similar structure. "Clustering'' is the process of differentiating a set of things into groups where each group has common features. Molecules can be clustered using a variety of techniques, such as common 2D and/or 3D features.

MATERIALS AND METHODS

Quantitative Structure Activity Relationship (QSAR)

QSAR is a process whereby the structures of a set of compounds are qualified and then compared to the numerical values of a biological activity or a physical property. structure information and the measured property or activity is then processed into a mathematical model of relationship. An important advantage of QSAR is that it models the *in vivo* situation since it is based on activity data. Calculation of Quantitative Structure – Activity Relationship (QSAR) and Quantitative Structure property Relationship values used to predict the activity of a compound from its structure.

Pass Prediction

Biological Activity Spectrum (BAS)

Biological Activity Spectrum (BAS) of a compound represents the pharmacological effects, physiological and biochemical mechanisms of action, specific toxicity which can be revealed in compounds interaction with biological system. Finding the most probable new leads. Selecting the most prospective compounds for high throughout screening from the set of available samples.

Mathematical Approach

n is the total amount of compounds in the training set;
ni is the total amount of compounds, that have the descriptor i;
nj is the total amount of compounds, that reveal the activity j;
nij is the amount of compounds, that have both the descriptor i and the activity j;
Pi = αi nij / αi nj is the estimate of a prior i probability of activity j;

Algorithm of prediction

For the compound under prediction structural descriptors are generated,
For each activity the following values are calculated;
Ui = αiArc sin {ni (2pij-1)}, Uoj = αiArc sin {ri (2pj-1)}
sj= sin(Uj/m)

RESULT AND DISCUSSION

Preparing molecular structure files

Two Dimensional Structures of the selected compounds were converted into 3D structures using the chemical structure drawing tool the chemsketch.The 3D structures were saved into SD files.The structure files of the selected compounds were sent through Internet for predicting the Biological properties are shown in the Table: 1

Table 1. Predicting the Biological properties

S.NO	NAME OF THE COMPOUND	BIOLOGICAL PROPERTY
6.	1-2-4-Triazole -3-Alanine	*NMDA receptor agonist*
7.	2- Amino-3 Benzylaminopropanic acid	Hydroxy tryptamine
8.	2- Amino-3 Benzylaminopropanic acid.2	Hydroxy tryptamine
9.	2-Amino-3-Phenylbutanic acid	Hydroxy trytamine
10.	2-AminoAdipicacid-hydrate2	Phosphatase inhibitor
11.	2-Aminoadiphicacid-hydrtate	NMDA receptor agonist
12.	2-Aminoheptanoic acid	NMDA receptor agonist
13.	2-Amino-N-Caprylic acid	NMDA receptor agonist
14.	2S-3R-2-Amino-3hydroxy-4methylpentonicacid	Phosphatase inhibitor
15.	2S-3R-2-Amino-3-methoxybutonicacid	Membrane integrity

Prediction of Cluster Analysis

The SD files of selected compounds were opened into TSAR. Molecular properties concerning to sterric, lipophilic and electronic nature of the molecules were calculated. Using cluster algorthims, the molecules were grouped based on their similarities in their molecular properties were shown in the Figure:1

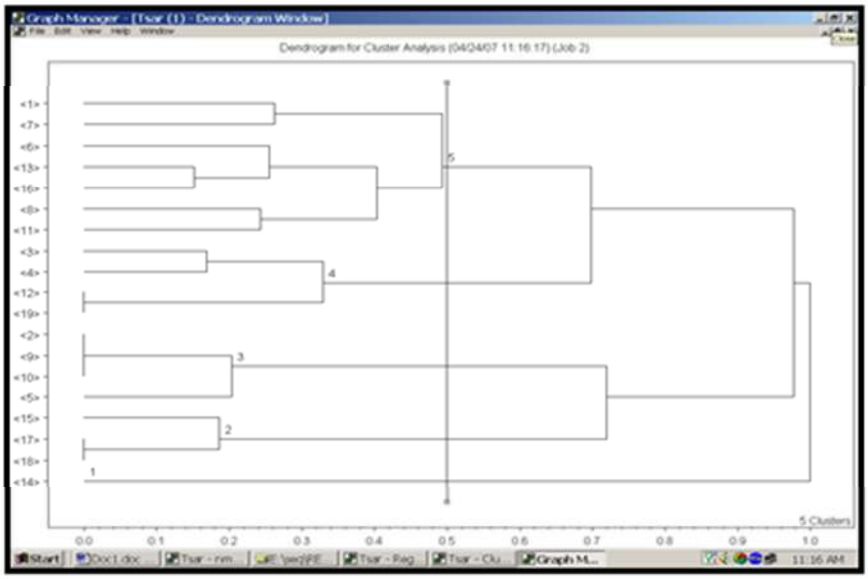

Figure 1. ***Clustering of nmda receptor glycine site agonist molecules***

Figure 2. ***Correlation matrix for nmda receptor glycine site agonist***

Correlation between molecular mass and NMDA receptor agonist property

A positive and negative correlation is done between molecular properties and NDMA receptors against property are shown in the Table: 2.

Table 2.

Correlation between NMDA receptor agonist activity and various molecular properties

Positive correlation	Negative correlation
Molecular volume logP	VAMP electronic energy
Hydrogen donor	Total molecular charge
Hydrogen acceptor	Heat of formation
VAMP molecular energy	Ionization potential
VAMP Mean polarizability	Total dipole

CONCLUSION

The selected compounds from Aphyllophorales can be divided into four major groups based on their Biological properties. NMDA receptor aganist are expected to promote the receptor function. The molecular properties

such as Hydrogen donor and Hydrogen acceptor and VAMP molecular energy showed a positive correlation. The correlation ship between certain molecular parameters and characteristic biological activity. This kind of analysis lends room for further modification of the molecules towards desired and improved biological activity.

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SP-21

STUDIES ON IN-VITRO BIODEGRADABILITY AND IT'S MECHANICAL PROPERTIES OF LDPE AND IT'S BLEND

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ABSTRACT

Samples of abiotically treated low density polyethylene (LDPE)(virgin and pro-oxidant additive added) is subjected to in-vitro degradation using a well defined bacterial culture of *Bacillus cereus* in shake flask culture. The LDPE films are first subjected to abiotic treatment with photo-oxidation (UV irradiation) and thermal-oxidation. The pretreated films were inoculated with the bacterial culture and the degradation is monitored for 12 months at various time intervals. The degradation of polymer is monitored by measuring the changes that occur in wettability and mechanical properties. The abiotic treatment introduces the formation of polar groups on the polymer surface. It initiates the bacterial adhesion. The biotic phase in progress showed the reduction in contact angle and increase in the surface energy. The film showed to lose its mechanical strength which was recorded in reduction of tensile strength of the bacterium treated samples. The spectroscopic studies reveal that the oxygenated products were taken up by the bacteria which in turn decrease the Carbonyl peak.

Keywords: ***LDPE, pro-oxidant additive, polymer, wettability, biotic.***

INTRODUCTION

Polyethylene as a thin film used as a packaging material. It has found its place in usage in many industries and business houses because of its excellent tensile strength, its resistance to microbes, low cost and easy availability [1]. In Landfill sites, it remains as it is due to its non-biodegradable nature. Albertson *et al* [2] studied the degradation of polyethylene in Sweden and he concluded that the degradation occurs at a rate of approximately 0.5 % of weight per annum. Daily about 600 tonnes of garbage is being piled at Vellalore dump yard, Coimbatore, Tamilnadu. According to a recent survey by the Central Pollution Control Board, plastic waste constitutes about 8.5 to 9.5 percent of the garbage, which ranges between 55 to 60 metric tonnes [3]. The inherent resistance of polyethylene to biological attack can be owing to its hydrophobic nature, higher molecular weight and the absence of any recognisable functional groups by microbes. The oxo-biodegradable additives contain specific transition metals (Mn, Fe and Co) as pro-oxidant. These compounds act as catalysts in speeding up the reactions of oxidative degradation with the overall reaction rate increased by several orders of magnitude. The products of the catalyzed oxidative degradation of plastics are precisely the same as for conventional plastics because other than the small amount of additive present, the plastics are indeed conventional plastics The disposal by degradation of these polymeric wastes into harmless and useful compounds is a challenging task to combat pollution. The extent of degradation of polymers depends on the nature of chemical process involved. The degradation process may be carried out by photochemical, thermal and biodegradation process. Out of these, biodegradation is much simpler, economic and occurs in normal conditions of waste disposal without any extra pre-requisites. The main attacking microorganisms like bacteria, actinomycetes, fungi etc., which are wide spread in the soil. Crabbe *et al*,[4] reported the polyurethane degrading soil fungi including *Fusarium solani, Curvularia senegalensis, Aureobasidium pillulans and Cladosporidium* sp. A number of bacteria were also claimed to degrade polyurethane namely, *Acinetobacter calcoaceticus, Arthrobacter globiformis, Pseudomonas aeruginosa, Pseudomonas cepacia and Pseudomonas putida* [5]. This study aims to identify the efficiency of *Bacillus cereus* strain Ma-Su (Bac) on low density polyethylene. In shake flask culture with the isolate, the in vitro polyethylene degradation was observed for 12 months and reported. Here the polymer is supplied to the organism as a sole source of carbon. The changes in its physical and mechanical properties at different time intervals were monitored.

