SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES AND EVOLUTION OF ITS ANTIOXIDANT ACTIVITIES

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In partial fulfilment for the award of the degree of

BACHELOR OF SCIENCE IN ZOOLOGY

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(Re-accredited with 'A^{+}' Grade by NAAC)

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CERTIFICATE

This is to certify that the project entitled **"Synthesis and Characterization of Silver Nanoparticles and Evolution of its Antioxidant Activities"** is submitted to **St. Mary's College (Autonomous), Thoothukudi** in partial fulfilment for the award of the degree of **Bachelor of Science in Zoology** and it is a project work done during the year 2021-2022 by the following students.

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DECLARATION

We do hereby declare that this thesis entitled, "**Synthesis and Characterization of Silver Nanoparticles and Evolution of its Antioxidant Activity**" submitted by us for the award of the degree of Bachelor of Science in Zoology is the result of our original independent research work carried out under the guidance of Dr. M. Paripooranaselvi M.Sc., M.Phil., B.Ed., Ph.D., SET., Assistant Professor, Department of Zoology, St. Mary's College (Autonomous), Thoothukudi and it has not been submitted elsewhere for the award of any other degree.

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CONTENTS

Oxidative stress is the major driving factor responsible for the initiation and progression of cancer, diabetes mellitus, cardiovascular diseases, neurodegenerative diseases, and inflammatory diseases among other syndromes (Severyn, *et al* 2009) The condition is brought by excessive generation of free oxygen and nitrogen species or their inefficient quenching in the cell. Free oxygen and nitrogen species are unstable molecules that are present in the environment (exogenous) and are also Oxygen is an essential element of the aerobic organisms for the production of energy. It is the key element in the human body, capable of combining with every other element leading to formation of essential components necessary for maintaining its regular metabolic activities. Oxygen regulates about 90% of the body function and plays a pivotal role in the respiration, gets absorbed by the blood stream in the lungs, transported to the cells and participates in complex processes of metabolic reactions involving enzymatic and non-enzymatic reactions with organic compounds catalyzed by ionizing radiations resulting in the formation of free radicals (Rajamani *et al.*, 2010). Free radicals have surplus free-floating electrons rather than having harmonized pairs and therefore unstable, but are highly reactive, moves freely through blood stream and in order to attain stability attacks nearby molecules including proteins, carbohydrates and nucleic acids damaging them by stealing their electrons

through a process called oxidation. Types of free radicals include the hydroxyl radical OH. the superoxide radical O. 2, the nitric oxide radical NO. and the lipid peroxyl radical LOO. (Chaitanya*, et al*., 2010). External sources like air pollutants, industrial chemicals, cigarette smoke, alcohol, oxidized poly unsaturated fats and cooked food (Bagchi, *et al.,* 1998) also contribute to the formation of free radicals leading to irreparable damage to the several organs, causing malfunctions. Diseases caused by free radical formation: The role of free radicals has been implicated in the development of at least 50 diseases. A few of them include arthritis, inflammatory diseases, kidney diseases, cataracts, inflammatory bowel disease, colitis, lung dysfunction; pancreatitis; drug reactions, skin lesions, and aging. Free radicals are also associated with liver damage due to alcohol consumption and the development of emphysema due to cigarette smoking. Aging is the prime mechanism oriented with the free radical accumulation in the humans as suggested in the Free Radical Theory of Aging (Harman, *et al*., 1956). A symptom of aging such as atherosclerosis is considered to be due to oxidation by free radicals. The primary site of free radical damage is the mitochondrial DNA. Damage to the mitochondrial DNA cannot be readily repaired and leads to the shutting down of mitochondria causing cell death and ageing (Speakman et al., 2004). Bombardment of free radicals with atoms of metals like mercury, lead, cadmium and even pesticides amplifies the production of free radicals several million times resulting in mitochondrial damage. Severe

mitochondrial damage in the cells leads to apoptosis occurs due to a cascade initiated by Bcl-2 proteins on the surface of mitochondria. Destruction properties of free radicals will not limit only to the process of aging but also plethora of diseases via various metabolic activities Accumulation of free radicals forms cataracts in the human eye. Scavenging of free radicals takes place in the eye, which gets hampered due to age-related insufficient production of antioxidant scavenging systems leading to the formation of an opaque spot on the eye lens causing blindness. (José, *et al*., 1991). Myocytes are the source of free radical accumulation in the heart. Free radicals damage proteins and calcium pumps on the sarcoplasmic reticulum, resulting in the accumulation of calcium. High levels of calcium cause erratical contraction of the myocytes causing arrhythmia (Marczin *et,al.,* 2003). Spread of arrhythmia to other cells disrupts heart beat, causing severe complications. Free radicals produced due to external sources especially radiation leads to cancer (Dreher, *et al*., 1996). Most of the radiation energy is taken by the cells, which is absorbed by the water causing one of its oxygenhydrogen covalent bonds to split and forms free radical. This free radical reacts with another molecule in microseconds of its generation attacks and injures the macro molecules of the cell such as DNA, disrupting its strands and causing mutations in its bases. (Curtis, *et al*., 2006). However, free radicals that are produced during combustion may last little longer in the lungs binding to other air pollutants leading to lung cancer (Lee, *et al*., 1999). Role of antioxidants in promoting

Human Health: Antioxidants are the molecules, capable of limiting the macro molecule oxidation of free radicals by terminating the chain reactions, which are the main source of free radical formation in the cell. The critical role of antioxidants in ameliorating the free radicals have been elaborately studied, still it is not clear whether the production of free radicals is the consequence or the cause of a disease. Broadly antioxidants are classified into two types. Enzymatic and non-enzymatic: The non-enzymatic antioxidants are again classified into hydrophilic and hydrophobic. Hydrophilic antioxidants can dissolve into blood and cytosol and react with free radicals. Hydrophobic antioxidants protect the cell membrane from lipid peroxidation, a mechanism by which free radicals degrade the membrane lipids (Muller *et al*., 2007). The role of antioxidants in scavenging the deleterious effects of free radicals is complex, which depend on the interactions of various metabolites and enzyme systems having synergistic and interdependent effects on one another (Chaudière et al., 1999). The performance level of antioxidants also depends on the concentration, reactive potentiality with the specific free radical, interaction and function with other antioxidant family members. (Vertuani *et, al*., 2004).

The field of nanotechnology is one of the most active research areas in modern materials science. Nanoparticles exhibit new or improved properties based on specific characteristics such as size, distribution and morphology. It deals with the materials whose structures exhibit significantly novel and

improved physical, chemical, and biological properties, phenomena and functionality due to their nano scaled size. Because of their size, nanoparticles have a larger surface area than macro-sized materials. The intrinsic properties of metal nanoparticles are mainly determined by size, shape, composition, crystallinity and morphology. Nanoparticles, because of their small size, have distinct properties compared to the bulk form of the same material, thus offering many new developments in the fields of biosensors, biomedicine and bio nanotechnology. Nanotechnology is also being utilized in medicine for diagnosis, therapeutic drug delivery and the development of treatments for many diseases and disorders.

Silver is well known for possessing an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes (Jiang *et al*., 2004). In medicines, silver and silver nanoparticles have an example application including skin ointments and creams containing silver to prevent infection of burns and open wounds (Duran *et al*., 2005), medical devices and implants prepared with silver-impregnated polymers (RO, 1999). In textile industry, silver-embedded fabrics are now used in sporting equipment (Klaus *et al*., 1999).

Nanoparticles can be synthesized using various approaches including chemical, physical, and biological. Although chemical method of synthesis requires short period of time for synthesis of large number of nanoparticles/

this method requires capping agents for size stabilization of the nanoparticles. Chemicals used for nanoparticles synthesis and stabilization are toxic and lead to non-ecofriendly byproducts. The need for environmental non-toxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as byproducts.

Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as provide natural capping agents. Moreover, use of plant extracts also reduces the cost of microorganisms isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms (Garima Singhal *et al*., 2011).

The smaller particles have higher antibacterial activities due to the equivalent silver mass content. With respect to the clinical applications of nanoparticle, microorganisms including diatoms, fungi, bacteria and yeast producing inorganic materials through biological synthesis either intra or extracellularly made nanoparticles more biocompatible (Guidelli *et al*., 2011).

IMPORTANCE OF SILVER NANOPARTICLES

- It is used for purification and quality management of air, biosensing, imaging, drug delivery system.
- Biologically synthesized silver nanoparticles have many applications like coatings for solar energy absorption and intercalation material for

electrical batteries, as optical receptors, as catalysts in chemical reactions, for biolabelling, and as antimicrobials.

- Though silver nanoparticles are cytotoxic but they have tremendous applications in the field of high sensitivity bimolecular detection and diagnostics, antimicrobials and therapeutics, catalysis and micro-electronics.
- It has some potential application like diagnostic biomedical optical imaging, biological implants (like heart valves) and medical application like wound dressings, contraceptive devices, surgical instruments and bone prostheses.
- Many major consumer goods manufacturers already are producing household items that utilize the antibacterial properties of silver nanoparticles. These products include nano silver lined refrigerators, air conditioners and washing machines.

APPLICATION OF NANOPARTICLES: Once materials are prepared in the form of very small particles, they change significantly their physical and chemical properties. In fact in nano-dimension, percentage of surface molecule compare to bulk molecule is high and this enhances the activity of the particle in nano dimension and therefore, the normal properties of the particle like heat treatment, mass transfer, catalytic activity, etc are all increases. But compare to non-metal nanoparticles, metal nanoparticles have more industrial application. Nanoparticles offer many new developments in

the field of biosensors, biomedicine and bio nanotechnology-specifically in the areas-

- Drug delivery
- As medical diagnostic tools,
- As a cancer treatment agent (Gold nanoparticles).

Ascidians are dominant organisms in many marine communities, having a wide geographic distribution. This ecological success is because of their ability to synthesize secondary metabolites, which possess an important defensive role against predation. They have increasingly become the target of natural products research. A natural product is a chemical compound or substance produced by a living organism - found in nature that usually has a pharmacological or biological activity for use in pharmaceutical drug discovery and drug designing. Research typically focuses on sessile organisms or slow moving animals because of their inherent need for chemical defences.

Many marine sedentary organisms produce components with unique structural pattern, for their chemical defence which do not occur in terrestrial plants. Sponges, bryozoans and tunicates are important source of new active principles for drug development.

Due to physical and chemical conditions of the marine environment, almost every class of marine organism exhibits variety of molecules with unique structural features, which are not found in terrestrial natural products.

Organisms with no apparent physical defence, like sessile organisms, are believed to have evolved chemical defences to protect themselves.

Marine organisms have been reported to be a rich source of biologically active compounds, especially ascidians which are most prominent sources of new compounds. The majority of metabolites reported from ascidians are derived from amino acids and it is an important source in drug discovery. They are considered as a nuisance as they grow on all underwater marine structures and are usually thrown away. Such discards may have a wealth of natural products. Marine organisms, especially those that are a nuisance to the environment like biofoulers can be usede for synthesis of nanoparticles.

The marine environment is an excellent source of novel chemicals, not found in terrestrial sources. According to Davidson, 1993; Faulkner, 2002 and Blunt *et al*., 2006 marine organisms such as ascidians, sponges and soft corals containing symbiotic microorganisms are a rich source of bioactive compounds. Dhorajiya *et al*., 2012 expressed that some of the compounds derived from marine organisms have antioxidant properties and anti-cancer activities, but they are largely unexplored.

Ismail *et al*., 2008 and Dellai *et al*., 2010 noted that since the few last decades, marine environment have been recognized to be a rich source of bioactive metabolites with varied biological and pharmacological activities. Chakraborty and Ghosh, 2010 suggested that bioactive peptides with novel

structures have also been shown in ascidians. Synthesis of nanoparticles from is lacking. As ascidians are available along the Tuticorin coast an attempt has been made to synthesize nanoparticles.

The objectives of the present study were to

* Collect the *Phallusia nigra* Savigny, 1816; and colonial ascidians *Didemnum psammatode* Sluiter, 1895
* Collect the seaweeds *Codium geppiorum* O.C. Schmidt, 1923 and *Dictyota ciliolata*
* Synthesize silver nanoparticles from *Phallusia nigra, Didemnum psammatode*, *Codium geppiorum* and *Dictyota ciliolata*
* Determine characterization of nanoparticles By Uv-Vis Spectrophotometer
* Study the chemical composition of the synthesized nanoparticles by using FTIR spectrometer
* Evaluate the *in-vitro* antioxidant activity of silver nanoparticles of *Phallusia nigra, Didemnum psammatode*, *Codium geppiorum* and *Dictyota ciliolata*

Kumaran *et al*., 2007 observed a statistical correlation between the antioxidant properties and phenolic contents of methanolic extracts of stem barks, root bark, leaves and fruits from *Morus alba*. Sreelatha and Padma 2009, studied that the extracts of *Moringa oleifera* both mature and tender leaves have potent antioxidant activity against free radicals, prevent oxidative damage to major biomolecules and afford significant protection against oxidative damage. According to Bhaskar and Balakrishnan, 2009 the extracts of the roots of *Carissa carandas* and *Pergularia daemia* possess antioxidant properties and could serve as free radical inhibitors or scavengers.

Sharma and Kumar, 2011 observed *in vitro* antioxidant activity of petroleum ether, ethanolic, and aqueous extracts of fruits of *Rubus ellipticus* using DPPH radical scavenging and reducing power assay. Beta hydroxy acid was used as a standard antioxidant for DPPH radical scavenging activity extracts of *Rubus ellipticus* fruits possess significant free radical scavenging and reducing power properties at concentration-dependent manner. Mahdi-Pour *et al*., 2012 observed the antioxidant activity of methanolic extracts of various parts of *Lantana camara*. According to Carbonera *et al*., 2014 supplementation of tilapia diet with ethanolic extract of acerola fruit residue resulted, in an improvement of the antioxidant capacity of the fillets. Nahid *et al*., 2017, evaluated the antioxidant, antimicrobial and phytochemical

constituents of the methanol extract of *Artemisia indica*. The powerful antioxidant activity is attributed to the greater amount of total phenol and flavonoid compound in the ethanolic leaf extract of *Memecylon umbellatum* as stated by Anbukkarasi *et al.,* 2017.

Trad *et al*., 2018 observed the butanolic extract of *Ephedra alte* had high phenolic contents and exhibited high antioxidant activity both *in-vitro* and *in-vivo*. Quispe *et al*., 2018 investigated antioxidant activity of the ethanolic extracts of peel, flowers, gel and root of *Aloe vera* as the capturing of the DPPH• and ABTS•+ radicals, while the iron-reducing antioxidant power (FRAP) was measured by spectroscopic methods. Keerthana and Visweswaran 2018 observed that the drug Seeraga choornam has promising therapeutic antioxidant activity when compared with the standard drug. This research work can help for medical practitioners to use this poly herbal compound for the treatment of cancer.

Yarnpakdee*et al.,* 2019 studied the extract of *Cladophoraglomerata* could be a source of alternative natural antioxidant in lipid based muscle food. Sharma *et al.,* 2019 determined *in-vitro* antiradical activity and *in-vivo* metabolism of polyphenol. Tekin and Küçükbay, 2020 the extracts of flowers of *Punica granatum* L. had high content of flavonoids and other phenolics with antioxidant activity. Arshan *et al*., 2020 observed the phytochemical analysis of extracts *Annona squamosa* linn leaves had glycosides, saponins, tannins, flavonoids, phenols, etc. In-vitro antioxidant activities clearly suggest

that methanol extract has higher antioxidant activity than the other extract due to a higher presence of phenolic and flavonodal constituents in the methanol extract.

Hamidinia *et al.,* 2008 stated that ethanolic extract of *Eudistoma viride* showed a promising antioxidant potential against free radical induced oxidative damage. Zhang *et al*., 2010 observed antioxidant activity of five polysaccharides extracted from five algae including one brown alga *Laminaria japonica*, one red alga *Porphyra haitanensis* and three green algae *Ulva pertusa*, *Enteromorpha linza* and *Bryopsis plumose.* Revathi *et al*., 2015 observed the maximum antioxidant activity in the methanol extract of *Hypnea valentiae* and antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. Priya *et al*., 2016 stated that the ethanolic extract of colonial ascidian, *Eudistoma viride* by DPPH method reveals a promising antioxidant potential against free radical induced oxidative damage. Elya *et al.,* 2018 according to them the methanol extract of the ascidian *Didemnum* sp. exhibited antioxidant activity. Praba*et al.,* 2020 stated that the antioxidant activity of two colonial ascidians such as *Eudistoma amplum* and *Polyclinum nudum*, were tested by DPPH scavenging test with concentrations ranging from 5 to 150 µg/ml based on the reduction of the maximum absorption of the DPPH radicals in the presence of an antioxidant compound.

Ekanayake *et al*., 2004 stated that the flesh and skin of *Eptatretus burgeri* (hag fish) showed higher DPPH radical scavenging activities when compared with commercial antioxidants. Palakkal and Ganesan 2005 tested antioxidant activity of the extract of *Macaranga peltata* and the correlation between the total phenolic content and antioxidant activity.

ANIMAL MATERIAL

Samples of simple ascidian *Phallusia nigra* Savigny, 1816 were collected from the under surface of the barges of Tuticorin harbor. The specimens of colonial ascidian *Didemnum psammathodes* were collected from the intertidal rocky shore area of Thoothukudi north break water Tamilnadu. The samples were washed with sea water to remove sand, mud and overgrowing organisms at the site collection, and then transported to laboratory. Identification up to the species level was carried out based on the key to identification of Indian ascidians by Meenakshi, 1997.

SYSTEMATIC POSITION

Phallusia nigra belongs to

Phylum : Chordata

Subphylum : Urochordata

Class : Ascidiacea

Order : Enterogona

Suborder : Phlebobranchia

Family : Ascidiidae

Genus : *Phallusia*

Species : *nigra*

Didemnum psammatode belongs to

Phylum : Chordata

Subphylum : Urochordata

Class : Ascidiaceae

Order : Enterogona

Suborder : Aplousobranchia

Family : Didemnidae

Genus : *Didemnum*

Species : *psammathodes*

Dictyota ciliolata belongs to

Class : Phaeophyceae

Order : Dictyotales

Family : Dictyotaceae

Codium geppiorum O.C.Schmidt, 1923 belongs to

Class : Ulvophyceae

Order : Bryopsidales

Family : Codiaceae

EXTERNAL APPEARANCE

Plate – 1 Individuals are oval or elongated, laterally compressed with the free edges thick and rounded. The size varies from 1.5 cm to 9.5 cm.

Attachment is by the posterior end or by one third of the posterior left side. In a few specimens the posterior basal part had a long flat creeping process for attachment. The anterior end narrows to a terminal branchial siphon. Atrial siphon is one third from the anterior end on the dorsal surface directed anteriorly. There are 8-10 branchial and 6-8 atrial lobes with ocelli in between them. The lobes are rounded without any tentacular fringe. The whole anterior part of the body is curved dorsally which is characteritic of the species so that the two apertures are quite close together. Test is firm, smooth, shiny and jet black in colour.

Plate -2 shows the colony of *Didemnum psammatode*. It is thin and soft. In the test spicules are few, but abundant ovoid faecal pellets are present. Live and preserved colonies are grey in colour.

Plate – 3 depicts *Codium geppiorum*. Cylindrical and irregularly forked branches of this species creep on the substrates, and attach to each other at any point of contact. The utricles are club-shaped with rounded tips, approximately 0.1-0.2 mm in diameter and slightly constricted at the upper portions. The plants are spongy, dark green, and are found on rocks or dead coral in subtidal zones along moderately wave exposed shorelines.

Dictyota ciliolata is shown in Plate – 4. Thalli are erect, to 8 cm tall, attached by means of a single stupose holdfast. Stolonoidal fibres are absent. Straps, 2-3 mm wide, are slender and dichotomously branched. The margins

are dentate, rarely smooth, while the surface is always smooth. The apices are rounded. The medulla and cortex are uniformly one-layered. Sporangia are single, scattered on both surfaces, but absent in the apical dichotomies. Sporangia, 95-110 μm in diameter, are borne on a single stalk cell and are not surrounded by a conspicuous involucrum. *Dictyota ciliolata* is characterised by its stupose holdfast, dentate margins and the absence of stolonoidal fibres, although the margins of some specimens can be nearly smooth (De Clerck *et al.*, 2002)

Preparation of Powder

The specimens were dried under shade. The dried animals were homogenized to get a coarse powder. The dried powder of the tunicate *Phallusia nigra* and *Didemnum psammatode, Codium geppiorum* and *Dictyota ciliolate* was used.

Synthesis of silver nanoparticles

Weighing 25 g of dry powder of *Phallusia nigra* was mixed with 100 ml sterile distilled water and filtered through Whatman No.1 filter paper (pore size 0.45 μm) and was further filtered through 0.22 μm sized filters. The extract was stored at 40° C for further experiments. The same procedure was followed for *Didemnum psammatode*, *Codium geppiorum* and *Dictyota ciliolata* also.

The aqueous solution of 1mM silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles. 10 ml of *Phallusia nigra* extract

was added into 90 ml of aqueous solution of 1 mM silver nitrate for reduction into Ag+ ions and kept for incubation period of 15 hours at room temperature. Here the filtrate act as reducing and stabilizing agent for 1 mM of AgNO3.

CHARACTERIZATION OF SILVER NANOPARTICLES:

UV-Vis Analysis:

The Ag nanoparticles were characterized in a Perkin-Elmer UV-VIS spectrophotometer, Lambda-19 to know the kinetic behavior of Ag nanoparticles. The scanning range for the samples was 200-800 nm at a scan speed of 480 nm/min. The spectrophotometer was equipped with "UVWinlab" software to record and analyze data. Base line correction of the spectrophotometer was carried out by using a blank reference. The UV-Vis absorption spectra of all the samples were recorded and numerical data were plotted in the "Origin 6.5".

FTIR analysis:

The chemical composition of the synthesized silver nanoparticles was studied by using FTIR spectrometer (Perkin-Elmer LS-55- Luminescence spectrometer). The solutions were dried at 75° C and the dried powders were characterized in the range 4000–400 cm-1 using KBr pellet method.

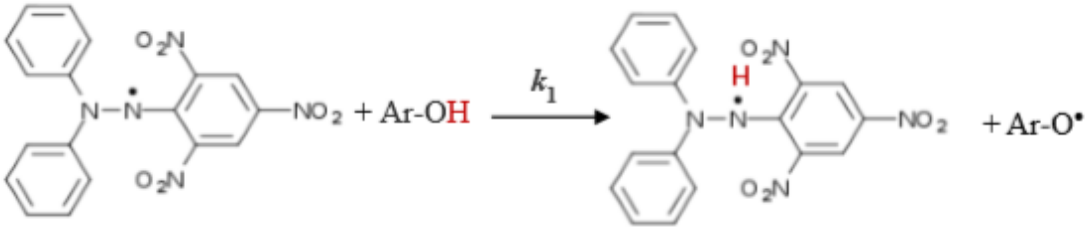
***DPPH (2,2-diphenyl- 1-picrylhydrazyl) radical scavenging* DPPH Assay**

The study of free radical in the antioxidant component is assayed by DPPH radical scavenging activity (Blois, 1958). This method is based on the

reduction of DPPH in methanol solution in presence of a hydrogen donating antioxidant resulting in the formation of the non-radical form DPPH-H. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Koleva, 2002). DPPH in methanol (0.1 mM) was prepared. To 3 ml of ethanolic extract of *Phallusia nigra* and *Didemnum psammathodes* at different concentrations (20, 40, 60, 80, 100 µg/ml), 1 ml of DPPH in methanol was added. After vigorous shaking the mixture was kept undisturbed at room temperature for 30 min. The absorbance was measured at 517 nm in a UVVis spectrophotometer. Ascorbic acid was used as the standard. A reagent blank was also run simultaneously.

While DPPH can accept an electron or hydrogen radical to become a stable, diamagnetic molecule, it can be oxidized only with difficulty and then irreversibly. DPPH shows a strong absorption band at 517 nm due to its odd electron and solution appears a deep violet colour, the absorption vanishes as the electron pairs off. The resulting decolorization is stoichiometric with respect to the number of electrons taken up. The alcoholic solutions of 0.5 mMare densely colored and at this concentration, the Lambert-Beer law is obeyed over the useful range of absorption (Blois 1958).DPPH assay is considered a valid accurate, easy and economic method to evaluate radical scavenging activity of antioxidants, since the radical compound is stable and need not be generated.

The DPPH method is the most frequently used assay for the evaluation of the free radical-scavenging capacity of plant extracts. The reaction mechanism involves the H transfer from a phenolic compound to the DPPH radical. Interaction of the DPPH radical (purple-coloured) with a phenolic compound, which is able to neutralize its free radical character, leads to the formation of yellow colorless hydrazine and the resulting effect can be quantified spectrophotometrically at 515 nm.



The H-transfer from a phenolic compound (AR-OH) to DPPH

The capabilityof scavenging DPPH radical was calculated using the following equation.

$$\text{Percentage of Inhibition} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \text{x } 100$$

Characterization of Ag nanoparticles:

UV-Vis Spectrophotometer Analysis:

AgNPs from the extract of *Phallusia nigra* and *Didemnum psammatode*, *Codium geppiorum* and *Dictyota ciliolata* were synthesized successfully. The UV-Vis absorption spectra of the Ag NP of *Phallusia nigra* was shown in Figure 1. Absorption spectra of Ag nanoparticles formed in the reaction media has absorbance maxima at 270 nm.

The UV-Vis absorption spectra of the Ag NP of *Didemnum psammatode* was shown in Figure 2. Absorption spectra of Ag nanoparticles formed in the reaction mixture in the range of 200-300 nm. The absorption spectrum has shown two peaks at 250 and 270 nm indicate the formation of silver nanoparticles using *Didemnum psammathodes*.

The UV-Vis absorption spectra of the Ag NP of *Codium geppiorum* was shown in Figure 3. Absorption spectra of Ag nanoparticles formed in the reaction media has absorbance maxima at 275 nm.

The UV-Vis absorption spectra of the Ag NP of *Dictyota ciliolata* was shown in Figure 4. Absorption spectra of Ag nanoparticles formed in the reaction mixture in the range of 250-300 nm. The absorption spectrum has

shown two peaks at 250 and 280 nm indicate the formation of silver nanoparticles using *Dictyota ciliolata*.

FTIR Analysis:

FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized. The FTIR spectrum of silver nanoparticles were shown in Figure 5 to 8.

The FT-IR spectra for Ag nanoparticles of *Phallusia nigra* revealed the presence of prominent peaks at 3424, 2923, 2106, 1627, 1422, 1384, 1120, 1021, 874, 675, 610, 513 and 466 cm^{-1} corresponding to different functional groups. The peak corresponds to 3424 cm^{-1} indicates N-H stretching (primary) functional group. The peak at 2923 cm^{-1} responds to C-H stretching of alkanes and alkyl groups and 1384 cm^{-1} indicates the C-H bending of alkanes. C-C multiple bond stretching of alkyne (mono-substituted) and aromatic functional groups were observed at 2106 and 1422 cm^{-1}. The carbonyl stretching groups such as acids, ketones and amides were noted at the peak of 1623 cm^{-1}. The plausible peaks at 1120and 1021 cm^{-1} at revealed the functional group of C-O stretching of esters and ethers. The following peaks at 874, 675 and 610 were indicated the C-X stretching halogen compounds. The peak at 468 cm^{-1} confirms the metal oxygen bond which evidenced the formation of Ag nanoparticles.

The spectra for Ag nanoparticles of *Didemnum psammatode* revealed the presence of prominent peaks at 3984, 3455, 2923, 1632, 1384, 1121, 1050, 639, 609 and 467 cm^{-1} corresponding to different functional groups. O-H stretching of alcohols and phenols were indicated from the peak at 3984 cm^{-1}. The peak corresponds to 3455 cm^{-1} indicates N-H stretching (primary) functional group. The peak at 2923 cm^{-1} responds to C-H stretching of alkanes and alkyl groups and 1384 cm^{-1} indicates the C-H bending of alkanes. C-C multiple bond stretching of alkyne (mono-substituted) and aromatic functional groups were observed at 2106 and 1422 cm^{-1}. The carbonyl stretching groups such as acids, ketones and amides were noted at the peak of 1632 cm^{-1}. The plausible peaks at 1121and 1050 cm^{-1} revealed the functional group of C-O stretching of esters and ethers. The following peaks at 639 and 609 were indicated the C-X stretching halogen compounds. The peak at 467 cm^{-1} confirms the metal oxygen bond which evidenced the formation of Ag nanoparticles.

The FT-IR spectra for Ag nanoparticles of *Codium geppiorum* revealed the presence of prominent peaks at 3134, 2361, 1628, 1400 and 670 cm^{-1} corresponding to different functional groups. The FT-IR spectra for Ag nanoparticles of *Dictyota ciliolata* revealed the presence of prominent peaks at 3133, 2362, 1627, 1384 and 839 cm^{-1} corresponding to different functional groups.

Screening of the *in-vitro* antioxidant activity of silver nano particles *Phallusia nigra* and *Didemnum psammatode, Codium geppiorum* and *Dictyota ciliolate* was assessed by DPPH radical scavenging activity.

The results of DPPH radical scavenging activity of different concentrations of ethanolic extracts of *Phallusia nigra* and *Didemnum psammatode* and standard ascorbic acid is given in Table 1 & 2. The results of DPPH radical scavenging activity of different concentrations of ethanolic extracts of *Codium geppiorum* and *Dictyota ciliolate* and standard ascorbic acid is given in Table 3 & 4. The radical scavenging effect was found to increase with increasing concentrations.

The antioxidant activity of silvernano particles of of *Phallusia nigra*, *Didemnum psammatode*, *Codium geppiorum* and *Dictyota* were assessed based on their ability to scavenge the DPPH free radicals. AgNPs of *Phallusia nigra* exhibited significant scavenging activity with 48.38%, 56.45%, 69.35%, 75.64% and 84.51% at 20, 40, 60, 80 and 100 µg/ml concentration respectively. Percentage of scavenging activity of *Didemnum psammatode* was 30.48%, 39.83%, 50.80%, 64.03% and 67.74% at 20, 40, 60, 80 and 100 µg/ml concentration respectively. Percentage of scavenging activity of *Codium geppiorum* was 35%, 41.61%, 49.67%, 59.35% and 72.09% at 20, 40, 60, 80 and 100 µg/ml concentration respectively. Percentage of scavenging activity of *Dictyota ciliolate* was 50%, 62.74%,

68.54%, 76.93% and 85.48% at 20, 40, 60, 80 and 100 µg/ml concentration respectively. silvernano particles of *Phallusia nigra* and silvernano particles of *Dictyota ciliolate* exhibited significant scavenging activity.

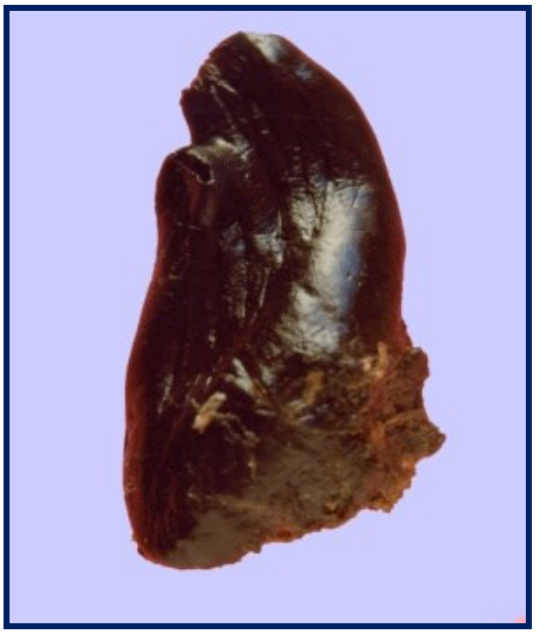


Plate - 1. *Phallusia nigra* Savigny, 1816

Plate - 2: *Didemnum psammatode*

Figure 1: UV-vis spectra for Ag nanoparticles of *Phallusia nigra*

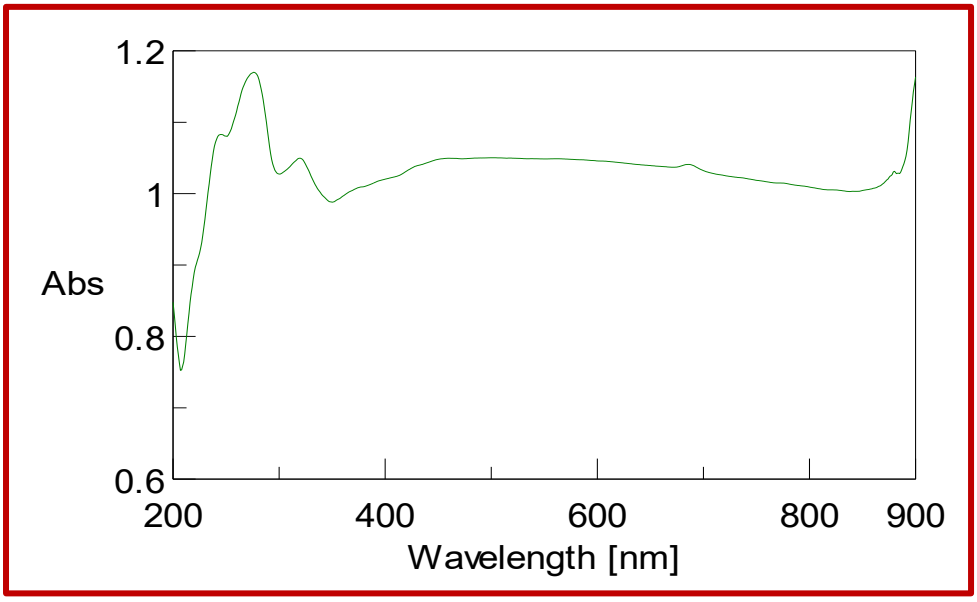


Figure 2: UV-vis spectra for Ag nanoparticles of *Didemnum psammatode*

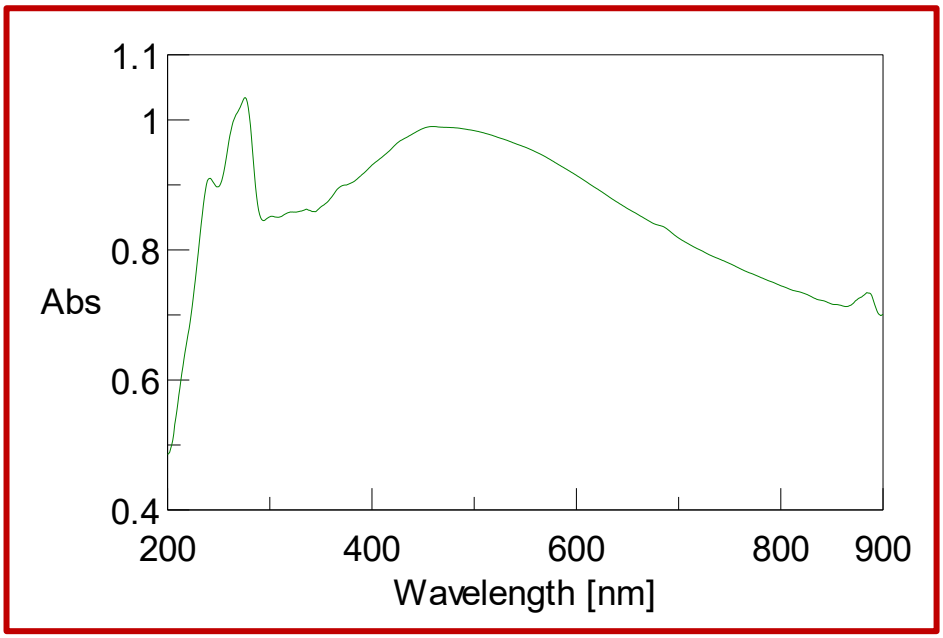


Figure 3: UV-vis spectra for Ag nanoparticles of *Codium geppiorum*

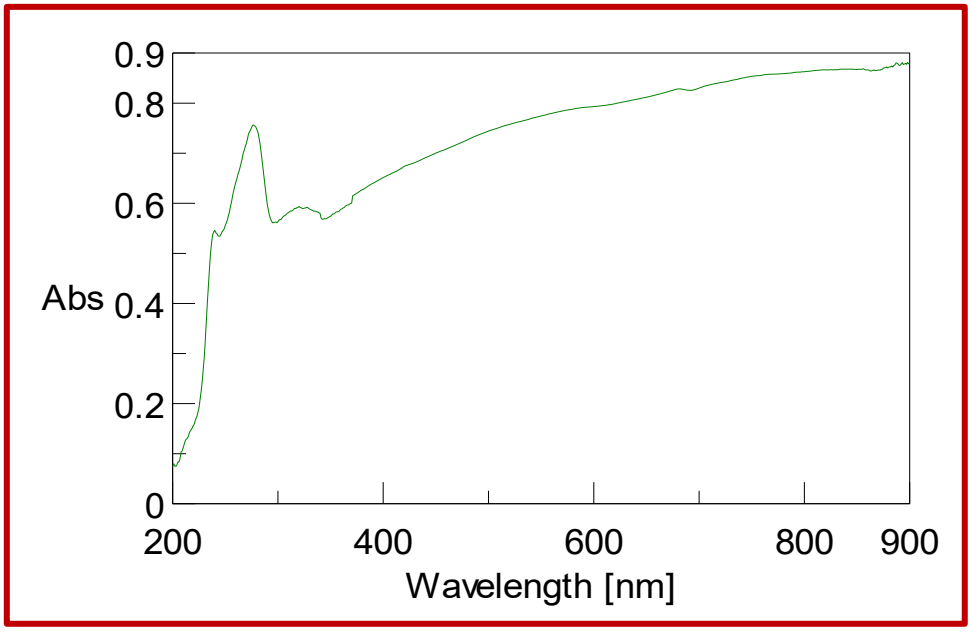


Figure 4: UV-vis spectra for Ag nanoparticles of *Dictyota ciliolata*

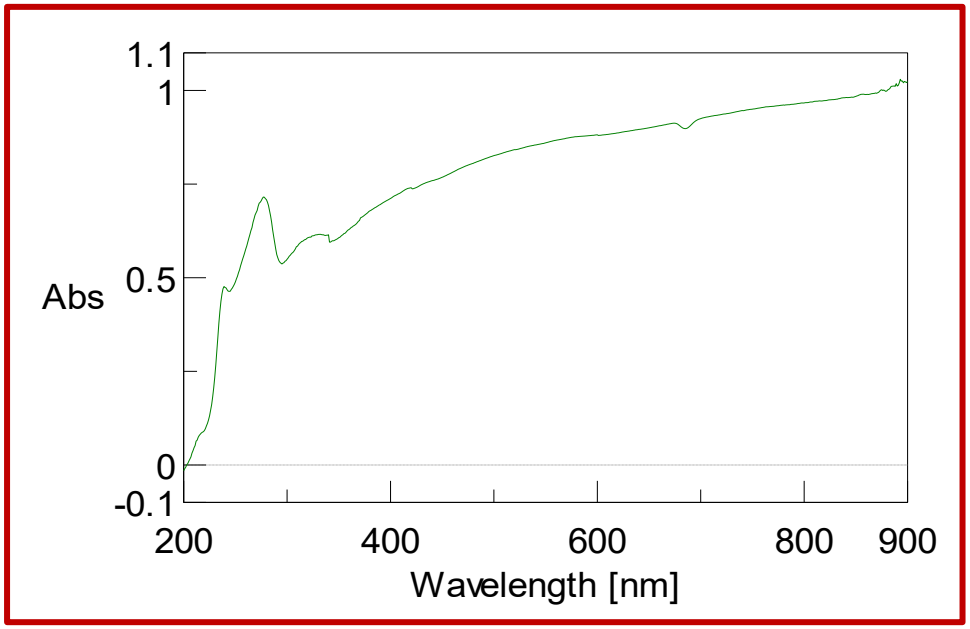


Figure 5: FTIR result for silver nanoparticles of *Phallusia nigra*

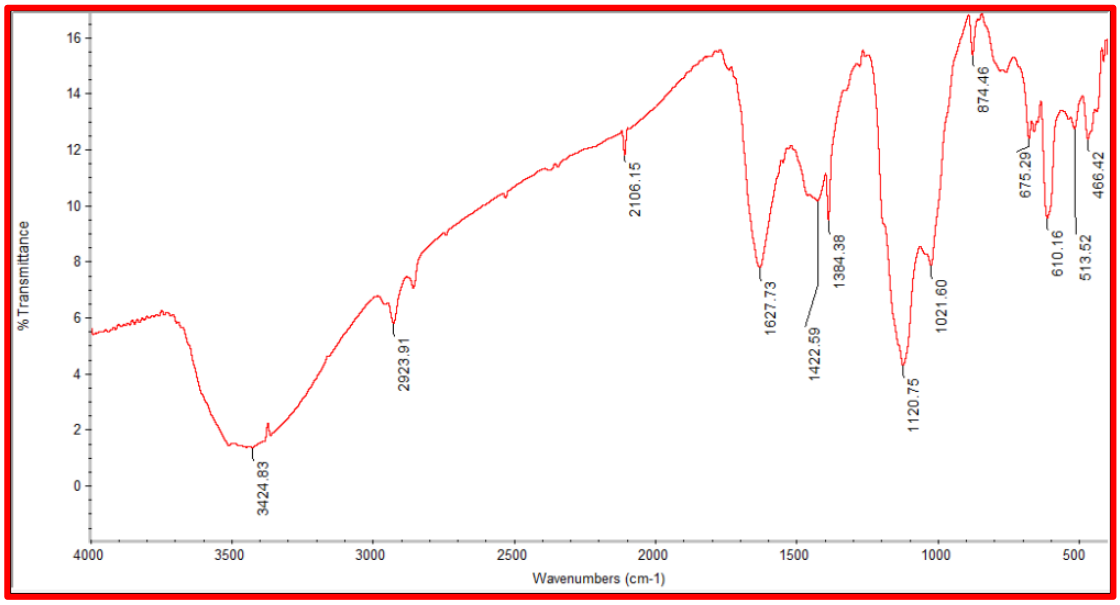


Figure 6: FTIR result for silver nanoparticles of *Didemnum psammatode*

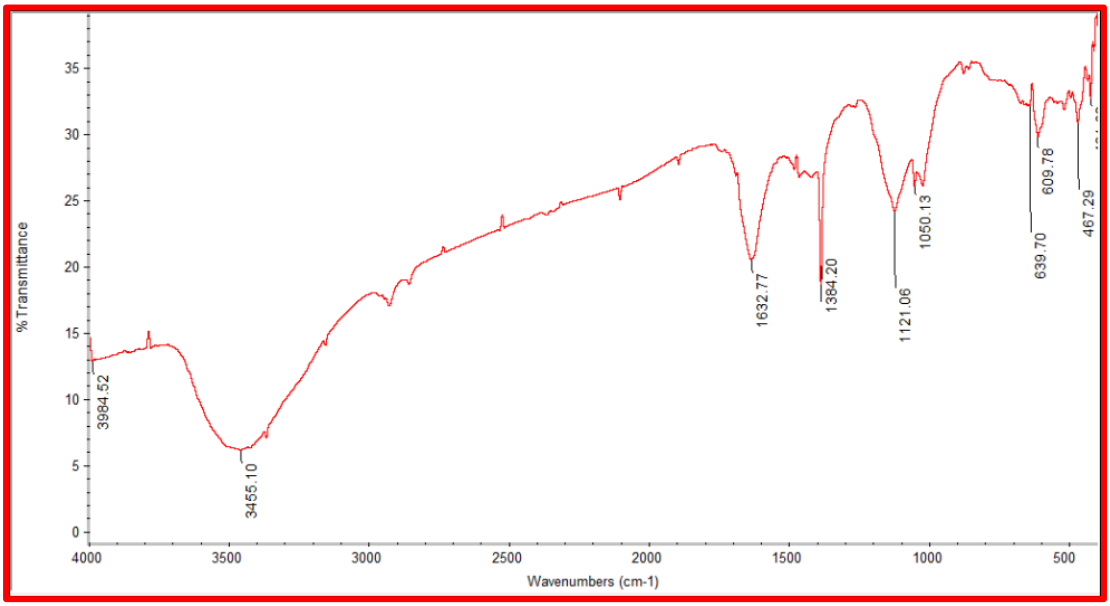


Figure 7: FTIR result for silver nanoparticles of *Codium geppiorum*

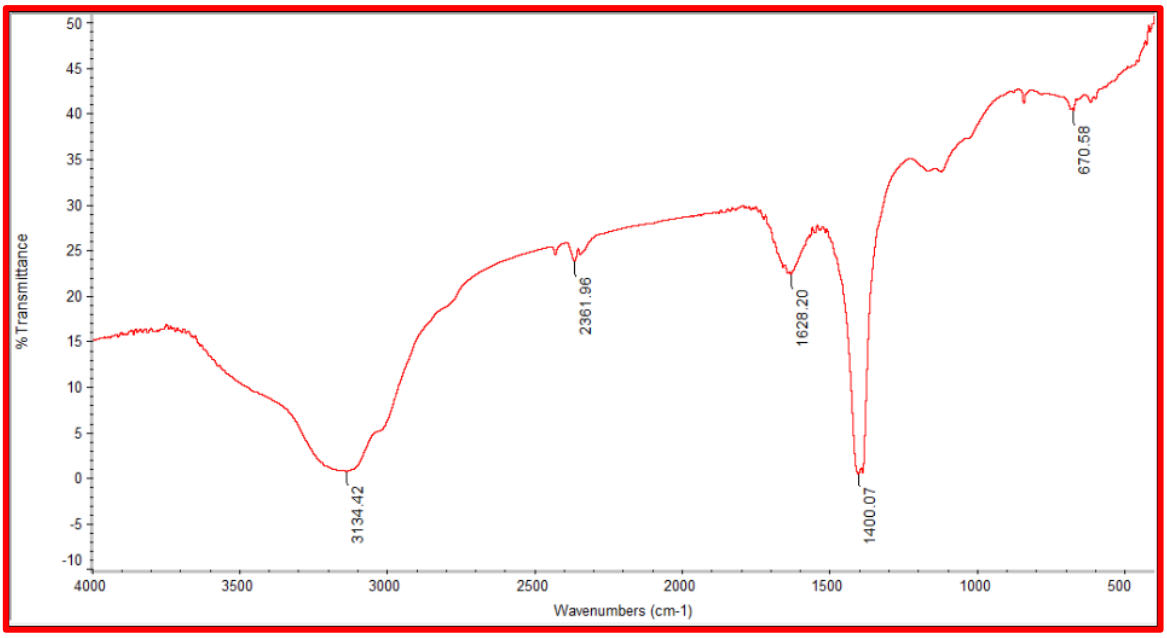


Figure 8: FTIR result for silver nanoparticles of *Dictyota ciliolata*

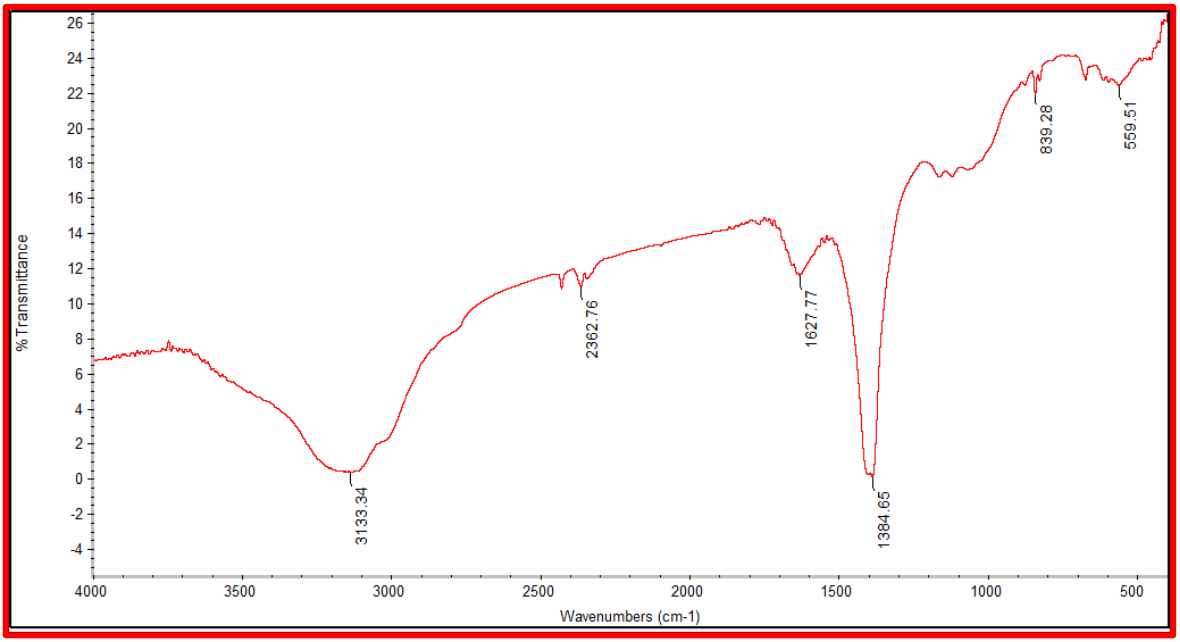


Table:1 In-vitro Anti oxidant activity of silver nanoparticles of *Phallusia nigra*