MATERIALS AND METHOD

Preparation of low density polyethylene film

Virgin LDPE films were prepared using a film blowing machine using an extruder (Gurusharan Polymer Make) and LDPE film was designated as PE and films containing oxo-biodegradable additive were designated as BPE followed by a numerical suffix indicating the amount of additive added. LDPE films containing 10% and 20% oxo-biodegradable additive were designated as BPE10 and BPE20 respectively.

Abiotic treatment: photodegradation and thermooxidation procedure

Fifty micrometer thick blown film of PE, BPE 10 and BPE20 were irradiated with 40 W UV-B lamps generating energy between 280 and 370 nm with a maxima at 313 nm in air at room temperature (30±1 °C) on open racks positioned 5 cm from the lamp [6]. Exposures were conducted uninterrupted, 24 h per day and the samples recovered in 21 days. The UV-treated samples were then kept for thermal aging in a hot air oven for 10 days at 70°C. The combined abiotic oxidation provides the initial changes in the polymer and it creates the effect of one year outdoor weathering [7].

Biotic treatment

Bacterial system

The bacterial culture of *Bacillus cereus* strain Ma-Su CECRI-1 (Bac) was isolated from Municipal compost yard Vellalore, Coimbatore, Tamilnadu (The 16S rRNA gene sequence of strain has been designated *Bacillus cereus* strain Ma-Su CECRI-1 and deposited in the GenBank database under Accession No. GQ 501070). The mineral medium-C [8] was used for the culturing medium. The colonies were preserved at 4°C in 2% agar slants of medium B (5 % malt extract, 0.3% yeast extract and distilled water; pH 7±0.2) [8].

Chemical disinfection of films

The disinfection procedure used with each pre-treated and un-treated rectangular film strips (20 X 2.5 cm) were placed in the fresh solution containing 7ml tween 80, 10 ml bleach and 983 ml of sterile water and stirring for 30-60 min. The films were removed and placed into a covered beaker filled with sterile water and stirred for 60 min at room temperature. The films were then aseptically transferred into ethanol solution 70% (Vol/Vol) and left for 30 min. The films were then placed into sterile petri dish and incubated at 45-50°C to dry overnight, allowed to equilibrate to room temperature [9]

In-vitro shake flask culture

Pre-weighted disinfected pre-treated films (20X2.5cm) were aseptically added to 100ml aliquots of filtered (0.22mm Millipore filter paper) medium - C. Log phase seed culture 3% (V/V) was added to these aliquots and they were kept under shaking at 180 rpm at 30°C (fig.1). The numbers of films taken were such that samples could be withdrawn after 1, 3, 6, 9 and 12 months. Also, all the samples prepared and run simultaneously in triplicate. Mineral medium-C containing polymer without the microorganism was maintained as positive controls [10]. Streak culture method was used for identifying any contamination in the medium or any possible changes to the morphology of the organisms. The growth of the bacterial culture containing polymer was monitored by counting the colony forming units (CFU). Fresh filtered Mineral medium-C was added after 6 months to make up. Three sets of abiotically treated polyethylene samples were analysed in each category of (a) PE, (b) BPE10 and (c) BPE20;

(a). PE

(i) Treated PE (TPE) unexposed to strain Bac [control] and (ii) Treated PE exposed to strain Bac (TPE+Bac)

(b). BPE10

(i) Treated BPE10 (TBPE10) unexposed to strain Bac [control] and (ii) Treated BPE10 exposed to strain Bac (TBPE10+Bac)

(c). BPE20

(i) Treated BPE20 (TBPE20) unexposed to strain Bac [control] and (ii) Treated BPE20 exposed to strain Bac (TBPE20+Bac)

Colony forming unit (CFU)

Bacterial culture growths for the 12 months were monitored by using plating method. In this method 1 ml of bacterial culture was serially diluted and inoculated on the surface of nutrient agar solid media in sterile

petridishes and incubated for 24 h. viable colony counts were obtained by appropriately diluting the sample [11] at different incubation periods. Colonies were counted by using digital colony counter (Scigenics, Chennai, India).

$$\textbf{Number of bacteria per mL} = \frac{Number\ of\ CFU}{Volume\ plated\ (ml) X\ total\ dilution\ used}$$

Fourier transformed infrared spectroscopy (FT-IR)

The surface chemical modifications which occur in LDPE films upon thermo-oxidation were investigated using FTIR spectroscopy. The FTIR spectra were recorded using a Thermo Nicolet, Avatar 370 spectrophotometer in the spectral range between 4000-400 cm^{-1}.

Mechanical strength test

Tensile tests were carried out at air-conditioned environment at 21°C and with a relative humidity of 65 %, and the samples were subjected to a crosshead speed of 100 mm/min. Changes in mechanical properties like tensile strength and elongation at break were performed on LDPE films according to ASTM 882-85 using INSTRON machine (no. 6021). Films of 100 mm length and 25 mm width were made as strips. The value is the average of five samples for each experiment.

RESULTS AND DISCUSSION

Pre-treatment initiated abiotic degradation

The abiotic pre-treatment involved the exposure of PE and BPE films to UV-B radiation for 21 days and thermal oxidation for 10 days. After this period, (PE and BPE samples after undergoing the light and heat treatment) the polymer is chemically modified and is more susceptible for microbial attack [13]. The catalytic degradation in the presence of transition metal in polyethylene has been due to its ability to generate free radicals on the surface of polyethylene, which later react with oxygen to generate carbonyl groups found evident in FTIR results [14, 15].

Microbial growth on polymer containing medium

The growth of bacteria as a function of biotic exposure time is presented in Fig.2. It has been suggested that carbon limitation may facilitate the slow growth on polyethylene films such as lignin [16]. Among the polyethylene films, TBPE20 enhances the growth of *B.cereus* (Bac) compared with the untreated LDPE sample. The culture flask containing TBPE20 inoculated with *Bacillus cereus* (Bac) showed 4.1 X 10^4 CFU/ml initially at 15 days of incubation and 5.8 X 10^3 CFU/ml after 12 months of incubation. The samples of TBPE10 initiate the colonies of 3.6 X 10^4 CFU/ml at 15 days of incubation and after one year it gradually increased to 5.3 X 10^4 CFU/ml. The TPE flask sample showed 2.8 X 10^4 CFU/ml initially at 15 days of incubation and 4.8 X 10^4 CFU/ml after 12 months of incubation. The pre-treatment with UV and thermal oxidation before incubation has increased the biodegradation by facilitating the bacterial growth in TBPE 20 than the TPE10. The bacterial growth in treated samples initially declines to 10^4 CFU/ml from 10^5 CFU/ml. This implies the bacteria will take some time period to acclimatise itself with the new environment. Then after 15 days it starts to utilize the oxidised smaller polyethylene fragments. In samples of TPE10 and TPE20 the growth found maximum till 60 days and continued a study state of growth. But in TBPE20 sample containing medium, the growth exhibits a steady increase in the number of bacteria till the end of the year. This is because the bacterium was capable of utilizing the polyethylene. It has also been reported that the microorganisms produce necessary oxidative and degradative enzymes for degrading and assimilating the polymeric carbon into their biomass and thus divide and grow in number [17, 18]. The pre-treated PE, BPE10 and BPE20 uninoculated samples act as control. All the flasks were monitored for contamination at regular time intervals by plating technique and no contamination was found.

FTIR studies

The FTIR spectra of pre-treated TPE, TBPE10 and TBPE20 control and with *B.cereus* (Bac) are shown in Fig. 3 a & b. The focus here are the 1720, 1740, 1640 and 3050-3550 cm^{-1}, corresponding to ketone carbonyl (-C0-); ester carbonyl (-COO-), internal double bonds (-C=C-) and hydroxyl (-OH-) respectively. The (fig.3 a) TPE, TBPE10 and TBPE20 [control samples] without bacteria (Bac) shows a marked increase of carbonyl groups at 1723 cm^{-1} after 12 months of degradation. It may be contributed due to the continuous

abiotic degradation initiated the free radical by the abiotic pre-treatment. But the PE, BPE10 and BPE20 samples with bacteria (Bac), clearly visualized the gradual increase and a little reduction of carbonyl groups at 1723 cm^{-1} due to bacterial utilization (fig. 3 b). The rate of decrease in carbonyl peak in TPE sample is much less than the rate of decrease in TBPE20 inoculated with bacteria indicating lower biodegradation. TBPE20 sample shows lower carbonyl index, and it also undergoes highest biodegradation. It implies the added pro-oxidant additive produces more carbonyl groups on the surface for bacterial attachment and also produces polyethylene chain session. FTIR as a tool for differentiating between abiotic and biotic degradation of LDPE has also been reported by Albertsson *et al* [2]. They have noted that samples stored in air increased their carbonyl index with time, but all samples in contact with soil showed a decrease of carbonyl index with time. Others have also observed a continuous increase in the amount of carbonyl compounds with exposure in an abiotic environment as against a decrease in the biotically aged samples [19, 20, 18, 9]. Albertson *et al* [19] and Weiland *et al* [21] observed a reduction in the carbonyl group after 650 days of incubation with a mixed fungal culture. Norrish type – II reaction leads to the formation of double bonds in the polymer chain Ester and keto carbonyls have also been reported as major products formed during abiotic oxidation of polymer under thermal oxidation or in the presence of enzymes such as oxidoreductase [2]. A negligible increase in carbonyl index was observed for all samples during the thermal pre-treatment at 70°C for 10 days similar to the observations made by Khabbaz *et al.* [22, 23]. These authors observed increase in carbonyl index after treatment at 100°C for 10 days [22]. This could be due to the formation of loose PE chain fragments during the heat & UV treatment and when later exposed to microbes underwent higher oxidative products leading to the formation of carbonyl and double bond. In the present study, the fraction of the terminal/vinyl double bond (910 cm^{-1}) was higher than that of internal double bond. Hence the surface modification by abiotic pre-treatment plays a vital role in bacterial attachment on PE films. It is also well correlated with the SEM micrographs and wettability results of this study. In the process of biodegradation of polyethylene, the abiotic oxidation process initiate's the process of initiating a free radical in the polymer chain. The oxidation of the polymer may obtain the oxygen from the dissolved oxygen or from the atmosphere and forms the carbonyl groups. These eventually form carboxylic groups, which subsequently undergo β-oxidation [2] and are totally degraded via citric acid cycle that is catalysed by microorganisms. Monitoring the formation or disappearance of acids (1715 cm^{-1}), ketones (1740 cm^{-1}) and double bonds (1640 and 915 cm^{-1}) using FTIR is necessary to elucidate the mechanism of the biodegradation process. Figure.3 b shows the FTIR spectra for pretreated PE , BPE10 and BPE20 as a function of time exposed to *Bacillus cereus* strain (Bac). The initial rise in the carbonyl peaks are attributed to the oxidation by the dissolved oxygen (abiotic factor). The prolonged exposure to organism leads to reduction in carbonyl index probably due to biodegradation (biotic) through Norrish – type mechanism or through the formation of ester.

Mechanical properties

The Fig. 4., shows the tensile strength of the pre-treated and untreated samples of PE before and after 12 months of biodegradation with *Bacillus cereus* (Bac).The initial tensile strength of 0-day treated (TPE) sample is of 15.96 MPa. After 12 months of incubation in media without microbe they did show any marked reduction and ends in 15.23 MPa. But in case of TPE with *Bacillus cereus* (Bac), they showed a little reduction to 14.01 MPa. The corresponding reduction in (fig 5) elongation at break was found at 412.41 from the initial value of 564.72. The PE blended with 10% additive, on 0-day TBPE10 showed 15.53 MPa with elongation at break at 682.2. After 12 months without bacteria in medium the sample exhibit 14.98 MPa tensile strength and 461.9 in elongation at break. The test samples inoculate with *Bacillus cereus* (Bac) showed the reduction of 13.65 Mpa of tensile strength and 231.52 in elongation at break. The PE added with 20% additive, on 0-day TBPE20 showed 15.91 MPa with elongation at break at 671.8. After 12 months (control) without bacteria in medium the sample exhibited 14.76 MPa tensile strength and 423.27 in elongation at break. The test samples inoculate with *Bacillus cereus* (Bac) showed the reduction of 11.06 Mpa of tensile strength and 362.7 in elongation at break. The abiotic exposure governs the reduction of its tensile strength slowly when compared to biotic tensile strength reduction [18]. The abiotic oxidation produces random chain session and it was further degraded by microbial oxydoreductase and catalase enzymes to render the polyethylene to lose its physical strength [24].

Figure 1. ***Experimental setup of shake flask culture***

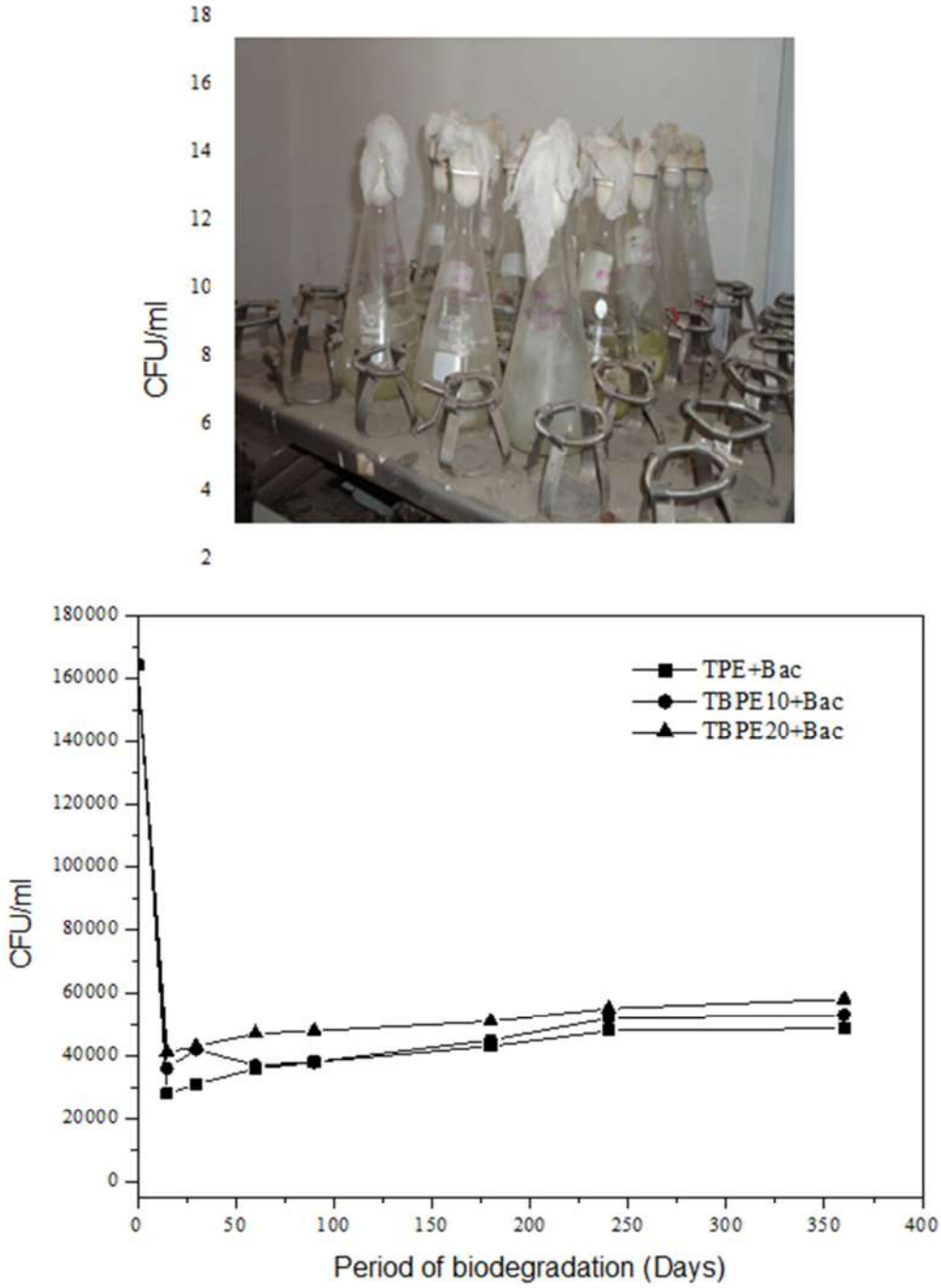

Figure 2. ***CFU count for various LDPE samples inoculated with Bacillus cereus (Bac)***