Concentration (µg/ml)	Absorbance	Percentage of Scavenging Activity
20	0.32	48.38
40	0.27	56.45
60	0.19	69.35
80	0.151	75.64
100	0.096	84.51
Ascorbic acid	0.044	92.90
Blank	0.62	-

Table:2 In-vitro Anti oxidant activity of silver nanoparticles of *Didemnum p*sammatode

Concentration (µg/ml)	Absorbance	Percentage of Scavenging Activity
20	0.431	30.48
40	0.381	39.83
60	0.305	50.80
80	0.203	64.03
100	0.200	67.74
Ascorbic acid	0.044	92.90
Blank	0.62	-

Table: 3 In-vitro Anti oxidant activity of silver nanoparticles of *Codium geppiorum*

Concentration (µg/ml)	Absorbance	Percentage of Scavenging Activity
20	0.403	35
40	0.362	41.61
60	0.312	49.67
80	0.252	59.35
100	0.173	72.09
Ascorbic acid	0.044	92.90
Blank	0.62	-

Table: 4 In-vitro Anti oxidant activity of silver nanoparticles of *Dictyota ciliolata*

Concentration (µg/ml)	Absorbance	Percentage of Scavenging Activity
20	0.310	50
40	0.231	62.74
60	0.195	68.54
80	0.143	76.93
100	0.090	85.48
Ascorbic acid	0.044	92.90
Blank	0.62	-

Figure:1 *In-vitro* Antioxidant activity of silver nanoparticles of *Phallusia nigra*

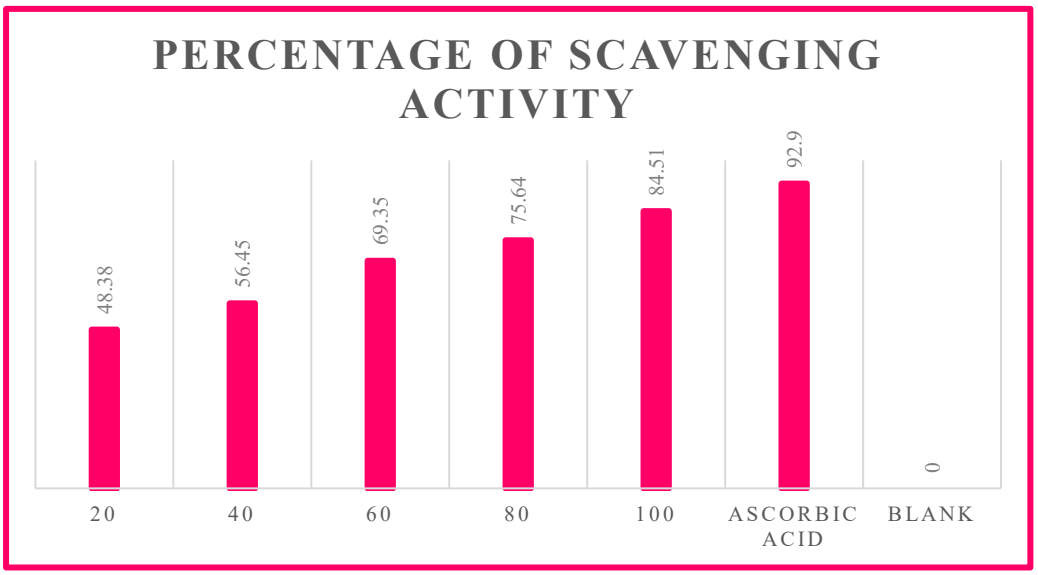


Figure:2 *In-vitro* Antioxidant activity of silver nanoparticles of *Didemnum psammatode*

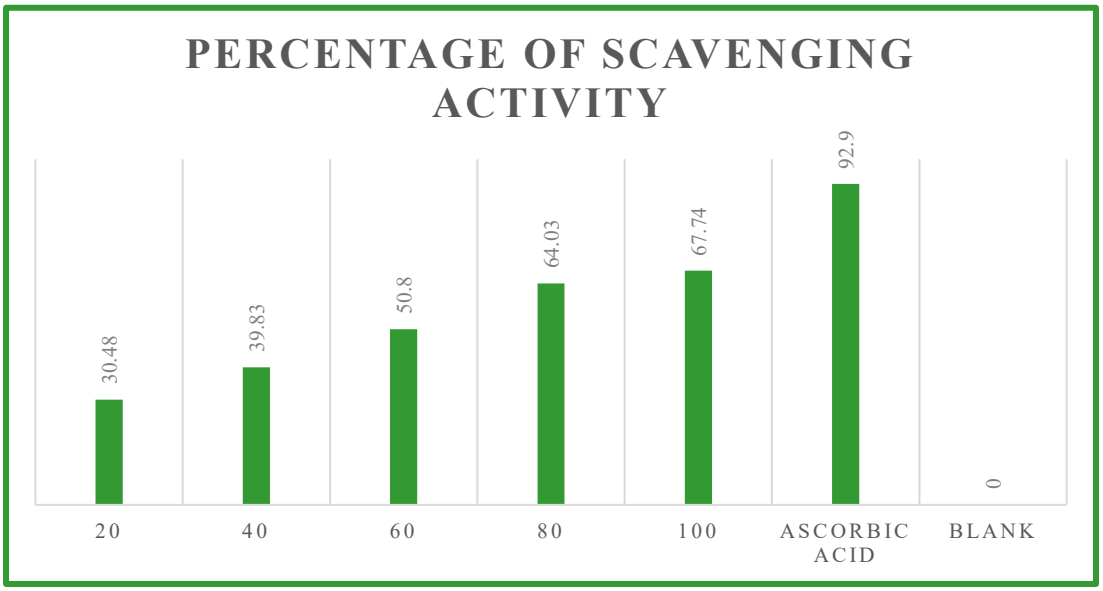


Figure: 3 *In-vitro* Antioxidant activity of silver nanoparticles of *Codium geppiorum*

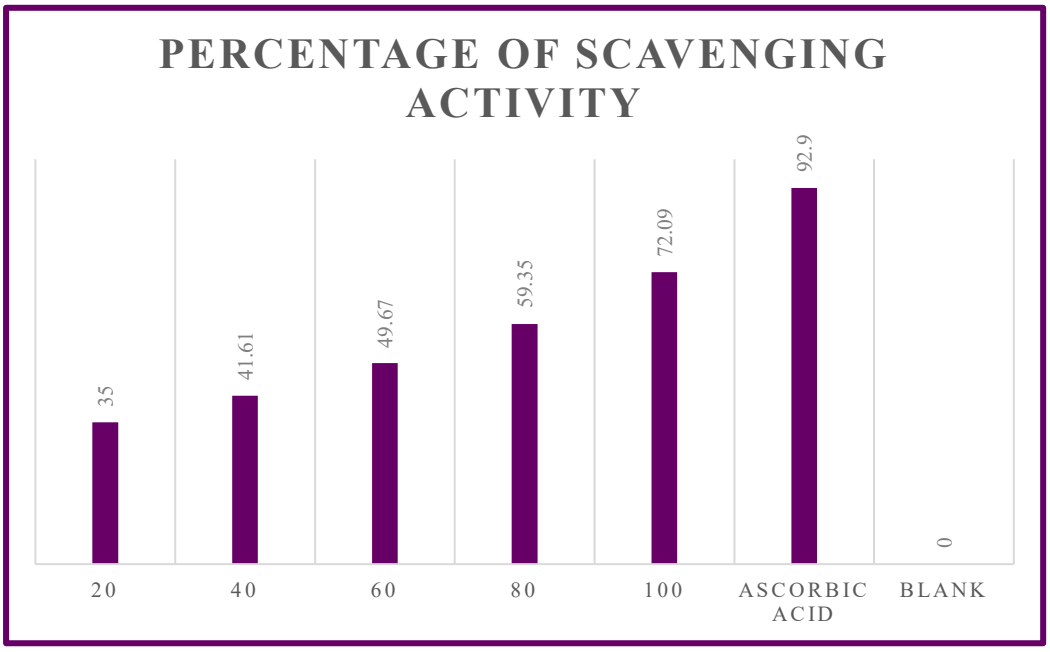
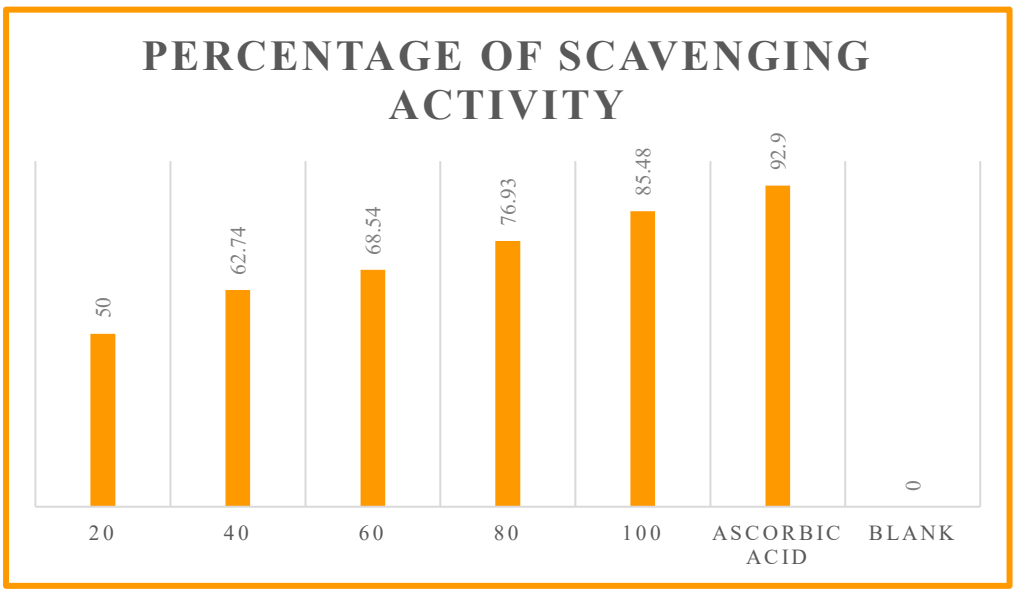


Figure: 4 *In-vitro* Antioxidant activity of silver nanoparticles of *Dictyota ciliolata*



The secondary metabolites of animal origins are gaining immense consideration recently due to their wide range of biological activities. Thus intake of the functional foods for their benefits has markedly increased with the awareness of their safety and nil side-effects. In this study an *in-vitro* antioxidant assay has been performed to evaluate the free radical scavenging properties of extracts of *Phallusia nigra*, *Didemnum psammathodes, Codium geppiorum* and *Dictyota*. The *in-vitro* antioxidant activity was dependent on the concentration of the extract.

According to Priya *et al*., 2016 the strongest antioxidant activity of ethanolic extract of *Eudistoma viride* may be due to the presence of flavonoids and phenolic compounds.High radical scavenging was observed in *Eudistoma viride*. The findings of the present study support the view of ascidians are promising sources of potential antioxidants and may be efficient in preventing agents in some other diseases. Roselin *et al*., 2018 suggested that the effectors include non enzymatic antioxidants like vitamin C, E, glutathione, thiol compounds, carotenoids and flavonoids.The present study clearly indicates that the different extracts of *Didemnum psammathodes* show strong antioxidant activity.

According to Esmaeili *etal*., 2015 samples with high level of phenolic content also contain flavonoids in great amount. The rich-flavonoid plants

could be a good antioxidant source that would help increase the overall antioxidant capacity of an organism and guard it against lipid peroxidation. Arshan *et al*., 2020 reported that the increased radical scavenging activity of the leaf extract could be due to the high content of phenolic compounds which were the main antioxidant components and their total contents were directly proportional to their antioxidant activity.

A preliminary chemical screening of the ethanolic extract of *Phallusia nigra* showed the presence of alkaloids, terpenoids, flavonoids, glycosides, phenolic compounds and tannins as stated by Priya *et al.,*2018. The reports of the current study are coincides with considerable DPPH scavenging activity which was better than the reports of earlier studies (Thakur and Singh 1965). Therefore, in this study, the presence of theflavonoids and phenols in all the tested extracts of *Phallusia nigra* might have contributed to the antioxidant activity.

Kumaran and Bragadeeswaran 2017 reported that the ethyl acetate extracts of ascidian *E. viride* and *D. psammathodes* showed antioxidant activity. Fractionation of the *Didemnum sp.* extract showed thatthe ethyl acetate fraction had the highest antioxidant activity. Further, fractionation of the ethyl acetate fraction by accelerated column chromatographyshowed that fraction VI had the highest antioxidant activity. The most active fraction contained alkaloids, steroids/triterpenoids, saponins, andglycosides. Antioxidants are the substances which inhibit oxidation, which has the ability

to remove the potentially damaging oxidizing agents in a living organism. Many phytochemicals present in the plants are able to reduce or prevent the oxidative damage to the human cells which can cause even cancer in humans. DPPH radical was used for evaluation of free radical scavenging and the total phenolic content was determined by the folin-Ciocalteaureagen. Polyphenols are responsible for the antioxidant activity, the obtained amount of total polyphenols in the extract indicated the extract to possess a high antioxidant activity as stated by Ganesan and Palakkal, 2019.

According to Sofna *and* Banjarnahor 2014 cardiovascular protective effect of flavonoids resembles in their antioxidant activity.Flavonoids as a strong antioxidant have also been used in certain study related to diabetes mellitus. The ability of flavonoids to interfere with cancer treatment has been tested in a series of flavonoids compound.The studies evaluating the antioxidant properties prompted by flavonoids have currently expanded to a wider range of therapeutic applications. Underlying the antioxidant properties of flavonoids have been concisely described.

According to literature survey, the antioxidant activity may be due to the presence of flavonoids. The simple ascidian *Phallusia nigra* and colonial ascidian *Didemnum psammathodes* contain flavonoids, which could be the reason for this significant antioxidant activity. The activity observed is in a very good correlation with the composition, where the most active extracts are those rich in polyphenol and flavonoids.

Conclusion

From the results, it was concluded that the AgNPs of *Phallusia nigra*, *Didemnum psammathodes, Codium geppiorum* and *Dictyota*. have appreciable antioxidant activity. The radical scavenging effect was found to increase with increasing concentrations.

* Antioxidant activity can be determined using other assays also.
* A further study on isolation, purification, structure determination and subsequent recognition of the novel mechanism of action of the clinically effective agent is suggested.
* Chemotherapeutic and spectroscopic techniques can lead to the development of new drugs.
* As the extract of *Phallusia nigra*, *Didemnum psammathodes, Codium geppiorum* and *Dictyota*. showed antioxidant activity, other species of ascidians and seaweeds can also be tried.

- The samples of the tunicate - *Phallusia nigra, Didemnum psammatode* and seaweed - *Codium geppiorum* and *Dictyota ciliolata* were collected and identified.
- The dried powder of the tunicates and seaweeds was used.
- Silver nanoparticles were synthesized using *Phallusia nigra, Didemnum psammatode Codium geppiorum* and *Dictyota ciliolata*
- The Ag nanoparticles were characterized in a Perkin-Elmer UV-VIS spectrophotometer.
- The chemical composition of the synthesized silver nanoparticles were studied by using FTIR spectrometer.
- Antioxidants are the molecules, capable of limiting the macro molecule oxidation of free radicals by terminating the chain reactions, which are the main source of free radical formation in the cell.
- The role of free radicals has been implicated in the development of at least 50 diseases. A few of them include arthritis, inflammatory diseases, kidney diseases, cataracts, inflammatory bowel disease, colitis, lung dysfunction pancreatitis drug reactions, skin lesions and aging.
- The marine environment is an excellent source of novel chemicals, not found in terrestrial sources. In the present study, *in vitro* antioxidant

activity of AgNPs of *Phallusia nigra, Didemnum psammatode* and seaweed - *Codium geppiorum* and *Dictyota* was assessed.

- The study of free radical in the antioxidant component is assayed by DPPH radical scavenging activity (Blois, 1958). Percentage of inhibition was calculated.

Abhishek R U, Mohana D C, Thippeswamy S, and Manjunath K. 2013. Antioxidant properties of some selected indian medicinal plants: Thier correlation with total phenolic contents,2013.International journal of green pharmacy.7(2).

Anbukkarasi M, Dhamotharan R and Janarthanam B. 2017. *In Vitro* Antioxidant Properties, Phenol and Flavonoid Content of *Memecylon umbellatum.* World Journal of Pharmaceutical Research.6 (3) 815-824.

Arshan MK, Magi F and Mishra R. 2020. Phytochemical screening and Antioxidant activity of different solvent extracts from *Annona squamosa* linn leaves. Asian journal of advances in research. 4 (1): 35-40.

Azmir 2013 technigues for extraction of bioactive comounds from plant materials journal of food engineering 117(4): 426-436.

Bagchi K, Puri S. 1998. Free radicals and antioxidants in health and disease. Eastern Mediterranean Health Journal. 4: 350-360.

Bagchi, K., Puri, S. 1998 Free radicals and antioxidants in health and disease. *Eastern Mediterranean Health Journal*, 4: 350- 360.

Baker, K. R. & Meydani, M. 1994. `Beta-carotene in immunity and cancer'. JOOt. Nutr., 3, p. 39-50.

Bendich, A. 1994. `Role of antioxidants in the maintenance of immune functions' in Frei, B. (ed.) Natural Antioxidants in Human Health and Disease. New York: Academic Press, p. 447-467.

Bendich, A. 1999. `Immunological role of antioxidant vitamins' in Basu, T. K., Temple, W. J. & Garg, M. L. (eds.) Antioxidants. in Human Health and Disease. Wallingford, U. K.: CAB International, 27-41.

Bhaskar H and Balakrishnan N, 2009. In vitro antioxidant property of Laticiferous plant species from western Ghats Tamilnadu, India.International Journal of Health Research. 2(2): 163-170.

Bhuvaneswari S, Deepa, Sripriya N, Prameela L. 2014. Antioxidant Activity and Phytochemistry of flowers from Tamil Nadu, India. International Journal of Research in Pharmaceutical Sciences 5(1):40-45.

Blois, M. 1958. Antioxidant Determinations by the Use of a Stable Free Radical. *Nature* **181,** 1199–1200

Blunt, J.W., Copp, B.R., Keyzers, R.A., Munro, M.H.G., and Prinsep, M.R. 2012. Marine natural products. *Natural Product Reports*. **29**(2): 144-222.

Buckman, R. (1996). What You Really Need to Know About Cancer. London: Macmillan Publishers Ltd.

Bushman, J. L. (1998). `Green tea and cancer in humans: a review of the literature'. Nutr. Cancer, 31, p. 151-159.

Chaitanya K V, Khan A A P, Spandana S M, Chakravarthi G P, Narasimhareddy P, Varaprasad B. 2010. Role of oxidative stress in

human health: an overview. Journal of Pharmacy Research, 3:1330-1333.

Chaitanya, K.V., Akbar Ali khan P., Sowmya Spandana M., Chakravarthi G.P., Narasimhareddy P., Varaprasad B. 2010. Role of oxidative stress in human health: an overview. *Journal of Pharmacy Research*, 3:1330-1333.

Chakraborty, S., and Ghosh, U. 2010.Oceans: a store of house of drugs-A review. *Journal of Pharmacy Research.***3**: 1293-1296.

Chaudière J, Ferrari-Iliou R. 1999. Intracellular antioxidants:from chemical to biochemical mechanisms. Journal of Food Chemistry Toxicol, 37: 949-962.

Chaudière, J., Ferrari-Iliou R.1999. Intracellular antioxidants:from chemical to biochemical mechanisms. *Food Chem.* Toxicol, 37: 949-962.

Cottier, H., Hodler, J. Kraft, R. 1995. `Oxidative stress: pathogenic mechanisms'. Forsch-Komplementarmed, 2, (5), p. 233-9.

Curtis RE, Freedman DM, Ron E, Ries LAG, Hacker DG, Edwards BK, Tucker MA, Fraumeni JF, 2000. New Malignancies Among Cancer Survivors.National Institutes of Health (NIH). 5(5302, 2006).

Curtis, R.E., Freedman, D.M., Ron, E., Ries, L.A.G., Hacker, D.G., Edwards B.K., Tucker, M.A., Fraumeni J.F, Jr. NIH .2006.Publ.vol. No. 05-5302..

Davidson, B.S. 1993. Ascidians: Producers of aminoacid derived metabolites. *Chemical Reviews*. **93**: 1771-1791.

Dellai, A., Deghrigue, M., Clary, A.L., Masour, H.B., Chouchane, N., Robert J., and Bouraoui, A. 2012. Evaluation of antiproliferative and antiinflammatory activities of methanol extract and its fractions from the Mediterranean sponge. *Cancer Cell International*. **12**: 18.

Dhorajiya, B., Malani, M., and Dholakiya, B. 2012. Extraction and preservation protocol of anti-cancer agents from marine world. *Chemical Sciences Journal*. CSJ-38.

Diplock, A. T. 1993. `Low dietary selenium and its relationship to human disease' in Parke, D. V., loannides, C. and Walker, R. (eds.) Food, Nutrition and Chemical Toxicity. London: Smith-Gordon, p. 395-402

Doss VA, Kalaichelvan P.T. 2012. In vitro antimicrobial and antioxidant activity screening of andrographispaniculata leaf ethanolic extract in Tamil nadu. International journal of pharmacy and pharmaceutical sciences. 4(1).

Dreher D, Junoda AF. 1996. Role of oxygen free radicals in cancer development. European Journal of Cancer, 32: 30-38.

Dreher, D., Junoda, A.F.1996. Role of oxygen free radicals in cancer development. *European Journal of Cancer*, 32: 30-38.

Eastwood, M. A. (1999). `Interaction of dietary antioxidants in vivo: how fruit and vegetables prevent disease? ' 0. J. Med., 99, p. 531-544.

Elya B, Yasman, Edawati Z, 2018.Antioxidant activity of the ascidian marine invertebrates, Didemnum sp. International Journal of Applied Pharmaceutics.10(1) 81-86.

Esmaeili K A, Taha M R, Mohajer S, Banisalam B, 2015. Antioxidant activity and total phenolic and flavonoids content of various solvent extracts from In vivo and in vitro grown trifoliumparatenseL.(Red Clover). Hindawi publishing corporation Bio Med research International. 11.

Fabiani, R., De Bartolomeo, A., Rosidnoli, P., Morozzi, G. (2001). `Antioxidants prevent the lymphocyte DNA damage induced by PMA-stimulated monocytes'. Nutrition and Cancer - An International Journal, 39, (2), p. 284-291.

Faulkner, D.J. 2002. Marine natural products. *Natural Product Reports*. **19**: 1- 48.

Gacche R N and Dhole N A., 2006. Antioxidant Potential of Selected Medicinal Plants Prescribed in the Indian Traditional System of Medicine. Pharmaceutical biology. 44(5) 389-365.

Gacche, R.N. Dhole, N.A. 2006. Antioxidant and Possible Anti-Inflammatory Potential of Selected Medicinal Plants Prescribed in the Indian Traditional System of Medicine. Pharmaceutical Biology. 44(6): 389-395.

Ganesan V, Palakkal S, 2019. Evaluation of Antioxidant Property of Extracts of Macarangapeltata by DPPH Free Radical Scavenging Activity. International Journal Of Pharmacy Pharmaceutical Reserch. 15(1).

Harman, D. 1956. Aging a theory based on free radical and radiation chemistry. *Journal of Gerontology,* 11: 298–300.

Harman, D. 1956. Aging: a theory based on free radical and radiation chemistry. Journal of Gerontology. 11: 298–300.

Ismail, H., Lemriss, S., Ben Aoun, Z., Mhadhebi, L., Dellai, A., Kacem, Y., Boiron, P., and Bouraoui, A. 2008. Antifungal activity of aqueous and methanolic extracts from the Mediterranean seacucumber, *Holoturia polii*. *Journal of Medical Mycology*. **18**: 23-26.

Jiang, C., Agarwal, R., Lu, J. (2000). `Anti-angiogenic potential of a cancer chemopreventive flavonoid antioxidant, silymarin: inhibition of key attributes of vascular endothelial cells and angiogenic cytokine secretion by cancer epithelial cells'. Biochem. Biophys. Res. Comm., 276, p. 371-378.

Jose V, Ferrera JS, Federico VP, Miguel A, Vicente A, José ME, José V, Jaime M. 1991. Senile cataract: a review on free radical related pathogenesis and antioxidant prevention. Archives of Gerontology and Geriatrics, 13:51-59.

José, V., Ferrera, J,S., Federico, V.P., Miguel, A., Vicente. A., José, M.E., José,V., Jaime, M. 1991 Senile cataract: a review on free radical related

pathogenesis and antioxidant prevention. *Archives of Gerontology and Geriatrics,* 13:51-59.

Kannaian U P N, Bhuvaneswari S, Sripriya N and Prameela L, 2014. Antioxidant activity of common plants of Northern Tamil Nadu, India. International Journal of Pharmacy and Pharmaceutical Sciences 6(4) 128-132.

Kaur, C. & Kapoor, H. C. 2001. `Antioxidants in fruit and vegetables: the millennium's health'. Int. J. Food Science and Technology, 36, (7), p. 703-725.

Keerthana R, Visweswaran S, Sivakkumar S, Maariappan A and Banumathi V, 2018. Evaluation o Antioxidant Activity of Seeraga Chooranam In-Vitro Assay (A Siddha Polyherbal Preparation).International Journal of Ayurveda and PharmaResearch. 6(7) 17 -23.

Khalaf N A, Shakya A K, Othman A A, Agbar Z E and Farah H, 2008. Antioxidant activity of some common plants. Turkish Journal of Biology. 32(1): 51-55.

Kim D, Jeong S and Lee Ch, 2003 .Antioxidant capacity of phenolic phytochemicals from various cultivars of plums, Food Chemistry, 81, 321-326.

Kumar N, Ahmad KK M, Dang R and Husain A, 2008. Antioxidant of propolis from Tamil Nadu zone. Journal of Medicinal Plants Research. 2(12); 361-364.

Kumaran A and Karunakaran R J, 2007. *In-vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. LWT- Food Science Technology. 40(2): 344-352.

Kumaran S N, and Bragadeeswaran S. 2017. In vitro antioxidant activity on colonial acidianseudistomaviride and DidemnumPsammathodes. International Journal of Pharmaceutical science and research. 8(7): 3170- 3179.

Lamson, D. W. & Brignall, M. S. 1999. `Antioxidants in cancer therapy; their actions and interactions with oncologic therapies. Altern Med. Rev. 4(5):304-29.

Lee IM, Cook NR, Manson JE. 1999. Beta-carotene supplementation and incidence of cancer and cardiovascular disease: Women's Health Study. Journal of the National Cancer Institute, 91(2102–2106).

Lee, I.M., Cook, N.R., Manson, J.E.,1999. Beta-carotene supplementation and incidence of cancer and cardiovascular disease: Women's Health Study. *J Natl Cancer Inst*, 91:2102–2106.

Lian, F., Li, Y., Bhuiyan M., Sarkar, F. H. 1999. `P-53-independent apoptosis induced by genestein in lung cancer cells'. Nutr. Cancer, 33, p. 125-131.

Liu X,Jia J, Jing X,and Li G. 2018. Antioxidant activities of extracts from sarcocarp of cotoneaster multiflorus, Journal of Chemistry, 2018, 7.

Mahdi-Pour B,Jothy S L,Latha L Y,Chen C and Sasidharan S, 2012. Antioxidant activity of methanol extracts of different parts of *Lantana camara*. Asian Pacific Journal of Tropical Biomedicine. 2(12): 960–965.

Manickam D, Ramamoorthi SKP, Udhayakumar M, Kumar SB, 2017. Antioxidant activity of traditional siddha formulation on ccl4 induced liver fibrosis in rats. International Journal of Pharmacy and Pharmaceutical science, 9(10): 81-85.

Manssouri M, Znini M and Majidi L , 2020. Studies on the antioxidant activity of essential oil and various extracts of *Ammodaucus leucotrichus* Coss. & Dur. Fruits from Morocco. Journal of Taibah University for Science. 14: 124-130.

Marczin N, El-HabashiN, Royston D. 2003. Free radicals and cardiac arrhythmias following coronary surgery: actors of the drama or bystanders of the spectacle? Acta Anaesthesiologica Scandinavica, 47: 639–642.

Marczin, N., El-Habashi. N., Royston, D.2003. Free radicals and cardiac arrhythmias following coronary surgery: actors of the drama or bystanders of the spectacle? Acta *Anaesthesiologica Scandinavica*, 47: 639–642.

Maria Carolina A, Da Silva, Selma R, Paiv, 2012. Antioxidant activity and flavonoid content of Clusiafluminensis Planch.&Triana. (Annals of the

Brazilian Academy of Science) Anais da Academia Brasileira de Ciencias. 84(3): 609- 616.

Meenakshi, V.K. 1997. Biology of few chosen ascidians. Ph.D., Thesis, Manonmaniam Sundaranar University, Tirunelveli, India.

Meydani, M. 2001. `Nutrition interventions in ageing and age-associated disease: healthy ageing for functional longevity'. Ann. NY Acad. Sci., 928, p. 226-235.

Mohankumar J B,Gladious A J and Velvizhi M, 2017. Antioxidant Content of Selected Medicinal Plants Used by Kaani Tribes of Kanyakumari District in Tamilnadu India. Journal of Food and Nutrition Research.5(3) 180-186.

Muller, F.L., Lustgarten, M.S., Jang, Y., Richardson, A., Van, R.H.2007. Trends in oxidative aging theories. *Free Radic Biol Med*, 43: 477- 503.

Nagabhushan, N. & Bhide, S. V. 1992. `Curcumin as an inhibitor of cancer'. J. American College of Nutrition, 11, p. 192-8.

Nahid A, Neelabh C and Navneet K. 2017. Antioxidant and antimicrobial potentials of Artemisiaindica collected from the Nepal region. Journal of Pharmaceutical Sciences and Research. 9(10):1822-1826.

Nie, G. J., Jin, C. F., Cao, Y. L., Shen S. R., Zhao, B. L. 2002. `Distinct effects of tea catechins on 6-hydroxydopamine-induced apoptosis in PC 12 cells'. Archives of Biochemistry and Biophysics, 397, (1), p. 84-90.

Niki, E., Yamamoto, Y., Komuro, E., Sato, K. 1991. `Membrane damage due to lipid oxidation'. American Journal Clinical Nutrition, 53, p. 201 S-5S.

Palakkal S and Ganesan V, 2018. Evaluation of Antioxidant Property of Extracts of *Macarangapeltata* by DPPH Free Radical Scavenging Activity. Asian Journal of Pharmaceutical and Health Sciences. 8(4).

Ponnusamy J, Prasad AD, PandikumarP, Ignacimuthu S, 2011. Antioxidant and free radical scavenging activities of common wild greens from Tiruvallur District of Tamil Nadu, India. Indian Journal of Natural Products and Resources. 2(2), 156-163.

Priya D S, Sankaravadivu S and Christy, 2016.Antioxidant activity of a colonial ascidian *Eudistoma viride* using DPPH method.European journal of pharmaceutical and Medical Research ejpmr.3(8) 427-429.

Priya S D, Christy S K H, Sankaravadivu S, Packiam S C, 2018. Antioxidant activity of the simple ascidian Phallusianigra of Thoothukudi coast. International Journal of Pharmaceutical Chemistry. 05(12).

Rajamani K, Manivasagam T, Anantharaman P, Balasubramanian T, Somasundaram ST, 2011. Chemopreventive effect of Padinaboergesenii extracts on ferric nitrilotriacetate (Fe-NTA)-induced oxidative damage in Wistar rats. Journal of Applied Phycology 23(2):257-263.

Rajamani, K., Manivasagam, T., Anantharaman, P., Balasubramanian, T., Somasundaram, S.T. 2010. Chemopreventive effect of Padina boergesenii extracts on ferric nitrilotriacetate (Fe-NTA)-induced oxidative damage in Wistar rats. *J. Appl. Phycol*, 10811-010-9564-0.

Reddy, C. V. K,Sreeramulu D, Raghunath M, 2010. Antioxidant activity of fresh and dry fruits commonly consumed in India.Food Research International 43(1):285-288.

Ren, M. Q., Kuhn, G., Wegner, J., Chen, J. 2001. `Isoflavones, substances with multibiological and clinical properties'. European Journal of Nutrition, 40, (4), p. 135-146.

Revathi D,Baskaran K and Subashini R, 2015.Antioxidant And Free Radical Scavenging Capacity of Red seaweed Hypneavalentiae from Rameshwaram Coast Tamil Nadu, INDIA.International Journal of Pharmacy and Pharmaceutical Sciences.7(8): 232-237.

Roselin K F, Veerabahu C, Meenaksh V K, 2018. Antioxidant studies of a colonial ascidian DidemnumPsammathodes. International journal of science, Engineering and Management. 3(4): 515-5117.

Severyn C J, Shinde U, Rotwein P. 2009. Molecular biology, genetics and biochemistry of the repulsive guidance molecule family. Biochemical Journal, 422:393-403.

Severyn, C.J., Shinde, U., Rotwein, P. 2009. Molecular biology, genetics and biochemistry of the repulsive guidance molecule family. *Biochem J*, 422:393-403.

Sharma R K, Micali M, Pellerito A, Santangelo A, Natalello S, Tulumello R and Singla R K, 2019. Studies on the determination of antioxidant activity and phenolic content of plant products in India (2000–2017). Journal of AOAC International. 102(5): 1407–1413.

Sofna D.S, C. N, 2014. Antioxidant properties of flavonoids. Medical Journal of Indonesia. 23(4): 239.

Speakman JR, Talbot DA, Selman C, Snart S, McLaren JS, Redman P, Krol E, Jackson DM, Johnson MS, Brand MD. 2004. Uncoupled and surviving: individual mice with high metabolism have greater mitochondrial uncoupling and live longer. Aging Cell, 3:87-95.

Speakman, J.R., Talbot, D.A., Selman, C., Snart, S., McLaren, J.S., Redman, P., Krol, E., Jackson, D.M., Johnson, M.S., Brand, M.D.2004. Uncoupled and surviving: individual mice with high metabolism have greater mitochondrial uncoupling and live longer. *Aging Cell,* 3:87-95.

Sree N V, P. Udaya Sri Y.V.V, Aswani Kumar, Rao N R, 2014. In-vitro Antioxidant and Antimicrobial Activities of Some Medicinal Plants grown in Western Ghats of India. IOSR Journal of Pharmacy. 4(7): 25-33.

Sriganth, I. N. P. & Premalatha, B. 1999. `Dietary curcumin with cisplatin administration modulates tumour marker indices in experimental fibrosarcoma'. Pharmacol. Res., 39, p. 175-179.

Stankovića N, Bernsteine N, 2016. Antioxidant Activity of Traditional Medicinal Plants from the Balkan Peninsula. NJAS - Wageningen Journal of Life Sciences. 78: 21-28.

Tekin Z, and Küçükbay F Z, 2020. Evaluation of phytochemical contents and antioxidant activity of pomegranate flower. Journal of the Turkish Chemical Society Section A: Chemistry. 7(1) 37–42.

Trad B A,Qudah M A A,Zoubi M A,Masri A A,MuhaidatR,QarJ,Alomari G and Alrabadi N I,2018.In-Vitro and In-Vivo Antioxidant Activity of the Butanolic Extract from the Stem of Ephedra Alte. Biomedical and pharmacology Journal.11(3) 1239-1245.

Vertuani S, Angusti A, Manfredini S. 2004. The antioxidants and pro-antioxidants network: an over. Journal of Current Pharmaceutical Design .10: 1677-1694.

Vertuani, S., Angusti, A., Manfredini, S. 2004. The antioxidants and proantioxidants network: an overview. *Curr Pharm Des,* 10: 1677-1694.

Zhang Z, Wang F, Wang X and Liu X. 2010. Extraction of the Polysaccharides from Five Algae and Their Potential Antioxidant Activity in vitro. Carbohydrate Polymers 82(1):118-121.

BIOMONITORING BY ASSESSING BIOLOGICAL OXYGEN DEMAND IN AND AROUND THOOTHUKUDI WATER BODIES

A Project submitted to

ST.MARY'S COLLEGE (AUTONOMOUS), THOOTHUKUDI

Affiliated to

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TIRUNELVELI

In partial fulfillment for the award of the degree of

Bachelor of Science in Zoology

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APRIL 2022

CERTIFICATE

This is to certify that the project entitled **Biomonitoring by assessing biological oxygen demand in and around Thoothukudi water bodies** is submitted to **St. Mary's College (Autonomous), Thoothukudi** in partial fulfillment for the award of the degree of **Bachelor of Science in Zoology** and it is a record of the work done during the year 2021-2022 by the following students.

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CONTENTS

INTRODUCTION

1. INTRODUCTION

Water is an indispensable component of human resource, which covers about 3/4th of the earth surface, among which the major part is contributed by Seas and Oceans (97%). (Malarvannan and Balamurugan, 2018). It is the culture environment for fish where they perform all their bodily functions. They are totally dependent upon water to breathe, feed and grow, excrete wastes, maintain a salt balance, and reproduce (Brönmark and Hansson, 2017). Water and water resources is very important for maintaining an adequate food supply and a productive environment for the all living organisms. As human populations and economies grow, global freshwater demand has been increasing rapidly. In addition to threatening the human food supply, water shortages severely reduce biodiversity in both aquatic and terrestrial ecosystems (Pimentel *et al.,* 2004).

Under the pretext of industrialization, though job opportunities abound and facilities and utilities become available, water is being polluted to a great extent. In contrast to natural sources, anthropogenic sources contribute heavily to water pollution. This calls for consistent monitoring of water quality. Untreated domestic sewage and industrial effluents being let out into the water bodies affect water utility for drinking, agricultural, recreational, and other purposes. Conversely, discharging toxic components into aquatic bodies cause harm to marine life, which in turn affects humans and the surrounding environment. Since water scarcity is on the rise, to make healthy drinking water available for

humankind in the present and future epochs, water quality assessment is indispensable (Navamuniyammal *et al.,* 2021).

Organic pollution of waterbodies by wastewater discharge from human activities (cities, farming, industry) affects humans and ecosystems worldwide through the global sanitation crisis. First, untreated urban sewage contains pathogens that cause a variety of diseases, including diarrhoea, globally the leading cause of illness and death (Pruss, A., *et al*., 2002). Second, accumulation of organic pollutants in rivers stimulates microbial growth, leading to oxygen depletion and disturbance of the entire river ecosystem (Sirota, J., *et al*., 2013).

Fresh water ecosystems are the most endangered ecosystems in the world and human activities have accelerated the process of degradation of these systems (Dudgeon *et al.,* 2006; Monroe *et al.,* 2009). Contamination of surface and groundwater by natural and anthropogenic input has put the aquatic fauna and human health at risk throughout the world (Akhtar *et al.,* 2021). In India the sources of drinking water include rivers, streams, lakes, ponds, wells, underground aquifers, reservoirs and springs (Shil *et al.,* 2019). Most of these systems are moderately polluted by agricultural waste, sewage, industrial waste and by human intervention (Jindal and Sharma 2011; Singh *et al.,* 2020). Natural factors like hydrological, climatic, atmospheric, lithological, topographical and anthropogenic factors like mining, livestock farming, sediment run-off, soil

erosion, heavy metal pollution etc. deteriorate the water quality badly (Uddin *et al.,* 2021).

The oxygen content of the open ocean and coastal waters has been declining for at least the past half-century, largely because of human activities that have increased global temperatures and nutrients discharged to coastal waters. These changes have accelerated consumption of oxygen by microbial respiration, reduced solubility of oxygen in water, and reduced the rate of oxygen resupply from the atmosphere to the ocean interior, with a wide range of biological and ecological consequences (Breitburg, D., *et al.,* 2018). The loss of dissolved oxygen from coastal areas and the open ocean is one the most important issues affecting the global marine environment. The extent of oxygen deficient areas known as “dead zones” is increasing in coastal areas while in the open ocean oxygen minimum zones are also expanding. The increase in dead zones is linked to coastal pollution while oxygen minimum zones are increasing because of climate induced rises in ocean temperature and strengthening water column stratification. The effect is the same; lower than normal oxygen concentrations that harm marine organisms and damage entire marine ecosystems (Boyle, S. 2020).

Water quality is the general descriptor of water properties such as its physical, chemical and biological characteristics. Monitoring and assessing the

quality of surface waters are critical for managing and improving its quality (Malarvannan and Balamurugan, 2018).

Biochemical oxygen demand (BOD) is a simple and practical indicator of the total organic content that is available to organisms plus any chemicals that spontaneously react with O_2. The procedure is straightforward: Water is incubated in sealed bottles, and the decrease in O_2 over time is monitored. If all the O_2 is consumed over the time of the incubation, the original water sample must be diluted and analysed again. During the incubation, naturally present heterotrophic organisms use O_2 to respire the organic carbon that is biologically available and any chemicals that spontaneously react with O_2 (e.g., sulphide) will also do so, allowing analysts to assess total BOD in wastewaters (Eaton *et al.*, 1995).

BOD directly affects the amount of dissolved oxygen in rivers and streams. The greater the BOD, the more rapidly oxygen is depleted in the stream. This means less oxygen is available to higher forms of aquatic life. The consequences of high BOD are the same as those for low dissolved oxygen aquatic organisms which become stressed, suffocate and die. Sources of BOD include leaves and woody debris; dead plants and animals; animal manure; effluents from pulp and paper mills, wastewater treatment plants, feedlots and food-processing plants; failing septic systems; and urban storm water runoff. The discharge of wastes with high levels of BOD can cause water quality problems such as severe dissolved oxygen depletion and fish kills in the receiving water bodies (Penn *et al.,* 2003).

Most pristine rivers will have a 5-day carbonaceous BOD below 1 mg/L. Moderately polluted rivers may have a BOD value in the range of 2 to 8 mg/L. Rivers may be considered severely polluted when BOD values exceed 8 mg/L (Connor and Richard, 2016). Municipal sewage that is efficiently treated by a three-stage process would have a value of about 20 mg/L or less. Untreated sewage varies but averages around 600 mg/L in Europe and as low as 200 mg/L in the US, or where there is severe groundwater or surface water infiltration (Verma, N., & Singh, A.K. 2013). The optimum BOD range for ocean water should be below 1-2 mg/L and for freshwater should be below 1 mg/L (Bhuyan *et al.,* 2020).

Hence in the present study we analysed seven water samples from different areas of Thoothukudi. The BOD values were expressed in milligrams of oxygen consumed per litre of sample during 5 days of incubation at 20°C, which is the degree of organic pollution of water.

OBJECTIVES

2. OBJECTIVES

The main objectives of the study are

- To determine the degree of water pollution and to understand the harmful effects of discharging pollutants and industrial effluents into water ecosystems of economically and traditionally important areas of Thoothukudi based on BOD (Biological Oxygen Demand) Index.
- To collect seven water samples from various stations of Thoothukudi and analyse the BOD values before and after 5 days of incubation.
- To document and tabulate the data.
- To provide suggestions to improve the water quality.