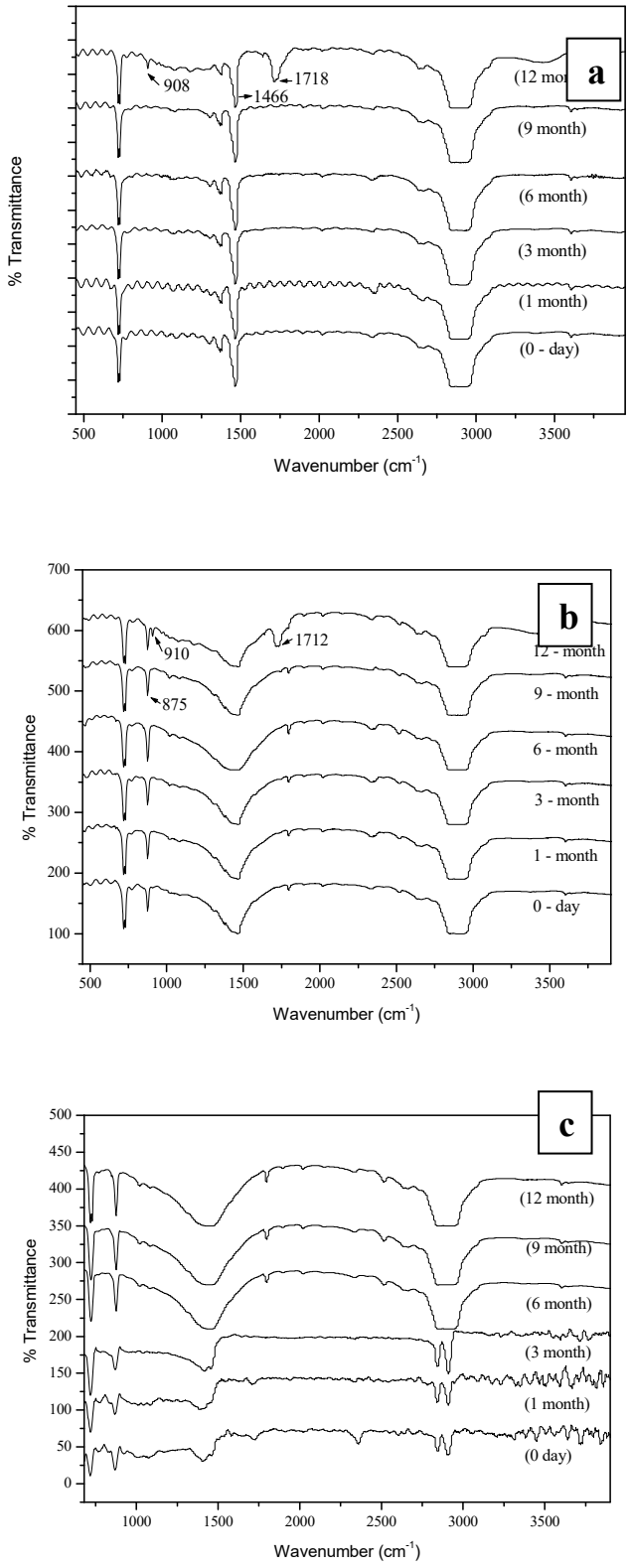

Figure 3.a. ***FTIR spectra of (a) pre-treated TPE, (b) TBPE10 and (c) TBPE20 unexposed to Bacillus cereus (Bac)***

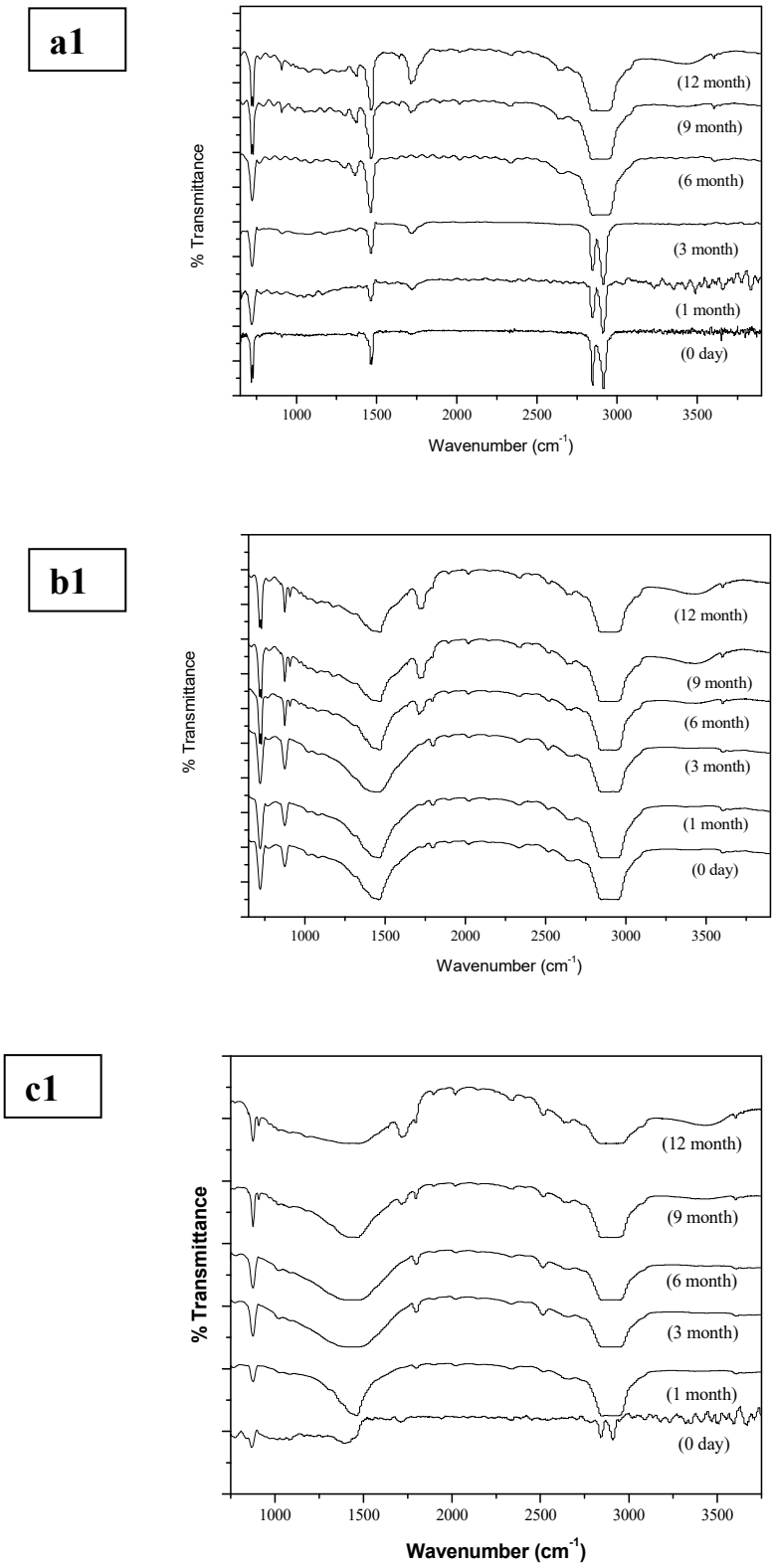

Figure 3.b. ***FTIR spectra of (a1) pre-treated TPE, (b1) TBPE10 and (c1) TBPE20 exposed to Bacillus cereus (Bac)***