REVIEW OF LITERATURE

3. REVIEW OF LITERATURE

Pollution load analysis of biological oxygen demand from household sources was studied by Parabang, Surono *et al.,* (2021). Based on the research results, it was found that the waste water generation per person per day was 90.96 litre with a BOD concentration of 45.63 mg/litre and a pollution load of 0.016 kg/person/day. The pollution load contained in household wastewater after being treated through the wastewater treatment plant has decreased to 0.007 kg/person/day.

The analysis of BOD (Biological Oxygen Demand) and COD (Chemical Oxygen Demand) contents in the water of around laying chicken farm was done by Abdullahi, A. B. *et al.,* (2021). The method used in the water BOD content test is the Winkler method. The results show that the BOD contents of water around the laying chicken farm was 7.99 mg/L on average. This research concluded that the BOD content of water around the laying chicken farm exceeds the threshold set by the government. The threshold for water BOD content is 2 mg/L.

Vigiak, Olga *et al.,* (2019) predicted biochemical oxygen demand in European freshwater bodies. The study showed that high BOD loadings to freshwater systems are mainly coming from anthropogenic sources, comprising domestic and livestock waste, industrial emissions, and combined sewer overflows.

Water pollution levels in the Suwung Estuary, Bali based on Biological Oxygen Demand was analysed by Saraswati, N.L.G.R.A. *et al.,* (2018). The BOD5 Samples were taken at all tide cycles, during ebb to high tide and high tide to ebb. The range of BOD5 values were: 0.84 mg/L - 9.47 mg/L during ebb to high tide and 0.96 mg/L - 8.75 mg/L during high tide to ebb. The result of BOD5 analysis showed that the water pollution level in the Suwung estuary was slightly contaminated in both tide conditions. The spatial distribution of BOD5 value tended to be higher around the cage aquaculture area, near the river, landfill area and around Benoa harbour.

Statistical evaluation of biochemical oxygen demand of river water was studied by Ghosh, Sourav *et al.,* (2018) reported that, far water BOD value came less than near water BOD value as the experiment have been performed. So, it can be concluded that far water system is more compatible for aquatic life.

Biological Oxygen Demand in Controlling Fish Production and Cost of Supplementary Feed towards better Sustainability of a Sewage-Fed Aquaculture System: A Case Study of East Kolkata Wetlands, West Bengal, India was done by Mukherjee, S., & Dutta, M. (2016). This study provides an insight about the effect of BOD on fish production towards better sustainability of this system.

Trichodina Sp. as bioindicator for evaluation of Biochemical Oxygen Demand (BOD5) in aquaculture fish farms (ponds) was studied by Al-Marjan,

K., & Abdullah, S. (2015). The aim of this study is to know the prevalence of the fish infestation by Trichodina sp. as bioindicator for evolution of the Biological Oxygen Demand (BOD5) from Ainkawa fish hatchery. The prevalence of fish infestation in each pond increased (57.5, 40, 27.5, 45, 15, and 42.5% respectively) with the increase of the values of BOD5 (9.2, 8.0, 5.7, 8.1, 2.9 and 8.0 mg/L, respectively).

Jouanneau, S., *et al.,* (2014) studied methods for assessing biochemical oxygen demand (BOD). This review has identified the main technological strategies designed to measure or to estimate the BOD parameter, used as an indicator of the biodegradability of organic matter. It focuses on the technological aspect of the assessment methods, on the nature of the performed measurement (real measurement or prediction) and on the pros and cons of them.

Pathologic effects of different biological oxygen demand levels on the juvenile catfish was studied by Mamacus, Ronan *et al*., (2013). A total of 40 catfish (*Clarias gariepinus*) were exposed for 96 hours to natural BOD levels of the wastewater treatment lagoons of a swine farm. After exposure, the gills, liver, spleen and kidney were examined and scored for lesions. Results of the study revealed an increasing lesion score with the rise in the BOD level, which is suggestive of the deleterious effects of high BOD levels on these organs.

Study of biochemical oxygen demand in Godawari river at Nanded city due to impact of industrial pollution was done by Deshmukh, J.U. *et al.,* (2012). It

concluded that the river water shows more concentration of BOD in Godawari river. In the month of April and May the water from old bridge and Wadgaon i.e., at Station-B and Station-C were unfit for public supply, drinking bathing, fish culture and irrigation.

Study on the changing law of dissolved oxygen and dissolved oxygen saturation in Baiyang Lake was done by Ma, Jianwei *et al.,* (2012). Following the comprehensive analysis, dissolved oxygen in Baiyang Lake was mainly affected by temperature, organic matter concentration and water-plants. The characteristics of photosynthesis were obvious in spring and summer and the oxidation in autumn and winter were obvious.

Ground water pollution calculated with the measurement of level of biological oxygen demand was investigated by Amit varale and Yashodhara varale (2012). The analysis indicated the level of BOD were generally higher in summer and winter than their levels in rainy season.

Dissolved Oxygen and Biochemical Oxygen Demand in the waters close to the Quelimane sewage discharge was estimated Moccuba, Jeremias (2010). The values of biochemical oxygen demand obtained are characteristic of unpolluted waters which suggest that the municipal effluents discharged into the estuary are negligible or are flushed away by the tides.

A study on optimum BOD levels for fish culture in wastewater ponds was done by Chattopadhyay, G.N. *et al.,* (1988). The results indicated 10–20 ppm to be the optimum BOD range for fish culture in wastewater ponds.

Tidal fluctuations in biological oxygen demand in exposed sandy beaches was analysed by Dye, A.H., (1980). Fluctuations in BOD of over two orders of magnitude were found with the highest occurring at or just after high tide and the lowest at low tide. Greatest fluctuations occurred at the higher tidal levels as well as near the surface of the substratum. Significant correlations between BOD and the degree of water saturation of the sand were found.

STUDY AREA

4. STUDY AREA

In this study seven water samples were collected from different stations of Thoothukudi town and the BOD values were determined by comparing the dissolved oxygen levels of water samples before and after 5 days of incubation in the dark by using Winkler's Method. The difference between the two DO levels represents the amount of oxygen required for the decomposition of any organic material in the sample and is a good approximation of the BOD level.

The pond water samples were collected from Teppakulam, Palyakayal and Authoor whereas the Sea water samples were collected from Therespuram beach, Harbor beach, Muthunagar beach and Tharuvaikulam beach.

Teppakula Mariamman Temple is located on the Heart of Thoothukudi City. Since the Presiding Deity is facing the Teppam or Pond hence the Name Teppakula Mari Amman. Teppakulam is a huge Sacred Tank in front of this Temple. Teppakulam is considered as the 'Place of cultural interest'. The Maha Kumbabishekam was performed in the year 1973. Thousands of Devotees visit this Temple during Aadi month. They prepare Kozhu in order to show their respect to the Presiding Deity.

Palayakayal is a Village in Srivaikundam Block in Tuticorin District of Tamil Nadu State, India. It is located 17 KM towards South from District headquarters Thoothukudi. People of this village are living in a peaceful manner.

The village has a very proud history with agriculture being the main profession of this village. This village also has many fisheries industries like V.V. Marine Products, Asvini Fisheries, etc.

Authoor is a panchayat town in Thoothukudi district. Many of the people of Authoor are farmers of betel leaf, banana and rice. The betel leaf from Authoor is famous for its quality in Tamil Nadu. The Thamirabarani river surrounds the town. The river flows through the area, forming a big pond on one end.

Muthunagar beach is located 5 kms towards south from Thoothukudi town. It is a local recreational beach and is one of the important fish-landing sites of Thoothukudi.

Tharuvaikulam Beach is located in Tharuvaikulam Village in Thoothukudi District. It is calm and sandy beach. Fishers from Tharuvaikulam have been quietly practising sustainable fishing for years and reaping profits - economical, social and environmental. According to marine researchers, Tharuvaikulam is the only village in the state that relies purely on gill nets to fish.

Therespuram is situated on the north of Thoothukudi, which is an important centre among the seven sea ports of South East coast in the bay of Mannar. Now it is fishing for sea shells and the central place for pearl fishing.

Harbour Beach is a well-maintained sandy beach in Thoothukudi. It is located close to the Harbour Guest House. There is also a park near it where people can stroll in the evenings amidst the refreshing sea breeze. The park is a

popular tourist spot today. The major industries like Power plant, Heavy water plant and Thoothukudi Thermal power station are located around this beach. Salt pans are also present near this beach.

Figure 1: Map showing stations were water samples were collected

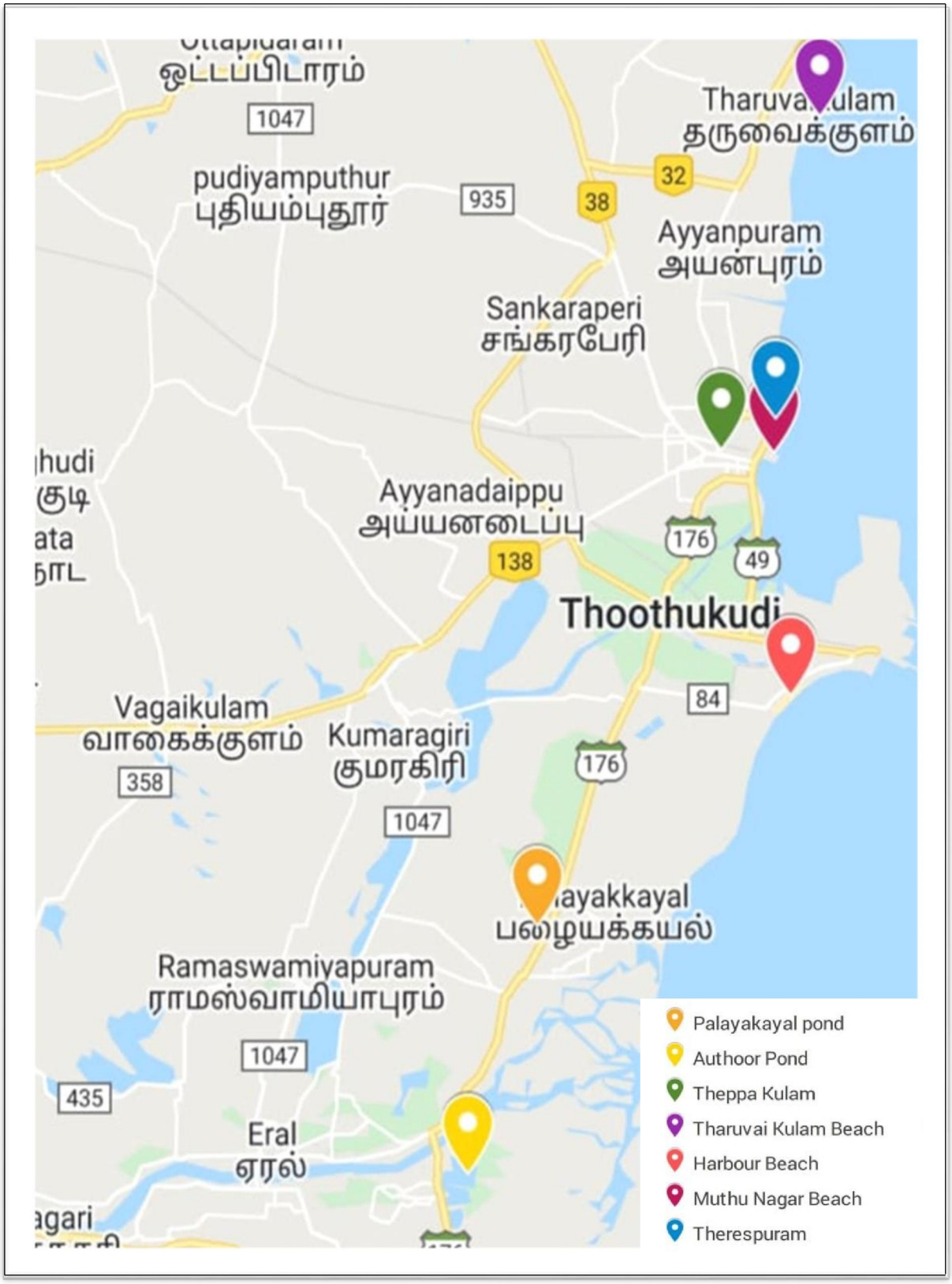


Figure 2: Teppakulam Pond

Figure 3: Palayakayal Pond

Figure 4: Authoor Pond

Figure 5: Harbour Beach

Figure 6: Therespuram Sea

Figure 7: Muthunagar Beach

Figure 8: Tharuvaikulam Sea

MATERIALS AND METHODS

5. MATERIALS AND METHODS

In this study Winkler's method is used to measure the dissolved oxygen content in the samples.

Glass wares used:

Burette, pipette, conical flask, measuring jar, burette stand, beaker, funnel, reagent bottle.

Chemicals used:

- Manganous Sulphate: 48g of Manganous sulphate dissolved in 100 mL of water.
- Alkali Mixture: 70g of Potassium hydroxide and 15g Potassium iodide are dissolved in distilled water and diluted to 100 mL
- Starch indicator: 1g of starch in 100 mL of boiled distilled water.
- Conc. H_2SO_4
- Sodium thiosulphate (0.025N): 3.102g of Sodium thiosulphate is dissolved in previously boiled distilled water and made up to 500ml and stored in brown bottle.

BOD measurement:

In a BOD bottle, 250 ml of sample water is taken and two samples are prepared. A sample is kept for DO analysis for day 1 and another sample is kept in a BOD incubator for 5 days at 20°C.

One mL of Alkali iodide is added to each sample bottle, followed by 1 mL of Manganous sulphate solution. The bottle is shaken well and allowed to sit for five minutes in order to settle the precipitate. Afterwards, 2 mL of concentrated H_2SO_4 is added, and the cap is placed on the bottle. The bottle is shaken until the precipitate is completely dissolved.

In a conical flask, 100 ml of sample is placed and titrated with standard sodium thiosulphate solution (0.025N) until the colour changes from dark yellow to light yellow. Next, a few drops of starch are added as an indicator. The titration is repeated until the colour of the solution becomes colourless or changes back to the original sample colour.

Volume of 0.025N Sodium thiosulphate consumed is recorded. The dissolved oxygen (DO) (in mg/L) is equal to the Sodium thiosulphate (0.025N) consumed.

After 5 days the water sample that is incubated at 20°C is taken out and then same procedure is repeated and the final DO value is obtained. The final

value obtained is less when compared to the initial DO value because the microorganisms present in the water sample consume the oxygen present.

In this way all the other water samples are titrated and the initial and the final DO values are tabulated. The results of each water samples are tabulated separately.

Amount of dissolved oxygen in the water sample can be calculated by the formula given below,

$$\text{Dissolved oxygen content (mg/L)} = \frac{\text{Titrant volume} \times 0.025 \times 8 \times 1000}{\text{Volume of the sample}}$$

0.025 = Normality of sodium thiosulphate

8 = Molecular weight of oxygen

From the dissolved oxygen content present in the sample calculated on the 1st day and 5th day, BOD value can be obtained by applying the following formula.

$$\text{BOD in water sample (mg/L)} = \frac{DO_I - DO_F \times 100}{V}$$

Where, DO_I = DO of the sample at the 1st day

DO_F = DO of the sample at the 5th day

V = Volume of the sample

RESULTS

6. RESULTS

Water samples from seven study areas were collected in and around Thoothukudi and BOD values were computed.

6.1 Pond Water Stations of Thoothukudi

Table 1: Estimation of Dissolved Oxygen content in pond water samples (2022)

S. No	Water sample	Volume of Sodium thiosulphate utilized (ml)	
		Day 1	Day 5
1.	Teppakulam pond water	3.5	0.6
2.	Palayakayal pond water	3.5	1.3
3.	Authoor pond water	2.05	1.45

6.1.1 Dissolved Oxygen and BOD levels of pond water samples in Thoothukudi:

1. Teppakulam Pond Water:

$$\text{Dissolved oxygen in the sample in Day 1 (mg/L)} = \frac{3.5 \times 0.025 \times 8 \times 1000}{100}$$

$$= 7 \text{ mg/L}$$

$$\text{Dissolved oxygen in the sample in Day 5 (mg/L)} = \frac{0.6 \times 0.025 \times 8 \times 1000}{100}$$

$$= 1.2 \text{ mg/L}$$

$$\text{BOD of Teppakulam pond water (mg/L)} = \frac{7 - 1.2 \times 100}{100}$$

$$= 5.8 \text{ mg/L}$$

2. Palayakayal Pond Water:

$$\text{Dissolved oxygen in the sample in Day 1 (mg/L)} = \frac{3.5 \times 0.025 \times 8 \times 1000}{100}$$

$$= 7 \text{ mg/L}$$

$$\text{Dissolved oxygen in the sample in Day 5 (mg/L)} = \frac{1.3 \times 0.025 \times 8 \times 1000}{100}$$

$$= 2.6 \text{ mg/L}$$

BOD of Palayakayal pond water (mg/L) $= \frac{7 - 2.6}{100} \times 100$

$= 4.4$ mg/L

3.Authoor Pond Water:

Dissolved oxygen in the sample in Day 1 (mg/L) $= \frac{2.05 \times 0.025 \times 8 \times 1000}{100}$

$= 4.1$ mg/L

Dissolved oxygen in the sample in Day 5 (mg/L) $= \frac{1.45 \times 0.025 \times 8 \times 1000}{100}$

$= 2.9$ mg/L

BOD of Authoor pond water (mg/L) $= \frac{4.1 - 2.9}{100} \times 100$

$= 1.2$ mg/L

Table 2: DO levels of Pond water samples in mg/L (2022)

Study Area	Dissolved Oxygen Level in Day 1 (mg/L)	Dissolved Oxygen Level in Day 5 (mg/L)
Teppakulam pond water	7.0	1.2
Palayakayal pond water	7.0	2.6
Authoor pond water	4.1	2.9

Chart 1: DO levels of Pond water stations in mg/L (2022)

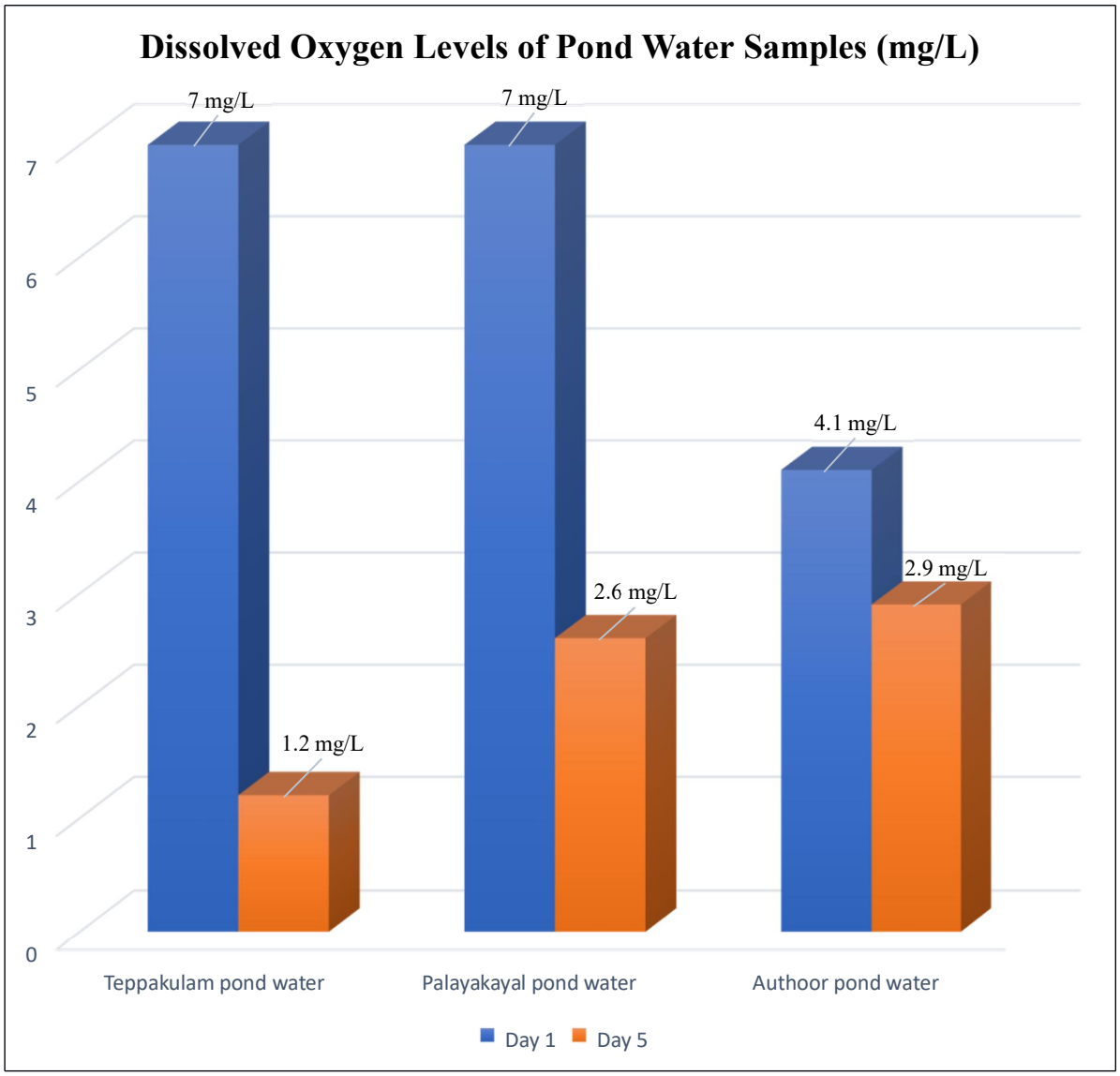
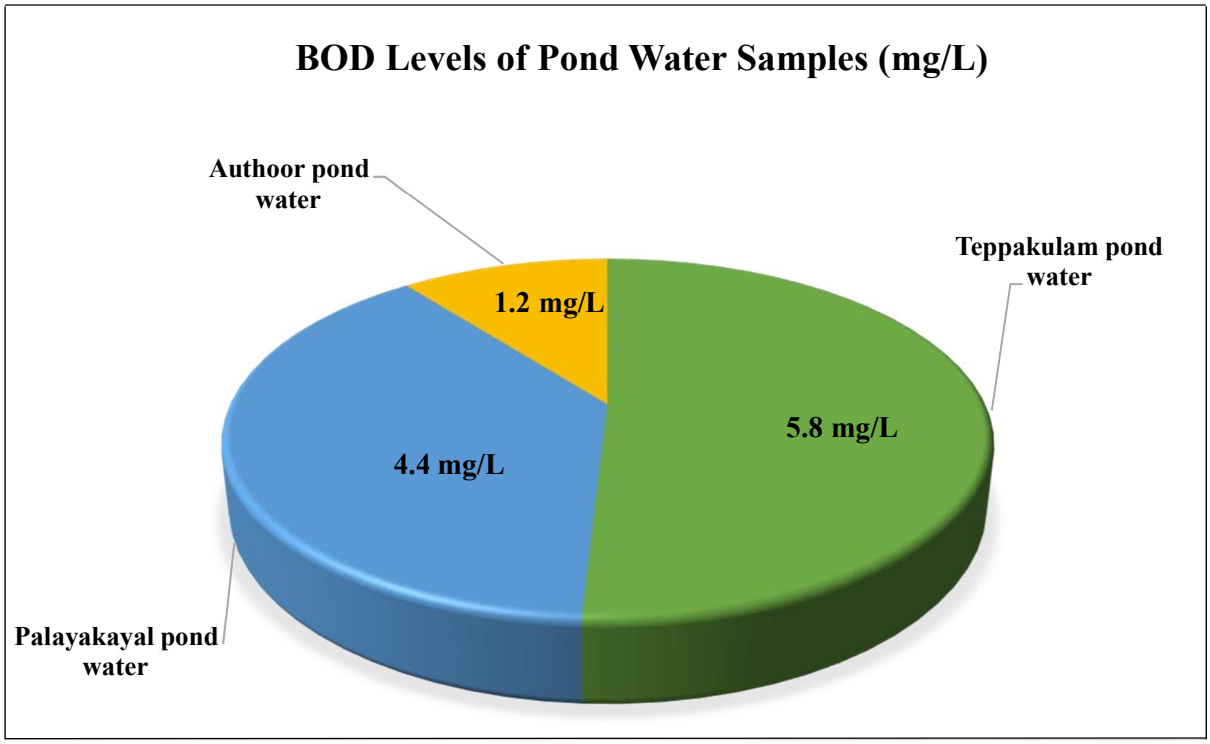


Table 3: BOD levels of Pond water stations in mg/L (2022)

Stations	BOD (mg/L)
Teppakulam pond water	5.8
Palayakayal pond water	4.4
Authoor pond water	1.2

Chart 2: BOD Level of Pond water stations in mg/L (2022)



6.2 Marine water stations of Thoothukudi

Table 4: Estimation of Dissolved Oxygen content in Marine water samples (2022)

S. No	Water sample	Volume of Sodium thiosulphate utilized (ml)	
		Day 1	Day 5
1.	Harbour Beach	5	1.5
2.	Therespuram Sea	4.1	0.9
3.	Muthunagar Beach	4	1.35
4.	Tharuvaikulam Sea	4.05	1.9

6.2.1 Dissolved Oxygen and BOD levels of various Marine water samples in Thoothukudi:

1. Harbour Beach Water:

$$\text{Dissolved oxygen in the sample in Day 1 (mg/L)} = \frac{5 \times 0.025 \times 8 \times 1000}{100}$$

$$= 10 \text{ mg/L}$$

$$\text{Dissolved oxygen in the sample in Day 5 (mg/L)} = \frac{1.5 \times 0.025 \times 8 \times 1000}{100}$$

$$= 3 \text{ mg/L}$$

$$\text{BOD of Harbour Beach water (mg/L)} = \frac{10 - 3}{100} \times 100$$

$$= 7 \text{ mg/L}$$

2.Therespuram Sea Water:

$$\text{Dissolved oxygen in the sample in Day 1 (mg/L)} = \frac{4.1 \times 0.025 \times 8 \times 1000}{100}$$

$$= 8.2 \text{ mg/L}$$

Dissolved oxygen in the sample in Day 5 (mg/L) $= \frac{0.9 \times 0.025 \times 8 \times 1000}{100}$

$= 1.8$ mg/L

BOD of Harbour Beach water (mg/L) $= \frac{8.2 - 1.8 \times 100}{100}$

$= 6.4$ mg/L

3. Muthunagar Sea Water:

Dissolved oxygen in the sample in Day 1 (mg/L) $= \frac{4 \times 0.025 \times 8 \times 1000}{100}$

$= 8$ mg/L

Dissolved oxygen in the sample in Day 5 (mg/L) $= \frac{1.35 \times 0.025 \times 8 \times 1000}{100}$

$= 2.7$ mg/L

BOD of Muthunagar beach water (mg/L) $= \frac{8 - 2.7 \times 100}{100}$

$= 5.3$ mg/L

4. Tharuvaikulam Sea Water:

$$\text{Dissolved oxygen in the sample in Day 1 (mg/L)} = \frac{4.05 \times 0.025 \times 8 \times 1000}{100}$$

$$= 8.1 \text{ mg/L}$$

$$\text{Dissolved oxygen in the sample in Day 5 (mg/L)} = \frac{1.9 \times 0.025 \times 8 \times 1000}{100}$$

$$= 3.8 \text{ mg/L}$$

$$\text{BOD of Tharuvaikulam water (mg/L)} = \frac{8.1 - 3.8 \times 100}{100}$$

$$= 4.3 \text{ mg/L}$$

Table 5: DO levels of Marine water samples in mg/L (2022)

Study area	Dissolved oxygen level in Day 1 (mg/L)	Dissolved Oxygen level in Day 5 (mg/L)
Harbour Beach	10	3
Therespuram Sea	8.2	1.8
Muthunagar Beach	8.0	2.7
Tharuvaikulam Sea	8.1	3.8

Chart 3: DO levels of Marine water stations in mg/L (2022)

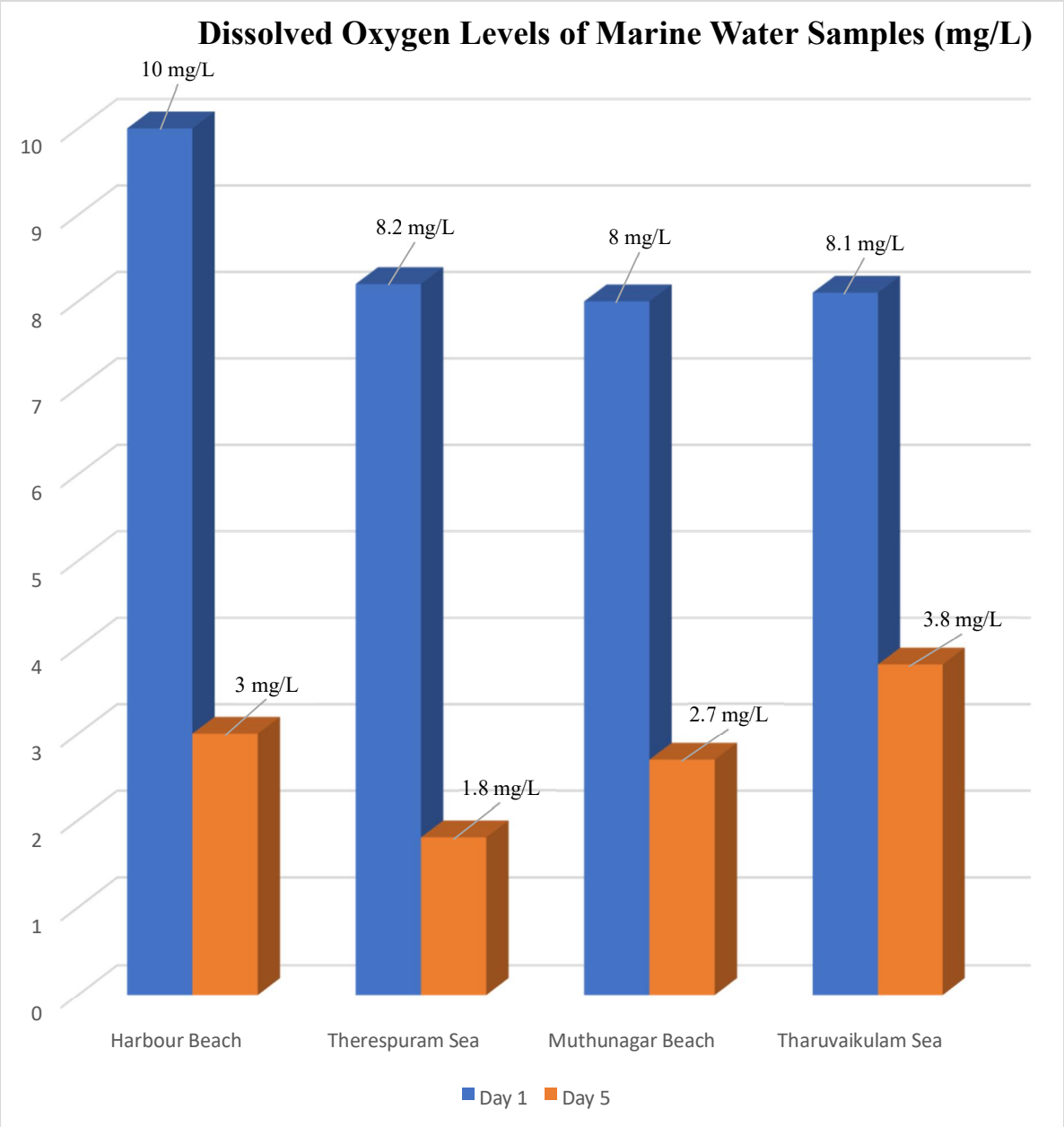
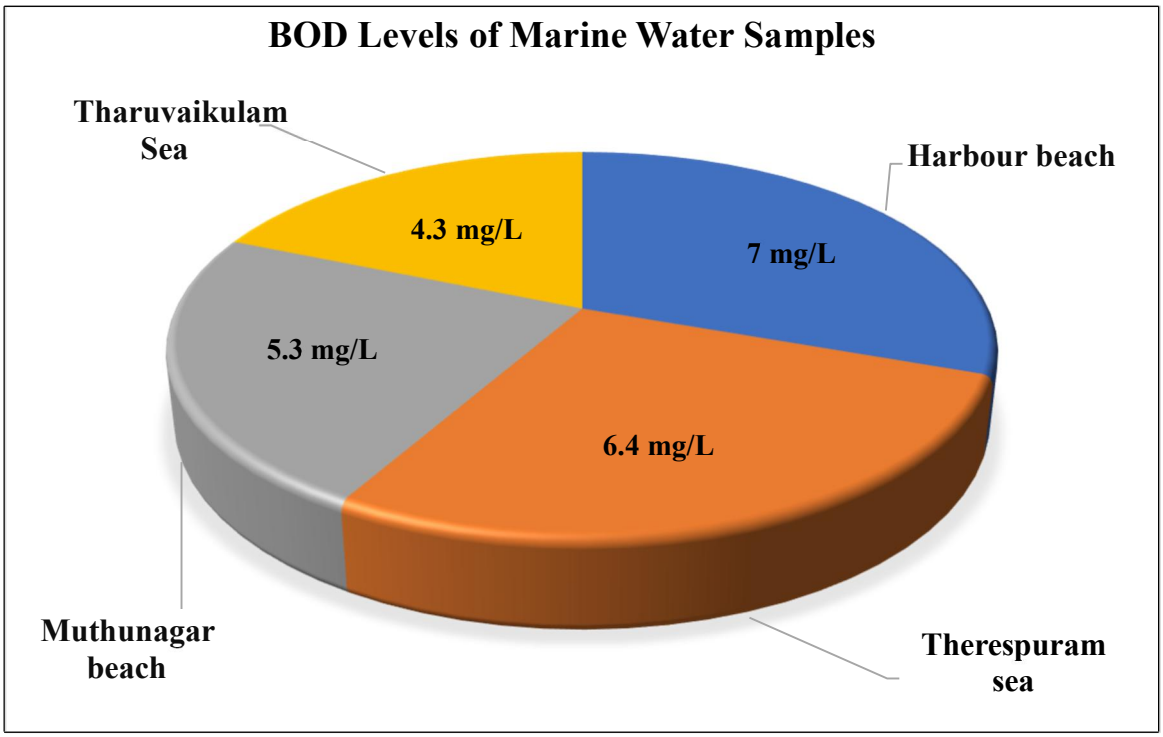


Table 6: BOD levels of Marine water stations in mg/L (2022)

Stations	BOD (mg/L)
Harbour Beach	7.0
Therespuram Sea	6.4
Muthunagar Beach	5.3
Tharuvaikulam Sea	4.3

Chart 4: BOD levels of Marine water stations in mg/L (2022)



DISCUSSION

7. DISCUSSION

Water is essential for life. From the time that primeval species ventured from the oceans to live on land, a major key to survival has been prevention of dehydration. The critical adaptations cross an array of species, including man. Without water, humans can survive only for days.

The oxygen in water which is available for species' use is called "dissolved oxygen," (DO). When organic matter from sewers, algal blooms, and other sources enters water, it is immediately broken down by bacteria, which requires some of the dissolved oxygen. When DO levels fall below a certain level, it adversely impacts aquatic life, sometimes causing mass fish kills. This, in turn, causes further problems because of the sheer number of decaying organisms requiring oxygen.

The biochemical oxygen demand (BOD) is a crucial environmental index for determining the relative oxygen requirements of wastewater, effluents, and polluted water. It refers to the quantity of oxygen required by bacteria and other microorganisms in the biochemical degradation and transformation of organic matter under aerobic conditions. The BOD is also interpreted as a measure of the concentration of organic material that can serve as a substrate to support the growth of microorganisms.

A BOD level of 1-2 ppm is considered very good. There will not be much organic waste present in the water supply. A water supply with a BOD level of 3-

5 ppm is considered moderately clean. In water with a BOD level of 6-9 ppm, the water is considered somewhat polluted because there is usually organic matter present and bacteria are decomposing this waste. At BOD levels of 100 ppm or greater, the water supply is considered very polluted with organic waste.

Higher BOD indicates more oxygen is required, which is less for oxygen demanding species to feed on, and signifies lower water quality. Inversely, low BOD means less oxygen is being removed from water, so water is generally purer. Cold water retains oxygen better than warmer water, so in summer months, dissolved oxygen is usually lower from the start. Unpolluted rivers usually have BOD levels below 1 part per million (equivalent to 1 mg/L), while untreated sewage has between 200 and 600 ppm.

Water pollution is a global challenge that has increased in both developed and developing countries, undermining economic growth as well as the physical and environmental health of billions of people.

Aquatic ecosystems have been affected by various types of contaminations around the globe in the recent few years. Heavy metals are one of the most common pollutants which have severely deteriorated the aquatic ecosystems due to their toxicity, abundance, persistence, and subsequent bio-accumulation. Their release in aquatic ecosystem is triggered by both natural and anthropogenic processes. Contamination of aquatic ecosystems with toxic heavy metals is an environmental problem of public health concern. Being persistent pollutants,

heavy metals accumulate in the environment and consequently contaminate the food chains. Accumulation of potentially toxic heavy metals in biota causes a potential health threat to their consumers including humans.

Tuticorin is the main coastal and industrial city present in the Gulf of Mannar region. Nowadays there is an abundant rise in the rate of industries and factories. This leads to the land pollution and the waste products released out from the factories are directed towards the seawater, pond and other water sources

We have collected three pond water samples and four sea water samples. Their BOD values are calculated and the results indicate the biological oxygen demand of the chosen samples.

The problem of pollution in the freshwater system in Thoothukudi is mainly due to industrialization and population expansion. Water quality is often overlooked in pond management, and poor water quality can lead to common problems, such as excessive algal blooms, overgrowth of plants, noxious smells, or dead and dying fish. In order to prevent these problems, an understanding of basic water chemistry and other physical parameters is necessary.

Teppakula Mariamman Temple is located in the heart of Thoothukudi. The Teppakulam pond belonging to this temple is also on the lower road. This pond was last dredged in the year 2017. Now the pond is littered with garbage without adequate maintenance. Thus, the pond is posing a major threat creating a health

disorder to the fishes living in them. The BOD value recorded here is 5.8 mg/L which is the highest value recorded among the pond water samples.

Palayakayal pond water is the second most polluted water among the pond water samples with the BOD value of 4.4 mg/L. The Thamirabarani river flows roughly east and enters the Gulf of Mannar of the Bay of Bengal near Palayakayal. This is pond water gets continuously polluted by the untreated sewage and organic waste from the villages on its banks.

Authoor is a green town, surrounded by rice paddies and banana trees. Authoor is famous for the Thamirabarani river surrounding the town. The river provides a majority of the water used for irrigation in and around the area. Authoor pond water shows the least amount of BOD of value 1.2 mg/L.

The coast of Tuticorin is sheltered by Srilanka and the coastal stretch extends upto 164 kms. The population of Tuticorin is 0.4 million which generates waste water of about 18 MLD. Tuticorin is becoming a hot spot polluted area owing to rapid industrialization and urbanization. Import of raw materials for the industries and exporting the products happens at harbour and is one of the busiest harbours of India. Such activities contribute lot of pollution load to the coastal area and almost entire basin. Due to the accelerated development activities the coastal area experience significant changes.

The present study shows that the highly polluted water sample among the sea water samples is from Harbour Beach with a value of 7.0mg/L. Near the Tuticorin

Harbour area major port activities take place. The main cause for the pollution of sea water in Harbour Beach is by industrial effluents that are directed towards the sea; that mainly includes dirt and gravel, masonry and concrete, scrap metal, oil and scrap lumber, chemical waste disposals and waste disposals from the nearby port.

The main cause for the pollution of sea water in Therespuram (BOD Value of 6.4 mg/L) is mainly due to the Buckle canal that bisects the town runs for 7.28 kilometres starting near the FCI godown area and drains into the sea at Therespuram. The Buckle Canal was constructed only to drain stagnant water from low-lying areas during rainy season. The letting of waste water into the shore of Therespuram through the Buckle Canal is causing serious health hazards to people. Residents of Therespuram feel the need for an immediate alternative to this problem, which has been prevailing for several years. The sewage outlet contains untreated domestic wastewater and solid wastes that are dumped into the sea. Fishing activities are also intensive.

Muthunagar beach is a commercial and recreational centre which attracts a lot of tourist and industrialists. This leads to the development of numerous recreational spots and resorts which automatically discharges plastic and other wastes near the coast. Shipping activities is also high near the coast and they contribute a considerable pollution load to the coast. The BOD value of Muthunagar beach was found to be 5.3 mg/L through our study.

In case of Tharuvaikulam sea water sample BOD showed lesser or minimum value (4.3 mg/L) compared to the other sea water samples. As per Census 2011, Tharuvaikulam has 1,743 households. In Tharuvaikulam, 88 percent of the people are dependent solely on fishing for livelihood and the remaining 12 percent depend partially on it. This area 13 km from Tuticorin town with major fishing activities has multi-day fishing gillnetters which needs 295 litres of diesel to catch one tonne of fish while multi-day trawlers consumes 513 litres to accomplish the same job. Fishing and pollution from fishing are the largest contributors to the decline in water quality in Tharuvaikulam.

Higher BOD indicates more oxygen is required, which is less for oxygendemanding species to feed on, and signifies lower water quality. The reason for high BOD in wastewater is due to the presence of dissolved organic solids. When the wastewater with high organic solids enters a fresh water body, the microorganism present in pure water will try to consume it as its primary food source. If the dissolved organic solids are minimal, they degrade it and convert into simple molecules with the help of dissolved oxygen present in water. If the BOD is much higher in wastewater the bacteria try to degrade it completely and they consume all dissolved oxygen for this process. Once the DO is completely exhausted, the other living organisms like protozoa, fishes, algae etc. will not get oxygen for breathing and they die immediately. In the absence of oxygen, no life is possible in water. Ultimately, the water body is not fit for any living organisms. Many industrial effluents have high BOD & its continuous discharge into any

water sources will make the water unfit for living things. This is the major reason for mass fish killings in water bodies. The pollution effect due to heavy metals will also cause damage to living organisms but the process is slow. Death due to devoid of DO is instant. Every wastewater with high BOD should be properly treated in biological treatment plant to completely eliminate the dissolved organics which causes serious adverse impacts on fresh water body.

From this study, it was inferred that in the pond water samples collected, Teppakulam and Palayakayal has the maximum level of BOD compared to Authoor pond water. In Sea water samples Harbour Beach and Therespuram has the highest levels of BOD compared to Muthunagar beach water sample and Tharuvaikulam sample.

Hence the study concluded that water pollution is mainly caused in a number of ways, the most common reason being city sewage, intense fishing and industrial waste discharge. So regular monitoring of the BOD is essential to protect the water sources against contamination in near future. Monitoring water quality is very important for maintaining ecosystem health and the livelihood of the population. It reflects the health of water. Therefore, best practices and efforts are needed to monitor and improve water quality.

SUMMARY

9. SUMMARY

- In the present study, three pond water samples and four sea water samples were collected in and around Thoothukudi and BOD levels were observed.
- The BOD values are calculated and tabulated.
- Seawater samples collected from Harbour Beach, Therespuram Sea, and Muthunagar Beach had predominantly high BOD values and the sample from Tharuvaikulam Sea had comparatively less BOD value.
- Among the pond water samples collected Teppakulam water and Palayakayal water had higher BOD values than Authoor pond water.
- It interpreted high levels of pollution and contamination.
- The higher BOD values indicate the growing threat to the species living in that particular environment and thereby affecting the people depending on these species for their livelihood.

CONCLUSION AND SUGGESTIONS

10. CONCLUSION AND SUGGESTIONS

As for the conclusion, the objective of this study was successfully achieved as the concentration of the BOD in water samples were measured. The result of this BOD experiment was interpreted and the data was also analysed. By referring to Table 3, Teppakulam and Palayakayal pond water samples had maximum BOD values. In Table 6, Harbour beach and Therespuram Sea water samples had maximum BOD values compared to Muthunagar beach sample and Tharvaikulam Sea water sample. This amount indicates that the area these water samples were taken are highly exposed to water pollution.

Some of the suggestions to improve the water quality in the pond water areas are given as follows:

- Pond water should be periodically tested to determine bacteria levels and to monitor the presence of any other non-visible problems.
- Overabundant growth of aquatic plants and algae should be prevented.
- Polluting activities should be strictly limited near the pond or in areas that drain into the pond.
- Ditches and grading should be used to divert polluted surface water away from the pond.

Some of the suggestions to improve the water quality of Sea water are given as follows:

- Treatment of sewage water should be carried out regularly. The three main stages of the wastewater treatment process, aptly known as primary, secondary and tertiary water treatment should be performed before letting them drain into the Sea.
- Excess chemical fertilizer eventually makes its way into the sea. Therefore, organic fertilizers which have moderate nutrient content should be used.
- Dumping of wastes and sewage by maritime and cruise ships in coastal waters should be banned. Ocean dumping of sludge and hazardous dredged material should be banned.
- Sensitive areas should be protected from development, oil drilling, and oil shipping and coastal development should be regulated.

BIBLIOGRAPHY

10. BIBLIOGRAPHY

Abdullahi, A. B., Siregar, A. R., Pakiding, W., & Mahyuddin. (2021). The analysis of BOD (Biological Oxygen Demand) and COD (Chemical Oxygen Demand) contents in the water of around laying chicken farm.

IOP Conference Series: Earth and Environmental Science, 788(1), 012155.

Akhtar, N., Ishak, M. I. S., Ahmad, M. I., Umar, K., Md Yusuff, M. S., Anees, M. T., Qadir, A., & Ali Almanasir, Y. K. (2021). Modification of the Water Quality Index (WQI) Process for Simple Calculation Using the Multi-Criteria Decision-Making (MCDM) Method: A Review. *Water*, 13(7), 905.

Al-Marjan, K., & Abdullah, S. (2015). Trichodina Sp. as Bioindicator for Evaluation of Biochemical Oxygen Demand (Bod5) in Aquaculture Fish Farms (Ponds). *Science Journal of University of Zakho*, 3(1), 27–31.