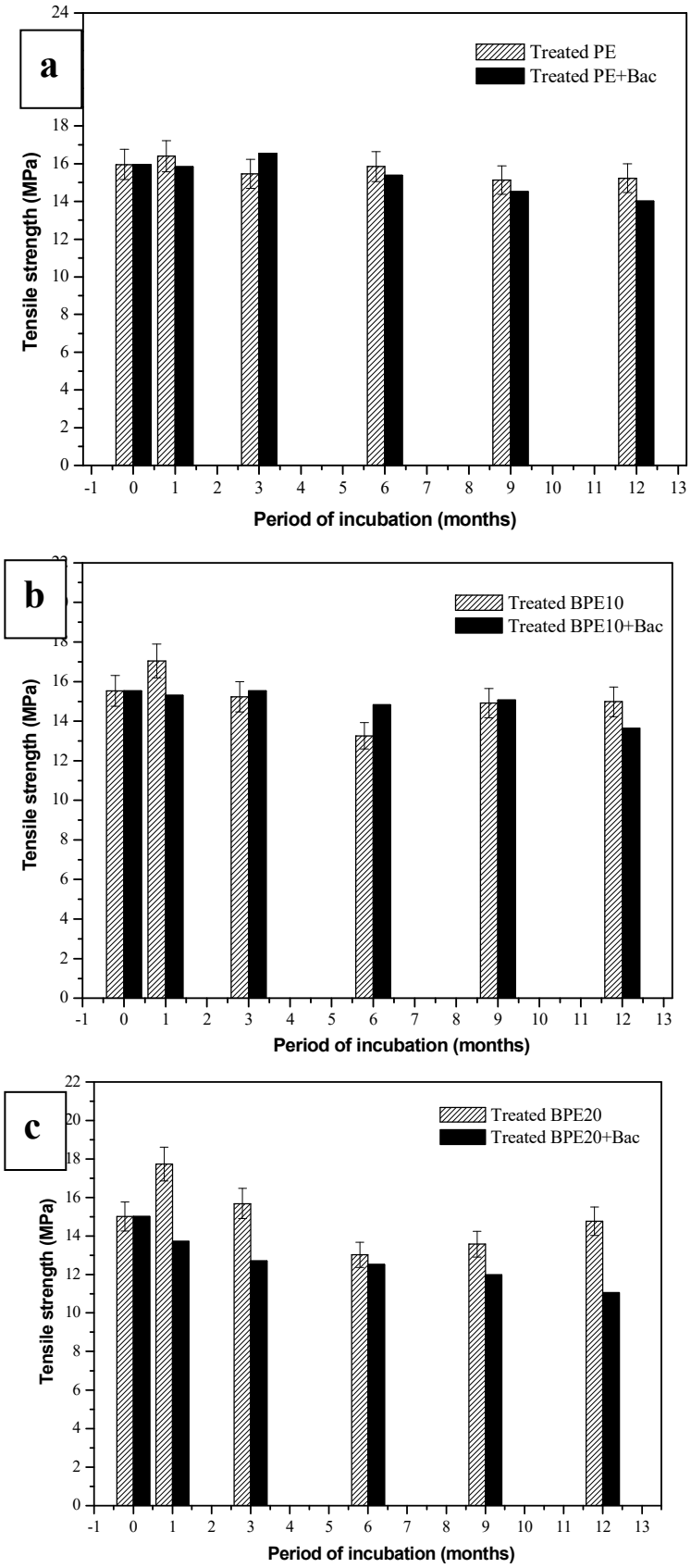

Figure 4. ***Variation in tensile strength of (a) pre-treated TPE, (b) TBPE10 and (c) TBPE20 samples unexposed (control) and exposed to Bacillus cereus (Bac).***

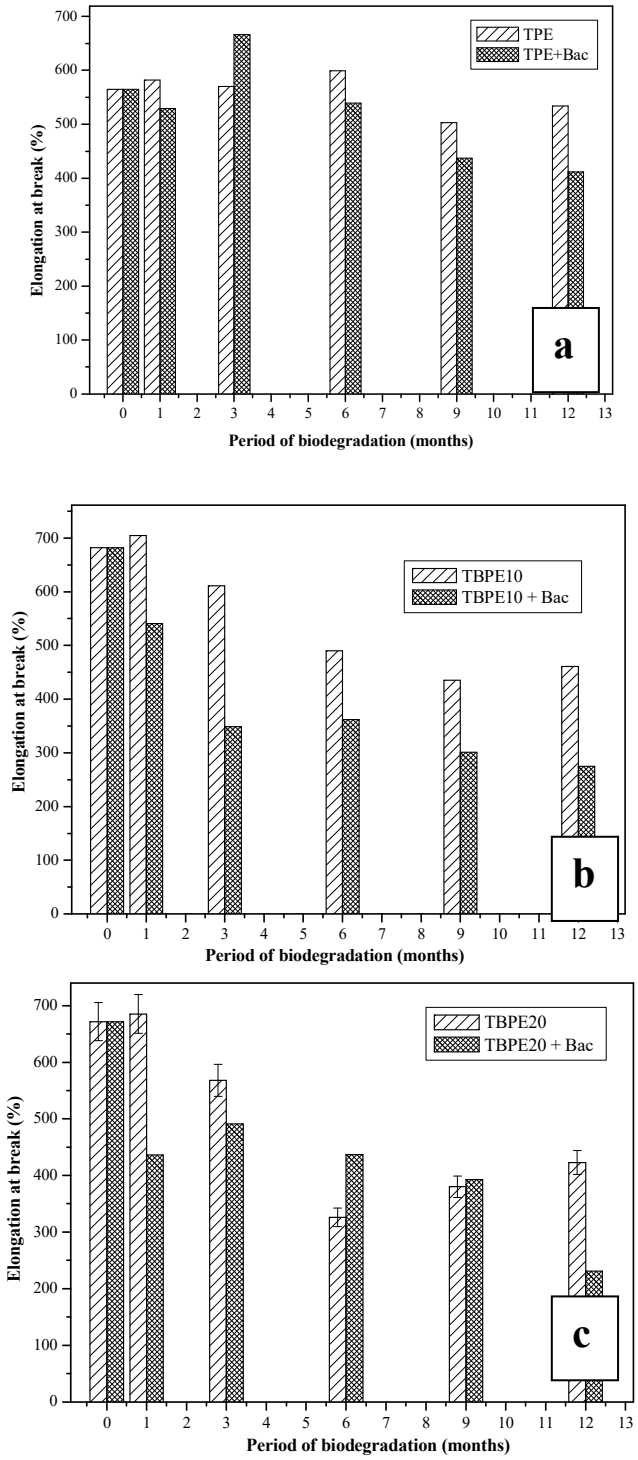

Figure 5. ***Percentage elongation of (a) pre-treated TPE, (b) TBPE10 and (c) TBPE20 samples unexposed (control) and exposed to Bacillus cereus (Bac).***

CONCLUSION

In conclusion the present study reveals that potential isolate of *Bacillus cereus* (Bac) from the municipal compost yard soil capable of degrading low density polyethylene. The strain exhibits the ability to utilize polyethylene as a carbon source. The introduction trace quantities of pro-oxidants initiate the abiotic degradation of polyethylene film in TBPE10 and TBPE20. The pre-treatment with UV and thermal oxidation of polyethylene films were capable of supporting the microbial growth. The abiotic phase leads to modify the carbon backbone and chain session simultaneously of the polymer. The oxygenated compounds and the low molecular weight hydrocarbons formed on the surface of the polymer as a result of abiotic treatment, are recognised by the bacterial strain (*Bacillus cereus* (Bac)), which is capable of utilizing it and grow in number. The little decrease in carbonyl group is due to biodegradation of polyethylene through Norrish (biotic) type mechanism [25]. As a result of bioerosion, the film exhibits (i) decrease in tensile

strength, elongation at break and contact angle;(ii) introduction of new polar groups such as –OH, C=O, COOH and COO- on the main chain of the polymer matrix. On absorption of energy in the form of light, the components present in the pro-oxidant additive forms free radicals. These species can combine with oxygen from air, that generates the introduction of polar groups such as –OH, C=O, COOH and COO- on the main chain of the polymer matrix. The presences of these groups were confirmed through FT-IR spectra. It leads to chain session in LDPE which alters the hydrophobic nature of the surface [26]. This phenomenon was relatively slower in PE films without pro-oxidant additive. Thus the new bacterial isolate *Bacillus cereus* Ma-Su (Bac) shows the potential of polyethylene biodegradation. The pro-oxidant added polyethylene seems to be highly susceptible for the isolate *Bacillus cereus* Ma-Su (Bac) attack as the pro-oxidants initiates the abiotic degradation process and makes the low molecular weight polyethylene available for microbial assimilation. These kinds of bacterial isolates and modified polyethylene will be helpful in the biodegradation of polyolefins in the near feature to overcome the plastic litter pollution.

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SP-22

SCREENING OF BIOACTIVE COMPOUNDS FROM MARINE ACTINOMYCETES

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ABSTRACT

Antibiotic drug resistance was increasing among pathogens in recent years, so there is an emergency in need of new drugs to be developed. Bioactive compound from the natural sources can be used as a hit molecule in the development of the new drug candidate against the drug resistant pathogens. In this study, eight isolates was obtained from marine sediment that was collected from van island of Tuticorin district, Tamil Nadu, India. The isolate S8 showed inhibitory effect against *Staphyloccus aureus* ATCC 6538 in both well diffusion and disc diffusion methods. From the molecular analysis, the antagonist isolate S8 was found to be *Streptomyces* sp. which was confirmed using the morphological and cultural characteristic and it was named as *Streptomyces* sp S8. The maximum antibacterial activity was obtained when the media consists of starch (2% w/v), tryptone (1% w/v), 3%-4% NaCl concentration and at the neutral pH. Purification of bioactive components was carried out using the chromatography techniques. A maximum absorbance of the partially purified compound from isolate S8 was at 260-270 nm respectively. FTIR and GC MS analysis also confirmed the presence of the carboxyl and keto functional groups revealed the presence of compounds with antimicrobial and anti-oxidant property.