Amit, V. & Yashodhara, V. (2012). Determination of BOD in the underground water. *Journal of Chemical and Pharmaceutical Research,* 4(10), 4601-4603.

Bhuyan, S., Istiak Ahamed, M., & Monika, D. (2020). The optimum range of ocean and freshwater quality parameters. *Annals of Marine Science, 4(1),* 019-020

Breitburg, D., Levin, L. A., Oschlies, A., Grégoire, M., Chavez, *et al.,* (2018). *Declining oxygen in the global ocean and coastal waters.* *Science*, 359(6371).

Brönmark, C., & Hansson, L. A. (2017). The Biology of Lakes and Ponds. *Oxford Scholarship Online*.

Connor, R. (2016). The United Nations World Water Development Report 2016: Water and Jobs, chapter 2: *The Global Perspective on Water.* *Paris: UNESCO.* Pg. 26.

Chattopadhyay, G., Saha, P., Ghosh, A., & Karmakar, H. (1988). A study on optimum BOD levels for fish culture in wastewater ponds. *Biological Wastes*, 25(2), 79–85.

Deshmukh, J.U. & Mali, R.P. & Ambore, N.E. (2012). Study of biochemical oxygen demand in Godawari river at Nanded city due to impact of industrial pollution. 28. 115-118.

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., Naiman, R. J., Prieur-Richard, A. H., Soto, D., Stiassny, M. L. J., & Sullivan, C. A. (2005). *Freshwater biodiversity: importance, threats, status and conservation challenges*. *Biological Reviews,* 81(02), 163.

Dye, A. (1980). Tidal fluctuations in biological oxygen demand in exposed sandy beaches. *Estuarine and Coastal Marine Science*, 11(1), 1–8.

Eaton, A. E., Clesceri, L.S., & Greenberg, A. E. (1995). Standard methods for examination of water and wastewater (19th ed.). Washington, DC: American Public Health Association.

Ghosh, S., Paul, M., Raha, A., Mukherjee, P., Bagchi, A., & Si, A. (2018). Statistical evaluation of biochemical oxygen demand of river water. *Advance Pharmaceutical Journal*, 3(4), 118–120.

Jindal, R., & Sharma, C. (2010). Studies on water quality of Sutlej River around Ludhiana with reference to physicochemical parameters. *Environmental Monitoring and Assessment*, 174(1–4), 417–425.

Jouanneau, S., Recoules, L., Durand, M., Boukabache, A., Picot, V., Primault, Y., Lakel, A., Sengelin, M., Barillon, B., & Thouand, G. (2014). Methods for assessing biochemical oxygen demand (BOD): A review. *Water Research*, 49, 62–82.

Ma, J. W., Liang, S. X., & Qin, Z. (2012). Study on the Changing Law of Dissolved Oxygen of Baiyang Lake in Autumn and Winter. *Advanced Materials Research*, 485, 318–322.

Malarvannan, A., & Balamurugan, P. (2018). Water quality study at Tuticorin harbour. *International Research Journal of Engineering and Technology (IRJET)* 5(4), 2730 – 2734.

Mamacus, Ronan., Marte, Benjamin Reuel & Paraso, Michelle Grace. (2013). Pathologic effects of different biological oxygen demand levels on the juvenile catfish. 29 – 35.

Moccuba, J.J (2010). Dissolved Oxygen and Biochemical Oxygen Demand in the waters close to the Quelimane sewage discharge. *The University of Bergen.*

Monroe, J. B., Baxter, C. V., Olden, J. D., & Angermeier, P. L. (2009). Freshwaters in the Public Eye: Understanding the Role of Images and Media in Aquatic Conservation. *Fisheries*, 34(12), 581–585.

Mukherjee, S., & Dutta, M. (2016). Biological Oxygen Demand in Controlling Fish Production and Cost of Supplementary Feed towards better Sustainability of a Sewage-Fed Aquaculture System: A Case Study of East Kolkata Wetlands, West Bengal, India. *International Journal of Waste Resources*, 6(2).

Navamuniyammal, M., Vidhya, R., Sivakumar, M., & Saravanan, K. (2021).

Surface Water Quality Analysis in Thamirabarani River Using Remote Sensing And GIS. *International Journal of Geoinformatics and Geological Science*, 8(1), 1–8.

O'Boyle, S. (2020). Oxygen Depletion in Coastal Waters and the Open Ocean. *Coastal and Deep Ocean Pollution*, 41–67.

Parabang, S. (2021). Pollution Load Analysis of Biological Oxygen Demand from Household Sources. *International Journal of Scientific and Research Publications (IJSRP)*, 11(4), 411–417.

Penn, M.R., Pauer, J.J., Mihelcic, J.R. (2003). Biological Oxygen Demand.

Environmental and Ecological chemistry, 2.

Pimentel, D., Berger, B., Filiberto, D., *et al.* (2004). Water Resources, Agriculture, and the Environmental. Ithaca. (NY): New York State College of Agriculture and Life Sciences, Cornell University.

Environmental Biology Report, 1-46.

Pruss, A., Kay, D., Fewtrell, L. & Bartram, J. (2002). Estimating the Burden of Disease from Water, Sanitation, Hygene at a Global Level. *Environ. Health Perspect*. 110, 537.

Saraswati, N.L.G.R.A. & Arthana, I.W. & Risuana, I.G.S. & Hendrawan, I.G. (2018). Water pollution levels in the Suwung Estuary, Bali based on Biological Oxygen Demand. *Biotropia*. 25, 223-230.

Shil, S., Singh, U. K., & Mehta, P. (2019). Water quality assessment of a tropical river using water quality index (WQI), multivariate statistical techniques and GIS. *Applied Water Science*, 9(7).

Singh, G., Patel, N., Jindal, T., Srivastava, P., & Bhowmik, A. (2020). Assessment of spatial and temporal variations in water quality by the application of multivariate statistical methods in the Kali River, Uttar Pradesh, India. *Environmental Monitoring and Assessment*, 192(6).

Sirota, J., Baiser, B., Gotelli, N. J. & Ellison, A. M. (2013). Organic-matter loading determines regime shifts and alternative states in an aquatic ecosystem. *Proc. Natl. Acad. Sci. USA.* 110, 7742–7.

Uddin, M. G., Nash, S., & Olbert, A. I. (2021). A review of water quality index models and their use for assessing surface water quality. *Ecological Indicators,* 122, 107218.

Verma, N., & Singh, A. K. (2013). Development of Biological Oxygen Demand Biosensor for Monitoring the Fermentation Industry Effluent. *ISRN Biotechnology*, 1–6.

Vigiak, O., Grizzetti, B., Udias-Moinelo, A., Zanni, M., Dorati, C., Bouraoui, F., & Pistocchi, A. (2019). Predicting biochemical oxygen demand in European freshwater bodies. *Science of The Total Environment*, 666, 1089–1105.

SILVER NANOPARTICLES FROM ASCIDIANS: SYNTHESIS, CHARACTERIZATION AND CORROSION INHIBITORY ACTION

A project submitted to

ST. MARY'S COLLEGE (Autonomous), **THOOTHUKUDI**

affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, TIRUNELVELI

In partial fulfilment for the award of the degree of

BACHELOR OF SCIENCE IN ZOOLOGY

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(Re-accredited with 'A^{+}' Grade by NAAC)

April -2022

CERTIFICATE

This is to certify that the project entitled **"*Silver Nanoparticles From Ascidians: Synthesis, Characterization And Corrosion Inhibitory Action*"** is submitted to **St. Mary's College (Autonomous), Thoothukudi** in partial fulfilment for the award of the degree of **Bachelor of Science in Zoology** and it is a project work done during the year 2021-2022 by the following students.

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DECLARATION

We do hereby declare that this dissertation entitled, "***Silver Nanoparticles From Ascidians: Synthesis, Characterization And Corrosion Inhibitory Action***" submitted by us for the award of the degree of Bachelor of Science in Zoology is the result of our original independent research work carried out under the guidance of Dr. M. Paripooranaselvi M.Sc., M.Phil., B.Ed., Ph.D., SET., Assistant Professor, Department of Zoology, St. Mary's College (Autonomous), Thoothukudi and it has not been submitted elsewhere for the award of any other degree.

Place: Thoothukudi

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CONTENTS

Corrosion is one of the most common phenomena that we observe in our daily lives. Corrosion is a natural process that causes the transformation of pure metals into undesirable substances when they react with substances like water or air. This reaction causes damage and disintegration of the metal starting from the portion of the metal exposed to the environment and spreading to the entire bulk of the metal. It is a spontaneous, irreversible process wherein the metals turn into a much more stable chemical compounds like oxides, sulphides, hydroxides, etc.

Corrosion is usually an undesirable phenomenon since it negatively affects the desirable properties of the metal. For example, iron is known to have good tensile strength and rigidity. However, when subjected to rusting, iron objects become brittle, flaky, and structurally unsound. On the other hand, corrosion is a diffusion-controlled process, and it mostly occurs on exposed surfaces.

Corrosion can be classified as an electrochemical process since it usually involves redox reactions between the metal and certain atmospheric agents such as water, oxygen, sulphur dioxide etc.

Metals placed higher in the reactivity series such as iron, zinc, etc. get corroded very easily than the metals placed lower in the reactivity series like gold, platinum and palladium do not corrode. The explanation lies in the fact

that corrosion involves the oxidation of metals. As we go down the reactivity series tendency to get oxidized is very low. Interestingly, aluminium doesn't corrode unlike other metals even though it is reactive. This is because aluminium is covered by a layer of aluminium oxide already. This layer of aluminium oxide protects it from further corrosion.

Factors Affecting Corrosion

- Exposure of the metals to air containing gases like CO_2, SO_2, SO_3 etc.
- Exposure of metals to moisture especially salt water
- Presence of impurities like salt (eg. NaCl).
- An increase in temperature increases corrosion.
- Nature of the first layer of oxide formed: some oxides like Al_2O_3 form an insoluble protecting layer that can prevent further corrosion. Others like rust easily crumble and expose the rest of the metal.
- Presence of acid in the atmosphere: acids can easily accelerate the process of corrosion.

Types of Corrosion

Some of the corrosion types include;

(i) Crevice Corrosion

Whenever there is a difference in ionic concentration between any two local areas of a metal, a localized form of corrosion known as crevice corrosion can occur. In a simple instance, this form of corrosion mostly occurs in confined spaces (crevices). Examples of areas where crevice

corrosion can occur are gaskets, the under surface of washers, and bolt heads. All grades of aluminium alloys and stainless steels also undergo crevice corrosion.

(ii) Stress Corrosion Cracking

Stress Corrosion Cracking can be abbreviated to 'SCC' and refers to the cracking of the metal as a result of the corrosive environment and the tensile stress placed on the metal. It often occurs at high temperatures.

Example: Stress corrosion cracking of austenitic stainless steel in chloride solution.

(iii) Intergranular Corrosion

Intergranular corrosion occurs due to the presence of impurities in the grain boundaries that separate the grain formed during the solidification of the metal alloy. It can also occur via the depletion or enrichment of the alloy at these grain boundaries.

Example: Aluminum-base alloys are affected by IGC.

(iv) Galvanic Corrosion

When there exists an electric contact between two metals that are electrochemically dissimilar and are in an electrolytic environment, galvanic corrosion can arise. It refers to the degradation of one of these metals at a joint or at a junction. A good example of this type of corrosion would be the degradation that occurs when copper, in a salt-water environment, comes in contact with steel.

Example: When aluminium and carbon steel are connected and immersed in seawater, aluminium corrodes faster and steel is protected.

(iv) Pitting Corrosion

Pitting Corrosion is very unpredictable and therefore is difficult to detect. It is considered one of the most dangerous types of corrosion. It occurs at a local point and proceeds with the formation of a corrosion cell surrounded by the normal metallic surface. Once this 'Pit' is formed, it continues to grow and can take various shapes. The pit slowly penetrates metal from the surface in a vertical direction, eventually leading to structural failure if left unchecked.

Example: Consider a droplet of water on a steel surface, pitting will initiate at the centre of the water droplet (anodic site).

(v) Uniform Corrosion

This is considered the most common form of corrosion wherein an attack on the surface of the metal is executed by the atmosphere. The extent of the corrosion is easily discernible. This type of corrosion has a relatively low impact on the performance of the material.

Example: A piece of zinc and steel immersed in diluted sulphuric acid would usually dissolve over its entire surface at a constant rate.

(vi) Hydrogen Grooving

This is a corrosion of the piping by grooves that are formed due to the interaction of a corrosive agent, corroded pipe constituents and hydrogen gas

bubbles. The bubbles usually remove the protective coating once it comes in contact with the material.

(vii) Metal Dusting

Metal dusting is a damaging form of corrosion that occurs when vulnerable materials are exposed to certain environments with high carbon activities including synthesis gas. The corrosion results in the break-up of bulk metal to metal powder. Corrosion occurs as a graphite layer is deposited on the surface of the metals from carbon monoxide (CO) in the vapour phase. This graphite layer then goes on to form meta-stable M_3C species (where M is the metal) that usually moves away from the metal surface. In some cases, no M_3C species may be observed. This means that the metal atoms have been directly transferred into the graphite layer.

(viii) Microbial Corrosion

Microbial corrosion which is also known as microbiologically influenced corrosion is a type of corrosion that is caused by microorganisms. The most common one is chemoautotrophs. Both metallic and non-metallic materials either in the presence or absence of oxygen can be affected by this corrosion.

(viii) High-temperature Corrosion

High-temperature corrosion as the name suggests is a type of corrosion of materials (mostly metals) due to heating. Chemical deterioration of metal can occur due to a hot atmosphere that contains gases such as oxygen, sulfur,

or other compounds. These compounds are capable of oxidizing the materials (metals in this case) easily. For example, materials used in car engines have to resist sustained periods at high temperatures during which they can be affected by an atmosphere containing corrosive products of combustion.

Some of the typical examples of corrosion as seen mostly in metals are given below.

1. Copper Corrosion

When copper metal is exposed to the environment it reacts with the oxygen in the atmosphere to form copper (I) oxide which is red in colour.

$2Cu_{(s)} + ½\ O_{2(g)} \rightarrow Cu_2O_{(s)}$

Cu_2O further gets oxidised to form CuO which is black in colour.

$Cu_2O_{(s)} + ½\ O_{2(g)} \rightarrow 2CuO_{(s)}$

This CuO reacts with CO_2, SO_3 and H_2O (present in the atmosphere to form $Cu_2(OH)_{2(s)}$ (Malachite) which is blue in colour and $Cu_4SO_4(OH)_{6(s)}$ (Brochantite) which is green in colour.

This is why we observe copper turning bluish-green in colour. A typical example of this is the colour of the statue of liberty which has the copper coating on it turning blue-green in colour.

2. Silver Tarnishing

Silver reacts with sulphur and sulphur compounds in the air give silver sulphide (Ag_2S) which is black in colour. Exposed silver forms Ag_2S as it

reacts with the $H_2S_{(g)}$ in the atmosphere which is present due to certain industrial processes.

$$2Ag_{(s)} + H_2S_{(g)} \rightarrow Ag_2S_{(s)} + H^+_2+(g)$$

3. Corrosion of Iron (Rusting)

Rusting of iron which is the most commonly seen example happens when iron comes in contact with air or water. The reaction could be seen as a typical electrochemical cell reaction. Consider the diagram given below.

Metals are widely used in human activities due to their excellent mechanical and electrical properties. In order to preserve the desired state of these metals, their preventive maintenance is a priority. Corrosion is probably the most common undesired phenomenon that leads metals to become weaker. This natural process originates from the electrochemical interaction of metals with the corrosive environment. Sulfides, oxides, and others are generated through reactions between the metal surface and the corrosive medium.

In the last few decades, the concept of “Green” has immensely influenced all the areas of science and technology, where the fascination for plant extracts as a metallic corrosion inhibitor has gotten significant attention. From 1960’s onward, a large number of synthetic organic compounds have been studied as anticorrosive materials in which heteroatoms such as C, N, O, and S were identified to be the prime reason for their corrosion inhibition ability. The selective adsorption of heteroatoms on the metal surface developed a

metal−electrolyte interface barrier and prevented corrosion. However, most of these inhibitors were expensive, harmful to humankind, and nonbiodegradable. Therefore, more attention was given to the development of naturally derived environmentally benign organic corrosion inhibitors. Apparently, every plant contains several active phytochemicals, and they are the ideal candidates to replace traditional toxic inhibitors. Reduced environmental hazards, easy availability, renewability, and simple extraction procedures made it the suitable one, and the intensity of works on different plant parts, such as seed, root, fruit, leaf, and so forth, and on various metals in different electrolytic media reveals the significance of the go green policy in scientific endeavor. Literature reports suggest that plant extracts have excellent corrosion inhibition efficiency, especially leaf extract, because of the presence of an abundant source of phytochemicals compared to other parts. Biofouling/biocorrosion comprises adsorption, colonization, and undesirable accumulation of molecules and organisms on the immersed substrate which may have a wide range of destructive effects on man-made structures in the aquatic environment. Biofouling is of serious concern globally in marine systems, causing considerable economic losses due to necessary maintenance and replacement operations of subsurface installations in marine technology. Among the several methods of corrosion control and prevention, the use of corrosion inhibitors is very popular. Corrosion inhibitors are substances that when added in small concentrations to corrosive

media decrease or prevent the reaction of the metal with the media. Inhibitors are added to many systems, namely, cooling systems, refinery units, chemicals, oil and gas production units, boiler, and so forth. Most of the effective inhibitors are used to contain heteroatom such as O, N, and S and multiple bonds in their molecules through which they are adsorbed on the metal surface. Industries depend heavily on the use of metals and alloys. One of the most challenging and difficult tasks for industries are the protection of metals from corrosion.

Corrosion is a ubiquitous problem that continues to be of great relevance in a wide range of industrial applications and products; it results in the degradation and eventual failure of components and systems both in the processing and manufacturing industries and in the service life of many components. Nevertheless, the popularity and use of synthetic compounds as a corrosion inhibitor is diminishing due to the strict environmental regulations and toxic effects of synthetic compounds on human and animal life. Consequently, there exists the need to develop a new class of corrosion inhibitors with low toxicity, eco-friendliness and good efficiency. Throughout the ages, plants have been used by human beings for their basic needs such as production of food-stuffs, shelters, clothing, fertilizers, flavors and fragrances, medicines and last but not least, as corrosion inhibitors. The use of natural products as corrosion inhibitors can be traced back to the 1930's when plant extracts of *Chelidonium majus* and other plants were used for the

first time in H2SO4 pickling baths (Sanyal, 1981). Corrosion can cause disastrous damage to metal and alloy structures causing economic consequences in terms of repair, replacement, product losses, safety, and environmental pollution. Due to these harmful effects, corrosion is an undesirable phenomenon that ought to be prevented. There are several ways of preventing corrosion and the rates at which it can propagate with a view of improving the lifetime of metallic and alloy materials. The use of inhibitors for the control of corrosion of metals and alloys which are in contact with aggressive environment is one among the acceptable practices used to reduce and/or prevent corrosion. Plants are sources of naturally occurring compounds, some with complex molecular structures and having different chemical, biological, and physical properties. The naturally occurring compounds are mostly used because they are environmentally acceptable, cost effective, and have abundant availability. These advantages are the reason for use of extracts of plants and their products as corrosion inhibitors for metals and alloys under different environment. Different plant extracts can be used as corrosion inhibitors commonly known as green corrosion inhibitors.

Nanoparticles, because of their small size, have distinct properties compared to the bulk form of the same material, thus offering many new developments in the fields of biosensors, biomedicine and bionanotechnology. Nanotechnology is also being utilized in medicine for

diagnosis, therapeutic drug delivery and the development of treatments for many diseases and disorders. Silver nanoparticles are one of the promising products in the nanotechnology industry. Silver nanoparticles can be synthesized by several physical, chemical and biological methods.

- It is used for purification and quality management of air, biosensing, imaging, drug delivery system.
- Biologically synthesized silver nanoparticles have many applications like coatings for solar energy absorption and intercalation material for electrical batteries, as optical receptors, as catalysts in chemical reactions, for biolabelling, and as antimicrobials.
- Though silver nanoparticles are cytotoxic but they have tremendous applications in the field of high sensitivity bimolecular detection and diagnostics, antimicrobials and therapeutics, catalysis and micro-electronics.
- It has some potential application like diagnostic biomedical optical imaging, biological implants (like heart valves) and medical application like wound dressings, contraceptive devices, surgical instruments and bone prostheses.
- Many major consumer goods manufacturers already are producing household items that utilize the antibacterial properties of silver nanoparticles. These products include nano silver lined refrigerators, air conditioners and washing machines.

Even though bioactive compounds with corrosion-inhibiting properties have been isolated from plants, they are exploited for various reasons. The marine environment is an excellent source of novel chemicals, not found in terrestrial sources. Marine organisms, especially those that are a nuisance to the environment like biofoulers can be screened for anticancer activity. Many marine sedentary organisms produce components with a unique structural pattern, for their chemical defense which does not occur in terrestrial plants.

Literature survey shows that anticorrosion work on ascidians is very few. In the present study, an attempt has been made to reduce the corrosion activity of the exposed surface and increase a material's corrosion resistance simple ascidian *Phallusia nigra* Savigny 1816; colonial ascidian *Didemnum psammatode* (Sluiter 1895) and seaweeds - *Codium geppiorum* O.C. Schmidt, 1923 and *Dictyota ciliolate.*

The objectives of the present study were to

- Collect the *Phallusia nigra* Savigny, 1816; and colonial ascidians *Didemnum psammatode* Sluiter, 1895
- Collect the seaweeds *Codium geppiorum* O.C. Schmidt, 1923 and *Dictyota ciliolata*
- Synthesize silver nanoparticles from *Phallusia nigra, Didemnum psammatode*, *Codium geppiorum* and *Dictyota ciliolata*
- Determine characterization of nanoparticles By Uv-Vis Spectrophotometer
- Study the chemical composition of the synthesized nanoparticles by using FTIR spectrometer
- Evaluate the corrosion inhibition activity of silver nanoparticles of *Phallusia nigra, Didemnum psammatode*, *Codium geppiorum* and *Dictyota ciliolata*

A review of literature is a comprehensive summary of previous research on a topic. The review of literature surveys scholarly articles, books, and other sources relevant to a particular area of research.

Thakur *et al.,* 2020 observed that Ag-doped ZnO shows a lower limit of detection as compared to pure ZnO for p-nitrophenol sensing. Bhuyar *et al*., 2020 observed that marine alga *Padina* species could be an alternative source for the production of Ag nanoparticles and are efficient antimicrobial compounds against both gram-negative and gram-positive bacteria which can be a promising material against infectious bacteria. Bhattacharya *et al.,* 2020 observed that the antimicrobial activity of synthesized nanoparticles against gram positive as well as gram negative, pathogenic bacteria i.e. *Pseudomonas aeruginosa* and *Staphylococcus aureus* species respectively. Zone of inhibition (ZOI) exhibited by *Pseudomonas aeruginosa* and *Staphylococcus aureus* for disc diffusion and well diffusion assay was around 10-22 mm and 9-12mm respectively. Sharma *et al.,* 2020 observed that zinc salts have been used as precursor and phytochemicals in the plant extract reduce the metal salt to lower oxidation state as well as stabilize the ZnO NPs. The morphological and physico-chemical properties of obtained NPs analyzed by various characterization techniques have been discoursed. Further, antimicrobial activity and potential photocatalytic application in terms of the

degradation of dyes have also been reviewed in addition to the toxicity aspects of these NPs on human beings and animals.

Rauvolfia tetraphylla (L.) seed extract was used to synthesize dark brown colored silver (Ag) and white colored zinc oxide nanoparticles. Synthesized nanoparticles were characterized by different spectroscopic analysis (XRD, XPS, and SEM with EDAX). Characterization results confirmed the particle morphology and structure. The synthesized Ag and ZnO NPs were analyzed against two gram positive and three gram negative bacteria (Vinay *et al.,* 2021).

Corrosion inhibition performance of the three new thiazole based pyridine derivatives on mild steel in 0.5 M HCl was studied using gravimetric, potentiodynamic polarisation, and electrochemical impedance techniques. Inhibition efficiency has direct relation with concentration and inverse relation with temperature (Singh, *et.al*., 1995). *Turbinaria ornata* extract was tested as green corrosion inhibitor on mild steel coupons in conc. HCl medium with an efficiency of 100% at 25gl−1 during 5min exposure. (Kuda, *et.al*., 2005). The effectiveness of two new pyridine derivatives namely N-(2-hydroxy benzylidene) pyridine-4-amine (HBPA) and N-(5-bromo-2-hydroxy benzylidene) pyridine-4-amine (B-HBPA) as corrosion inhibitors for X70 steel in 2 M HCl solution was examined using weight loss, electrochemical and quantum chemical calculations (Quraishi, *et.al*.,2007).

The efficiency of polyacrylamide on corrosion inhibition of C-steel in 1.0 M HCl solution was evaluated by means of electrochemical impedance spectroscopy, potentiodynamic polarization and mass-loss measurements and a very good concordance was obtained from the three techniques (Kowalski*, et.al.,* 2007). The inhibition of the corrosion of mild steel by ethanol extract of *Musa sapientum* peels in H_2SO_4 has been studied using gasometric and thermometric methods (Eddy and Ebenso, 2008).

Two new organic compounds were tested experimentally as inhibitors for mild steel in NaOH in presence of NaCl by electrochemical and hydrogen evolution techniques. Results demonstrated that the two inhibitors show an adsorption on steel surface according to Langmuir adsorption isotherm. The inhibition efficiency increases with increasing inhibitor concentrations to attain a maximum value at 1.0 mM for compound I and at 6.0 mM for compound II, respectively. (Ameer *et.al.,* 2010). The corrosion inhibition of mild steel in 1.0 M Hcl solution by four Schiff bases was investigated using weight loss and electrochemical measurements and quantum chemical calculations (Ahamada, *et.al*.,2010).

A new phenanthroline derivative, 2-mesityl-1H-imidazo[4,5-f] [1,10] phenanthroline was synthesized and characterized by elemental analysis, FT-IR, 1HNMR, and 13CNMR spectra. MEIP was evaluated as corrosion inhibitor for carbon steel in 0.5 M H_2SO_4 solution using gravimetric and UV−visible spectrophotometric methods at 303−333 K. Results obtained

show that MEIP is a good inhibitor for mild steel in H_2SO_4 solution. The inhibition efficiency was found to increase with increase in MEIP concentration but decreased with temperature, which is suggestive of physical adsorption mechanism (Obot., *et.al*.,2011). A new inhibitor, 6-bromo-(2,4-dimethoxyphenyl) methylidene] imidazo [1,2-a] pyridine-2-carbohydrazide (DMPIP) was evaluated as a corrosion inhibitor for Mild Steel in 0.5 M HCl solution at 303–323 K using potentiodynamic polarization and electrochemical impedance spectroscopic techniques. Both the techniques confirmed an increase in inhibition efficiency with the concentration of DMPIP but decrease with temperature (Obot *et.al*.,2011).

Extract of *Andrographis paniculata* showed better inhibition performance on Carbon Steel in HCl Solution (Singh *et al*., 2012). Anti-corrosion activities of *Origanum compactum* on carbon steel in 0.5 M sulfuric acid were studied using weight loss and electrochemical methods (Fadel, *et al*., 2013). The corrosion inhibition behavior of carbon steel in 0.5 M sulfuric acid in the presence *Origanum compactum* extracts have been studied using the weight loss and electrochemical methods (Fadel *et.al*., 2013).

The alcoholic extracts of eight plants namely *Lycium shawii*, *Teucrium oliverianum*, *Och-radenus baccatus*, *Anvillea garcinii*, *Cassia italica*, *Artemisia sieberi*, *Carthamus tinctorius* and *Tripl-eurospermum auriculatum* grown in Saudi Arabia were studied for their corrosion inhibitive effect on mild steel in 0.5 M HCl media using the open circuit potential, Tafel plots

and A.C.impedance methods (Al - otaibi *et al*., 2014). Ketosulfone has been evaluated as a green corrosion inhibitor for mild steel in 1 M HCl medium by chemical and electrochemical methods at various concentrations and temperature. The adsorption of the inhibitor on the mild steel surface in acid solution was found to obey the Langmuir adsorption isotherm (Prasanna, *et.al.,* 2014). The inhibition properties of phytic acid on Q235 mild steel corrosion in 0.5 M H_2SO_4 was estimated using electrochemical techniques (Maduabuchi, *et.al.,* 2014). Adsorption and inhibition mechanism and the efficiencies of natural products which are used as eco-friendly corrosion inhibitors for various metals and alloys in different acid media (Khan *et al.,* 2015). Inhibition of carbon steel corrosion in 0.5 M hydrochloric acid solutions at 298 K by β-cyclodextrin modified natural chitosan was investigated by weight loss measurement, potentiodynamic polarization, electrochemical impedance spectroscopy scanning electron microscopy and energy dispersive spectroscopy (Liu, *et al*., 2015).

AgNPs/chitosan was characterized using Fourier transformed infrared (FTIR), EDS, and SEM. The results obtained show that AgNPs/chitosan is an effective cathodic type inhibitor particularly at higher temperature and protects the metal surface by formation of a protective film. (Moses, *et.al.,* 2016). The inhibition effect of animal glue towards the corrosion of aluminum and two aluminum-silicon alloys in 0.1 M NaOH solution was investigated using potentiostatic polarization, electrochemical impedance

spectroscopy, cyclic voltammetry and potentiodynamic anodic polarization techniques (Abdallah *et al*., 2016). The corrosion inhibition performance of the synthesized chitosan Schiff bases inhibitors for mild steel in 1 M HCl solution was studied by electrochemical impedance spectroscopy and potentiodynamic polarization (Khan *et al*., 2017).

Polysaccharides made by microorganisms show anti-corrosive properties, *Lactobacillus fermentum* Ts produce EPS (exopolysaccharides) ,which serves as corrosion inhibitor for mild steel. (Ibryamova, *et al*., 2018). Effect of Fungal Glycolipids produced by a mixture of sunflower oil cake and pineapple waste as green corrosion inhibitors was studied by Rashad *et.al*., 2018. The corrosive inhibitory efficiency of Algerian *Lactobacillus stoechas* oil on C38 carbon steel in 1 M HCl medium by hydrodistillation and characterized by GC and GC/MS was studied using gravimetric and electrochemical methods (Belarbi *et al.,* 2018). Plant materials are ideal green candidatures to replace traditional toxic corrosion inhibitors (Verma *et al*., 2018). The heterogeneous ring compounds bearing larger electronegativity atoms (i.e., N, O, S, and P), polar functional groups and conjugated double bonds are the most effective inhibitors (Li *et.al.,*2018). Seed extracts of *Piper guineense* were assessed for anticorrosion on aluminum coupon with weight percentage composition of Al>95% and 3x1.5x0.1cm in size. Anticorrosion effects of the extracts was studied using gravimetric and potentio dynamic polarization techniques, while the antifungal potency of ethanol, methanol,

cold water and hot water extracts respectively against the corrosion-associated (Koch *et.al.,* 2018).

The adsorption and anti-corrosion effects of butanolic extract of the aerial parts of *Veronica rosea* were investigated towards the corrosion of copper in 1 M HNO_3 aqueous by the weight loss technique and potentiodynamic polarization (Ouache *et al.,* 2019). *Ceriops tagal* extract showed 95% corrosion inhibition against 1 M HCl attack on mild steel at 303 ± 1 K, which declined over other concentrations and temperatures, where AAS (atomic adsorption spectrometric) produced 82% inhibition at 600 ppm. UV-visible spectroscopy analysis revealed the formation of an inhibitor metal complex.

The influence of three newly synthesized oxadiazole derivatives on the corrosion inhibition of mild steel in 0.5 M HCl solution was studied using mass loss and electrochemical techniques. The corrosion rate decreased with increasing concentration of inhibitors and increased with an increase in temperature of the medium. Adsorption of all the three inhibitors obeyed the Langmuir isotherm model (Du, *et.al.,* 2020).

The concentration of Cr_2O_3 nanoparticles increases with a decrease in the rate of corrosion, which confirmed an increase in the efficiency of inhibition (Sharma, 2021). The anticorrosion behavior of the synthesized polymers on pure aluminium in 0.7 M HCl was studied by potentiodynamic polarization electrochemical impedance spectroscopy and weight loss

methods. (Devakumarab., *et.al.,* 2021). The synergistic inhibition effect of Tween-80 and 1,2,3,4-tetrahydroacridines, including 9-(4-chlorophenyl)-2-methyl-1,2,3,4-tetrahydroacridine and ethyl 9-(4-chlorophenyl)-1,2,3,4-tetrahydroacridine-2-carboxylate on mild steel corrosion in 1 M HCl was investigated by using weight loss tests, electrochemical techniques, and surface morphology. (Tang, *et.al.,* 2021). A new inhibitor, 6-bromo-(2,4-dimethoxyphenyl) methylidene] imidazo [1,2-*a*] pyridine-2-carbohydrazide was evaluated as a corrosion inhibitor for Mild Steel in 0.5 M HCl solution at 303–323 K using potentiodynamic polarization and electrochemical impedance spectroscopic techniques (Vranda, 2021).

The effect of *Ceratonia siliqua* L. pulp corrosion inhibition on carbon steel has been studied by gravimetric testing and electrochemical methods. The inhibition results achieved revealed that the aqueous extract with gallic acid had a good anticorrosion activity with an inhibition rate of 91.32% at 3 g/l for a temperature of 323 K. Potentiodynamic polarization was performed in 1 M HCl without and with different concentrations of *Ceratonia siliqua* extracts clearly proves that inhibitor extracts behave as mixed type. (Ghazi *et.al*., 2022).

ANIMAL MATERIAL

Samples of simple ascidian *Phallusia nigra* Savigny, 1816 were collected from the under surface of the barges of Tuticorin harbor. The specimens of colonial ascidian *Didemnum psammathodes* were collected from the intertidal rocky shore area of Thoothukudi north break water Tamilnadu. The samples were washed with sea water to remove sand, mud and overgrowing organisms at the site collection, and then transported to laboratory. Identification up to the species level was carried out based on the key to identification of Indian ascidians by Meenakshi, 1997.

SYSTEMATIC POSITION

Phallusia nigra belongs to

Phylum : Chordata

Subphylum : Urochordata

Class : Ascidiacea

Order : Enterogona

Suborder : Phlebobranchia

Family : Ascidiidae

Genus : *Phallusia*

Species : *nigra*

Didemnum psammatode belongs to

Phylum : Chordata

Subphylum : Urochordata

Class : Ascidiaceae

Order : Enterogona

Suborder : Aplousobranchia

Family : Didemnidae

Genus : *Didemnum*

Species : *psammathodes*

Dictyota ciliolata belongs to

Class : Phaeophyceae

Order : Dictyotales

Family : Dictyotaceae

Codium geppiorum O.C.Schmidt, 1923 belongs to

Class : Ulvophyceae

Order : Bryopsidales

Family : Codiaceae

EXTERNAL APPEARANCE

Plate – 1 Individuals are oval or elongated, laterally compressed with the free edges thick and rounded. The size varies from 1.5 cm to 9.5 cm. Attachment is by the posterior end or by one third of the posterior left side. In a few specimens the posterior basal part had a long flat creeping process for attachment. The anterior end narrows to a terminal branchial siphon. Atrial siphon is one third from the anterior end on the dorsal surface directed anteriorly. There are 8-10 branchial and 6-8 atrial lobes with ocelli in between them. The lobes are rounded without any tentacular fringe. The whole anterior part of the body is curved dorsally which is characteritic of the species so that the two apertures are quite close together. Test is firm, smooth, shiny and jet black in colour.

Plate -2 shows the colony of *Didemnum psammatode*. It is thin and soft. In the test spicules are few, but abundant ovoid faecal pellets are present. Live and preserved colonies are grey in colour.

Plate – 3 depicts *Codium geppiorum.* Cylindrical and irregularly forked branches of this species creep on the substrates, and attach to each other at any point of contact. The utricles are club-shaped with rounded tips, approximately 0.1-0.2 mm in diameter and slightly constricted at the upper portions. The plants are spongy, dark green, and are found on rocks or dead coral in subtidal zones along moderately wave exposed shorelines.

Dictyota ciliolata is shown in Plate – 4. Thalli are erect, to 8 cm tall, attached by means of a single stupose holdfast. Stolonoidal fibres are absent. Straps, 2-3 mm wide, are slender and dichotomously branched. The margins are dentate, rarely smooth, while the surface is always smooth. The apices are rounded. The medulla and cortex are uniformly one-layered. Sporangia are single, scattered on both surfaces, but absent in the apical dichotomies. Sporangia, 95-110 μm in diameter, are borne on a single stalk cell and are not surrounded by a conspicuous involucrum. *Dictyota ciliolata* is characterised by its stupose holdfast, dentate margins and the absence of stolonoidal fibres, although the margins of some specimens can be nearly smooth (De Clerck *et al.*, 2002)

Preparation of Powder

The specimens were dried under shade. The dried animals were homogenized to get a coarse powder. The dried powder of the tunicate *Phallusia nigra* and *Didemnum psammatode, Codium geppiorum* and *Dictyota ciliolate* was used.

Synthesis of silver nanoparticles

Weighing 25 g of dry powder of *Phallusia nigra* was mixed with 100 ml sterile distilled water and filtered through Whatman No.1 filter paper (pore size 0.45 μm) and was further filtered through 0.22 μm sized filters. The extract was stored at 40° C for further experiments. The same procedure was

followed for *Didemnum psammatode*, *Codium geppiorum* and *Dictyota ciliolata* also.

The aqueous solution of 1mM silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles. 10 ml of *Phallusia nigra* extract was added into 90 ml of aqueous solution of 1 mM silver nitrate for reduction into Ag+ ions and kept for incubation period of 15 hours at room temperature. Here the filtrate act as reducing and stabilizing agent for 1 mM of AgNO3.

CHARACTERIZATION OF SILVER NANOPARTICLES:

UV-Vis Analysis:

The Ag nanoparticles were characterized in a Perkin-Elmer UV-VIS spectrophotometer, Lambda-19 to know the kinetic behavior of Ag nanoparticles. The scanning range for the samples was 200-800 nm at a scan speed of 480 nm/min. The spectrophotometer was equipped with "UVWinlab" software to record and analyze data. Base line correction of the spectrophotometer was carried out by using a blank reference. The UV-Vis absorption spectra of all the samples were recorded and numerical data were plotted in the "Origin 6.5".

FTIR analysis:

The chemical composition of the synthesized silver nanoparticles was studied by using FTIR spectrometer (Perkin-Elmer LS-55- Luminescence

spectrometer). The solutions were dried at 75° C and the dried powders were characterized in the range 4000–400 cm-1 using KBr pellet method.

AgNPs of ascidians used for the corrosion tests were taken. Small plates of carbon steel (10 × 15 mm) were used for corrosion tests, polished using silicon carbide grinding paper with grit P400, degreased with sodium carbonate, rinsed in distilled water, and dried with ethanol. After that, the samples were weighed accurately using an analytical balance (Kern KB, KERN & SOHN GmbH, Balingen, Germany). They were then suspended entirely in glass vessels that contained 25 mL of seawater, without and with AgNPs of ascidians. The corrosion experiments were examined by the gravimetric method for 3 days at room temperature (22 ± 2 °C). After the corrosion time ended, samples were taken out, washed with distilled water, dried at room temperature, and weighed accurately. The surface morphology of the carbon steel, before and after corrosion tests, was examined using a digital optical microscope (MT4096, USB, Media-Tech, Bratislava, Slovakia, magnification 300×).

Ascidians – *Phallusia nigra*, *Didemnum psammatode* and seaweeds - *Dictyota ciliolate*, *Codium geppiorum* and seawater used for the corrosion tests were taken. Small plates of carbon steel (10 × 15 mm) were used for corrosion tests, polished using silicon carbide grinding paper with grit P400, degreased with sodium carbonate, rinsed in distilled water, and dried with

ethanol. After that, the samples were weighed accurately using an analytical balance (Kern KB, KERN & SOHN GmbH, Balingen, Germany). They were then suspended entirely in glass vessels that contained 25 mL of seawater, without and with natural seaweeds (10 g) (Figure 1). The corrosion experiments were examined by the gravimetric method for 7 days at room temperature (22 ± 2 °C) using one sample for each test. After the corrosion time ended, samples were taken out, washed with distilled water, dried at room temperature, and weighed accurately. The surface morphology of the carbon steel, before and after corrosion tests, was examined using a digital optical microscope (MT4096, USB, Media-Tech, Bratislava, Slovakia, magnification 300×).

Characterization of Ag nanoparticles:

UV-Vis Spectrophotometer Analysis:

AgNPs from the extract of *Phallusia nigra* and *Didemnum psammatode*, *Codium geppiorum* and *Dictyota ciliolata* were synthesized successfully. The UV-Vis absorption spectra of the Ag NP of *Phallusia nigra* was shown in Figure 1. Absorption spectra of Ag nanoparticles formed in the reaction media has absorbance maxima at 270 nm.

The UV-Vis absorption spectra of the Ag NP of *Didemnum psammatode* was shown in Figure 2. Absorption spectra of Ag nanoparticles formed in the reaction mixture in the range of 200-300 nm. The absorption spectrum has shown two peaks at 250 and 270 nm indicate the formation of silver nanoparticles using *Didemnum psammathodes*.

The UV-Vis absorption spectra of the Ag NP of *Codium geppiorum* was shown in Figure 3. Absorption spectra of Ag nanoparticles formed in the reaction media has absorbance maxima at 275 nm.

The UV-Vis absorption spectra of the Ag NP of *Dictyota ciliolata* was shown in Figure 4. Absorption spectra of Ag nanoparticles formed in the reaction mixture in the range of 250-300 nm. The absorption spectrum has

shown two peaks at 250 and 280 nm indicate the formation of silver nanoparticles using *Dictyota ciliolata*.

FTIR Analysis:

FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized. The FTIR spectrum of silver nanoparticles were shown in Figure 5 to 8.

The FT-IR spectra for Ag nanoparticles of *Phallusia nigra* revealed the presence of prominent peaks at 3424, 2923, 2106, 1627, 1422, 1384, 1120, 1021, 874, 675, 610, 513 and 466 cm^{-1} corresponding to different functional groups. The peak corresponds to 3424 cm^{-1} indicates N-H stretching (primary) functional group. The peak at 2923 cm^{-1} responds to C-H stretching of alkanes and alkyl groups and 1384 cm^{-1} indicates the C-H bending of alkanes. C-C multiple bond stretching of alkyne (mono-substituted) and aromatic functional groups were observed at 2106 and 1422 cm^{-1}. The carbonyl stretching groups such as acids, ketones and amides were noted at the peak of 1623 cm^{-1}. The plausible peaks at 1120and 1021 cm^{-1} at revealed the functional group of C-O stretching of esters and ethers. The following peaks at 874, 675 and 610 were indicated the C-X stretching halogen compounds. The peak at 468 cm^{-1} confirms the metal oxygen bond which evidenced the formation of Ag nanoparticles.

The spectra for Ag nanoparticles of *Didemnum psammatode* revealed the presence of prominent peaks at 3984, 3455, 2923, 1632, 1384, 1121, 1050, 639, 609 and 467 cm^{-1} corresponding to different functional groups. O-H stretching of alcohols and phenols were indicated from the peak at 3984 cm^{-1}. The peak corresponds to 3455 cm^{-1} indicates N-H stretching (primary) functional group. The peak at 2923 cm^{-1} responds to C-H stretching of alkanes and alkyl groups and 1384 cm^{-1} indicates the C-H bending of alkanes. C-C multiple bond stretching of alkyne (mono-substituted) and aromatic functional groups were observed at 2106 and 1422 cm^{-1}. The carbonyl stretching groups such as acids, ketones and amides were noted at the peak of 1632 cm^{-1}. The plausible peaks at 1121and 1050 cm^{-1} revealed the functional group of C-O stretching of esters and ethers. The following peaks at 639 and 609 were indicated the C-X stretching halogen compounds. The peak at 467 cm^{-1} confirms the metal oxygen bond which evidenced the formation of Ag nanoparticles.

The FT-IR spectra for Ag nanoparticles of *Codium geppiorum* revealed the presence of prominent peaks at 3134, 2361, 1628, 1400 and 670 cm^{-1} corresponding to different functional groups. The FT-IR spectra for Ag nanoparticles of *Dictyota ciliolata* revealed the presence of prominent peaks at 3133, 2362, 1627, 1384 and 839 cm^{-1} corresponding to different functional groups.

Anticorrosion activity is shown in Table 1 and Figure 1 – 4. Weight loss and Rate of corrosion is calculated by using the following formulae.

Weight loss = Initial weight - Final weight

$$\text{Rate of Corrosion} = \frac{\text{Weight Loss}}{\text{Initial weight}} \times 100$$

The results presented in Table 1 indicate anticorrosion property in the medium of seawater with ascidians and seaweeds. The surface morphology images show that the corrosion reaction does not take place homogeneously over the surface of carbon steel in the absence of ascidians and seaweeds in seawater.

Weight loss in 24 hours was calculated. It was higher 1.0155 g for *Didemnum psammatode* and the rate of corrosion was 5.05 %. Minimum weight loss was noted as 0.4306 for *Phallusia nigra* and the rate of corrosion was 2.23 %.

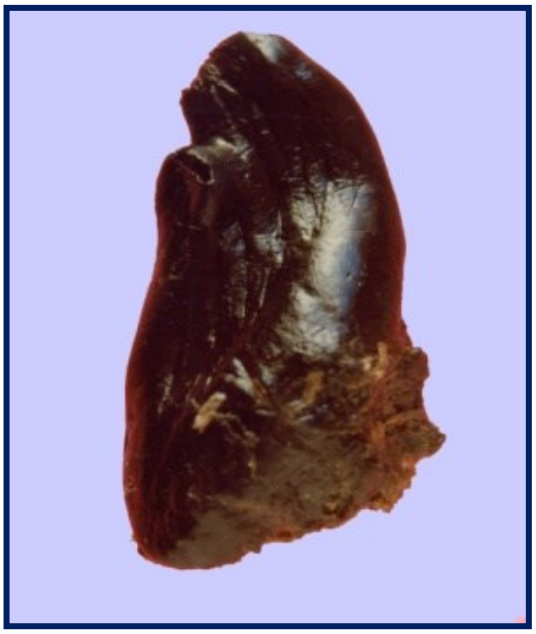


Plate - 1. *Phallusia nigra* Savigny, 1816

Plate - 2: *Didemnum psammatode*

Figure 1: UV-vis spectra for Ag nanoparticles of *Phallusia nigra*

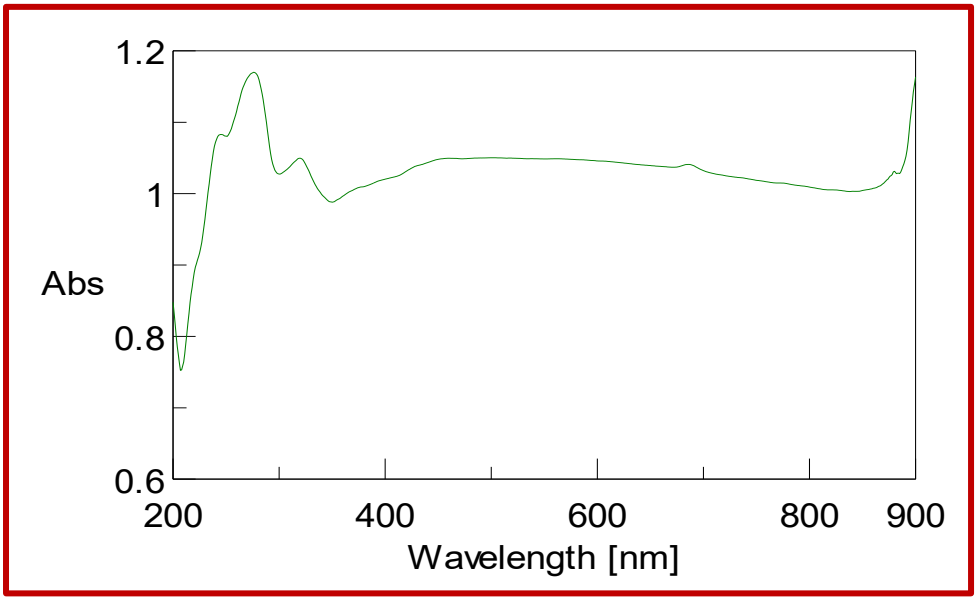


Figure 2: UV-vis spectra for Ag nanoparticles of *Didemnum psammatode*

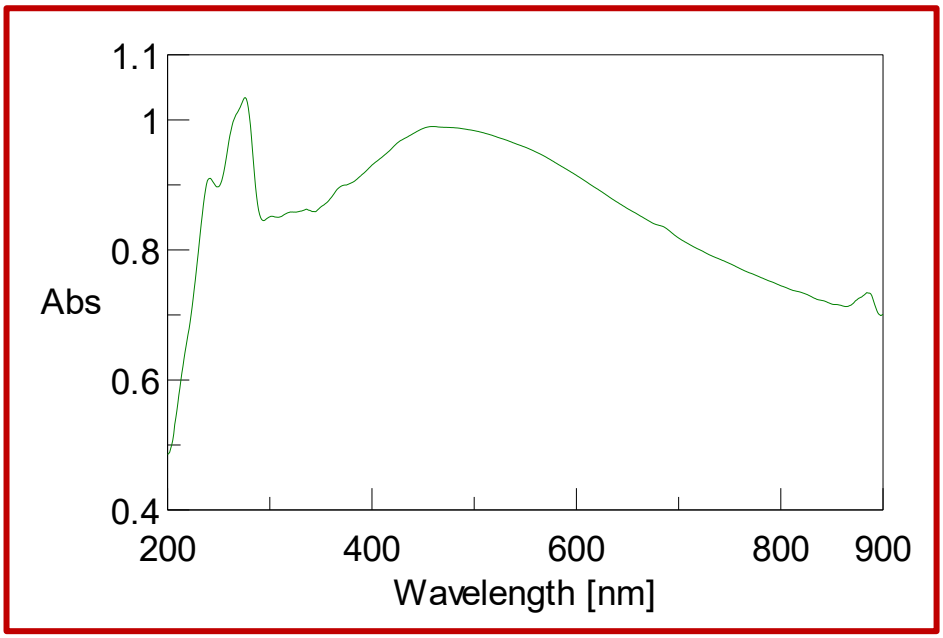


Figure 3: UV-vis spectra for Ag nanoparticles of *Codium geppiorum*

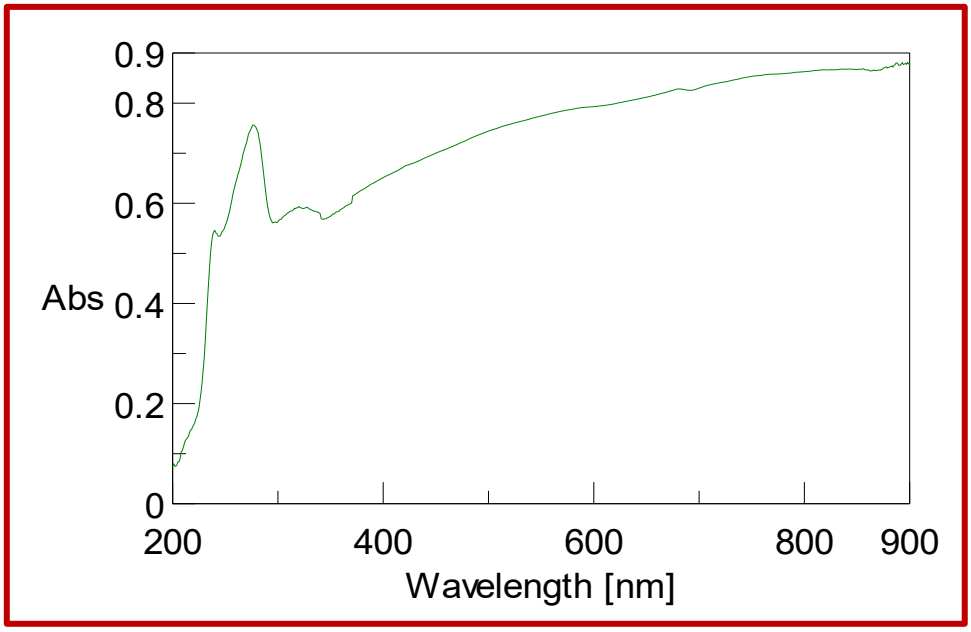


Figure 4: UV-vis spectra for Ag nanoparticles of *Dictyota ciliolata*

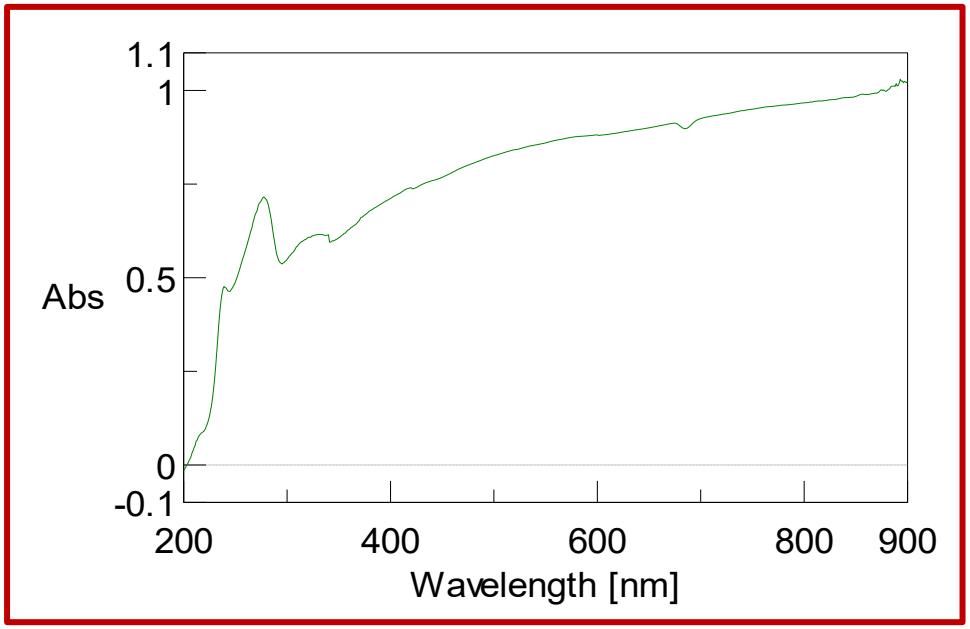


Figure 5: FTIR result for silver nanoparticles of *Phallusia nigra*

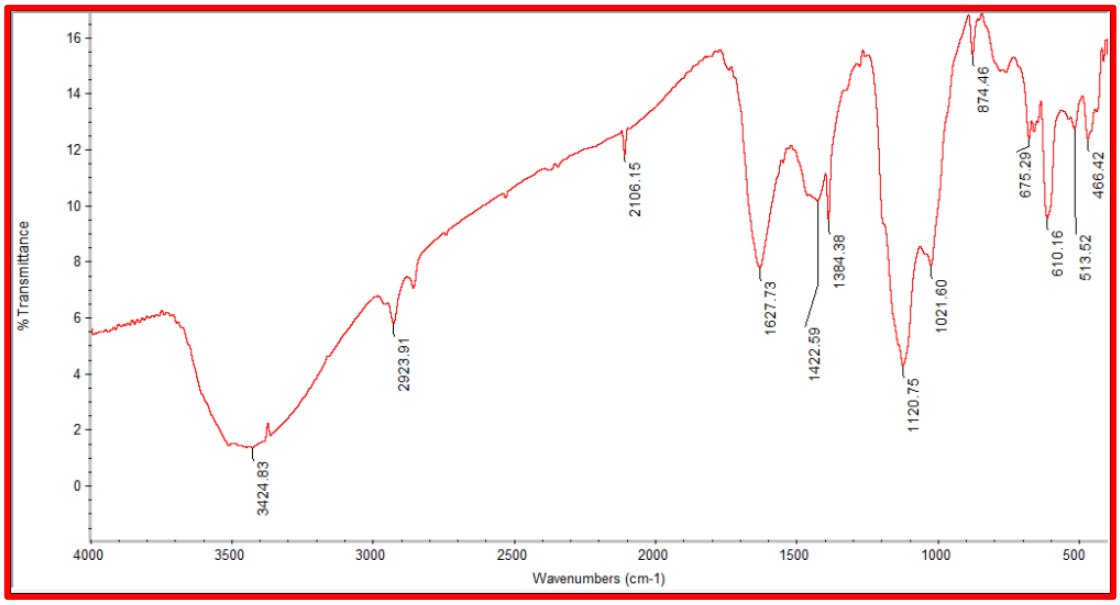


Figure 6: FTIR result for silver nanoparticles of *Didemnum psammatode*

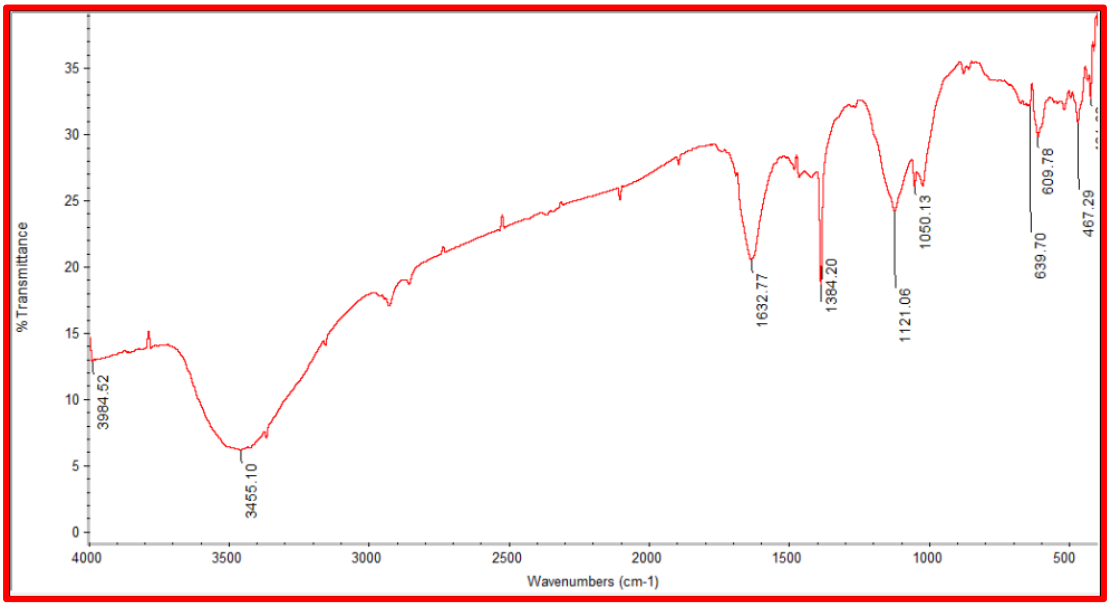


Figure 7: FTIR result for silver nanoparticles of *Codium geppiorum*

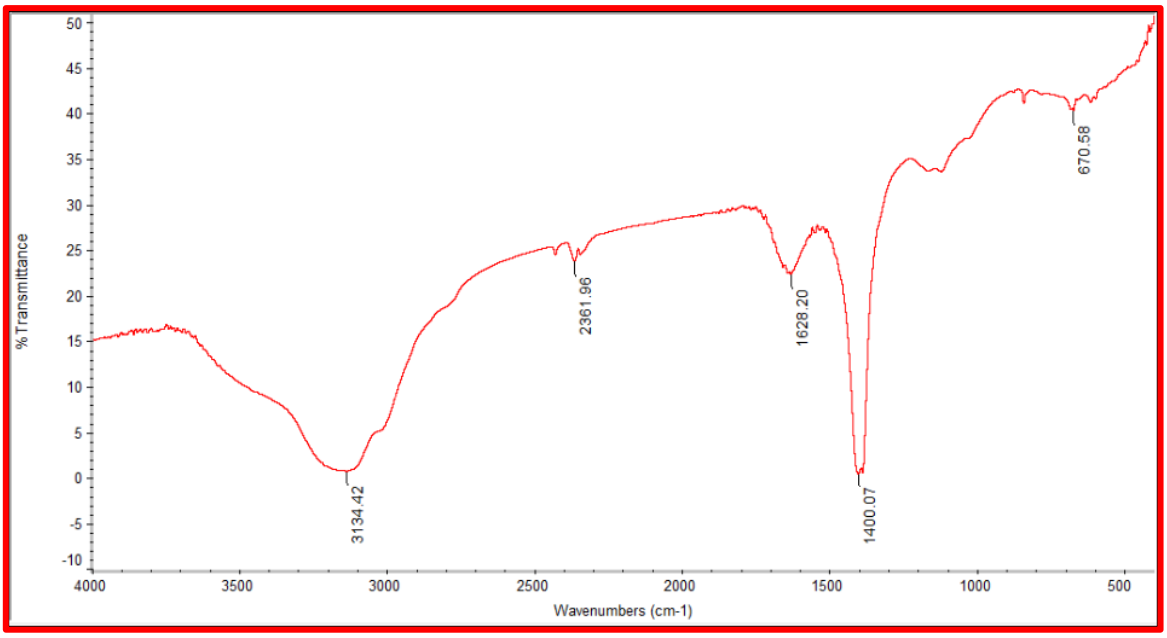


Figure 8: FTIR result for silver nanoparticles of *Dictyota ciliolata*

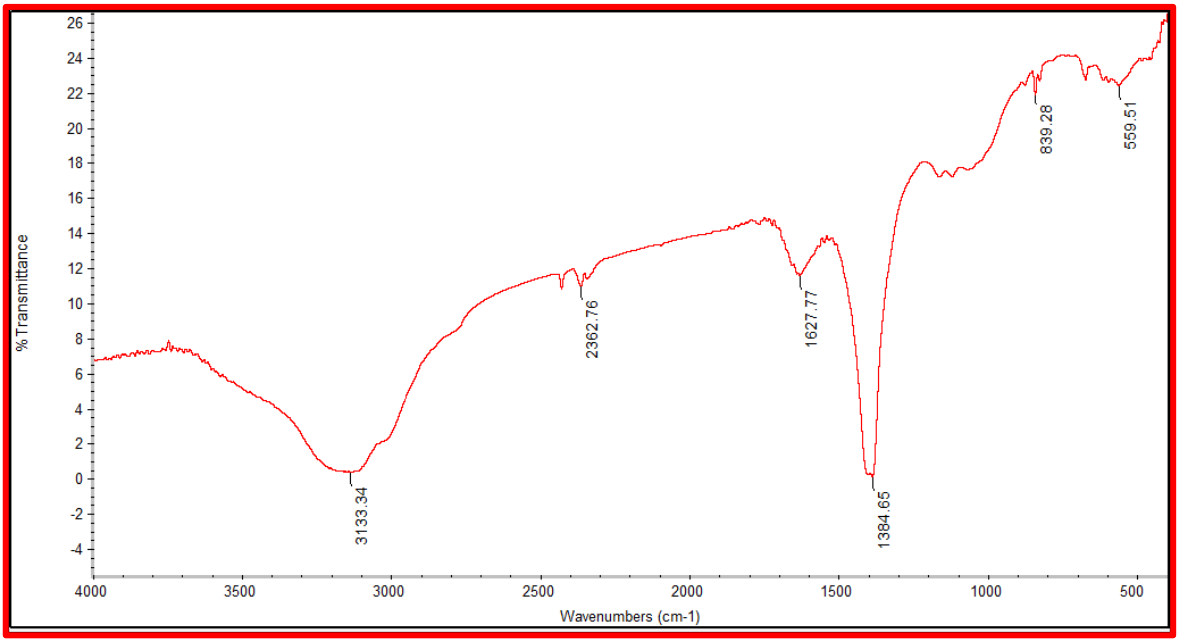


Table 1: Anti-corrosion activity of silver nanoparticles of *Phallusia nigra*, *Didemnum psammatode*, *Codium geppiorum* and *Dictyota ciliolate*

S. No.	Samples	Weight of inhibitor (g)	Weight of plate before immersion (g)	Weight of plate after immersion for 24 hours (g)	Weight loss in 24 hrs (g)	Rate of Corrosion in 24 hrs (%)	Weight of plate after immersion for 72 hours (g)	Weight loss in 72 hrs (g)	Rate of Corrosion in 72 hrs (%)
1	*Phallusia nigra*	0.06	19.2626	18.8320	0.4306	2.23	18.2767	0.9859	5.11
2	*Didemnum psammatode*	0.06	20.0995	19.0840	1.0155	5.05	17.7027	2.3968	11.92
3	*Codium geppiorum*	0.06	19.0820	18.2072	0.8748	4.58	17.0469	2.0351	10.66
4	*Dictyota ciliolata*	0.06	19.8026	19.1905	0.6121	3.09	18.4269	1.3757	6.94
5	Blank	0.06	19.5526	18.8446	0.708	3.62	18.1209	1.4317	7.22

Figure 1: Effect of silver nanoparticles of *Phallusia nigra*, *Didemnum psammatode*, *Codium geppiorum* and *Dictyota ciliolate* in Weight Loss in 24 Hours

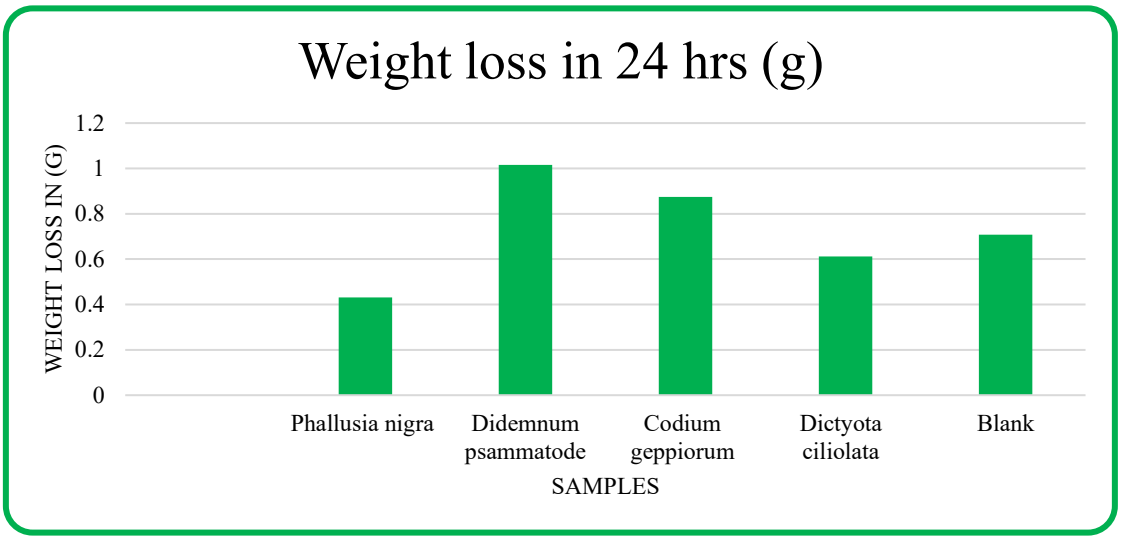


Figure 2: Effect of silver nanoparticles of *Phallusia nigra*, *Didemnum psammatode*, *Codium geppiorum* and *Dictyota ciliolate* in Rate of Corrosion in 24 Hours

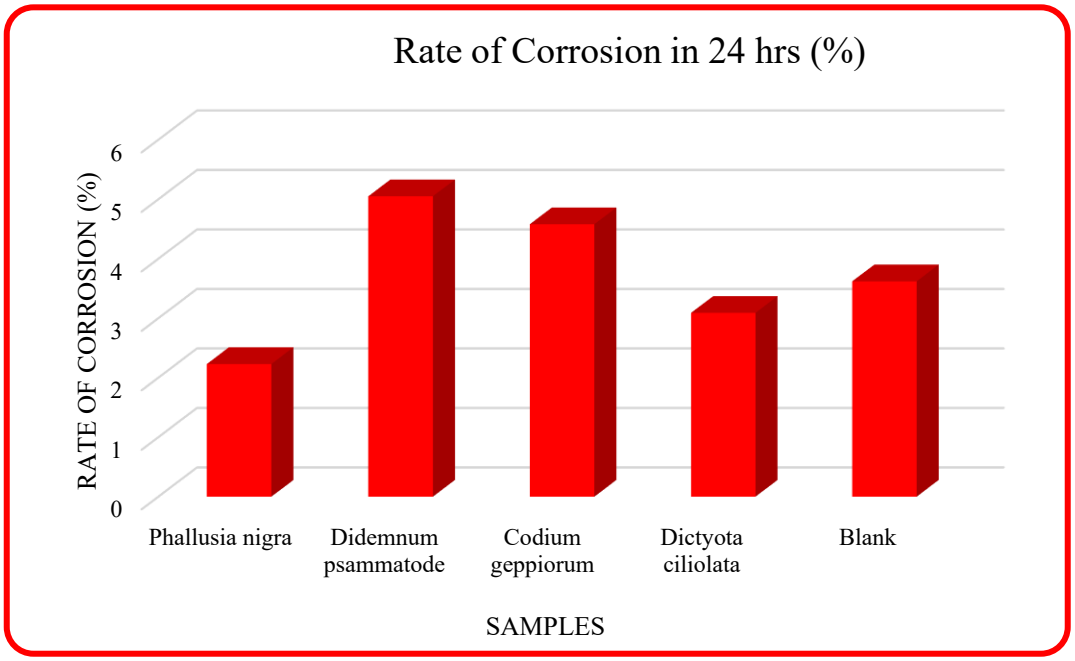


Figure 3: Effect of silver nanoparticles of *Phallusia nigra*, *Didemnum psammatode*, *Codium geppiorum* and *Dictyota ciliolate* in Weight Loss in 72 Hours

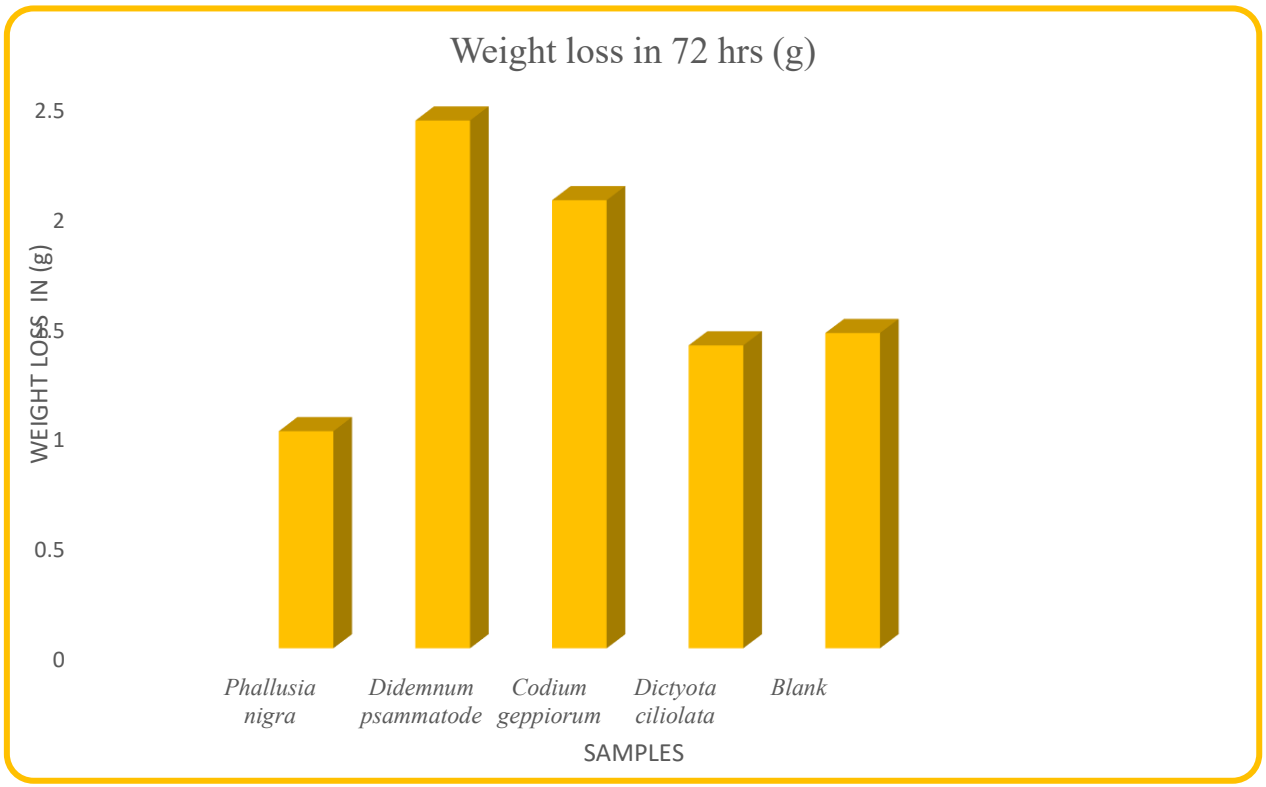
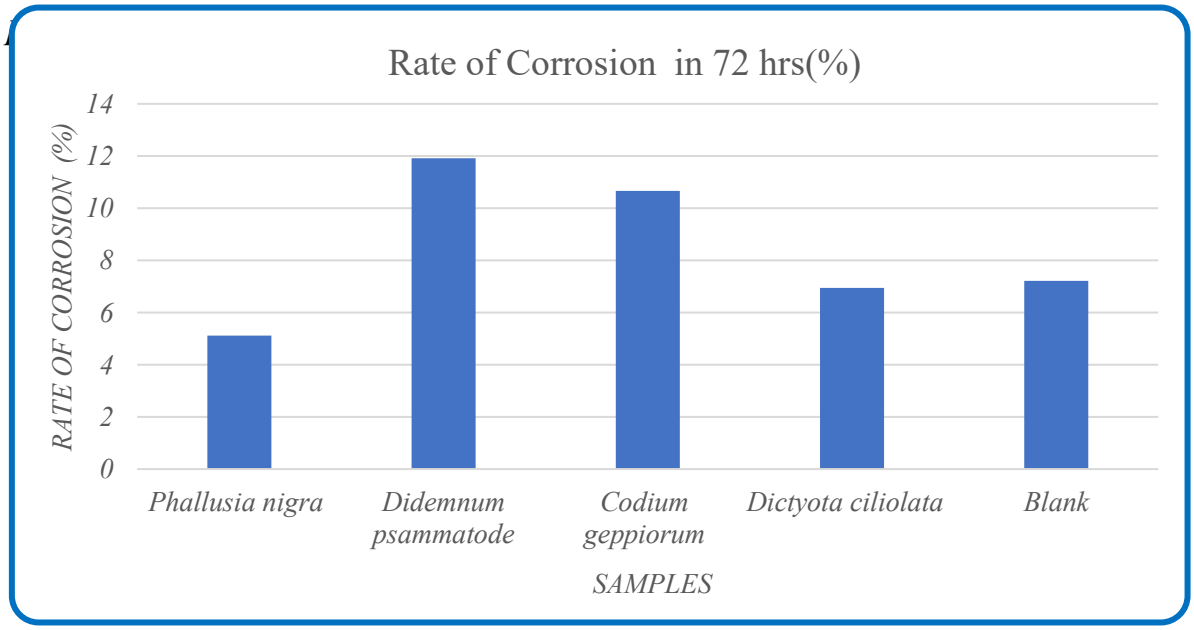


Figure 4: Effect of silver nanoparticles of *Phallusia nigra*, *Didemnum psammatode*, *Codium geppiorum* and *Dictyota ciliolate* in Rate of Corrosion in 72 Hours



Tannins, organic amino acids, alkaloids, and organic dyes of plant origin have good corrosion-inhibiting abilities. Plant extracts contain many organic compounds, having polar atoms such as O, P, S, and N. These are adsorbed on the metal surface by these polar atoms, and protective films are formed and various adsorption isotherms are obeyed.

The corrosion inhibition of mild steel through the studied plants may be attributed to the adsorption of the phytochemicals containing O, N or p-electrons in their molecules as these atoms are regarded as centers of adsorption onto the metal surface.

Extract of plant inhibit the corrosion of carbon steel in 0.5 H_2SO_4 and the inhibiting effect of this latter increases with increase of inhibitor concentration. The inhibition is due to adsorption of the inhibitor molecules on the steel surface and blocking its active sites (Verma *et al*., 2018).

It can be seen that seaweeds protected the surface of carbon steel remarkably well in comparison to the solution without seaweeds (Caprarescu et al., 2020; Dancila, and Fierascu, 2019).

Having confirmed the corrosion inhibition effectiveness of these plants extracts, further detailed investigation for each plant extract through inhibitive assay guided isolation using surface analytical techniques will

enable the characterization of the active compounds in the adsorbed layer and assist in identifying the most active compounds.

The AgNPs of tunicates - *Phallusia nigra* and *Didemnum psammatode,* seaweeds - *Codium geppiorum* and *Dictyota ciliolata* have showed promising corrosion inhibition properties for mild steel in seawater. On comparing the percentage inhibition efficiencies of these four extracts could serve as effective corrosion inhibitors for mild steel.

Further investigations to assess the corrosion morphology and to isolate and confirm the active compounds responsible for the inhibition of mild steel corrosion are required. Corrosion inhibitory property can be assessed using various media.

Anti-fungal, anti-bacterial, anti-viral and anti-cancerous activity of the Ag nanoparticles may be investigated in details.

Various ascidian species can be used to synthesize Ag nanoparticles to find a more non-toxic and economical method of synthesis.

Nanoparticles conjugate may be applied in various cancer cells to observe any anti-cancerous potential of the protein-NP conjugate.

- The samples of the tunicate - *Phallusia nigra, Didemnum psammatode* and seaweed - *Codium geppiorum* and *Dictyota ciliolata* were collected and identified.
- The dried powder of the tunicates and seaweeds was used.
- Silver nanoparticles were synthesized using *Phallusia nigra, Didemnum psammatode Codium geppiorum* and *Dictyota ciliolata*
- The Ag nanoparticles were characterized in a Perkin-Elmer UV-VIS spectrophotometer.
- The chemical composition of the synthesized silver nanoparticles were studied by using FTIR spectrometer.
- To evaluate the corrosion efficiency of carbon steel in the absence and the presence of natural seaweeds in seawater, corrosion rate and protection degree were calculated. Corrosion rate (CR, g/m2 h) for the samples was calculated

Abdallah, M., Kamar, E., Eid, S., and El-Etre, A.Y. 2016. Animal glue as green inhibitor for corrosion of aluminum and aluminum-silicon alloys in sodium hydroxide solutions. *Journal of Molecular Liquids*. **220**: 755 - 761.

Ahamada, I., and Quraishia, M.A. 2010. Thermodynamic, and quantum chemical investigation of some Schiff bases as corrosion inhibitors for mild steel in hydrochloric acid solutions. *Corrosion **52**(3): 933-942.*

Ameer, M.A., and Fekry, A.M. 2010. Inhibition effect of newly synthesized heterocyclic organic molecules on of steel in alkaline medium containing chloride. *International Journal of Hydrogen Energy.* **35**(20): 11387-11396.

Belarbi, N., and Derga, F., Bachari K., 2018. Study of anti-corrosion activity of Algerian L. Stoechas oil on C38 carbon steel in 1 M HCl medium. *International Journal of industry chemistry*. **9**: 115 - 125.

Caprarescu, S.; Modrogan, C.; Purcar, V.; Dancila, A.M.; Fierascu, R.C. Study of Corrosion Efficiency of Mild Steel Using Commercial Liquid Additives for Fish in Natural Water. Rev. Chim. 2019, 70, 1140–1143.

Caprarescu, S.; Purcar, V.; Modrogan, C. Commercial aqueous additives used as eco-friendly corrosion inhibitors for carbon steel in natural seawater. Rev. Roum. Chim. 2020, 65, 77–82. 8.

De Clerck, O., H.R. Engledow, J.J. Bolton, J.J. Anderson and E. Coppejans 2002. Twenty marine benthic algae new to South Africa, with emphasis on the flora of Kwazulu-Natal. *Botanica Marina.* 45:413-431.

Devakumarab, J., and Rameshd, V. 2021. Materials Today: Proceedings Anticorrosion activities of polymethacrylic acid and grafted/modified polymethacrylic acid on pure aluminium in acid median .**42**(2): 1184-1194.

Du, H., Xie, Y., Zhang, H., Chima, A., Tao, M., and Zhang W. 2020. Oxadiazole-Functionalized Fibers for Selective Adsorption of Hg2+. *Industrial & Engineering Chemistry Research.* **59** (29): 13333-13342.

Eddy, N.O., and Ebenso, E.E. 2008. Adsorption and inhibitive properties of ethanol extracts of Musa sapientum peels as a green corrosion inhibitor for mild steel in H2SO4*," African Journal of Pure and Applied Chemistry.**2(6):*** 046–054.

Eswaraiah, G., Peel, K.A., Krupanidhi, S., Kumar R.B., and Venkateswarulu, T. 2019. Studies on phytochemical, antioxidant, antimicrobialanalysis and separation of bioactive leads of leaf extract from the selected mangroves. *Journal King Saudy University. -Science*. **32**: 842–847.

Fadel1, F., Hmamou1, D.B., Salghi1, R., Chebli1, B., Benali2, O., Zarrouk3, A., Ebenso4, E.E., Chakir5, A., and Hammouti b. 2013. Antifungal Activity and Anti-Corrosion Inhibition of OriganumCompactum Extracts. *International Journal of electro chemical science*.**8**: 11019 – 11032.

G. Wranglen, Introduction to Corrosion and Protection of Metals, vol. 236, Chapman & Hall, London, UK, 1985.

Gao, X., Liu, S., Lu, H., Gao, F., and Ma, H. 2015. Corrosion Inhibition of Iron in Acidic Solutions by Monoalkyl Phosphate Esters with Different Chain Lengths. *Industrial and Engineering Chemistry Research*.**54(**7): 1941–1952.

Gaylarde, C.C., and Gaylarde, P.M. 2005. A Comparative Study of the Major Microbial Biomass of Biofilms on Exteriors of Buildings in Europe and Latin America. *Industrial Biodet. Biodeg* **55**: 131-139.

Ghazi, M., and Zefzoufi, H. 2022. Elattari Corrosion Inhibition of Carob Pod Pulp (Ceratonia siliqua L.) on Carbon Steel Surface C38 in Hydrochloric Acid. *Journal of Bio- and Tribo-Corrosion* .**8** (31): 2022.

Hadi, S., Afriyani, H., Qudus, H.I. and Noviany. 2016. The anticorrosion activity of dibutyltin (IV) and diphenyltin (IV) dihydroxybenzoate compounds towards HRP mild steel in NaCl. *Journal of Chemical and Pharmaceutical Research*. **8**(8): 975-980.

Al – otaibi, M.S., Al – Mazraa, S.A., Khan, M., Mousa, A.A., and

Alkathalan, H.Z. 2014. Corrosion inhibitory action of some plant extracts on the corrosion of mild steel in acidic media. *Arabian journal of chemistry*. **7**: 340-346.

Ibryamova, S., Ivanova, R., and Ignatova-Ivanova, T.S. 2018. Study Of the Anticorrosion Effect of Exopolysaccharides Produced by Lactobacillus Fermentum Ts Cultivated on Different Carbohydrates. *Research Journal of Pharmaceutical, Biological and Chemical Sciences.* **9**(6): 366.

Khan, G., Basirum, W.J., Kazi, S.N., and Rehman, M.A. 2017. Electrochemical investigation on the corrosion inhibition of mild steel by Quinazoline Schiff Base compounds in hydrochloric acid solution. *Journal of Colloid and Interface Science.*

Khan, G., Newaz, K.S., Basirun, W.J., Ali, H.B.M., Faraj, F.L., and Khan, G.M. 2015. Application of Natural Product Extracts as Green Corrosion Inhibitors for Metals and Alloys in Acid Pickling Processes, *International Journal of Electrochemical Science*. **10**: 6120 - 6134.

Koch, G.H., Brongers, M.P.H., Thomas, N.G., Virman, Y.P., and Payer, J.H. 2018. Corrosion cost and preventive strategies in United States Supplement to Material Performance, 1-12. *Journal of Applied & Environmental Microbiology*. **6**(2): 37-41.

Kowalski, D., Ueda, M., and Ohtsukat, T. 2007. The effect of counter ani-ons on corrosion resistance of steel covered by bi-layered polypyr-role film.

Anticorrosion Activity of a Polyacrylamide with High Molecular Weight on C-Steel in Acidic Media, *Corrosion science.* **49**(2): 3442-3452.

Kuda, T., Tsunekawa, M., Goto, H. and Araki, Y. 2005. Antioxidant properties of four edible algae harvested in the Noto Peninsula. Japan. J. Food Comp. Anal. **18** :625–633.

Li, H.J., Zhang, W. and Wu, Y.C. 2018. Alkaloids-Their Importance in Nature and Human Life. Anti-Corrosive Properties of Alkaloids on Metals

Liu, Y., Zou, C., Yan, X., Xiao, R., Wang, T., and Li, M. 2015. β-Cyclodextrin Modified Natural Chitosan as a Green Inhibitor for Carbon Steel in Acid Solutions. *Industrial and Engineering Chemistry Research*. **54**(21): 5664−5672.

Maduabuchi, A., Emeka, E., Liu, L., Li, Y., and Wang, F. 2014. Corrosion Inhibition of Q235 Mild Steel in 0.5 M H2SO4 Solution by Phytic Acid and Synergistic Iodide Additives. *Industrial and Engineering Chemistry Research*. **53**(18): 7670–7679.

Moses, M., Gerengi, H., Kaya, T., and Saviour, A. 2016. OrcidPerformance Evaluation of a Chitosan/Silver Nanoparticles Composite on St37 Steel Corrosion in a 15% HCl Solution. *Journal Logo, ACS Sustainable Chemistry Engineering.* **5**(1): 809–820.

Obot, B., Obi-Egbedi, O.N., and Eseola, A.O. 2011. Anticorrosion Potential of 2-Mesityl-1H-imidazo[4,5-f] [1,10] phenanthroline on Mild Steel in

Sulfuric Acid Solution: Experimental and Theoretical Study. *Industrial and Engineering Chemistry Research.* **50(**4): 2098–2110.

Ouache, R. 2019. Phytochemical compounds and anti-corrosion activity of Veronica rosea. Nation Library of Medicine. *National center of Biotechnology information*.**33**(9): 1374 – 137.

Prasanna, B., Praveen, M., Mokshanatha, B., Hebbar N., Vnkatarangaiah, T., Venkatesha‖, and Tandon, H.C. 2014. Ketosulfone Drug as a Green Corrosion Inhibitor for Mild Steel in Acidic Medium. *Industrial and Engineering Chemistry Research.* **53**(20): 8436–8444.

Quraishi, M.A., Rafiquee, M.Z.A., Khan, S., and Saxena, N. 2007. Corrosion inhibition of aluminium in acid solutions by some imidazoline derivatives. *Journal of Applied Electrochemistry*.**37**(10): 1153–1162.

Sathyabama, J., Susai, R., Arokia, S.J., and John, A.A. 2008. "Methyl orange as corrosion inhibitor for carbon steel in well water," *Indian Journal of Chemical Technology.* **15**: 462–466.

Sharma, U.R., and Sharma, N. 2021. Green Synthesis, Anti-cancer and Corrosion Inhibition Activity of Cr2O3 Nanoparticles. *Biointerface Research in Applied Chemistry platinum open Access Journal.* **11**(1): 8402 - 8412.

Singh, A., Ebenso, E.E., and Quraishi, M.A. 2012. Corrosion Inhibition of Carbon Steel in HCl Solution by Some Plant Extracts. Green

Approaches to corrosion Mitigation. *International Journal of Corrosion*.**10**(1): 897430.

Singh, D.D.N.T.B., and Gaur, B. 1995. The role of metal cations in improving the inhibitive performance of hexamine on the corrosion of steel in hydrochloric acid solution. *Corrosion Science* **37**(6): 1005–1019.

Stanger, R., Midland, S.L., Sims, J.J., McCollum, T.G., Mark, N.E.M., Leger, E., Jerkins, K., Mills, M.B., Pallansch, R.L., Roger, G.R., and Veter, P. 2005. Immun. Antifungal Activity and Anti-Corrosion Inhibition of Origanum Compactum Extracts: *International Journal of Electrochemical Science*.**8**(9): 11019-11032.

Tang, X., Wang, H., Liu, C., Zhu, X., Gao, W., and Yin, H. 2021. Direct Growth of Hexagonal Boron Nitride Nanofilms on Stainless Steel for Corrosion Protection. *ACS Applied Nano Materials* **4**(11): 12024-12033.

Verma, C., Ebenso, E. E., Bahadur, I., and Quraishi, M. A .2018. An overview on plant extracts as environmental sustainable and green corrosion inhibitors for metals and alloys in aggressive corrosive media. *J. Mol. Liq.* **266**: 577– 590.

Vranda, K., Venugopal, P.P., Kumari, P.D.R., and Chakraborty, D. [b]2021. Effective inhibition of mild steel corrosion by 6-bromo-(2,4-dimethoxyphenyl) methylidene] imidazo [1,2-a] pyridine-2-carbohydrazide in 0.5 M HCl: Insights from experimental and computational study. *Journal of Molecular Structure*. **1232**: 130074.

A STUDY OF ACTINOMYCETES FROM THE SALT PAN SOIL

A project submitted to

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In partial fulfilment for the award of the degree of

BACHELOR OF SCIENCE IN ZOOLOGY

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(Re-accredited with 'A+' Grade by NAAC)

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CERTIFICATE

This is to certify that the project entitled ***"A Study of Actinomycetes from the Salt pan soil"*** is submitted to **St. Mary's College (Autonomous), Thoothukudi** in partial fulfilment for the award of the degree of **Bachelor of Science in Zoology** and it is a project work done during the year 2021-2022 by the following students.

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DECLARATION

We do hereby declare that this dissertation entitled, "***The Study Of Actinomycetes from the Salt pan soil***" submitted by us for the award of the degree of **Bachelor of Science in Zoology** is the result of our original independent research work carried out under the guidance of **Dr. R. Sri Priya M.Sc., Ph.D.,** Assistant Professor, Department of Zoology, St. Mary's College (Autonomous), Thoothukudi and it has not been submitted elsewhere for the award of any other degree.

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INTRODUCTION

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Saltpans and salt lakes have been reported to harbour high number of taxonomically diverse halopilic microorganism, which differ in salt requirement and metabolic capabilities. Their inhabitants make these unique ecosystems fascinating to study. Saltpans are sites where different ions, including metals, become concentrated and halopilic bacteria evolve, suppressing the less halophilic and halotolerent forms. There is hardly a hypersaline niche in nature that is not occupied by some halophiles. Cultured microorganisms represent only a minor component in the existing diversity of salt pan because of the difficulty in culturing most of the microbial assemblage.

Halophilic microorganisms or the salt loving bacteria are extermophiles, found as normal inhabitants of highly saline environments. They are mainly represented, by the halobacteria (extremely halophilic aerobic Archaea), the moderate halophiles (Bacteria and some methanogens) and several eukaryotic algae. These extremophilic microorganisms are already used for some biotechnological processes, for example halobacteria are used for the production of bacteriorhodopsin, and the alga *Dunaliella* is used in the commercial production

of β- carotene. The potential applications of halophiles include the production of polymers (polyhydroxyalcanoates and polysaccharides), enzymes, and compatible solutes. These extremophiles are also being used in enhanced oil recovery, cancer detection, drug screening and biodegradation of residues and toxic compounds (Ventosa and Neito, 1995)

Halophilic bacteria thrive under high salt environments such as solar salterns, salt lakes and salt mines which contain large populations of these organisms. The metabolic patterns of halophiles are diverse when compared to their terrestrial counterpart and obligate halophiles. Recently many reports have been published on the production of pigments from halophilic bacteria and its applications. Studies have also demonstrated several biotechnological potentials of halophilic bacteria such as pigments, exopolysaccharides, biopolymers, biosurfactants, enzymes, compatible solutes, antioxidants, antimicrobial compounds and anti-tumor agents.