Keywords: ***Marine Environment, Streptomyces sp, Staphylococcus aureus, antibacterial activity***

INTRODUCTION

Multidrug resistance pathogens emerged due to continuous exposure of antibiotics in treatment against pathogens and further resistant was enhanced by self-medication, utilizing antibiotics for other purpose rather than health care system. New and effective lead compounds from natural resources have to be discovered hastily in order to compete against multidrug resistant pathogens. In general, two third of the earth surface was covered by marine environment. Marine environment has the resources for the discovery of numerous natural products with potential pharmaceutical properties and these marine natural products are derived from animals, plants and microorganisms (Aneiros and Garateix, 2004). The biomass of the marine sediments predominantly constitutes the microorganisms which are playing a key role in nutrient cycling, metabolism of pollutants and secondary metabolite production. Antibiotics from natural source, can be either bactericidal or bacteriostatic and most of them are derived from bacteria or fungi (Fenical and Jenson, 2006). *Streptomyces* are the filamentous, Gram-positive, spore forming bacteria that can synthesize different kind of biologically important therapeutic compounds. The members belonging to the *Streptomyces* from the unexploited marine sediment harbors the unexplored compound with unique chemical diversity for pharmaceutical lead compounds when compared to the terrestrial microbes. The antimicrobial natural products biosynthesized by the marine *Streptomyces* have immense applications in the human and veterinary medicine to treat infectious diseases caused by MDR pathogens.

MATERIALS AND METHODS

Isolation and screening of the antagonistic isolate

The marine sediment sample was collected from Van Island, Tuticorin district of Tamilnadu, India. The standard serial dilution method was done to isolate actimomycetes strains in the Actinomycetes isolation medium. The screening of the isolate with antibacterial activity was carried out using well diffusion and disc diffusion method against *Staphylocuss aureus* ATCC 6538 culture.

Morphological characteristics of isolate S8

After antibacterial activity, the cultural characteristics of the isolate S8 was studied in different ISP (International *Streptomyces* Project) medium. Various ISP media (ISP1-ISP7) were used to identify the morphological characteristics of isolate S8 (Shirling and Gottleib, 1966). The different ISP media culture plates were incubated at room temperature for 5-7 days. Utilization of various carbon (1% w/v) and amino acid (0.5% w/v) sources by isolate S8 was studied by using the basal medium.

Molecular Characterization of the isolate S8

Genomic DNA isolation

Genomic DNA from the isolate S8 was isolated according to the method described by Hopwood *et al* (1985). The spore suspension of about 10 µl was inoculated in 100 ml of NDYE broth and incubated 30°C at 120 rpm for 36 h. Cells were harvested and centrifuged 6000 rpm for 20 min at 4°C. After centrifugation, the cell pellet was suspended in 5 ml of sucrose TE-buffer and incubated at 37° C for about 1 h. About 1.2 ml of 0.5 M EDTA (pH 8.0) was added to the microfuge tube and incubated at 30° C for 5 min. Then 0.7 ml of 10 % SDS was added and incubated at 37° C for 2 h. After incubation 6.0 ml of Tris- saturated phenol was added and kept for 5 minutes incubation at room temperature. The cells were centrifuged at 6000 rpm for 20 min at 4°C. Equal volume of Phenol/Chloroform/Isoamyl alcohol (25:24:1) was added and the tubes were centrifuged till the two layers separated from each other. The upper aqueous phase was transferred to another tube with twice the volumes of ice cold isopropanol was added. The mixture was then centrifuged at 6000 rpm for 10 min at 4°C. 1.0 ml of 70% ethanol was added to the pellet and the tubes were centrifuged at 6000 rpm for 10 min at 4°C. The DNA pellets were allowed to air dry at room temperature for 30 min and 30 µl of TE buffer containing 10 µg/ml RNase A was added and stored at 4°C.The 16S rRNA gene of the isolate S8 was amplified by using universal primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGTTACCTTGTTACGACTT-3'). The PCR mixture contained 10X PCR buffer (5 µl) (Sigma Aldrich, India), dNTP (1.2 µl), forward primer (4 µl), reverse primer (4 µl), Taq DNA polymerase (0.5 µl) (Sigma Aldrich, India), genomic DNA (0.2 µl) as a template and finally de-ionized water (33.3 µl) to a final volume of 50 µl. PCR amplification was carried out with an initial denaturation of 5 min at 94ºC followed by 31 cycles of 30 seconds denaturing at 94ºC, 1 min annealing at 54ºC and 2 min extension at 72 ºC, followed by 20 min final extension at 72 ºC. The PCR product was sequenced and the obtained sequence was compared with the other sequence in NCBI using the BLAST programme.

Media optimization for the antagonistic isolate S8

Optimization of carbon source

In order to determine the suitable carbon source, seven different carbon sources such as sucrose, glucose, fructose, mannitol, xylose, lactose, starch and maltose were supplemented at 2% w/v to the basal medium. The effect of medium without carbon source was also determined. The effective carbon source was selected based on antibacterial activity of the bioactive compound synthesized by the antagonistic isolate. The antibacterial activity was defined as units of activity per milliliter (U/ml), in which IU was defiend as 1.0 mm clearing zone around the antibiotic disc.

Optimization of Nitrogen source

Five different organic nitrogen sources were used in this study to identify the effective nitrogen source for the production of bioactive compounds. The nitrogen source was added individually at 1% w/v to the flasks with optimized carbon source at 2% w/v respectively. The production of bioactive compound without nitrogen source was also studied. The suitable nitrogen source was selected based on antibacterial activity of the bioactive molecule synthesized by the antagonistic isolate.

Optimization of NaCl Concentration

The suitable NaCl concentration was identified by optimizing the NaCl concentration that ranges from 1% to 6% w/v respectively. The NaCl source was added individually from 1% to 6% w/v to the flasks with optimized carbon source at 2% w/v and nitrogen source at 1% w/v respectively . The effect of medium without NaCl was also checked. The suitable nitrogen source was selected based on antibacterial activity of the bioactive molecule synthesized by the antagonistic isolate.

Optimization of Suitable pH

The suitable pH of the medium was selected by ranging the pH from acidic to alkaline condition (pH5 to pH 9). The suitable pH source was selected based on antibacterial activity of the bioactive compound synthesized by the antagonistic isolate.

Mass fermentation of the isolate S8

The Antagonistic isolate S8 was cultured in the optimized fermentation medium which contains 20.0 g Starch, 1.0g Tryptone , 4.0g NaCl to the basal media that consists of 1.0 g K_2HPO_4, 1.0 g $MgSO_4.7H_2O$, 0.01 g $FeSO_4.7H_2O$, 1.0 g $CaCo_3$ and 1.0 g KH_2PO_4 in 1000 mL of distilled water at pH 7.0 respectively (Saranya et al., 2017). The fermentation media was incubated at 28ºC for 7 days. The fermented broth was centrifuged to remove the cells. The cell- free culture filtrate was then extracted with equal volume of ethyl acetate. The extracted solution was concentrated by evaporation. Concentrated bioactive compound after evaporation was once again dissolved in ethyl acetate and the crude extract was subjected to chromatographic separation and then it is characterized by different spectroscopic methods.

Purification and characterization of bioactive compound from the isolate S8

Thin layer chromatography (TLC)

Thin layer chromatographic profiling was used for the analysis of crude compound obtained from the marine isolate S8. The crude compound was spotted on the baseline of the silica gel plates (activated at 100°C for 15 minutes) at 1.0 cm and then allowed to dry at room temperature. Then the plates were placed in pre-saturated TLC chamber with different mobile phase and different concentration. Finally the suitable mobile phase for separation of bioactive compounds obtained from marine isolate S8 was identified. The fraction obtained as a result of TLC was subjected to further spectroscopic analysis.

UV-Visible spectral analysis

The absorption spectrum of the bioactive compound obtained from the marine isolate S8 was determined in the UV-VIS region by using a UV-VIS Spectrophotometer – UV-1800 Shimadzu. In X-axis, Wavelength was plotted and in Y-axis, the absorbance intensity of the bioactive compound obtained from marine isolate S8 was plotted.

FT-IR spectroscopic analysis

The FT- IR spectra of the bioactive compound obtained from the marine isolate S8 was measured on Shimadzu FT-IR 8400S series instrument. The FT- IR spectrum was recorded in the region that ranges from 500 to 5000 cm^{-1} respectively. The intensity was denoted in X-axis and wavelength was given in Y-axis of the FT-IR Spectra.

GC-MS analysis

The fraction that exhibits inhibitory effect was analyzed in GC-MS (GC-Trace Ultra Ver 5.0, MS DSQ II Thermo Scientific, MA, and USA). The column was used in a dimension of 30 mm with the DB35-MS capillary standard non-polar column. The source used was electron Bombardment (EI) ion source at 70 ev. The carrier gas used in this study was helium at a flow rate of 1.0 ml/min. The initial oven temperature was at 50ºC and but it was gradually increased to 260ºC at 10ºC/ min. The spectrum was scanned in the range that varies from 50-650 amu.

RESULT AND DISCUSSIONS

A total of eight isolates were obtained by isolation in Actinomycetes isolation media. Among the eight isolates, the supernatant of the isolate S8 exhibited antibacterial activity of about 9 mm against *Staphylococcus aureus* by well diffusion method. From the disc diffusion method, a zone of inhibition of about 18 mm was observed.