Antioxidants are natural substances which protects the body from free radicals. It helps to prevent oxidation, which can cause cell damage, thereby boosts immune function and possibly reduce the risk for infections, cardiovascular diseases and cancer. Carotenoids, vitamin-C, vitamin-E, selenium, flavanoids and polyphenols are the most common antioxidants. Many marine organisms including micro and macroalgae are known to produce antioxidant substances. However, the

production of antioxidant substances from pigmented halophilic microbes are less reported. In this regard, the present work was aimed to isolate pigmented halophilic bacteria and to study its antioxidant activity.

Halophilic microorganisms which live in saline environments throughout of their different adaptation mechanisms produce metabolites with great potential. Some of its biomolecules has been studied and applied in industrial processes, such as exopolyssaccharides, carotenoid pigments, bacteriorhodopsin etc. beside certain enzymes especially hydrolases (pectinases, amylases, proteases, lipases, etc.) are important. Recent researches on halophilic microorganisms and their biomolecules has increased around the world. Saline environments such as saline lakes or saline soils are excellent sources for isolation of halophilic microorganisms. However, few saline environments have been studied in depth in order to evaluate the special characteristics of halophilic biomolecules. In this review, the importance of halophilic microorganisms for biotechnological industries, methods for their isolation; techniques for physiological, taxonomical and molecular characterization have been highlighted so as to establish them as important source for enzyme production (Garcia et al., 2015). Because of the growing interest in the study of secondary metabolites from marine environments these hypersaline ecosystems could be highly promising habitats for the discovery of microorganisms capable of

producing novel and useful bioactive compounds. These extremophiles have been reported to have antibacterial activity (Kamat and Kerkar, 2011)

Another major thrust recently is isolation of genes for salt stress resistance which can be used for mitigating salt stress in crop varieties. The halophilic organisms are major resources of genes that confer salt resistance. The extremophiles have a wide range of adaptive mechanisms that makes them to tolerate the extreme environmental conditions. The molecular mechanism behind the salt tolerance is governed by a number of genes that are present in these organisms. These microorganisms can be hampered for their genes, that can be used for genetic engineering of plants for salt stress resistance. Salinity stress is one of the major factors negatively affecting growth and productivity in living organisms including plants and bacteria resulting in significant losses worldwide. Therefore, it would be fruitful to develop salinity stress tolerant useful species and also to understand the mechanism of stress tolerance that simulate the production of bioactive osmotic compatible solute which are of great significance to cope with hostile salt stress conditions, and to have industrial and pharmaceuticals applications as well. A prerequisite for molecular studies is the identification of genes involved in the accumulation of compatible solutes. It becomes important to investigate organisms that harbor these genes, by isolating halophilic / halotolerant bacteria (Das et al., 2015).

Actinomycetes are Gram positive or Gram variable, filamentous bacterial species which are abundantly found in the halophilic environment. They are regarded as one of the great significant group of soil microbial population. These have been isolated and characterized from several marine samples, including sediments obtained from deep-sea, even from greatest depth-marine trench, and also in the surrounding regions of hydrothermal vents. Halophilic actinomycetes are gaining interest because of their wide economic importance. They are also sources of different biologically active secondary metabolites such as vitamins, nutritional materials, herbicides, antibiotics, pesticides, anti-parasitic and enzymes like cellulose and xylanase used in waste treatment. Only 1-3% *Streptomycete* antibiotics have been discovered and remaining 97-99% are yet to be analysed. The current study is designed to isolate and characterize Actinomycete species from the salterns.

The current work focuses on isolation of halophilic/halotolerant bacterial species and their characterization. The isolated microorganisms can be identified and characterized for the adaptive mechanisms involved in salt tolerance.

OBJECTIVES

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- To isolate Actinomycetes from the soil samples from salterns
- Morphological characterization of the isolated Actinomycte strains
- Biochemical characterization of the isolated Actinomycete strains
- To test their salt tolerance capacity

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Hypersaline environment provides an excellent medium for natural microbial communities which serve as a potential source of pharmaceutical substances. Salt is widely present in the earth. Almost 73% of earth is covered with marine water which contains 25% of common salts. A hypersaline environment such as salt pans and salt lakes have high salt concentration and ph. The salt pan provides a diversity of different environmental conditions of alkalinity, salinity, temperature, pH and nutrition. Hlophilic organisms grow between 0.5 and 3.0 M salt concentration. Extreme environments are the best source of bioactive compounds producing halophilic microbes. Halophilic microorganisms can be used for industrially important enzymes and numerous bioactive compounds. Halophilic bacteria also produce secondary metabolities, extracellular polysaccharides, proteins, enzymes, amylase, cellulose and aminoacids etc. These environments are favourable for halophiles whose occurrence becomes visible due to the pigment production.

Halophilic bacteria are commonly found in natural environments containing significant amounts of NaCI such as inland salt lakes and evaporated sea-shore pools, as well as environments such as curing brines, salted food products and saline soils. Adaptation is an evolutionary process through which an organism

develops ability to live in its habitat (Dobzhansky et al.,1968). Environmental changes shift the balance of the complex microbial communities by favoring some populations and restricting others, through mechanisms such as microbial competition for nutrients , antibiosis and by selecting the most suitable organism to environmental stress. Most halophilic and all halotolerant organisms expend energy to eliminate salt from their cytoplasm to avoid protein aggregation (Welsh, 2000).

Halophiles can thrive not only in those small salt water bodies such as salterns and lakes but also in large oceans and benthic floors of the sea. Halophilic bacteria are some of the best examples of environmental adaptation.With a heavy salt presence in the surrounding area, these microbes have developed metabolic conditions to their survival. Some may be found within fluid inclusions in salt crystals (Nortan and Grant 1988) and other produce compatible solutes to adapt high salt stress conditions. The requirement for sodium chloride plays a major role in the metabolism of extremely halophilic bacteria, but they also require magnesium. As the growth of halophiles is predominately dictated by the concentration of salt in the water, most moderate and slight halophilic bacteria do not require magnesium (Grant et al.,2001).

The halobacteria tolerate the heavy concentrations of salt by the group of enzyme called the haloenzymes. Haloenzymes have unique features rarely finding

in other enzymes. The optimum activity and stability of haloenzymes occur at a high NaCl concentration and they need NaCl to maintain their structure. They show high resistance against denaturation and can do the enzymatic function in low water or non aqueous medium (Madern et al.,2000; Setati 2010). Halophilic enzymes are divided into three categories (1) intracellular enzymes which are not directly in contact with the ionic concentration of the surroundings , (2)membrane bound enzymes (carrier proteins)which are in direct contact with the cytoplasmic content as well as the outside medium and (3)extracellular enzymes which are directly exposed to the saline medium (Ventosa et al.,1998). The enzyme from halophiles and haloalkaliphiles cannot only withstand higher NaCl concentrations but actually the salt is required for the function and stability of the enzymes (Madern et al.,2000)

Culture dependent studies have been done to isolate halophiles from different hypersaline habitats ranging from solar salterns to deep salt mines (Birbir M ,et al.,2004) (Ara ,L et al.,2013).Solar saltpans are found all around the world and provide ideal settings for halophilic and halotolerant microbes (Gupt et al., 2015). Halophiles have wide range of biotechnological potential in industry, example biosurfactant production, biopolymers in oil recovery, protease and amylases in detergent industry, poly-beta hydroxyalkanoate as biodegradable

plastic, exopolysaccharide and bioremediation of contaminated hypersaline brines, etc.,(Kanekar et al.,2012).

Actinomycetes is a nontaxanomic term for a group of commom soil microorganisms sometimes called "Thread or hairy bacteria". They are known for decomposing more resistant organic materials such as chitin, a complex sugar found in the outer skeleton of insects and elsewhere. Actinobacteria are a well recognized type of bacteria due to their ability to produce antibiotics. Examples of antibiotics from actinobacteria include streptomycin, streptothricin,and actinomycin. Actinomycetes are gram-positive mycelial bacteria,known to produce a wide variety of industrially and medically relevant compounds antibiotics, chemotherapeutics, fungicides, herbicides and immunisuppressants (Stackebrandt et al.,1997).

Actinobacteria are Gram-positive or Gram-variable microorganisms with high G+C content which have a rigid cell wall that contains muramic acid. Most are chemoorganotrophs and some of them are halophiles. Recently, members of actino bacteria were raised to the taxonomic rank of a phylum which is one of the major phyla in the domain Bacteria, as inferred from its branching pattern in the 16S rRNA gene tree (Garrity and Holt 2001 ; Ludwig and Klenk 2005). The phylum actinobacteria includes phenotypically diverse microorganisms which

show diverse morphological properties that range from cocci to highly differentiated mycelia (Goodfellow et al., 2012).

The life cycle of these organisms include spores and mycelial growth in the majority of the species, the spore formation includes separation at different stages of aerial hyphae. Thus the plasma membrane is invaded and the inner cell wall breaks down and thickens to form a thick wall. During the process of sporulation process. A thick wall is formed around each one of the spores due to the presence of new material. This sporulation process occurs due to the fragmentation of hyphae or due to spore formation.In the soil or environmental conditions the spores are capable of surviving for a longer period of time.In these conditions,the spores germinate to form germ tubes;these tubes further develop to become mycelium. They have a cell wall that covers the organisms, this cell wall is made up of amino acids,amino sugars,and sugars.It also contains a peptidoglycan layer that is made up of diaminopimelic acid. These are heterophic organisms that depend on other organisms for survival. To break down the material that is present in the environment these organisms produce enzymes such as cellulases that help to break down the sugars, proteases help to break down the protein molecules, keratinases help to break down the keratin material and amylases help to break down the starch. Some of the species of actinomycetes bacteria can have symbiotic relationships with the leguminous plants for survival (Hirvonen et.al).

Morphological similarities between actinomycetes and fungi has been partially attributed to adaptation to the same habitat.

For the majority of these species, spore formation is characterized by septation at several intervals of the aerial hyphae. This involve the invagination of the plasma membrane as well as a break in the inner wall. The hyphal wall then starts to thicken to form a thick wall. According to a number of studies, the sporulation process may occur through fragmentation of the hyphae or endogenous spore formation. In the cases where the spores are formed through fragmentation/subdivision of the hyphae, the spores formed may be covered by a sheath even after fragmentation. However, this is not the case with all species.

Many of the antibacterial are considered to have antibacterial property because of the plethora of antimicrobial compounds they produced. Investigations on the antimicrobial properties of actionomycetes can be divided into two groups (A) Those dealing with the bacteriostatis and bact icidal activities of these organisms, and (B) Those concerned primarily with their bacteriolytic properties.In both cases,the possible practical applications have been kept in mind. The hope of isolating specific antibacterial substances potentially useful for the control of infectious diseases of man, animals, or plants has been general. Interest in the lytic agents is dominated by the possibke use of bacterial lysates for vaccination purposes (Maurice Welsch, 1942).

The actinomycetes exits in various habitats in nature. The terrestrial ones from the soil have been extensively used for the production of secondary metabolites used to human. Marine salt pans are important ecological niches which is rich in halobacteria. These bacteria tolerate and thrive in salt concentrations ranging from 0.5 to more than 5M in which only very few other organisms are able to survive. Many actinomycete bacteria have been found to inhabit hypersaline environments like salt pans, salterns and sea bed. The actinomycetes from the salpans are a potential resource for antibacterial metabolites. There are numerous reports of isolation and characterization of bacteria from the hyper saline environments.

The halophilic actinobacteria are widely distributed in saline, hypersaline terrestrial and aquatic habitats. In general, different pretreatment methods such as incorporation of specific antibiotics were used to prevent the growth of bacteria and fungi, and alteration in the composition of the cultivation medium was specific for the isolation of rare actinomycetes. Humic acid and different concentrations of vitamin agar medium were the best base for the selection of rare halophilic actinomycetes (Tang YW, et al., 1999).

Isolation of them does not demand special enrichment techniques , supplementing the isolation media with salt would be enough, but very few species have been isolated and characterized till date (Kanekar et al., 2012). The phylum

actinobacteria includes phenotypically diverse microorganisms which show diverse morphological properties that range from cocci to highly differentiated mycelia (Goodfellow 2012). The halophilic actinobacteria are widely distributed in saline ,hypersaline terrestrial and aquatic habitats. Isolation of them does not demand special enrichment techniques (Kanekar et al., 2012), supplementing the isolation media with salt would be enough. Halophilic actinomycetes require higher concentrations of salt for their growth and are classified into moderate (15% NaCl) and extreme (30% NaCl) halophiles. They survive through two mechanisms – "high-salt-in" and "low-salt, organic-solutes-in" – for the protection of intercellular proteins in the presence of salts similar to potassium chloride and production of organic acids, which will directly alter the intracellular enzyme levels (Quillaguaman J, et al., 2010).

Actinobacteria are widely distributed in aquatic and terrestrial habitats, including extreme habitats, such as the hypersaline environments which are widely distributed on our planet (Ventosa et al., 2008). The halophilic and halotolerant actinobacteria exhibited interesting diversity in terms of distribution in different habitats. There are several reports on inhabitance of them in diverse hypersaline environments such as hypersaline soils, saline salt lakes, solar salterns, salt mines, salted food products, and in some unexpected places as brines deep in the sea, on

plants that excrete salts from their leaves, and on ancient wall paintings (Chen et al. 2007 ; Xiang et al., 2008 ; Sabet et al., 2009 ; Guan et al., 2011).

Actinobacteria are one among the dominant species found in the halophilic environments. The halophilic and halotolerant *Actinobacteria* as accommodated in different taxa, they show a remarkable range of different morphologies from organisms that from cocci (e.g.Ruania and Serinicoccus), short rods (e.g.cellumonas), irregular rods (e.g.Microbacterium and Salinibacterium), rods and cocci (e.g.Arthrobacter), and mycelia that fragment into cocoid and rod like elements (e.g. Nocardiopsis). Others show more extensive morphological differentiation ranging from those which reduce extensively branched substrate hyphae that bears spores (e.g.Micromonospora) to those that form a stable branched mycelium that carries aerial hyphae which differentiate into short or long chains of spores (e.g.Actinopolyspora and Streptomyces). In general, spores are non motile. Currently, the number of species names that have been validity published as halophilic and halotolerant actinobacteria is very large and is growing exponentially due to the recent efforts to characterize microorganisms from hypersaline environments.

Numerous actinomycetes have been isolated from soils worldwide. As a group, these microorganisms have a variety of genes encoding for many useful bioactive compounds, such as antibiotics, antitumor agents and

immunosuppressive agents (Bérdy, 2005). Unfortunately, conventional bioprospecting of soil actinomycetes, the microbial group that was the most significant source of new antibiotics in the twentieth century, largely resulted in the rediscovery of already known compounds (e.g., Walsh, 2003; Taubes, 2008; Fischbach and Walsh, 2009). A study on diversity of actinomycetes under salt and alkaline environments showed the relationship of actinomycetes (especially halophilic actinomycete strains) to cations such as Na+ , K+ , and Mg2+ to be very complex (Jiang et al., 2006).

Bacteria from marine salt pans of varying salinities of 220 to 395 psu were isolated during the peak salt harvesting season and screening to evaluate their antibiotic producing potential. It was reported that, a total of 119 bacteria were screened on 12 different solid media supplemented with either natural salt or sea water or distilled water to check their substrste utilization and salinity requirement. Based on their morphological variations, 94 isolates were further screened for their antagonistic properties, against 20 different clinical pathogens.Thirty one isolates were found to produce antibacterial compounds of which, 21 showed bacterial action and one was bacteriostatic while 9 isolates were broad spectrum antibiotic producers.

Numerous methodologies have been adopted to isolate actimycetes from the marine sediments. Nine selective culture media were used for counting

actinomycetes in marine water and sediments. The presence of glycerol and asparagines in the media favoured the growth of these microorganisms. Thermic treatment of sediment samples resulted in a selective reduction of the non-actinomycetal heterotrophic microflors. However, the same treatment of water samples had a negative effect on the actinomycetal flora. Actinomycetes in water and sediment represented 3-4% and 5-6% of the total flora, respectively (Barcina et al. 1987).

MATERIALS AND METHOD

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Collection of sample:

Samples for the experiments were collected from the Saltpans in Sathya Nagar, Thoothukudi District. Salt samples were collected from the salt Bundhs in the salt pans approximately 35cm below the surface. Water samples were also collected from the salterns in autoclaved bottles.

Characterization of culture:

Isolation of halophilic bacteria by serial dilution:

1 gram of salt sample was taken into a test tube and mixed with 10 ml of distilled water. The salt sample was serially diluted till 10^{-6} dilutions and the 200 µl of the sample from each dilution was spread plated to obtain single colony isolates.

Plating of culture:

The nutrient agar / actinomycete isolation agar was prepared and was autoclaved at 121°C for 15 minutes. The medium was poured into petridish and allowed to solidify. The samples were plated on the medium by spread plating.

100 µl of the samples from each dilution (10^{-1} and 10^{-6}) was plated spreading the technique. Plates were incubated at 37°C till the growth of the colonies.

Selective isolation of Actinomycete strains:

From the salt samples, microorganism were isolated by serial dilution followed by spreading and restreaking. The plates were incubated at 37°C till visible colonies appeared.

Isolation of pure bacterial colonies:

Single colonies were picked and streaked to obtain single pure colonies. Pure colonies were incubated at 37°C for 24 hours and stored at 4^0C for further studies.

Characterization of isolates:

Morphological characteristics of colonies such as shape, size, colour, pigmentation, gram staining were recorded

Gram staining:

Gram staining was performed for isolated colonies according to standard procedure. A smear of bacterial cells was prepared on a clean glass slide by gentle heat fixation. Heat fixed smear filled with crystal violet solution for one minute. The smear was washed with distilled water and then gram iodine was added.

Smears were washed with 95% decolourisation and cleaned with water. Finally safranin was used as counter stains for 60-80 seconds and washed with water. Then observed under a microscope.

Biochemical characterization:

Catalase test:

Slants of bacterial culture in nutrient agar were made and the catalase test was performed. Two drops of hydrogen peroxide was added to the 24 hours bacterial culture. The immediate evolution of the gas bubbles indicates the production of catalase enzyme by the isolates and hence considered catalase positive (Hadioetomo 1990).

NaCl tolerance test:

For endurance experiments involving NaCl concentrations, nutrient agar was used as the basic medium and supplemented with the following NaCl concentrations: 3% ,5%, 7% ,8% . After plating of the isolated strains, the plates were incubated at 37°C for a day. Based on the growth of bacteria on each concentrations, they were considered as halotolerant or halophilic bacteria.

Phosphate solubilization:

Bacterial isolates was screened invitro for their phosphate solubilizing activity using potato dextrose rose Bengal agar. The cultures were streaked on the agar. Plates are incubated at 37°C for 24 hours. The growth of the bacterial colony indicates a positive result for phosphate solubilization (Martin, 1950).

Screening for Hydrolytic enzyme production:

Bacterial isolates were screened for their hydrolytic enzyme production like protease and amylase.

Protease production activity:

Bacterial isolates were screened for the ability to produce proteolytic enzymes in skim milk agar (SM medium). The medium was poured into a sterilized petridish and the isolated bacterial strain was streaked on the surface of the medium. Formation of a clear zone around the colonies is indicative of protease production.

Starch hydrolysis activity:

About 20 ml of starch medium was poured into the sterilized petridish. The isolate was streaked and incubated for 24 hours. At the end of the incubation period, two or three drops of Iugol's iodine solution were added on the surface of

the medium. A clear zone around the area of growth indicates starch hydrolysis activity of the isolates.

RESULTS

ISOLATION OF BACTERIA

Bacterial strains were isolated from the soil sample from the saltpan in the study area mentioned (Fig. 1). The bacterial cultures were isolate by serial dilution. The diluted cultures were plateded on Actinomycete isolation agar. The plates had many bacterial colonies of diverse genus and species. In order to obtain Actinomycetes from the soil sample Actinomycete isolation agar was used and the plates were incubated at 37°C for 7 days (Fig. 2). Six individual colonies were taken for further studies from the dilution where distinct single colonies were observed. A patch of the colonies were maintained and the colonies were named as S1, S2, S3, S4, S5 and S6 (Fig. 3).

GRAM STAINING

Gram staining was done on the isolates S1, S2, S3, S4, S5 and S6 (Fig. 4) (Table 2). The strains S1, S2, S5 and S6 were Gram positive and S3 and S4 were Gram negative organisms.

COLONY MORPHOLOGY:

The bacterial isolates were characterized for the colony morphology such as shape, colour, margin, elevation and opacity and also characterized for cellular morphology using student microscope.

The morphology of the bacterial colonies and their colours were observed and tabulated in (Table 1). The colonies had varied morphology. The strains S1 and S6 were white, S2 and S5 were dull white colonies. The strain S5 were irregular and S1, S2, S3, S4 and S6 were circular. The margins of the colony were entire in S1, S2, S3, S4, S6, and S5. The elevation of the colony were raised in S1, S2, S4 and S6 and flat in S3 and S5. The colonies S1, S2, S3, S4, S5 and S6 were opaque (Table 1) (Fig.2)

HALOTOLERENCE TEST:

To check if the bacteria were halotolerent, the bacterial isolates S1, S2, S3, S4, S5, S6 were done in duplicates. The colonies were patched on Nutrient agar medium supplement with 3%, 5%, 7% and 8% (Fig 5). The bacterial isolates grew will on the medium containing 5% of NaCl and there was growth retardation on medium containing 7% NaCl (Fig. 5). They were not able to tolerate 8% of NaCl. Hence these isolates are probably mildly halotolerent actinobacterial strains (Table 3).

BIOCHEMICAL ANALYSIS OF ISOLATED BACTERIA:

Biochemical tests were performed on the bacterial isolates using Actinomycete isolation agar. Tests like Catalase test, Phosphate solubilisation test, Protease production test and Starch hydrolysis tests were performed.

CATALASE TEST:

Catalase test is done to identify strains that produce the catalase enzyme. The isolates S1, S2, S3, S4, S5 and S6 were used for catalase test. To the overnight grown bacterial strains few drops of hydrogen peroxide was added. A brisk effervescence was observed in all the test cultures, showing that all the strains produced catalase enzyme (Fig. 6) (Table 4).

PHOSPHATE SOLUBILIZATION TEST:

Phosphate solubilisation ability of bacteria can be detected by culturing the isolates on potato dextrose rose Bengal agar plate. Growth on the medium confirms their phosphate solubilisation activity. The isolates S1, S2, S3, S4, S5 and S6 were checked for phosphate solubilisation. The strains S1, S2, S3, S4, S5 and S6 grew

well on the Dextrose rose Bengal agar medium, implicating that these colonies could solubilize phosphate (Fig.7) (Table 5)

HYDROLYTIC ENZYME PRODUCTION

PROTEASE PRODUCTION TEST

To check the ability of the actinobacterial strains to produce protease enzyme,the colonies were patched on the skimmed milk (SM) agar medium.The colonies S1, S2, S3, S4, S5 and S6 were used for the analysis, The colonies grew on the SM agar medium and there was a zone of clearance observed around the colonies (Fig. 8) (Table 6). This showed that the isolates produce the protease enzyme.

STARCH HYDROLYSIS TEST:

The starch hydrolysis test is performed to confirm the production of the enzyme amylase by the bacterial isolates. The isolates that produced amylase enzyme can hydrolyse the starch present in the Starch agar and show a zone of clearance around their colony when iodine is added. The test was performed on 6 colonies S1, S2, S3,S4, S5 and S6 (Fig. 9) (Table 7) A zone of clearance was observed around the colonies S1, S2, S3, S5 and S6. Indicating that these produce the amylase enzyme. The colony S4 was negative for starch hydrolysis test.

Table 1. Morphology of the isolates

Isolates	Colour	Shape	Margin	Elevation	Opacity
S1	White	Circular	Entire	Raised	Opaque
S2	Dull white	Circular	Entire	Raised	Opaque
S3	Yellow	Circular	Entire	Flat	Opaque
S4	Yellow	Circular	Entire	Raised	Opaque
S5	Dull white	Irregular	Filiform	Flat	Opaque
S6	White	Circular	Entire	Raised	Opaque

Table 2. Gram staining of bacterial isolates

Isolates code	Gram staining
S1	Positive
S2	Positive
S3	Negative
S4	Negative
S5	Positive
S6	Positive

Table 3. Halotolerance test of isolated bacteria

Concentration	3% of NaCl	5% of NaCl	7% of NaCl	8% of NaCl
Growth level	+++	++	-	-

Note, - = no growth, + = weak level of growth, ++ = medium level of growth, +++ = high level of growth

Table 4 Catalase enzyme production of bacterial isolates

Isolates	Catalase test
S1	Positive
S2	Positive
S3	Positive
S4	Positive
S5	Positive
S6	Positive

Table 5 Phosphate solubilisation test in bacterial isolates

Isolates	Phosphate solubilization
S1	Positive
S2	Positive
S3	Positive
S4	Positive
S5	Positive
S6	Positive

Table 6 Protease enzyme production of bacterial isolates

Isolates	Protease production
S1	Positive
S2	Positive
S3	Positive
S4	Positive
S5	Positive
S6	Positive

Table 7 Starch hydrolysis test

Isolates	Starch hydrolysis
S1	Positive
S2	Positive
S3	Positive
S4	Negative
S5	Positive
S6	Positive

Fig.1.Site of sample collection –Sathya Nagar , Thoothukudi.

Fig .2A

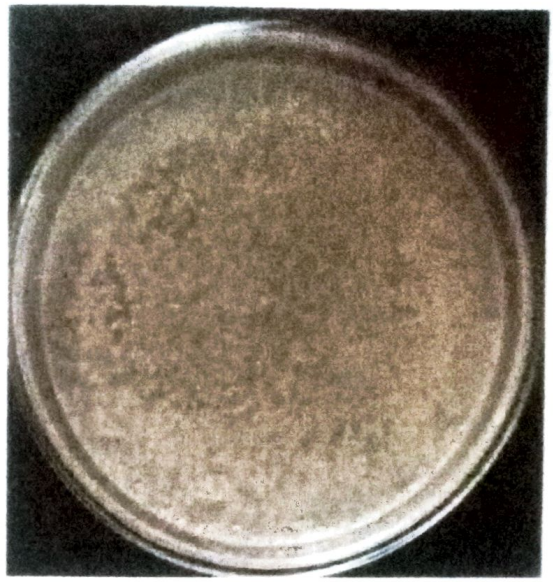


Fig .2B

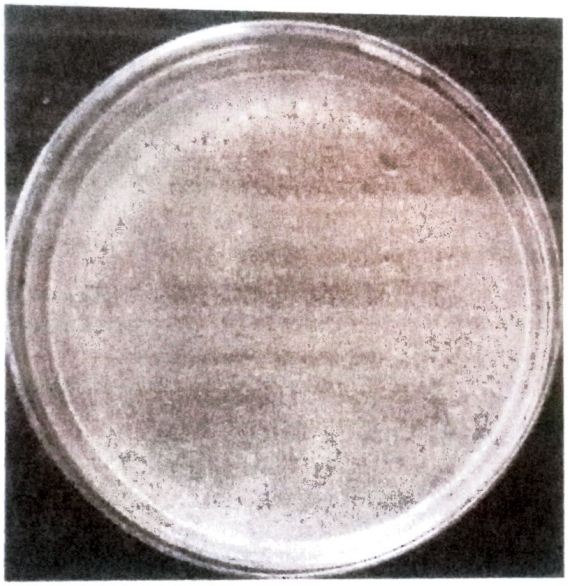


Fig . 2C

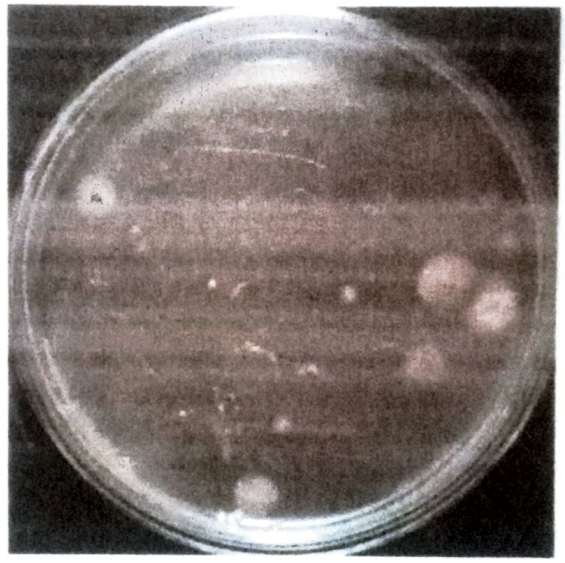


Fig . 2D

Fig.2. Serial dilution of bacterial colonies obtained from soil samples on Actinomycetes Isolation Agar

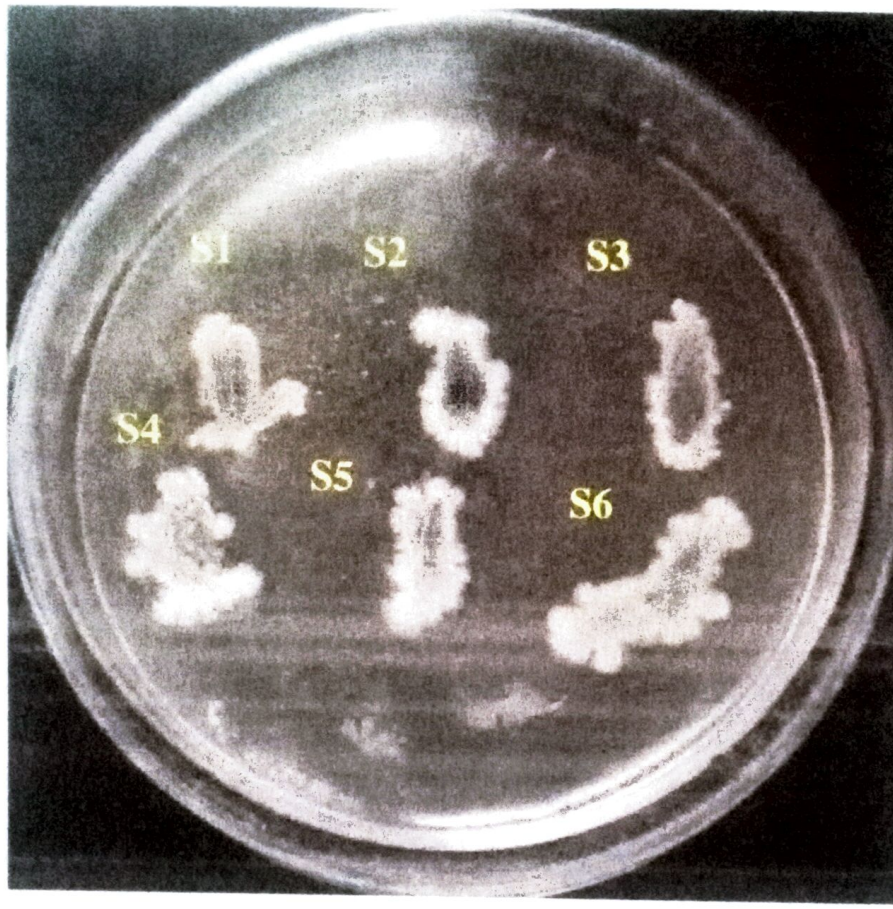


Fig . 3

Fig.3.Pure culture of bacterial colonies on Actinomycetes Isolation Agar

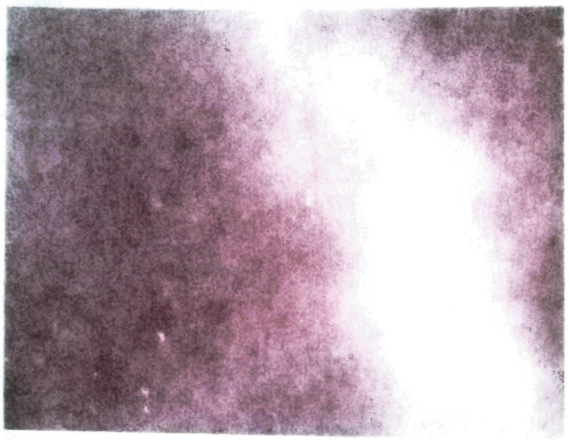
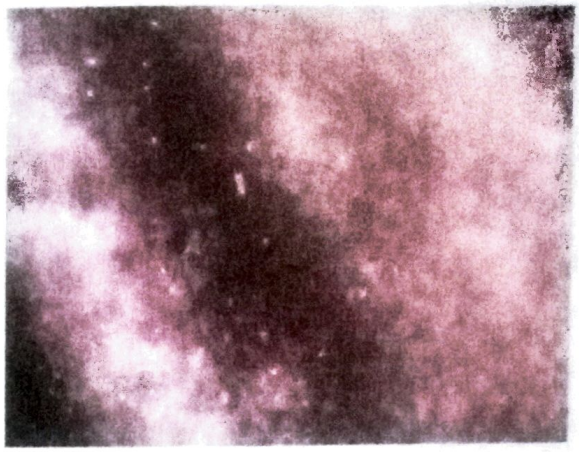


Fig : 4A



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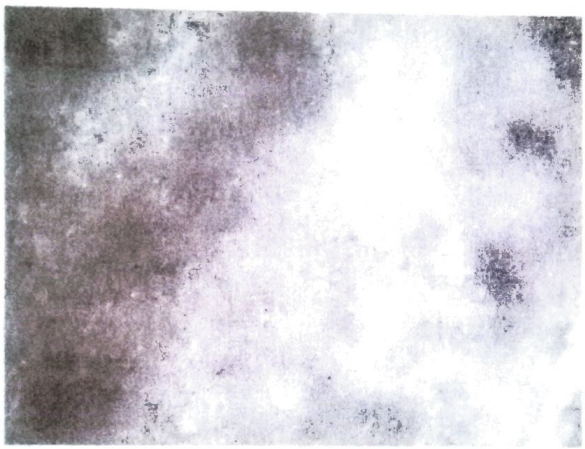

Fig .4C

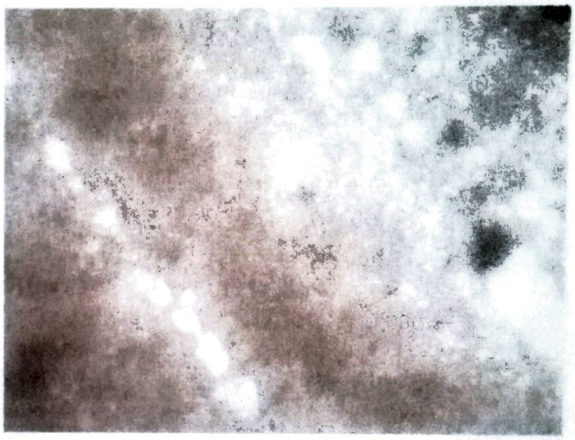

Fig : 4D

Fig .4E

Fig .4F

Fig. 4.Gram-staining of the bacterial isolates

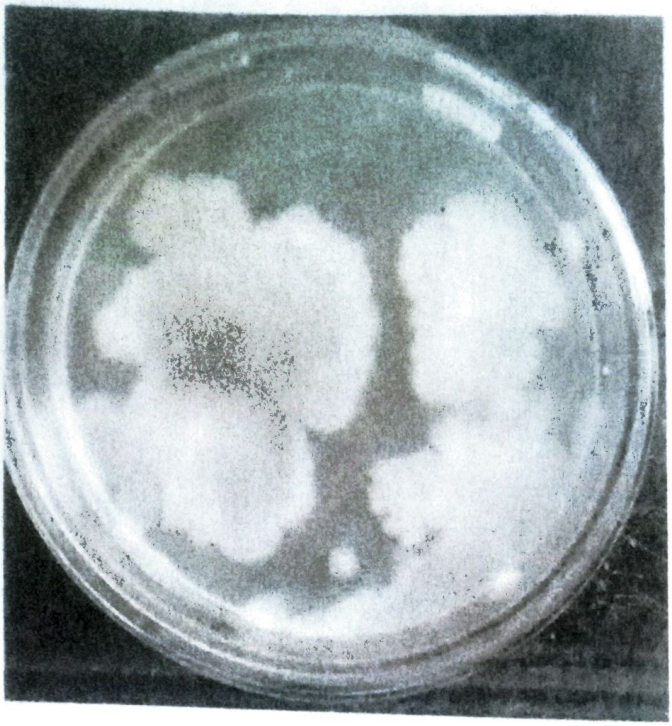

Fig . 5A 3%NaCl

Fig . 5B 5%NaCl

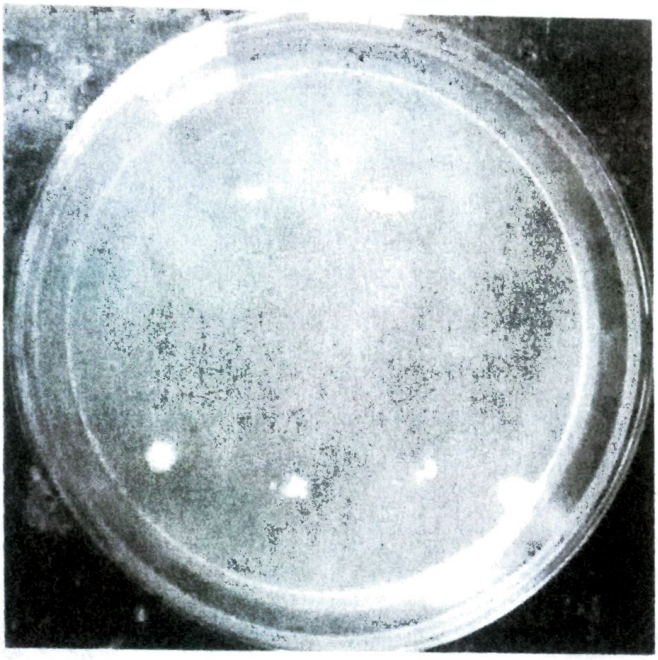

Fig . 5C 7%NaCl

Fig . 5D 8%NaCl

Fig. 5 Halotolerance test for the bacterial strains

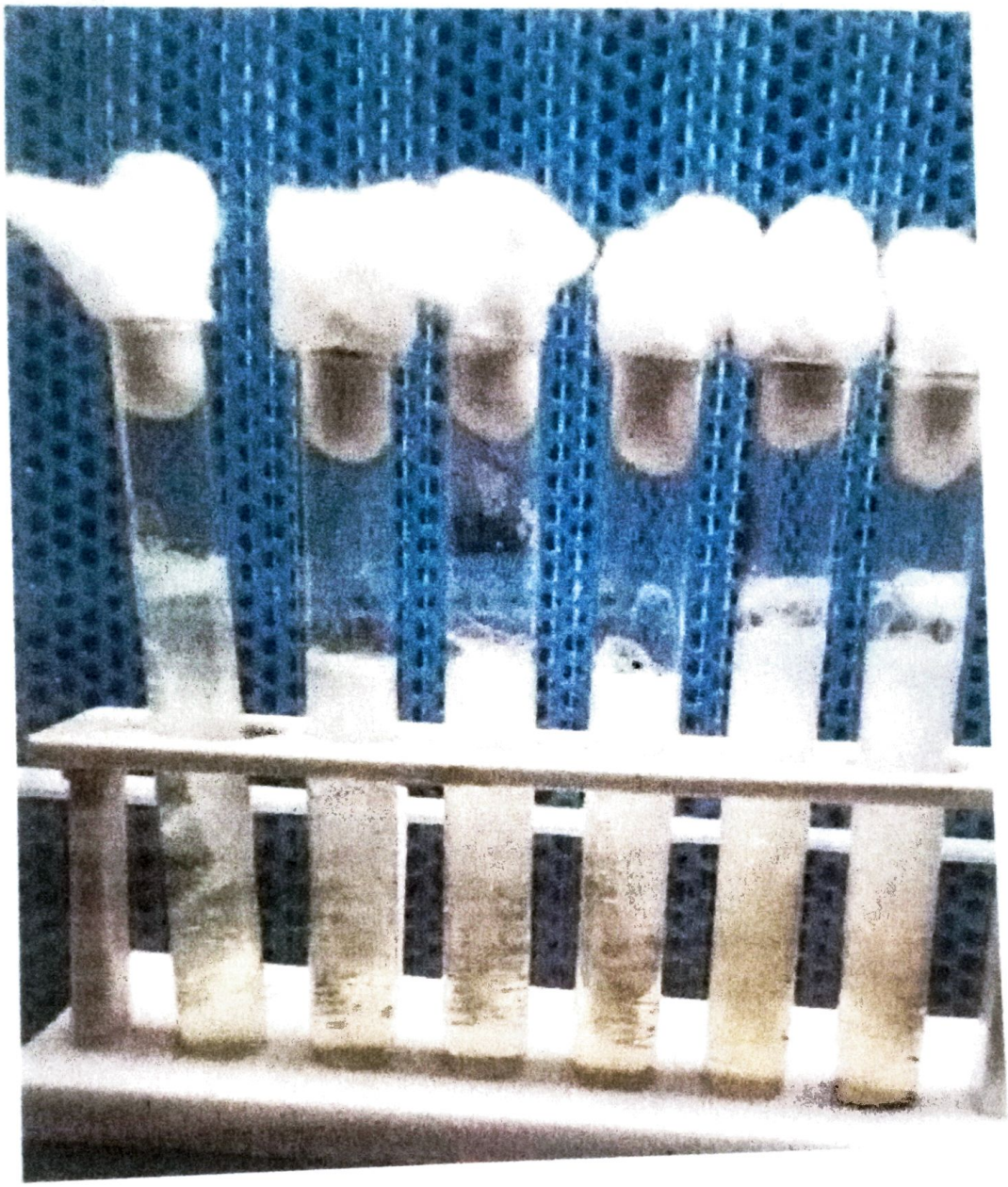

Fig.6. Catalase test of the isolated bacterial strains

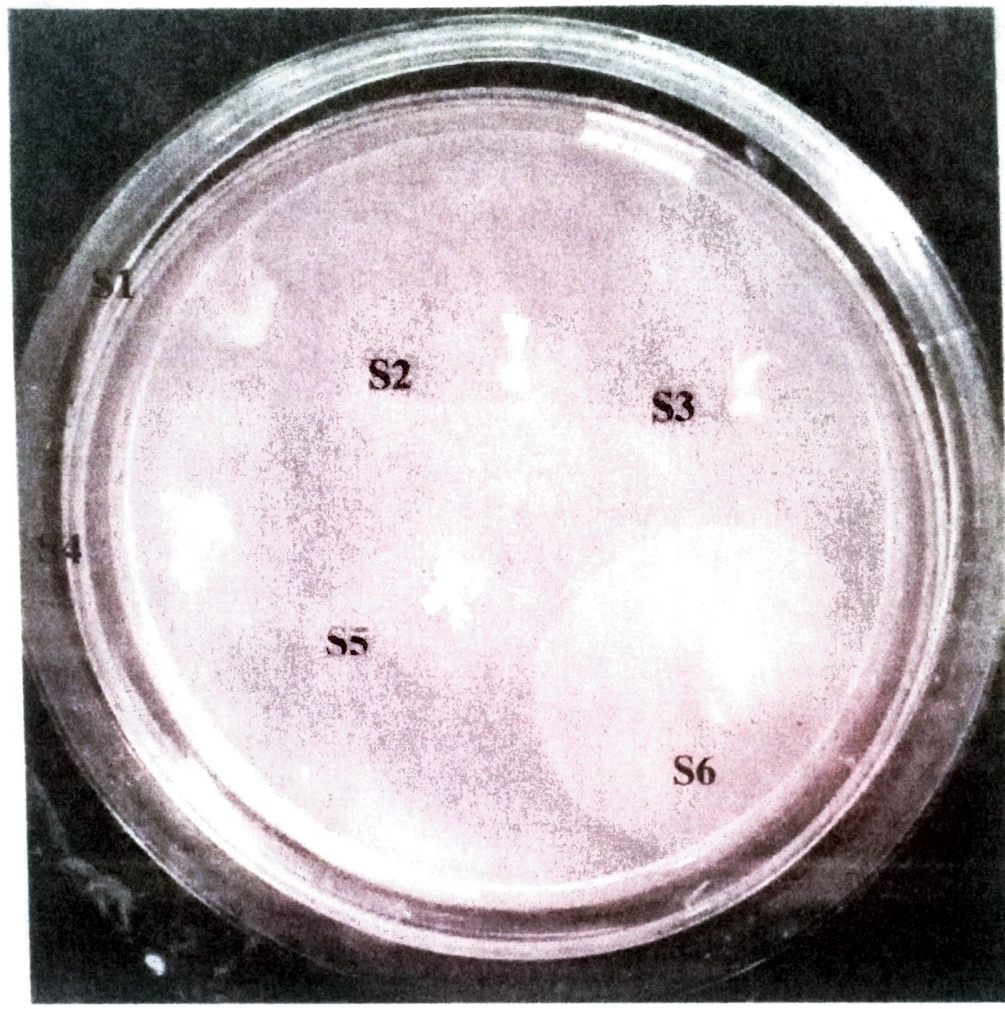

Fig . 7. Phosphate solubilisation test

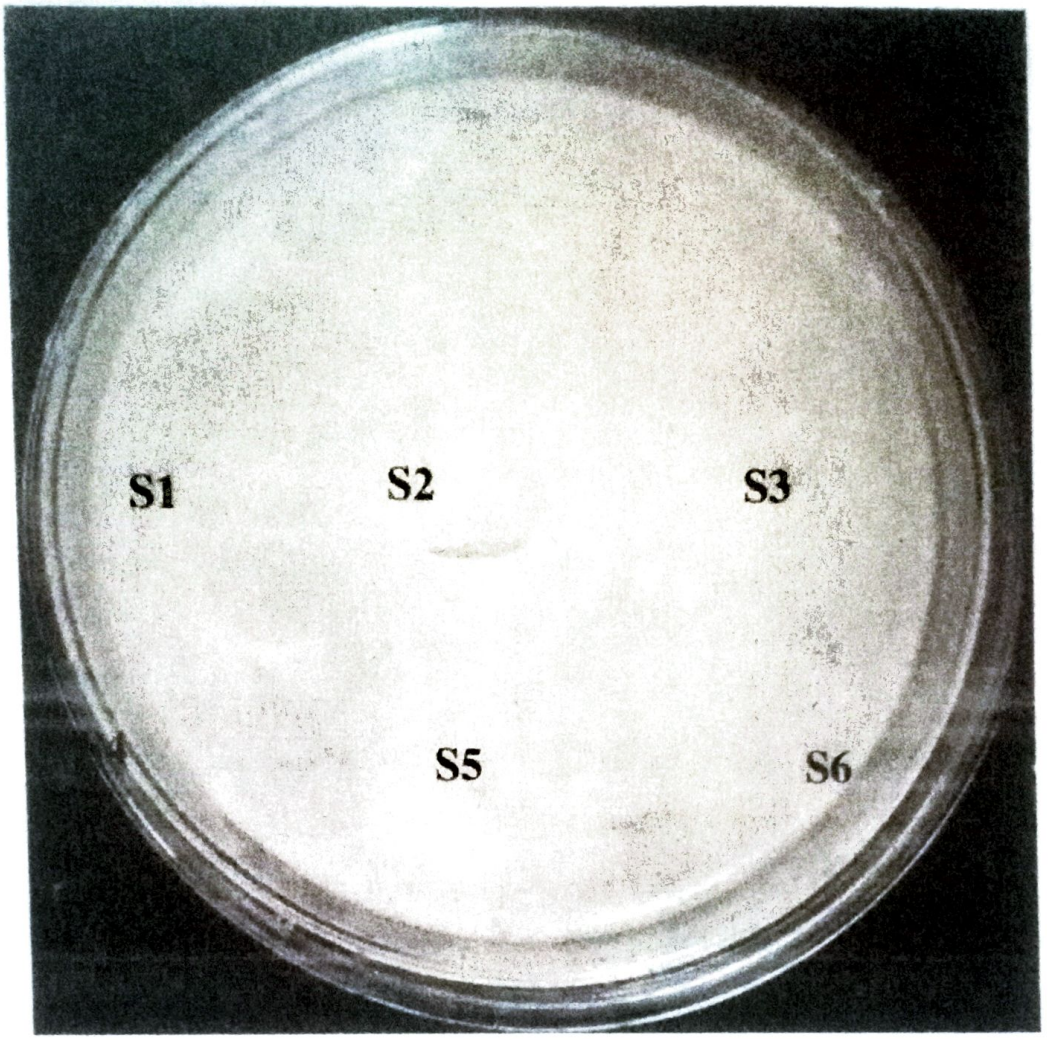

Fig . 8. Protease production test

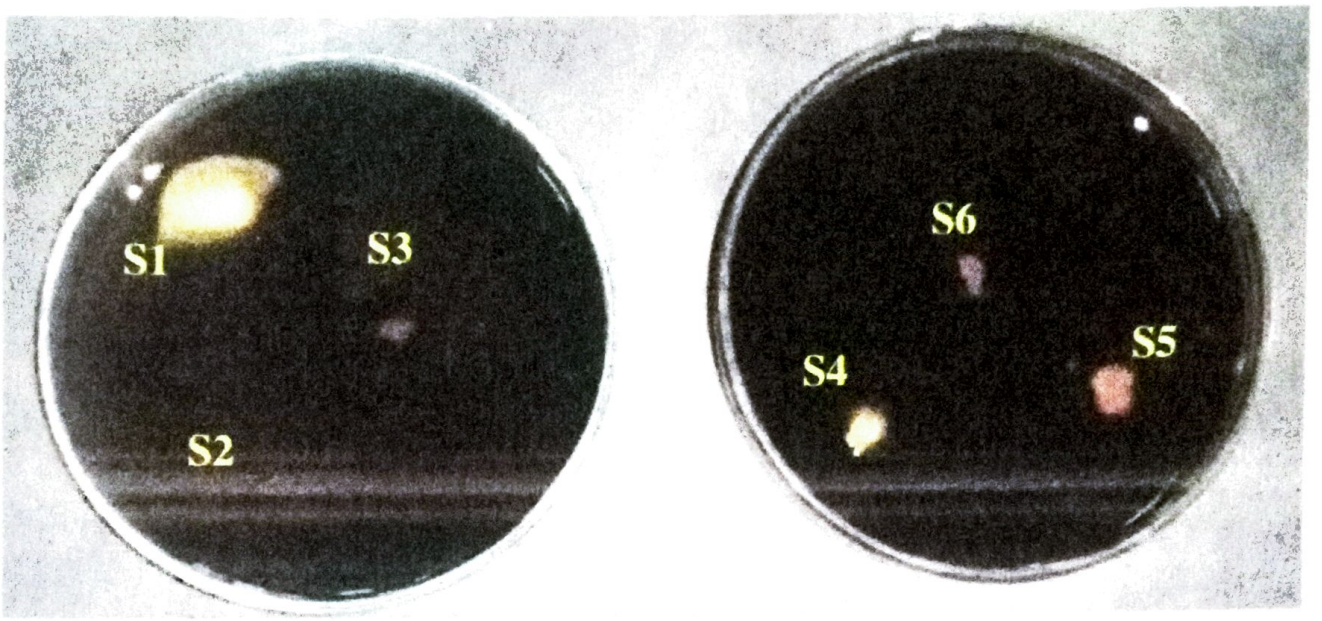

Fig. 9. Starch hydrolysis test

DISCUSSION

DISCUSSION

Actinomycetes, which occur in both terrestrial and aquatic habitats, are among the most common groups of gram-positive microorganisms in nature. Actinomycetes decompose organic matter and display antagonism against other bacteria and fungi, with which they compete for nutrients. Actinomycetes have incredible abilities to survive under extreme conditions in their natural environment and have long been the focus of scholarly attention and have been harnessed as valuable sources of natural compounds, such as antibiotics, enzymes, and vitamins. More than 90 percent of chemotherapeutic antibiotics have been isolated from actinomycetes (Newman et al., 2007; Demain, 1999).

The halophiles are named after the Greek word for 'salt-loving' are extremophiles that thrive in high salt concentrations. It can live, grow and reproduce in salty concentrations. In recent past, the use of halophilic forms in industrial applications has been increased. On this aspect, the present study was carried out to identify many halophilic forms and their activities from Sathya nagar salt pan environment.

Generally, most of the studies that focus on the screening of antibiotic producing actinomyctes are done in a neutral medium (pH 7). In those studies Actinomycetes that are halophilic were not selected and hence, these extremophiles could have been missed out (Trenozhnikova and Azizan, 2018)

The objective of the current study was to isolate halophilic actinobacterial strains. Hence salt samples from Salterns were used to isolate the organisms. Initially the colonies were isolated on Actinomycete selection Agar. On the Actinomycete selection medium, many halophilic bacterial strains were identified. For the analysis a few of the colonies which grew on actinomyctes isolation Agar. Morphological and biochemical characterization of the colonies were done. The bacterial colonies showed diverse morphological characteristics as indicated from variation in shape, colour, margin , elevation and opacity. On the basis of their gram reaction S1,S2,S5,S6 were gram positive. Some of the colonies also produced a wide range of bioactive compounds such as enzymes protease, amylase, catalase etc.,

Actinomycetes are an indispensable part of modern medical science. These are found in nearly all habitats. Actinomycete from marine environment was first discovered from the salt molds of St. Padenbur (Nadson, 1903). The need for antimicrobials is going up day by day due to emergence of new pathogens or due

to drug resistance, so efforts are to be taken to discover newer and potent antimicrobials to combat emerging diseases.

Dhanasekaran, (2014) isolated two potential actinomycetes from saltpan of Mumbai. It showed resistance to salt concentration upto 10-15%. It also showed potential anti infective activity againt various drug resistant human bacterial and fungal pathogens. In this study, we have isolated actinomycete strains from the salt samples by selectively culturing the samples on Actinomycetes isolation agar.

Roshan et al., (2013) performed salt tolerance activity by using different concentrations of NaCl with suitable media. 4% NaCl was optimized for maximum growth for the isolates. Our results are in accordance with previous studies. In our study, 3-7% of NaCl was optimized for maximum growth for the bacterial strains. Roohi et al., (2012) evaluated the growth of halophilic bacteria at 5 to 40 % salt concentration. In this study, we have performed halotolrance test from isolated bacteria. Growth was observed in the plates supplemented with 7% NaCl and 8% NaCl completely inhibited the growth of the strains. Hence it can be concluded that the isolated strains are moderately halophilic bacteria.

Pathak et al., (2012) revealed that the enzyme protease activity was measured at different NaCl concentrations. Protease activity was recorded at wide range of NaCl concentrations. Sannchez-Porro et al., (2002) showed that the

culture collection strains tested for hydrolytic activities most of them were negative for the production of the protease enzymes. Our results are in accordance with previous study. In our study, all isolated were negative for protease production.

Soni et al., (2013) revealed that the microbes had the ability to solubilize phosphate in the presence of high salt. They found treated seeds had showed significant increase in germination percentage in the presence of salt in the media depicting their ability of solubilize the phosphate even in the presence of salts and facilitating the seeds to germinate. In this study the isolates S1, S2, S3, S4, S5 and S6 were able to solubilize phosphate at higher rates. In this present study, the isolated bacterial strains solubilised phosphate, produced catalase and showed starch hydrolytic activity. These strains have to the further characterized by 16s rRNA to identify their genus and species. Analyse of more colonies have to be done to identify a strains that could be used efficiently for bioprospecting. Antimicrobial activity of the characterized strains have to be performed.

SUMMARY

SUMMARY

- Six Putative actinomycete bacterial strains were isolated from Salt pan soil by selection on Actinomycete isolation agar.
- All the isolates were found to be gram positive
- Morphological characterization of the colonies was done.The colony morphology was circular, flat, entire and some are irregular too.
- Actinomycete strains were identified by using Nalidixic acid & Actinomycete isolation agar.
- Halotolerance test were performed to identify bacterial tolerance.
- In biochemical test, catalase test was performed.
- Hydrolytic enzyme production test such as protease and starch hydrolytic test were performed.
- Phosphate solubilization test were performed .

CONCLUSION AND SUGGESTIONS

CONCLUSION

Salt pans are a good model for studying the ecological succession of organisms ranging from microbes to avifauna. Keeping in view of their importance economically, halophilic microorganisms have many advantages. First, most of them can grow at high salt concentrations, minimizing the risk of contamination. Second, they are easy to grow, and their nutritional requirements are simple. It is well known that actinomycetes are the most economically and biotechnologically valuable prokaryotes. It is believed that the halophilic and halotolerant actinobacteria hold a prominent position due to their biodiversity and potentiality to produce novel compounds. Several bio-molecules produced by these halophilic organisms,i.e. enzymes, halocins (halobacterial proteins with antibiotic activities), exopolysaccharides etc., show biological activity in harsh conditions. The halophilic microorganisms represent a rich source of novel products (enzymes, surfactants, biopolymers) for biotechnological applications. Discovery of enzymatic abilities of halophilic microorganisms can accelerate the industrial processes and meet the increasing demands for biocatalysts.

SUGGESIONS FOR FUTURE

The identification of the bacterial genera has to be done by 16s rDNA sequencing. It will be interesting to know more about genetic of halophilic enzymes and in a future performed studies of cloning, recombinant DNA or over-expression of halophilic enzymes, contributing to generation of new enzymes appropriate for modern biotechnological industries.

BIBLIOGRAPHY

BIBLIOGRAPHY

Abdel-Mageed. W.M, Milne. B.F., Wagner. M., Schumacher. M., Sandor. P., Pathom-aree W(2010). Dermacozines, a new phenazine family from deep-sea dermacocci isolated from a Mariana Trench sediment. Org Biomol Chem; 8: 2352-2362.

Ammar .Y.B., Matsubara.T., Ito.K., Iizuka.M., Limpaseni.T., Pongsawasdi. P., Minamiura (2002) . Characterization of a thermostable levansucrase from Bacillus sp. TH4-2 capable of producing high molecular weight levan at high temperature. J Biotechnol 99:111-119.

Anjali.S., Sejal.R., Preeti.S (2013). Screening of efficient halotolrant phosphate solubilizing bacteria and their effect on seed germination under saline conditions. Journal of Scientific and Innovative Research Vol. 2(5): 932-937.

Anupama .P., Kshipra .B (2012). Alkaline protease production,extraction and characterization from alkaliphilic *Bacillus licheniformis* KBDLA:A Lonar Soda lake isolate. Indian Journal of Experimental Biology. Vol. 50pp 569-576.

Austain, B(1988). Methods in Aquatic Bacteriology. A Wiley -Interscience Publication.. 222-231.

Phenol Degrading Halophilic Bacterium *Halomonas organivorans*. Vol. 50pp 569-576.

DasSarma, S. and Robb, F.T. eds., (1995). *Archaea: A laboratory manual. Halophiles*. Cold Spring Harbor Laboratory Press.

De Lourdes M., Perez. D., Garcia. M.T., Mellado, E(2013). Halophilic bacteria as a source of novel hydrolytic enzymes. 3, 38-51[CrossRef][PubMed].

Debananda. S.N., Kshetri. P., Sanasam. S., Nimaichand. S (2009). Screening, identification of best producers and optimization of extracellular proteases from moderately halophilic alkali thermo tolerant indigenous actinomycetes. World Appl Sci J; 7: 907-916.

DeLong, D.C. (1996). Defining Biodiversity. *Wildlife Soc. Bull.*,24: 38-749.

Demain.A.L (1999). Pharmaceutically active secondary metabolites of microorganisms. Applied microbiology and biotechnology;52(4):455-463

Dobzhansky.T., Hecht. M.K, Steere.W.C (1968). On some fundamental concepts of evolutionary biology. Evol Biol 2:1–34.

Dyall-Smith. M., Tang. S. L., & Bath. C. (2003). Haloarchaeal viruses: how diverse are they?. *Research in microbiology*, *154*(4), 309-313.

Elazari-Volcani, B., (1972). A ciliate from the Dead Sea. Nature 154: 335–336.

Fischbach.A., Walsh.T (2009). Antibiotics for Emerging Pathogens. Science. Vol 325 (5944):1089-1093.

Flannery. W.L. (1956) Current status of knowledge of halophilicbacteria., *bacteriol. Rev.* 2049.

Garrity .G.M, Holt .J.G (2001). The road map to the manual. In: Boone C (ed) Bergey's manual of systematic bacteriology, vol 1, 2nd edn. Springer, New York, pp 119–155

Gibbons, N.E (1974). Family V. Halobacteriaceae ram. nov., in Bergey's Manual of Determinative Bacteriology (Buchanan, R.E. and Gibbons, N.E., 'Eds.) 8th ed., pp. 269-273.

Grant. W.D., Kamekura. M., Mc Genity .T.J., Ventosa. A (2001). Class III. Halobacteria class. nov. In: Boone DR, Castenholz RW, Garrity GM (eds) Bergey's manual of systematic bacteriology, vol 1. Springer, New York, pp 294–334.

Guan.T.W., Wu.N., Xia.Z.F., Ruan.J.S., Zhang.X.P., Huang.Y., Zhang.L.L (2011). *Saccharopolyspora lacisalsi* sp. nov., a novel halophilic actinomycete isolated from a salt lake in Xinjiang, China.Extremophiles 15:373–37.

Gupta. S., Sharma.P., Dev .k ., Srivastava. M., Sourirajan .A(2015). A diverse group of halophilic bacteria exist in Lunsu, a natural salt water body of Himachal Pradesh, India. SpringerPlus, 4, 274[CrossRef][PubMed].

Houda.B., Ridha.A., Neji.G., Abdelghani.S., Emno .A (2009) . Isolation and Characterization of Moderately Halophilic Bacteria from Tunisian Solar Saltern.Curr Microbiol. Vol.60:157-161

Jiang. H.L., Tay. S.T., Maszenan .A.M., Tay .J.H (2006). Physiological traits of bacterial strains isolated from phenol-degrading aerobic granules. FEMS Microbiol Ecol 57:182–191.

Kanekar. P.P, Kanekar. S.P., Kelkar .A.S., Dhakephalkar .P.K (2012) . Halophiles–Taxonomy, diversity, physiology and applications. In: Satyanarayana T, Johri BN, Prakash A (eds) Microorganisms in environmental management: microbes and environment. Springer, Dordrecht, pp 1–34.

Kizhekkedathu.N.N., Prema.P (2005). Mangrove actinomycetes as the source of ligninolytic enzymes. Actinomycetologica; 19:40-47.

Kushner .D.J., Kamekura.M (1988). Physiology of halophilic eubacteria. In: Rodriguez-Valera F (ed) Halophilic bacteria, vol 1. CRC Press, Boca Raton, pp 109–140.

Kuznetsov .V.D., Zajtseva. T.A., Vakulenko.L.V., Flippova.S.N (1992). *Streptomyces albiaxialis* sp. nov. a new oil hydrocarbon degrading species of thermo- and halotolerant streptomyces. Mikrobiologiya 61:84–91.

Larsen. J.L., (1984). *Vibrioangillarum* :Influence of temperature, pH, NaCl concentration and incubation time on growth. Journal of Applied Bacteriology Vol. 57(2):237-246.

Asian Journal of Global Health. Vol. 7.Online Journal.

Madern. D., Ebel.C., Zaccai. G (2000). Halophilic adaptation of enzymes . Extremophiles 4:91-98.

Maloney. K.N., MacMillan. J.B., Kauffman. C.A., Jensen. P.R., DiPasquale. A.G., Rheingold A.L (2009). Lodopyridone, a structurally unprecedented alkaloid from a marine actinomycete. Org Lett 2009; 11:5422.

Martin, J. P (1950). Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. *Soil science*, *69*(3), 215-232.

Maskey. R.P., Li F., Qin .S., Fiebig. H.H., Laatsch. H (2003). Chandrananimycins A approximately C: production of novel anticancer antibiotics from a marine Actinomadura sp. isolate M048 by variation of medium composition and growth conditions. J Antibiot 2003b; 56:622-634.

Mevarech. M., Frolow. F., Gloss. L.M (2000). Halophilic enzymes: proteins with a grain of salt. Biophys Chem 86:155–164.

Moreno. M.L., Perez. D., Garcia. M.T., Mellado. E (2013). Halophilic bacteria as a source of novel hydrolytic enzymes. Life 3:38–51.

Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. Journal of natural products. 2007;70(3):461-477.

Norberg .P., Hofsten. B (1969). Proteolytic enzymes from extremely halophilic bacteria. J Gen Microbiol 55:251-256.

Norton. C.F., Grant. W.D (1988). Survival of halobacteria within fluid inclusions in salt crystals. J Gen Microbiol 134:1365–1373.

Novitsky. T.J., Kushner .D.J (1976). *Planococcus halophilus sp.*nov., a facultatively halophilic coccus. Int J Syst Bacteriol 26:53-57.

Pandey.A., Nigam.P., Soccol.C.R., Soccol.V.T., Singh. D., Mohan.R (2000). Advances in microbial amylases. Biotechnol Appl Biochem 31:135–152.

Procopio.R.E., Silva.I.R., Martins.M.K., Azevedo.J.L., Araujo. J.M (2012). Antibiotics produced by Streptomyces. Braz J Infect Dis 2012; 16:466-471.

Quillaguaman.J., Guzman.H., Van-Thuoc. D., Hatti-Kaul. R(2010). Synthesis and production of polyhydroxyalkanoates by halophiles: current potential and future prospects. Appl Microbiol Biotechnol; 85:1687–1696.

Ray.L., Suar.M., Pattnaik.A.K., Raina.V (2013). *Streptomyces chilikensis sp. nov ., a halophilic Streptomycete* isolated from brackish water sediment. Int J Syst Evol Microbiol 63:2757-2764.

Roohi. A., Ahmed.I. Iqbal. M., Jamil. M. (2012). "Preliminary isolation and characterization of halotolerant and halophilic bacteria from salt mines of Karak, Pakistan." Pak J Bot 44(SI 1), 365-370.

Sabet. S., Diallo. L., Hays. L., Jung. W., Dillon. J.G (2009). Characterization of halophiles isolated from solar salterns in Baja California, Mexico. Extremophiles 13:643–656

Subramani.R., Aalbersberg .W (2012). Marine actinomycetes: an ongoing source of novel bioactive metabolites. Microbiol Res; 167:571–580.7-63.

Tang.Y.W., Persing.D.H(1999). Molecular detection and identification of microorganisms. In: Murray PR (editor in chief), Manual of Clinical Microbiology. Washington DC, USA: ASM Press; 215–244.

Tango, M.S.A. and M.R. Islam. (2002). Potential for extremophiles for biotechnological and petroleum applications. *Energy Sources*, 24: 543-559.

Taubes, Gary. "The bacteria fight back." (2008): 356-361.

Tiwari. K., Gupta. K.R (2012). Rare actinomycetes: a potential storehouse for novel antibiotics. Crit Rev Biotechnol; 32:108–132.

Trenozhnikova, L., & Azizan, A. (2018). Discovery of actinomycetes from extreme environments with potential to produce novel antibiotics. *Central Asian Journal of Global Health*, 7(1).

Ventosa .A., Nieto. J.J., Oren.A (1998). Biology of moderately halophilic aerobic bacteria. Microbiol Mol Biol Rev; 62:504–544.

Ventosa, A.; Marquez, M.C.; Ruiz-Berraquero, F.; Kocur, M (2010).*Salinicoccus roseus* gen. nov., a new moderately halophilic Gram-positive coccus. *Syst. Appl. Microbiol.* 13, 2933.

Ventosa.A (2006). Unusual micro-organisms from unusual habitats: hypersaline environments. In: Logan NA, Lappin-Scott HM, Oyston PCF (eds) Prokaryotic diversity: mechanisms and significance. Cambridge University Press, Cambridge, pp 223–253.

Wadilee .S., Kageyama. R., Takabe.H., Halophilic.T (2016). Micro organism resources and their applications in industrial and environmental biotechnology. AIMS Microbiol. 2016, 2, 42-54[CrossRef].

Walsh. J, Maillard .A., Russell. C., Charbonneau. R., Bartolo.G (2003). Activity and mechanisms of action of selected biocidal agents on Gram positive and negative bacteria.Journal of Applied Moicrobiology. Vol 94(2):240-247.

Whitman .W.B., Goodfellow. M., Kämpfer. P., Busse HJ., Trujillo M.E., Ludwig. W., Suzuki .K.I (2012). Parte A (eds) Bergey's manual of systematic bacteriology, vol 5, 2nd edn, The Actinobacteria, Part B. Springer, Phylum XXVI. Actinobacteria phyl. nov. In: New York, pp 33–34

Xiang .W., Guo .J., Feng .W., Huang. M., Chen. H., Zhao. J., Zhang .J., Yang. Z., Sun. Q (2008). Community of extremely halophilic bacteria in historic Dagong Brine Well in south western China. World J Microbiol Biotechnol 24:2297–2305.

Yasuhara-Bell. J, Lu Y (2010). Marine compounds and their antiviral activities. Antivir Res; 86:231–240.

Zvyagintseva, I. S., Poglazova, M. N., Gotoeva, M. T., & Belyaev, S. S. (2001) Effect on the medium salinity on oil degradation by Nocardioform bacteria. *Microbiology*, 70(6), 652-656

A Comparative Study on Bioactive Substances, Antibacterial and Antioxidant Activities of *Penaeus monodon, Palaemon adspersus* and *Azadirachta indica* From Punnaikayal Coast.

A Project submitted to

ST. MARY'S COLLEGE (Autonomous), THOOTHUKUDI.

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

In partial fulfilment for the award of the degree of

Bachelor of Science in Zoology

By

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DEPARTMENT OF ZOOLOGY

ST.MARY'S COLLEGE(AUTONOMOUS), THOOTHUKUDI

APRIL 2022

CERTIFICATE

This is to certify that the project entitled **A Comparative Study on Bioactive Substances, Antibacterial and Antioxidant Activities of *Penaeus monodon, Palaemon adspersus* and *Azadirachta indica* From Punnaikayal Coast** is submitted to **St. Mary's College (Autonomous), Thoothukudi** in partial fulfilment for the award of the degree of **Bachelor of Science in Zoology** and it is a record of the work done during the year 2021-2022 by the following students.

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CONTENTS

1. INTRODUCTION

The oceans stand out as a key component of the biosphere, called to accommodate much of the increasing demands for resources (food, water, and energy) required to meet future projected global human population growth (Duarte *et al.*, 2020). The Gulf of Mannar can be found in the Indo – Pacific Region. The Gulf of Mannar Biosphere Reserve is located on the south eastern tip of India and is near Sri Lanka. The Gulf of Mannar was established as a biosphere reserve in 1989 by the Indian Government and the State of Tamil Nadu (Marine Conservation Society, 2009). The Gulf of Mannar Biosphere Reserve (GoMBR) encompasses 21 island. The 21 islands between Rameshwaram and Thoothukudi as well as the entire Gulf of Mannar were declared as marine national park in 1986 for the purpose of protecting marine wild life and its environment (Upreti and Shanmugaraj 1997).

The bio resources present in the marine ecosystem have potent biomolecules which includes many natural organic compounds. These compounds are reported to have biological activities like anti–bacterial, antifungal, antioxidant etc (Rajamanikandan *et al.*, 2011). Environmental pollution is a natural consequence of human activities. It is also the result of natural processes. (Brine *et al.*, 1992).

Three parts of India is surrounded by ocean and its inner land is also very much rich with ponds, lakes and lagoons. Fisheries sector plays an important role in nutrition, socio economic development and poverty alleviation of a large number of population. The proper utilization of those water resources in terms of research in chitin and chitosan can bring the economic and academic prosperity of the nation. Crustacean shell wastes contain about 30-40% protein; 30-50% CaCO3 and 20-30% of Chitin.Shrimp cells contain a huge amount of Chitin (20-30%) which is an expensive ingredient used in many foods, cosmetics and pharmaceutical products. (Suparna and Poernomo 1992).

Chitin is the second most abundant natural polysaccharide after cellulose and is present in the crustacean exoskeleton, insects and fungi (Blackwell and Walton1989). The shellfish industry generates a huge amount of shell waste which usually cause environmental nuisance. Alternatively, this waste can be utilized as an economic source of chitin and its derivative chitosan. Chitin and Chitosan are considerably versatile and promising bio materials. Chitosan, the deacetylated chitin derivative, is a more useful and interesting bio active polymer. Despite its biodegradability, it can be chemically modified to produce derivatives, which have varied applications in the biomedical field .It is a very wellknown biopolymer with increased properties of biodegradable, biocompatible and biomedical. It is an excellent natural polymer that synthesized from chitin through

cationic process. It is an important biopolymer driven by alkaline deacetylation process, which is used in the entire field including agriculture, food, environmental, pharmaceutical, biomedical and clinical (Badawy *et al*., 2020).

The shell fish industry is operative among all the coastal countries and contributes hugely to the food delicacies. During the processing of prawns, shrimps and lobsters mostly the meat is taken, while the shell and head portions are generated as wastes. This results in the generation of a huge amount of waste throughout the world. It is estimated that the shell- fish industry produces about 60000-80000 tons of waste. The disposal of such an enormous amount of waste has become a serious environmental concern (Muzzareli *et al.,* 1986). Although these wastes are biodegradable but the rate of degradation of a large amount of waste generated per processing operation is comparatively slow (Prashanth *et al.,* 2007). This results in accumulation over time and the ads to environmental concerns as they not only produce obnoxious smell but also attract pathogenic insects, flies and rodents, thus creating an unhygienic atmosphere. The immediate solution to this problem seems to be quick recycling of the crustacean shells generated and extraction of commercially viable substances to be further used in other applications (Roberts *et al.,* 2008). As we know the shell and head wastes of crustaceans contain chitin, proteins and minerals. So by demineralization and deproteinizing the wastes chitin can be obtained (Jiang *et al.,* 2003). Chitin can be

used for various economic applications. Production of chitin within the country can reduce the present dependency on import for this valuable raw material. If a nominal care is taken, it is possible to produce both chitin and chitosan within the existing process line of the shrimp processing plants. The products can either be marketed locally or exported. Therefore this research aimed at developing appropriate field supported techniques for the commercial manufacture of chitin and chitosan from shrimp shell wastes. Chitosan and chito oligosaccharides possess various biological activities including hypocholesterolemic, antimicrobial, antiinflammatory, antioxidant and Angiotensin I converting enzyme (ACE) inhibitory activities and so on, which are all correlated with their structures and physicochemical properties. (Wenshui *et al.,* 2010). Chitosan can act in synergy to enhance the antimicrobial potential of other antimicrobial (Joraholmen *et al.,* 2020) .Additionally, chitosan-based coatings and packaging increased the shelf life of fruits (Ishkeh *et al*., 2021).

In this present study to evaluate the comparative study on bioactive substances, antibacterial activity and antioxidant activities of *Penaeus monodon, Palaemon adspersus* and *Azardirachta indica* from Punnaikayal coast.

OBJECTIVES

The objectives are the present study are,

- To extract chitin and chitosan from *Penaeus monodon, Palaemon adspersus* and *Azadirachta indica.*
- To compare the antimicrobial activity of ethanolic and methanolic extract of *Penaeus monodon, Palaemon adspersus* and *Azadirachta indica* on *Bacillus cereus (BC), Vibrio cholerae (VC)* and *Pseudomonas aeruginosa (PA).*
- To determine the antioxidant activity of methanolic extract of *Penaeus monodon, Palaemon adspersus* and *Azadirachta indica.*
- To estimate the moisture content of *Penaeus monodon, Palaemon adspersus* and *Azadirachta indica.*
- To determine the FT-IR analysis of *Penaeus monodon, Palaemon adspersus* and *Azadirachta indica*

2. REVIEW OF LITERATURE

Chitosan is natural, nontoxic, copolymer of glucosamine and N-acetyl glucosamine prepared from chitin by de-acetylation, which in turn, is a major component of the shells of crustaceans. It is found commercially in the waste products of the marine food processing industry (Khanafari *et al.*, 2008);(Limam *et al.*, 2011). Various chemical modifications have been investigated to try and improve chitosan's solubility and thus to increase its range of applications (Park *et al.*, 2010); Zhang *et al.*, 2010). Considering this limitation, researchers are now concentrating on conversion of chitosan into oligosaccharides (Mohan *et al.*, 2012). Recent studies on chitosan de-polymerisation have drawn considerable attention, as the products obtained are more water-soluble. Beneficial properties of chitosan and its oligosaccharide include: antitumour (Quan *et al.*, 2009); neuroprotective (Pangestuti *et al.*, 2010); antifungal and antibacterial (Fernandes *et al.*, 2008); (Wang *et al.*, 2007); and anti-inflammatory (Yang *et al.*, 2010). In addition chito-oligosaccharides (COS) are very promising compounds for use as natural antioxidants in biological systems (Fernandes *et al.*, 2010). The antimicrobial activity of chitin, chitosan and their derivatives against different groups of microorganisms, such as bacteria, yeast, and fungi, has received considerable attention in recent years. (Khanafari *et al.*, 2008);(Li *et al.*, 1992);(Limam *et al.*,

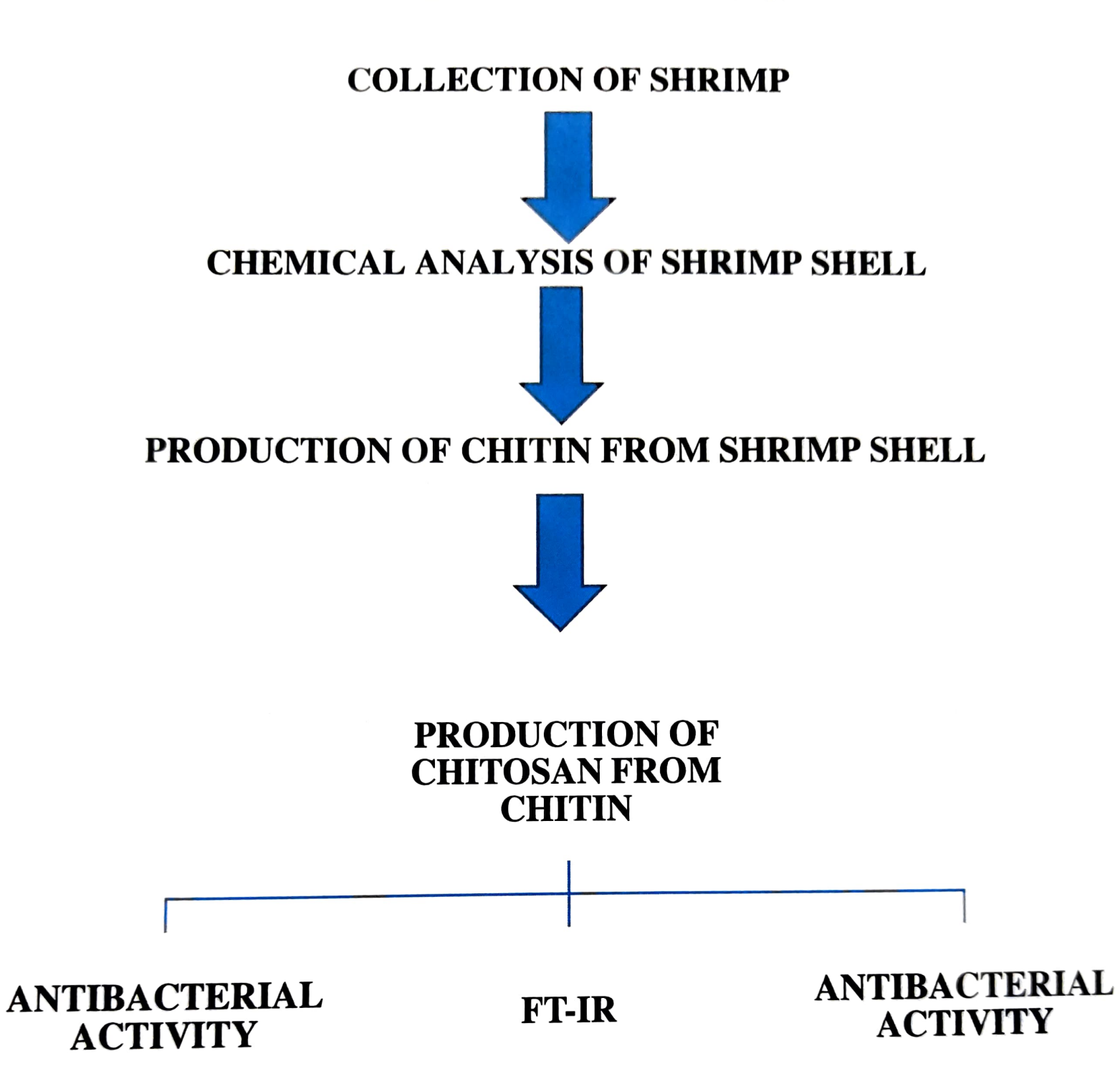
2011). The antimicrobial activities of chitosan are greatly dependent on its physical characteristics, most notably molecular weight (Mv) and degree of De-acetylation (DD). Chitosan with a higher degree of de-acetylation tends to have a higher antimicrobial activity (Aharya *et al.*, 2005). Some authors have reported that chitosan is more effective than chito-oligosaccharides (COS) in inhibiting growth of bacteria; for example, water insoluble chitosan exhibited higher antimicrobial effect against E. coli than COS (Qin et al., 2006). In recent years, various investigators have observed the antioxidant activity of chitosan derivatives. There has been increasing interest in finding 2 natural antioxidants, since they can protect the human body from free radicals and retard the progress of many chronic diseases. (Kinsella, *et al.*, 1993). In general, the natural antioxidants mainly constitute a broad range of compounds including phenolic compounds nitrogen compounds and carotenoids (Velioglu *et al.*, 1998). However, the antioxidant activities of several biological polysaccharides have recently been described. The antioxidant activity of chitosan and its derivatives also has attracted the most attention (Yin, *et al.*, 2002). Numerous bacteria and fungi are highly pathogenic causing various infectious diseases (Chistiakov *et al.*, 2007). The increasing economic and social concern to decrease the use of antibiotics and other therapeutic chemicals has encouraged more environmentally friendly approaches to control diseases (Torrecillas *et al.* 2007). Interestingly, chitin and chitosan have

been investigated as an antimicrobial agents against a wide range of target organisms like algae, bacteria, yeasts and fungi in experiments involving *in vivo* and *in vitro* interactions in different forms (solutions, films and composites) (Goy *et al.,* 2009). The antimicrobial activity of chitosan can be enhanced by complexing it with suitable materials. Encapsulation of plant essential oils in chitosan-based coatings is gaining interest in agriculture research due to the antimicrobial properties associated with these volatile organic compounds (VOCs). Recently, several different plant essential oils have been incorporated into chitosan, and their antimicrobial activity was shown against a wide range of microbes (Rabea *et al.*, 2003). Some bioactive components can also be recently, microemulsion associated with chitosan was also assessed for its potential in the tropical treatment of vulvovaginal candidiasis (Oliverie *et al.,* 2021). It was reported that an antimicrobial peptide chitosan conjugate was synthesized by the grafting of an antimicrobial peptide, anoplin, to chitosan polymers. The antimicrobial activity of the conjugate is better than anoplin.

Antioxidant property is an important characteristic and greatly significant for natural life. Numerous biological roles of antioxidants are documented like anti-mutagenicity, anti-carcinogenicity and anti-aging among others. The influence of antioxidants on DPPH radical scavenging was due to their hydrogen giving facility (Thakran *et al.,* 2010). The favorable advantages of chitosan and its

oligosaccharides enhance its uses in the therapy as anticancer (Quan *et al.*, 2009), neuroprotective (Pangestuti and Kim, 2010) against microorganisms and fungi (Fernandez-Kim , 2004) (Wang *et al.*, 2007) and anti-inflammatory (Yang *et al.*, 2010).

EXPERIMENTAL DESIGNS



3. MATERIALS AND METHODS

P.monodon, commonly known as - Giant Tiger Prawn, generally has a light pink hue and *P. adspersus* were obtained from the local market at Punnaikayal. *P. monodon and P. adspersus* inedible parts including head, body shells and tails were removed from the whole body for extraction of chitosan.

The chemicals used in the extraction process consists of:

1. Hydrochloric acid (1.25N to 1.5 N): for 100 ml stock solution, 10.8 ml HCl is measured and the volume made up to 100 ml with distilled water
2. Sodium Hydroxide (0.5% w/v): 0.5 gm NaOH per 100 ml distilled water
3. Sodium Hydroxide water
4. Sodium Hydroxide (42% w/v): 42 gm NaOH per 100 ml distilled water.
5. 1% Acetic Acid: 1ml acetic acid per 100 ml distilled water.

Procedure:

To extract chitosan, 10 grams of prawn shell waste as raw material was collected. After washing it properly, the prawn shells were under sunlight. Then we proceeded with the determination process by adding 1.5N HCl at room temperature for 1hour. The spent acid was distracted and the shells were repeatedly washed with distilled water until the pH is neutral. The demineralized shells were then de-proteinized with 0.5% NaOH at 100^0 C for 30 minutes. This

method helped to weaken the protein tertiary structure of the shells. Protein solution was removed and washed thoroughly with distilled water and the pH was checked. The de-proteinization process was again repeated for that, 3% NaOH was added to the sample at 100^0 C for 30 minutes. After draining the residual protein along with the effluents, the sample once again washed and the pH was observed till it was approximately near to neutral. This step also helped in de-colourization of the shells here the chitin slurry was obtained. The excess water was removed and chitin cake was formed. The chitosan was prepared by de-acetylation of chitin by treating with 42% aqueous NaOH at 95^0 C for 1.5 hour. (Galed *et al.*, 2005.) After de-acetylation the alkali was drained off and washed thoroughly with distilled water until the pH is less than 7.5 and then dried at ambient temperature (30+/- 2^0 C).

Verification of the Chitosan produced:

1. Quality of the chitosan produced was checked by a solubility test with 1% Acetic acid. Chitosan dissolves completely in 1% Acetic acid. For the estimation of chitosan produced we took the sample out of the storage and weighed few flakes of prawn shells. Then the sample was put inside a clean beaker and 10 to 20ml of 1% acetic acid was added to it. The solution was kept in BOD shaker for 30 to 40 minutes. Then the sample was taken out and weighed carefully.

2. It is known that highly benzolyzed chitin is soluble in (DMSO) Dimethyl Sulphoxide Chitosan being organic in nature should be completely soluble in DMSO. We also analyzed the solubility of chitosan in DMSO as a quality check parameter. We observed that 15 mg of grinded shells were completely dissolved in 30 ml of DMSO solution.
3. The quality check parameters however confirmed that the chitosan obtained from deacetylation of *P. monodon* shells was of superior activity and quality than that obtained from *P. adspersus* shells and hence was used in the corresponding experiments devised.

COLLECTION, PROCESSING AND EXTRACTION OF NEEM

The medicinal herb selected for this study was *Azadirachta indica* which was collected in and around Punnaikayal. The collected leaves were shade dried at room temperature to reduce the moisture content. The leaves were then powdered and sieved. 2 grams of the ground herbal powder was suspended in 10 ml methanol and incubated overnight. The supernatant was filtered twice using Whatman No.1 filter paper and the filterate was further used for antibacterial activity.

Antibacterial Activity:

Bacterial Strains:

The reference strains of pathogens used to test antibacterial activity are

Bacillus cereus (BC)

Vibrio cholerae (0139) VC (0139)

Pseudomonas aeruginosa (PA)

Broth Culture:

A broth culture is a nutrient solution in which bacteria are being grown.

Broth Medium:

Nutrient broth – 1.3 g

Distilled water – 100 ml

2 to 3 ml of sterilized broth medium was taken in a sterilized culture tube. The inoculating loop was flamed and cooled for few minutes. A loopful of each strain was transferred into the individual culture tubes and incubated at room temperature.

Muller-Hinton Agar medium:

Beaf infusion	–	30 g
Casein acid hydrolysate	–	17 g
Agar	–	17 g
Distilled water	–	1000 ml

Muller Hinton agar was suspended in 1000 ml of distilled water and the pH was adjusted to 7.4 ± 0.2. The medium was boiled to dissolve completely and sterilized by autoclaving at 120°C for 15 minutes.

Agar diffusion technique

The antibacterial activity of Ethanol and Methanol extract of *P. monodon, P. adspersus* and *A. indica* was determined by the standard agar well diffusion assay by using the technique of (Perez *et al.*, (1990). Petri plates were prepared by pouring approximately 20 ml of Muller Hinton Agar Medium and allowed to solidify. After solidification, culture of each microbial strain was swabbed to sterile cotton on the surface of medium. Sterilized paper discs prepared from Whatmann No. 1 were used for loading ethanol and methanol extract. The paper discs were loaded with different concentrations viz 10 mg/10 µl, 1 mg/10 µl and 0.1 mg/10 µl. The plates were incubated for 24 hours at 37°C and solvent control was performed in each case. Areas of inhibited microbial growth were observed as clear zone around the paper disc after 24 hours.

Antioxidant Activity:

Measurement of DPPH Radical scavenging activity:

The ability of the samples to annihilate the DPPH radical (1, 1-diphenyl-2-picrylhydrazyl) was investigated by the method described by (Blois, 1958). Stock

solution of compound was prepared to the concentration of 10 mg/ml. Different concentration of the extracts (50 µg, 100 µg, 150 µg & 200 µg) of samples were added, at an equal volume to methanolic solution of DPPH (0.1 mM). The reaction mixture is incubated for 30 minutes at room temperature and the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic acid was used as standard control. The capability of scavenging DPPH radical was calculated using the following equation.

$$\% \text{ of Inhibition} = \frac{(Control - Test)}{Control} \times 100$$

Moisture Content:

Moisture content of the samples were determined on wet basis. The sample were kept in an oven for 1 hour at 100˚C.The percentage moisture content was the difference between the weights of the wet and oven dried samples and expressed as

$$\text{Moisture content (\%)} = \frac{Wet\ weig\ ht - Dry\ weig\ ht}{Wet\ weig\ ht} \times 100$$

FT-IR:

The spectra of the chitosan samples were measured in the spectral range from 400 cm^{-1} to 4000 cm^{-1} using nicolet impact -410, Fourier Transform- Infrared spectrometer in transmittance mode with a resolution of 4 cm^{-1}.

4. RESULT

4.1 Effect of crude ethanolic extract of *P. monodon* on bacterial strains:

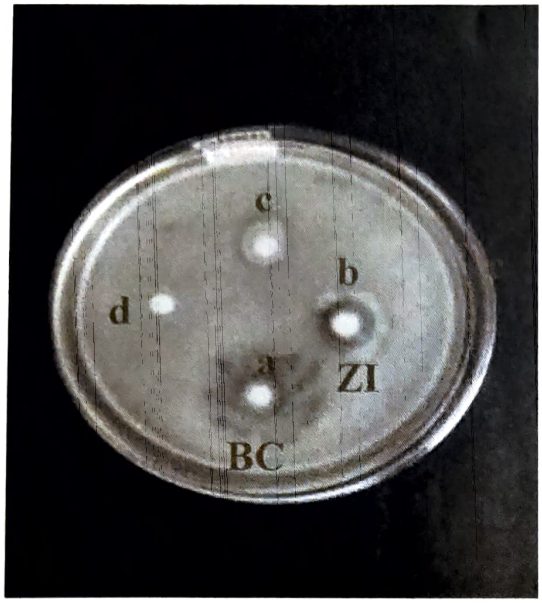
All the bacterial strains were sensitive to ethanolic extract of *P. monodon* (Plate 1). Clear zone of inhibition with 17.5 mm, 15.0 mm and 12.5 mm radius at 10 mg/ 10 µl, 1.0 mg/10 µl and 0.1 mg/10 µl respectively was observed in *P. aeruginosa*. *B. cereus* was sensitive with zone of inhibition 16.0 mm radius at 10 mg/10 µl. *V. cholerae* (0139) showed zone of inhibition with 14.0 mm radius at 10 mg/10 µl, 13.0 mm radius at 1.0 mg/10 µl and 12.5 mm radius at 0.1 mg/10 µl concentrations (Table 1). The mean zone of inhibition were calculated as 12.8, 13.1 and 15 in *BC, VC* (0139) and *PA* respectively (Fig 1).