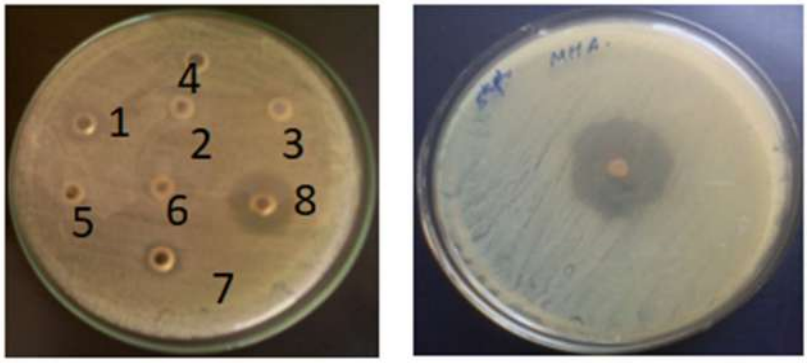

Fig 1: ***Antibacterial activity of the isolate S8 by Well diffusion and disc diffusion method.***

Morphological characteristics of the isolate S8

Morphological characteristics of the isolate S8 were determined in various ISP media (International *Streptomyces* Project media). Their morphological characters are listed below in table 1.

Medium	Aerial mycelium	Substrate mycelium	Diffusible pigment	Shape &growth	Elevation
ISP 1	Grey	Yellow	Yellow	Round	Not Elevated
ISP 2	Grey	Yellow	Yellow	Rough, irregular	Elevated
ISP 4	Grey	Greyish yellow	Yellow	Round	Elevated
ISP 6	Grey	Yellowish orange	Grey	Round	Not Elevated
ISP 7	Yellow	Yellow	Yellow	Round	Not elevated

Table 1: ***Morphological characteristic of the isolate S8***

Growth characteristics of the isolate S8 in different carbon sources and amino acid sources has been listed in table 2 & 3.

S.NO	Name of the carbon source	Growth characteristics
1	Mannose	Moderate growth
2	Rhamnose	Moderate growth
3	Dextrose	High growth
4	Maltose	Moderate growth
5	Adonitol	No growth
6	Raffinose	No growth
7	Melibinose	No growth -
8	Arabinose	Moderate growth
9	Lactose	High growth
10	Dulcitol	No growth
11	Inulin	No growth
12	Galactose	High growth
13	Fructose	High growth

Table 2: ***Growth characteristic of isolate S8 in different carbon source.***

S.NO	Name of the amino acid	Growth characteristics
1	Aspartic acid	High growth
2	Cystine	High growth
3	Alanine	High growth
4	Glumatic acid	Moderate growth

5	Leucine	No growth
6	Phenylalanine	High growth
8	Glumatic acid	High growth

Table 3: ***Growth characteristic of isolate S8 in different amino acid source.***

The isolate S8, grows only in the low concentration of phenol, which is only at 0.1% whereas it doesn't grow when the concentration was increased (Table 3). From this it is clear that the isolate S8 will tolerate phenol concentration up to 0.1 % only.

S.NO	Phenol concentration (%)	Growth characteristics
1	0.1%	Medium growth
2	0.2%	No growth
3	0.5%	No growth
4	1%	No growth

Table 4: ***Growth Characteristic of the isolate S8 in different phenol concentration.***

Molecular characterization of isolate S8

Genomic DNA was isolated and the 16S rRNA gene of the isolate S8 was amplified by universal primers and amplified product was sequenced. From the BLAST analysis, it was found to be *Streptomyces* species and named as *Streptomyces* sp S8 respectively. The *Streptomyces sp* S8 was having 95.80% identity with *Streptomyces sp* MK45 chromosome (CP034539), 91.22% identity with *Streptomyces sp* 21142 genome assembly and 91.22% identity with *Streptomyces ceolicolar* A3(2) complete genome respectively.

Thin layer chromatography

After mass fermentation crude compound was obtained from the marine isolate. Two bands were visualized when chloroform and methanol was used in 4:1 ratio. After the separation by TLC, two fractions were collected and antibacterial activity was done. The fraction with the antibacterial activity was taken for the further spectroscopic analysis.

UV-Visible Spectroscopic analysis

From the UV-Visible analysis, the maximum absorption of the bioactive compound obtained from marine isolate S8 was found to be at the wavelength of about 260 nm to 270 nm respectively.

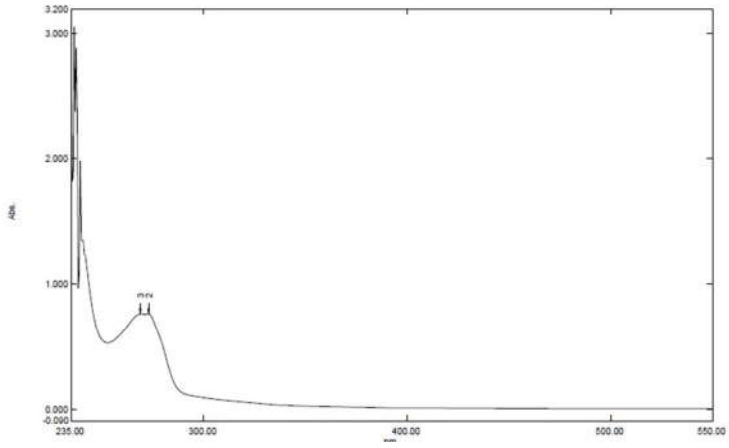

Fig 2: ***UV-Vis analysis of isolate S8***

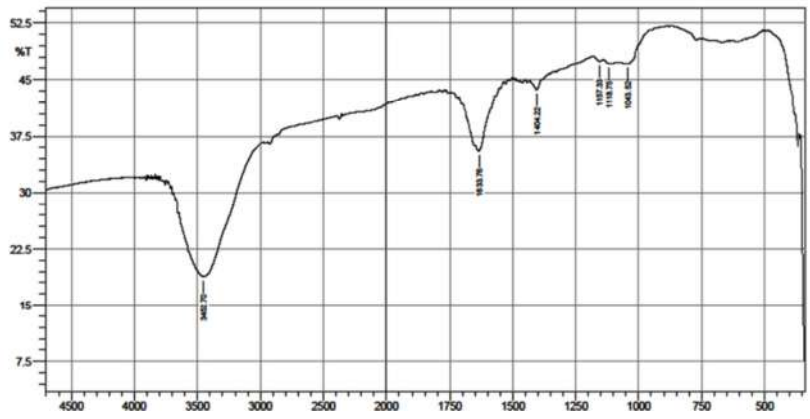

Fig 3: ***FT-IR analysis of the bioactive compound from isolate S8***

From the FTIR analysis, the presence of functional groups such as Carboxyl and Keto group was confirmed (John Coates., 2000).

Optimization of Carbon source

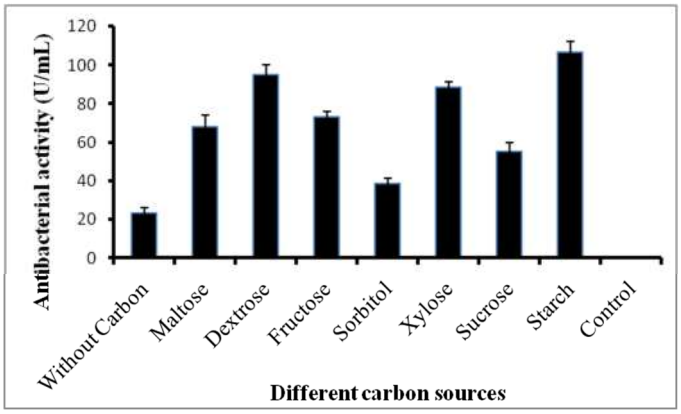

Fig 4: ***Optimization of different carbon source***

Seven different carbon sources were utilized to obtain the suitable carbon source for the enhanced antibacterial activity. Out of seven sources, starch recorded the highest antibacterial activity followed by Dextrose, Xylose, Fructose and Maltose. The media without carbon source exhibited minimum inhibitory effect. Ethyl acetate disc was used as negative control. Similarly Rakesh *et al.,* 2014 also reported that starch was the suitable carbon source for maximum antibacterial activity. It was also reported that there was increase in antimicrobial activity, when the concentration of starch was increased.

Optimization of Nitrogen source

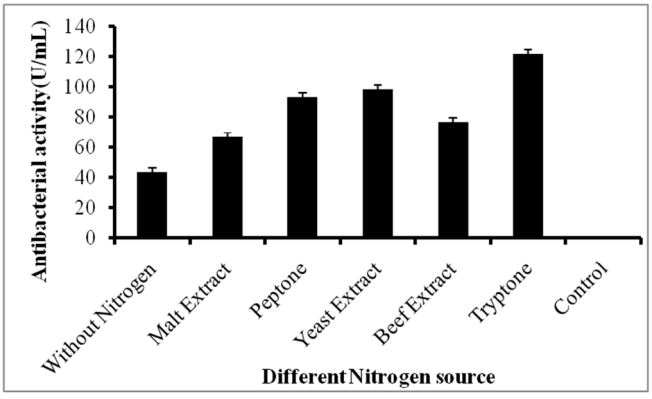

Fig 5: ***Optimization of different nitrogen source***

Tryptone was selected as a suitable nitrogen source among the five different nitrogen source used in this study. Tryptone followed by yeast extract and peptone exhibited the highest antibacterial activity when compared to the other nitrogen sources. Malt extract and Beef extract exhibited the moderate activity when compared to other nitrogen sources respectively. There was a minimum activity in the media without nitrogen source. Kiranmayi *et al*., 2011 reported that the tryptone at 0.25% w/v, enhances the bioactive compound production in the actinomycetes *Pseudonocardia* species VUK-10 which exhibited broad spectrum antibacterial activity.

Optimization of NaCl Concentration

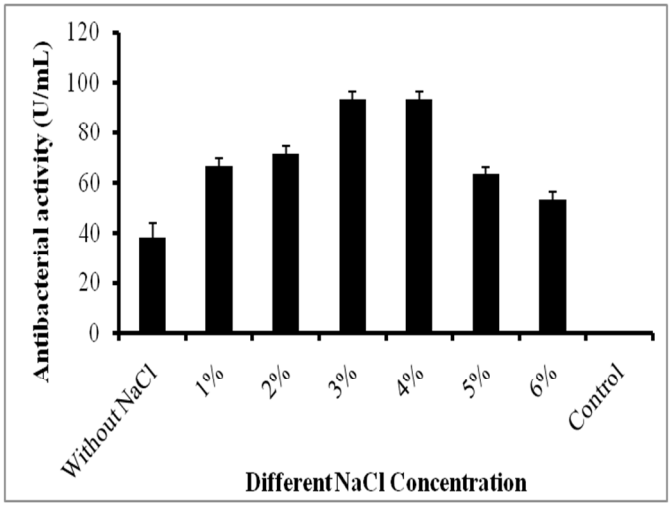

Fig 6: ***Optimization of NaCl Concentration***

The NaCl concentration was varied in the range of 1% to 6% concentration respectively. The maximum antibacterial activity was obtained in the range of 3% to 4% w/v concentration. The media without NaCl concentration exhibited low antibacterial activity against *Staphylococcus aureus* ATCC 6538. Zhang et al., 2016 observed that the bioactive compound production by *Streptomyces lacrimifluminis* was maximum at the 4% NaCl concentration respectively.

Optimization of Suitable pH

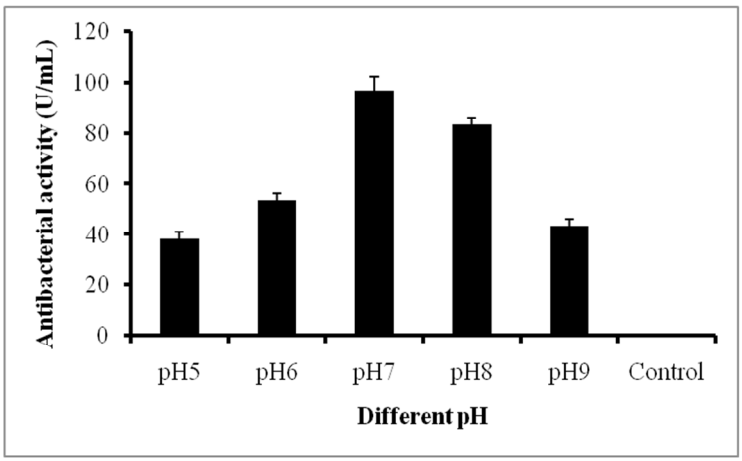

Fig 7: ***Optimization of suitable pH***

The pH of the medium have the influence in the antibacterial activity. In this study, the pH was varied from acid to alkali conditions, but the inhibitory activity was marked highest at the neutral pH only. Similar to this study, *Kiranmayi et al., 2011* reported that the bioactive compound production by the *Streptomyces violates* 22 and *Pseudonocardia sp*. VUK-107 has maximum antibacterial activity at the neutral pH 7.0.

GC-MS analysis

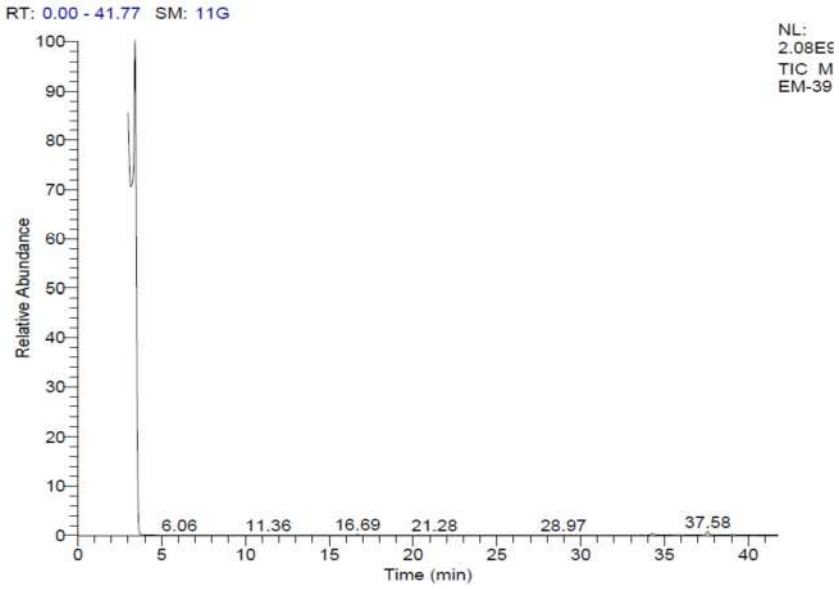

Fig 8 : ***Chromatogram of partially purified compound obtained from Streptomyces sp S8.***

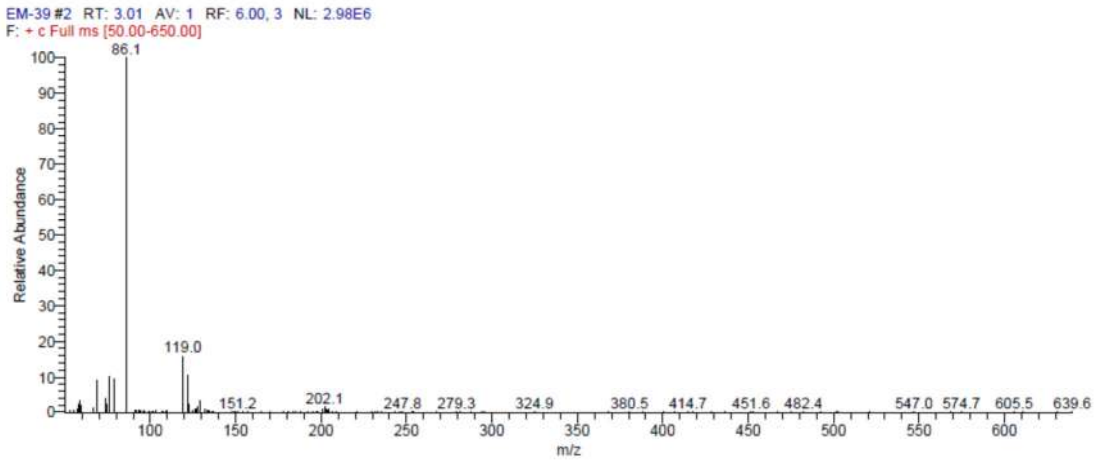

Fig 9: ***Mass spectrum of partially purified compound obtained from Streptomyces sp S8.***

GC-MS analysis was done to the partially purified bioactive compound obtained from the *Streptomyces* sp S8. The peak was obtained at the retention time of 3.01 min respectively and the mass spectrum of the peak at 3.01 min reveals the presence of various number of compounds namely Benzoic acid,4-2(acetylamine)ethoxy, 2-Methylbenzoic acid, 2-diethylaminoethyl ether, Napthalene and Benzamide. The benzoic acid derivatives exhibited strong antioxidant properties against the superoxide radicals (Velika and Kron. 2013). Napthalene and its derivatives were found to have inhibitory effects against most of the pathogens that causes disease in human beings (Rokade and Sayyed, 2009). The benzamide derivatives from endophytic *Streptomyces* Y1M67086 exhibited both antibacterial and antioxidant activity (Xueqiong *et al.,* 2015).

CONCLUSION

From this study, it was concluded that partially purified compound obtained from marine derived *Streptomyces* sp S8 can serve as a lead or hit molecule for the future drug development process.

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