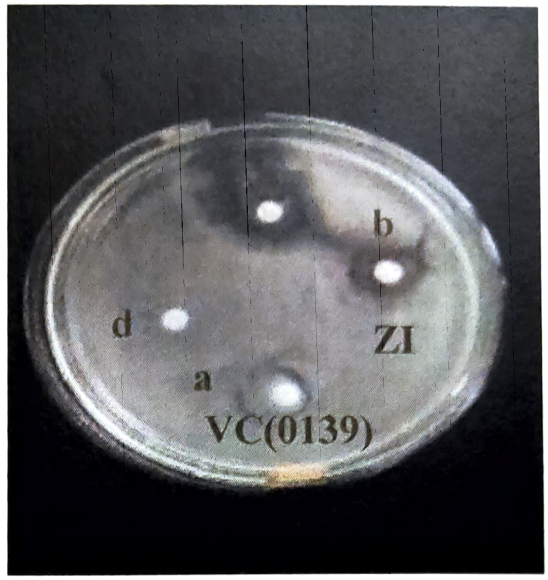
4.2 Effect of crude methanolic extract of *P. monodon* on bacterial strains:

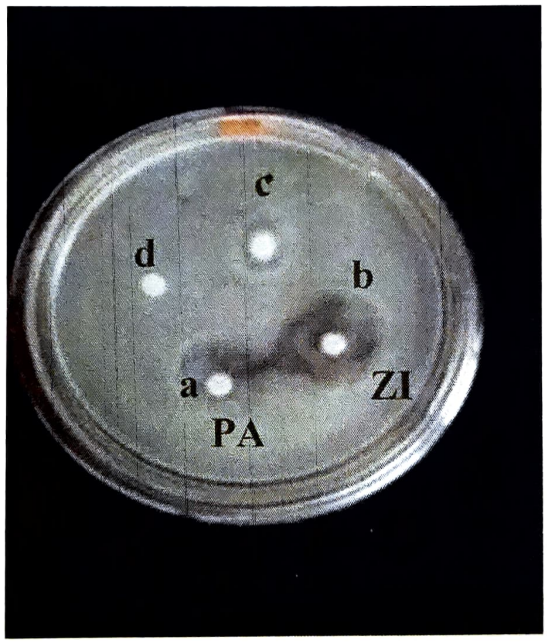
In *P. monodon* methanolic extract, the zone of inhibition were measured as follows. The zone of inhibition extended up to 21.0 mm radius at 10 mg/10 µl, 13.0 mm radius at 1.0 mg/10 µl and 10.5 mm radius 0.1 mg/10 µl in *P.aeruginosa, V. cholerae* (0139) showed zone of inhibition with 18.5 mm, 15.0 mm and 7.5 mm radius at 10 mg/10 µl, 1.0 mg/10 µl and 0.1 mg/10 µl. *B.cereus* was sensitive with zone of inhibition ranged from 8.0 mm to 16.5 mm radius (Plate 2 & Table 2).

Plate – 1

Antibiotic agar plates showing antibacterial activity of Ethanolic extract of *Penaeus monodon* on bacterial strains







Zone of inhibition mm (Radius)

+ = 7-10 mm

++ = 10-13 mm

+++ = 13-18 mm

++++ = 18-21 mm

Table - 1

Activity of ethanolic extract of *Penaeus monodon* against bacterial stains

Bacterial stains	**10 mg/10 µl**	**1 mg/ 10 µl**	**0.1 mg/ 10 µl**	**Control**
BC	+++	++	++	-
VC(0139)	+++	+++	++	-
PA	++++	+++	+++	-

Zone of inhibition mm (Radius)

\+ = 7-10 mm

++ = 10-13mm

+++ = 13-18mm

++++ = 18-21mm

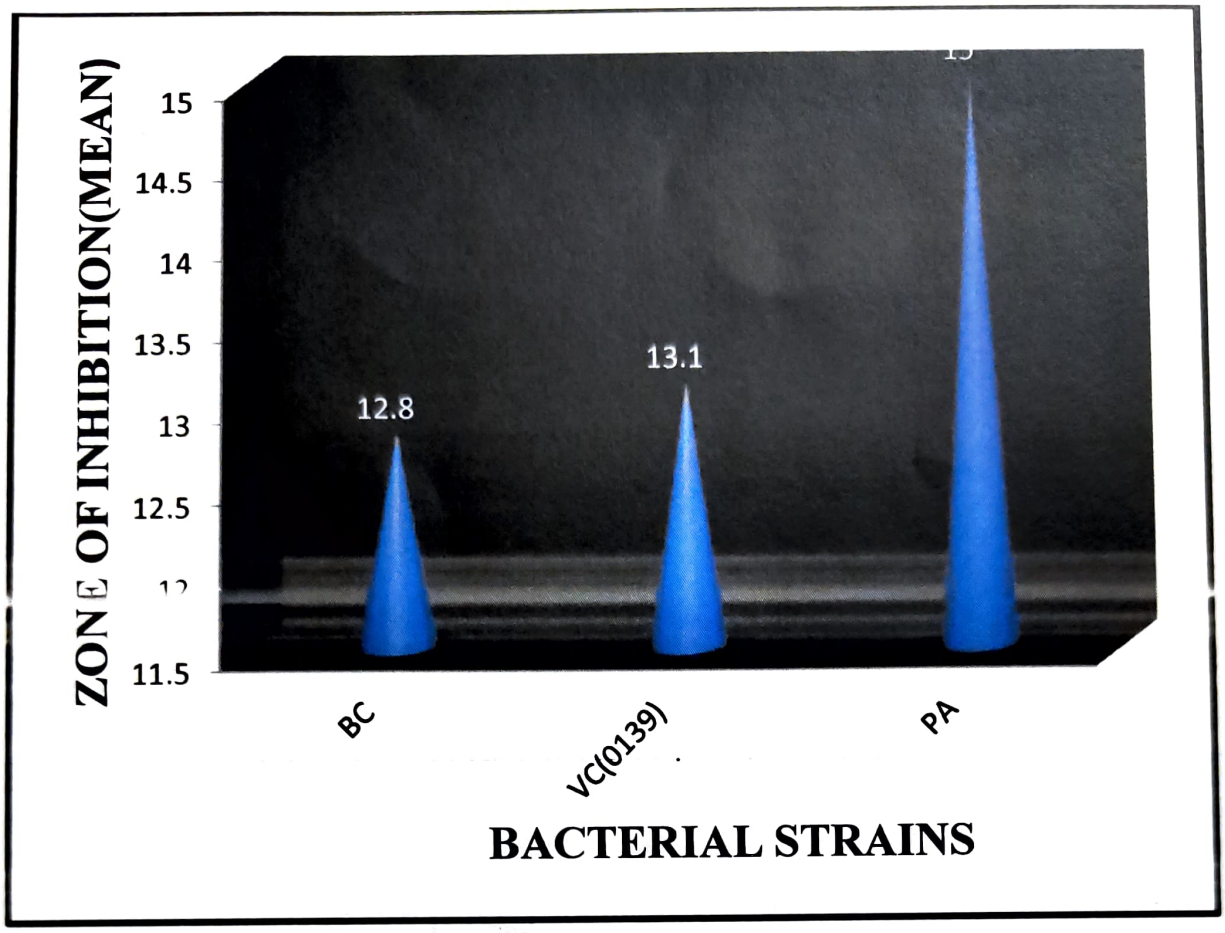
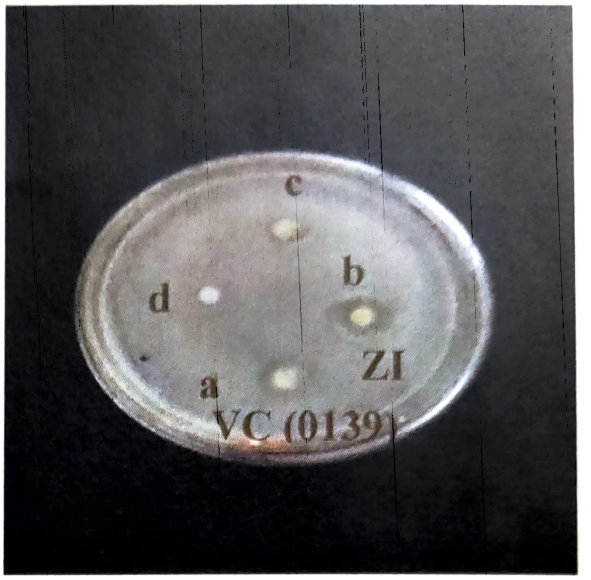
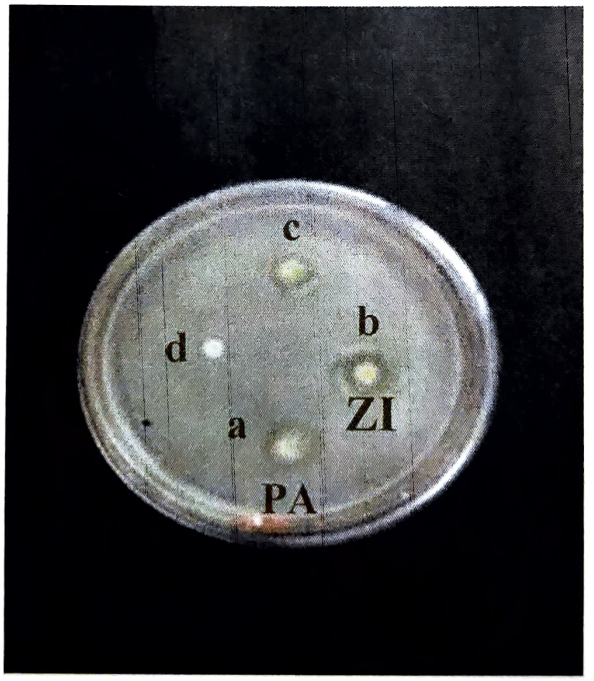


Fig: 1 Antibacterial activity of ethanolic extract of *Penaeus monodon*

Plate - 2

Antibiotic agar plates showing antibacterial activity of Methanolic extract of *Penaeus monodon* on bacterial strains





Zone of inhibition mm (Radius)

+ = 7-10 mm

++ = 10-13 mm

+++ = 13-18 mm

++++ = 18-21 mm

Table – 2

Activity of methanolic extract of *Penaeus monodon* against bacterial stains

Bacterial stains	**10 mg/10 µl**	**1 mg/ 10 µl**	**0.1 mg/ 10 µl**	**Control**
BC	+++	++	+	-
VC (0139)	++++	+++	+	-
PA	++++	+++	++	-

Zone of inhibition mm(radius)

\+ = 7-10 mm

++ = 10-13mm

+++ = 13-18mm

++++ = 18-21mm

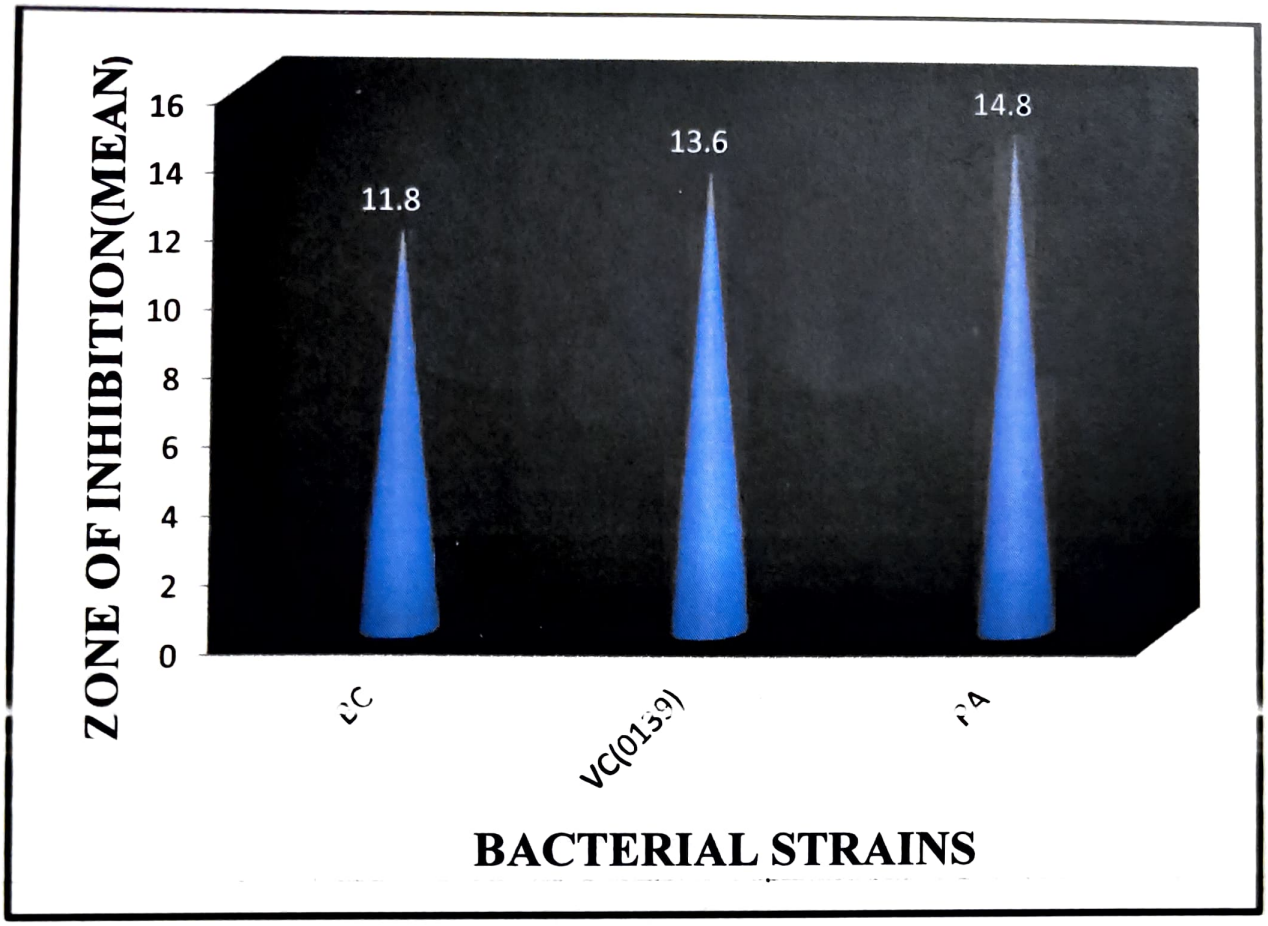


Fig: 2 Antibacterial activity of methanolic extract of *Penaeus monodon*

mean zone of inhibition were calculated as 11.8, 13.6 and 14.8 in *BC, VC* (0139) and *PA* respectively (Fig 2).

4.3 Effect of crude ethanolic extract of *P. adspersus* on bacterial strains:

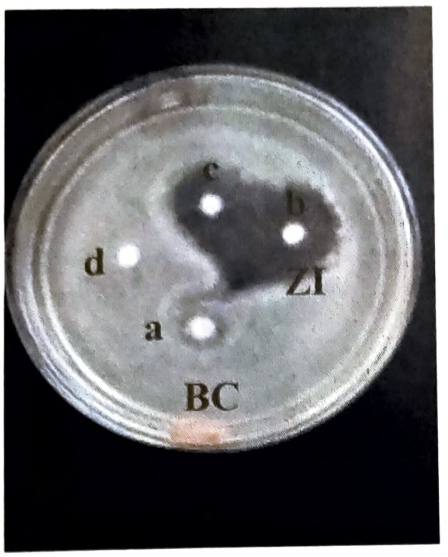
Ethanolic extract of *P. adspersus* exhibited the growth of all the bacterial strains tested (Plate 3) In in *P. aeruginosa*, the extract produced the zone of inhibition with 16.0 mm, 15.0 mm and 12.5 mm radius at 10 mg/10 µl, 1.0 mg/10 µl and 0.1 mg/10 µl respectively. The zone of inhibition ranging from 10.0 mm to 1.0 mm radius in *V. cholerae* (0139). *B.cereus* showed zone of inhibition with 13.5 mm, 12.5 mm and 10.0 mm at at 10 mg/10 µl, 1.0 mg/10 µl and 0.1 mg/10 µl respectively (Table 3). The mean zone of inhibition were calculated as 12.0, 12.0 and 14.5 in *BC, VC* (0139) and *PA* respectively (Fig 3).

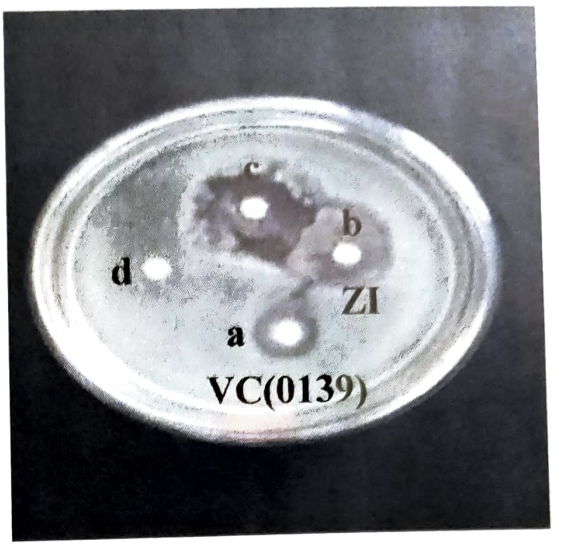
4.4 Effect of crude methanolic extract of *P. adspersus* on bacterial strains:

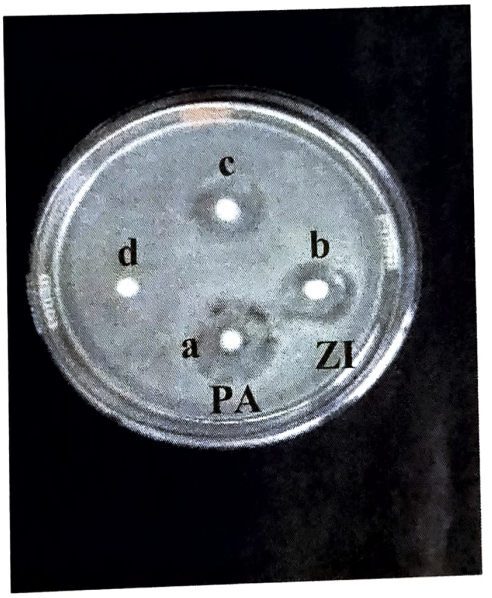
Methonolic extract of *P. adspersus* inhibited the growth of all the bacterial strains at all the concentrations (Plate 4). The zone of inhibition extended up to 14.5 mm at 10 mg/10 µl in *V. cholerae* (0139). The extract produced zone of inhibition ranging from 7.5 mm to 14.0 mm in *P.aeruginosa. B.cereus* was found to be sensitive with zone of inhibition 12.5 mm, 11.0 mm and 8.5 mm radius at 10 mg/10 µl, 1.0 mg/10 µl and 0.1 mg/10 µl respectively (Table 4). The mean zone of

Plate - 3

Antibiotic agar plates showing antibacterial activity of Ethanolic extract *Palaemon adspersus* on bacterial strains







Zone of inhibition mm (Radius)

+ = 7-10 mm

++ = 10-13 mm

+++ = 13-18 mm

++++ = 18-21 mm

Table – 3

Activity of ethanolic extract of Palaemon adspersus against bacterial stains

Bacterial stains	**10 mg/10 µl**	**1 mg/ 10 µl**	**0.1 mg/ 10 µl**	**Control**
BC	++	++	+	-
VC (0139)	+++	++	+	-
PA	+++.	+++	++	-

Zone of inhibition mm (Radius)

\+ = 7-10 mm

++ = 10-13mm

+++ = 13-18mm

++++ = 18-21mm

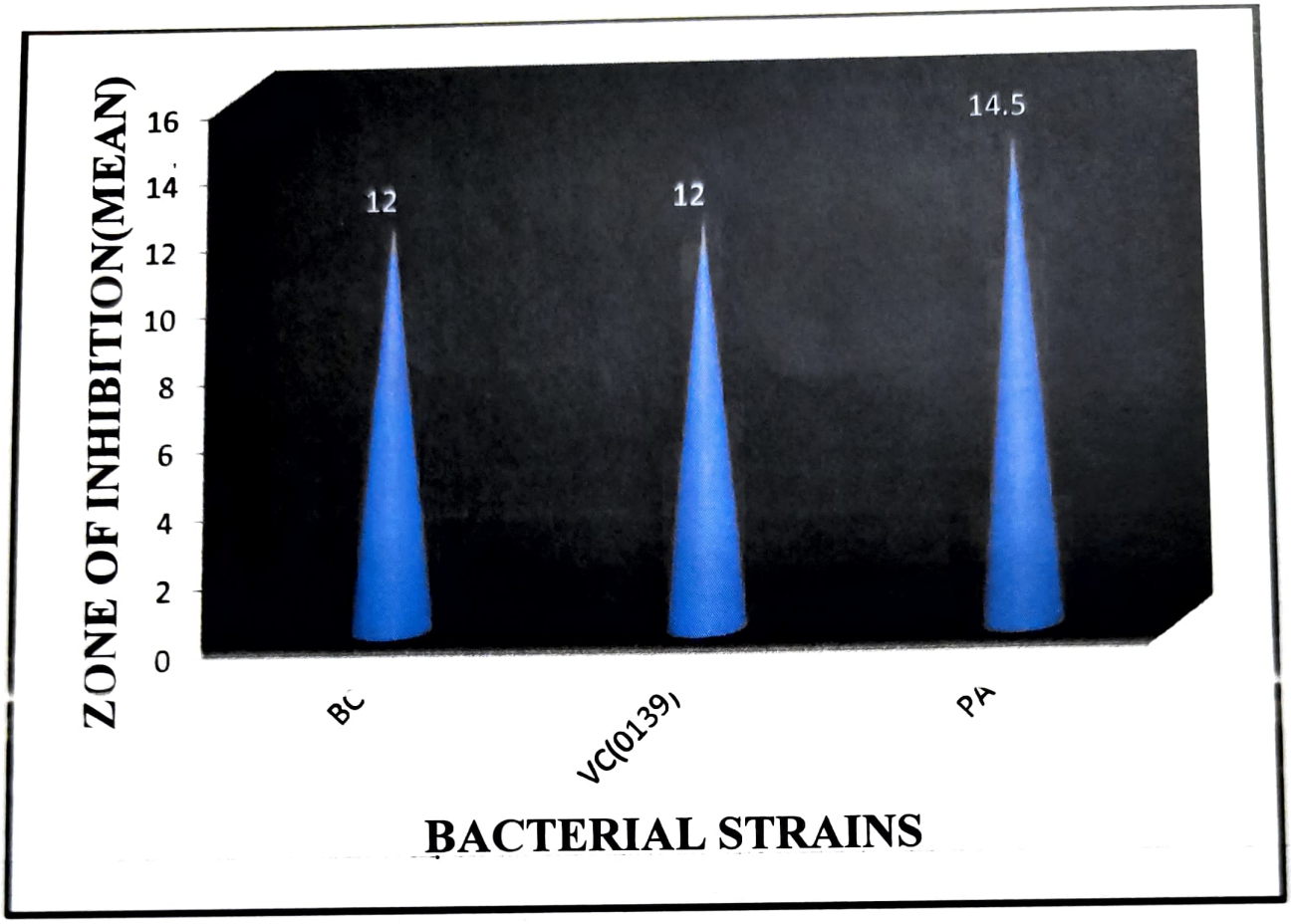
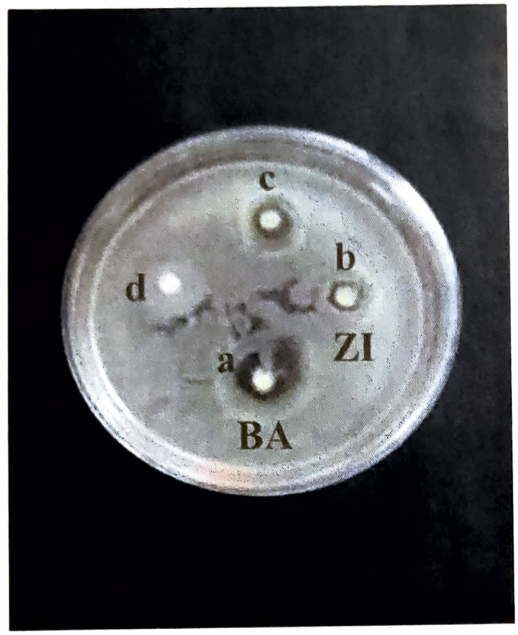
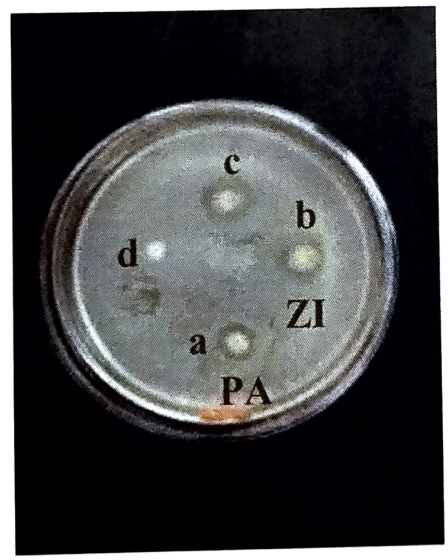


Fig: 3 Antibacterial activity of ethanolic extract of *Palaemon adspersus*

Plate - 4

Antibiotic agar plates showing antibacterial activity of Methanolic extract of *Palaemon adspersus* on bacterial strains





Zone of inhibition mm (Radius)

+ = 7-10 mm

++ = 10-13 mm

+++ = 13-18 mm

++++ = 18-21 mm

Table - 4

Activity of methanolic extract of *Palaemon adsperus* against bacterial stains.

Bacterial stains	**10 mg/10 µl**	**1 mg/ 10 µl**	**0.1 mg/ 10 µl**	**Control**
BC	++	++	+	-
VC (0139)	+++	+++	++	-
PA	+++	++	+	-

Zone of inhibition mm (Radius)

\+ = 7-10 mm

++ = 10-13mm

+++ = 13-18mm

++++ = 18-21mm

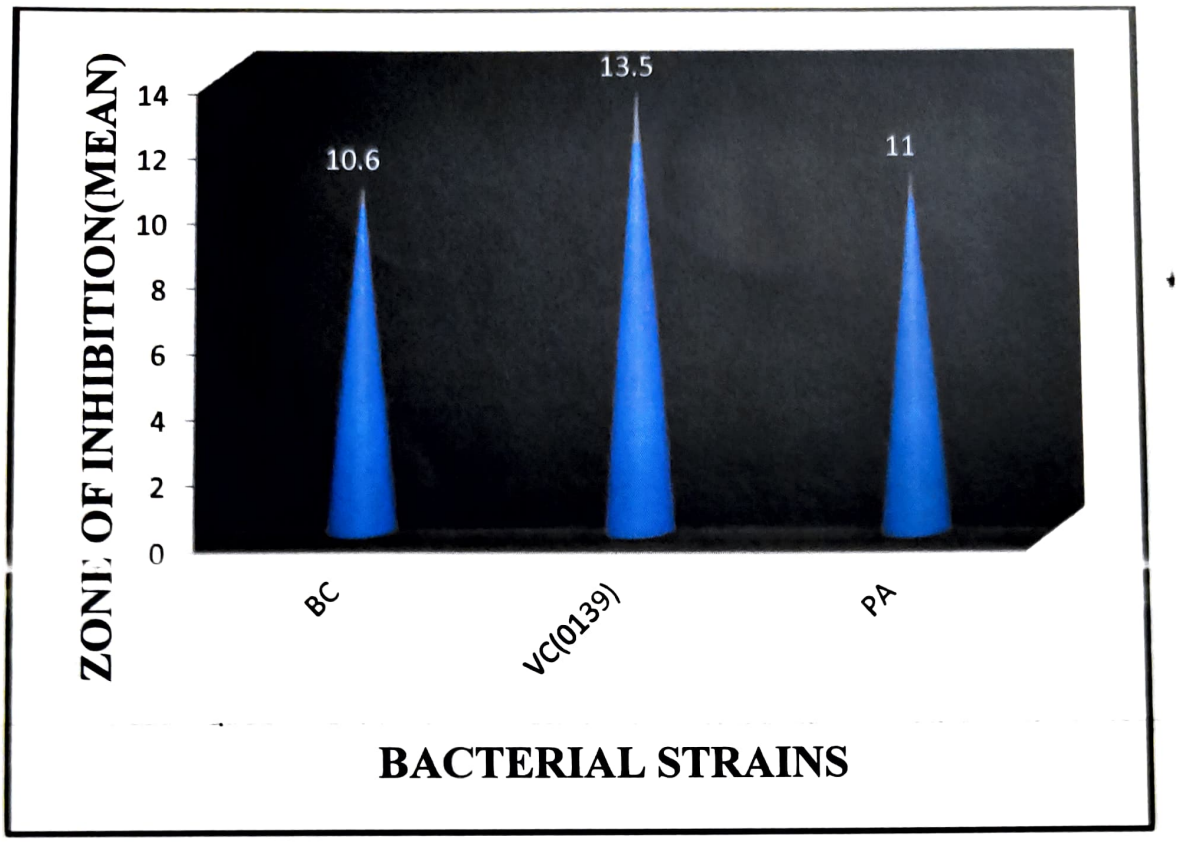


Fig: 4 Antibacterial activity of methanolic extract of *Palaemon adspersus*

inhibition were calculated as 10.5, 13.5 and 11.0 in *BC, VC* (0139) and *PA* respectively (Fig 4).

4.5 Effect of crude ethanolic extract of *A. indica* on bacterial strains:

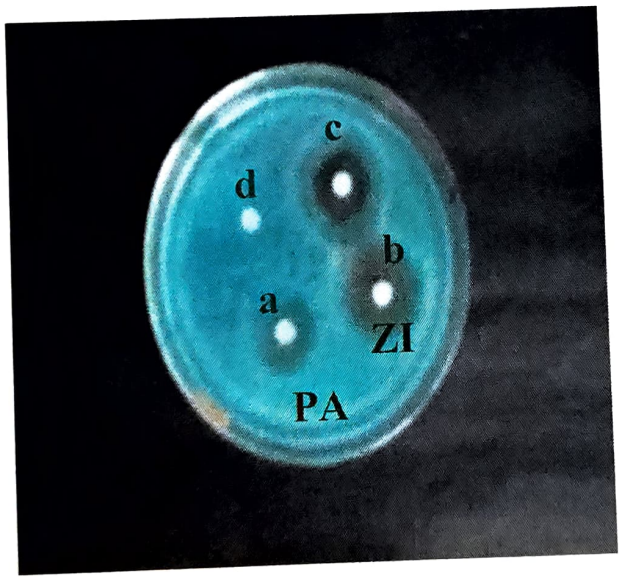
Ethanolic extract of *A. indica* showed inhibiting activity against bacterial strains tested (Plate 5). Clear zone of inhibition with 17.5 mm, 13.5 mm and 12.5 mm at 10 mg/10 µl, 1.0 mg/10 µl and 0.1 mg/10 µl respectively was observed in *V. cholerae* (0139). The zone of inhibition extended up to 15.0 mm, 12.5 mm and 11.5 mm radius at 10 mg/10 µl, 1.0 mg/10 µl and 0.1 mg/10 µl in *B. cereus.P. aeruginosa* was sensitive with zone of inhibition ranging from 7.5 mm to 15.0 mm radius (Table 5). The mean zone of inhibition were calculated as 13.0 14.5 and 11.6 in *BC, VC* (0139) and *PA* respectively (Fig 5).

4.6 Effect of crude methanolic extract of *A. indica* on bacterial strains:

Methanolic extract of *A. indica* showed zone of inhibition with 14.0 mm, 12.5 mm and 10.0 mm radius in *P. aeruginosa* at 10 mg/10 µl, 1.0 mg/10 µl and 0.1 mg/10 µl. *V. cholerae* (0139) was sensitive with zone of inhibition 12.5 mm, 11.0 mm and 10.0 mm radius at 10 mg/10 µl, 1.0 mg/10 µl and 0.1 mg/10 µl concentrations respectively. *B. cereus* was sensitive with zone of inhibition ranging

Plate - 5

Antibiotic agar plates showing antibacterial activity of Ethanolic extract of *Azadirachta indica* on bacterial strains



Zone of inhibition mm (Radius)

+ = 7-10 mm

++ = 10-13 mm

+++ = 13-18 mm

++++ = 18-21 mm

Table - 5

Activity of ethanolic extract of *Azadirchta indica* against bacterial stains

Bacterial stains	**10 mg/10 µl**	**1 mg/ 10 µl**	**0.1 mg/ 10 µl**	**Control**
BC	+++	++	++	-
VC (0139)	+++	+++	++	-
PA	+++	++	+	-

Zone of inhibition mm (Radius)

+ = 7-10mm

++ = 10-13mm

+++ = 13-18mm

++++ = 18-21mm

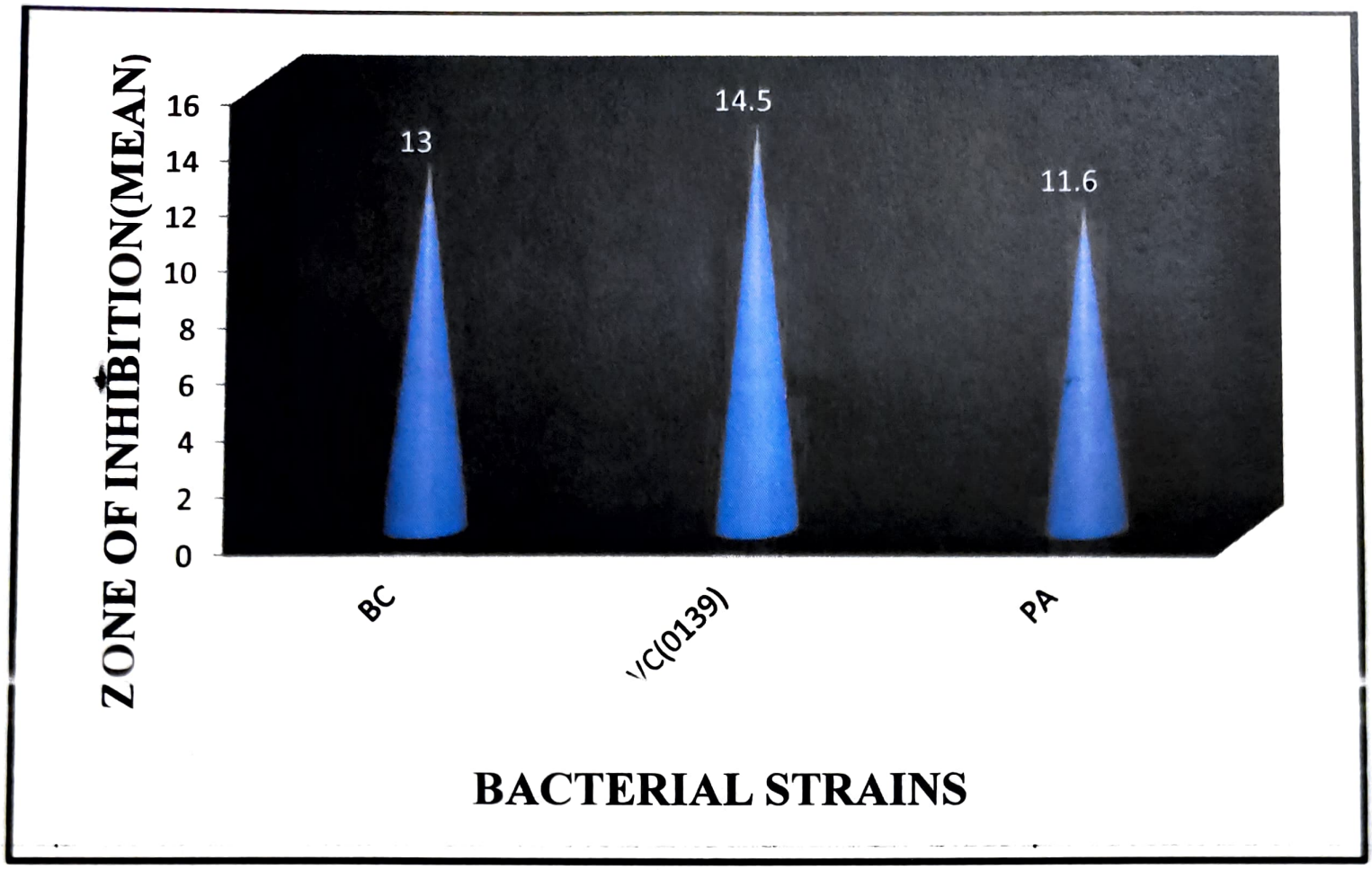
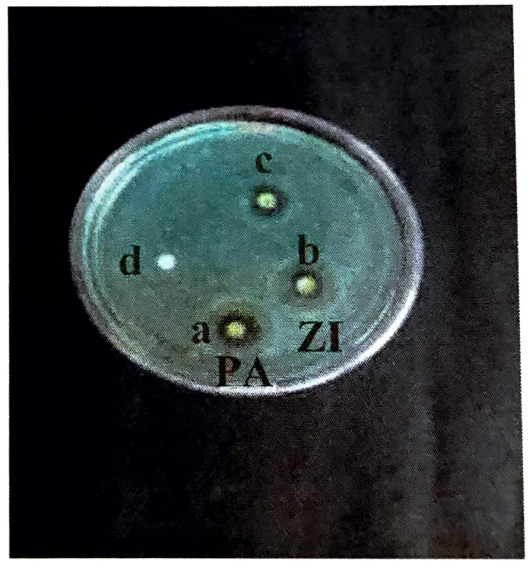


Fig: 5 Antibacterial activity of ethanolic extract of *Azadirachta indica*

Plate – 6

Antibiotic agar plates showing antibacterial activity of Methanolic extract of *Azadirachta indica* on bacterial strains



Zone of inhibition mm (Radius)

+ = 7-10 mm

++ = 10-13 mm

+++ = 13-18 mm

++++ = 18-21 mm

Table - 6

Activity of methanolic extract of *Azadirchta indica* against bacterial stains.

Bacterial stains	**10 mg/10 µl**	**1 mg/ 10 µl**	**0.1 mg/ 10 µl**	**Control**
BC	++	+	+	-
VC (0139)	++	++	+	-
PA	+++	++	+	-

Zone of inhibition mm (Radius)

+ = 7-10mm

++ = 10-13mm

+++ =13-18 mm

++++ = 18-21mm

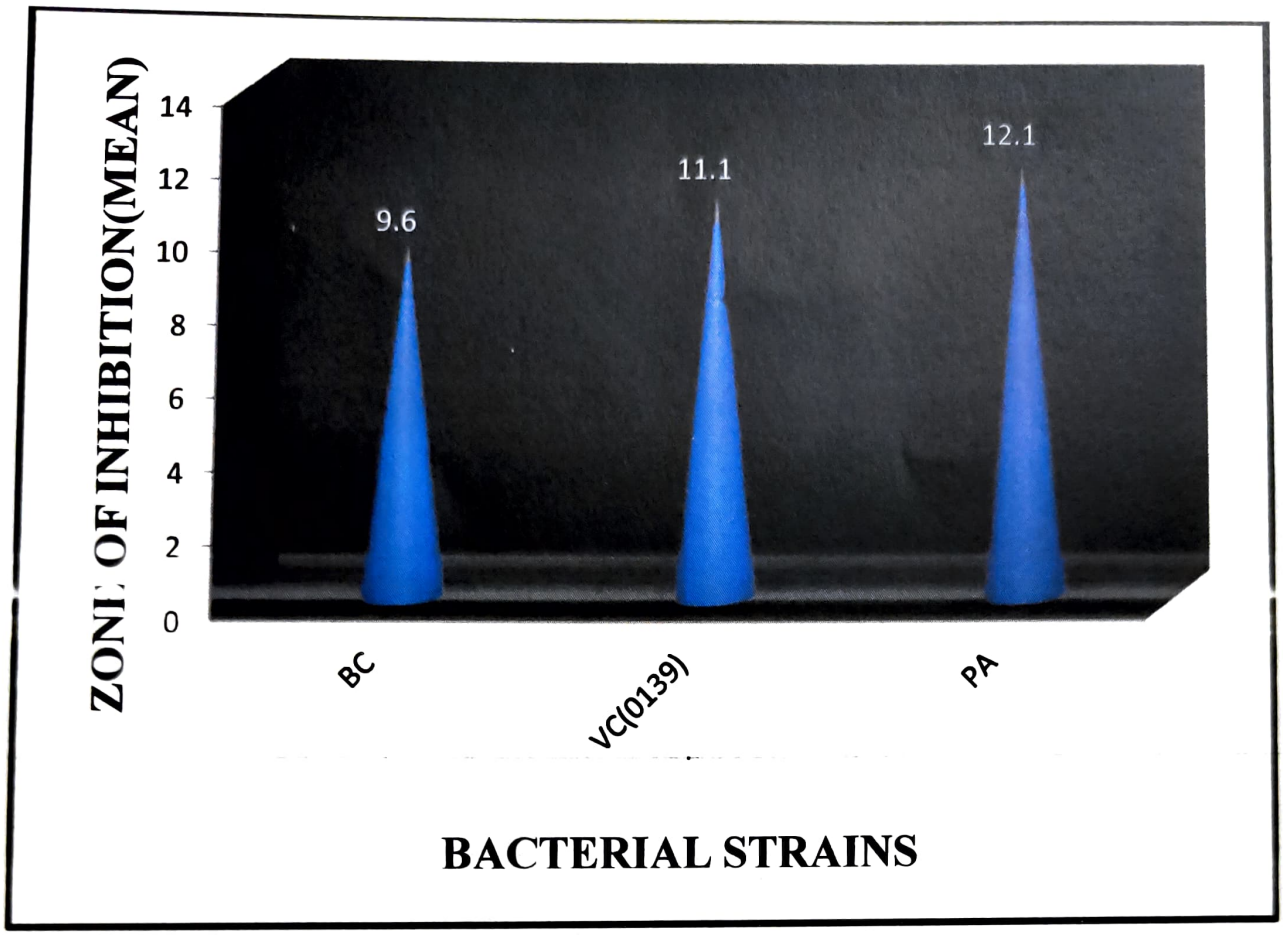


Fig: 6 Antibacterial activity of methanolic extract of *Azadirachta indica*

from 7.5 mm to 11.5 mm radius (Plate 6 & Table 6). The mean zone of inhibition were calculated as 9.6 11.1 and 12.1 in *BC, VC* (0139) and *PA* respectively (Fig 6).

Antioxidant Activity:

The antioxidant activity of *P. monodon* ,*P. adspersus* ,*A. indica* and positive control (Ascorbic acid) was assessed based on their ability to scavenge the DPHH free radicals .The free radical scavenging activity of methanolic extract of *P.monodon* ,*P. adspersus* and *A. indica* was evaluated .The methanolic extract of *P. monodon* exhibited the strong antioxidant activity with 80.2% ,85.45%, 89.3% and 99.5% at 50 µg ,100 µg,150 µg and 200 µg respectively (Fig 7 & Table 7). The methanolic extract of *P.adspersus* showed antioxidant activity with 85.76%, 86.7%, 87.87% and 91.5% respectively (Fig 8 & Table 8).Methanolic extract of *A.indica* exhibited the antioxidant activity with 84.2%, 86.44%,87.7% and 93.5% respectively(Fig 9 & Table 9).

Moisture content:

The Moisture contents of *P.monodon* was 77%.The Moisture contents of *P.adspersus* was 79%. The Moisture content is high in *P.monodon* .The Moisture content may vary depending on season, relative humidity and intensity of sunlight (Table 10 & Fig 10).

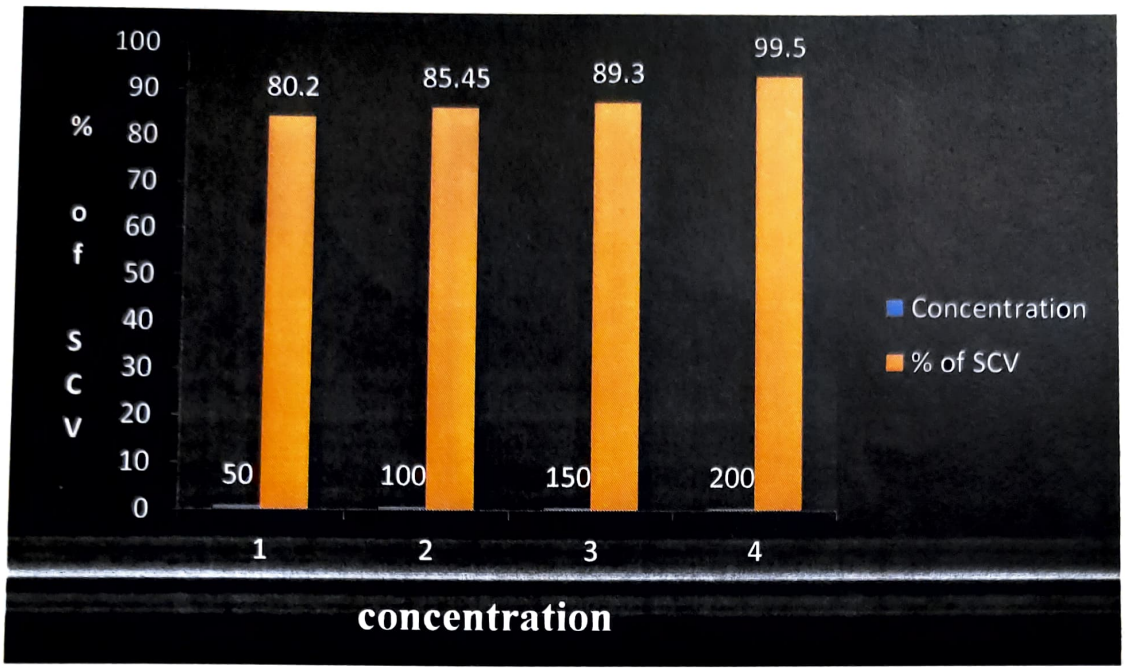


Fig: 7 Antioxidant activity of methanolic extract of *Penaeus monodon*.

Table - 7

Antioxidant activity of methanolic extract of *Penaeus monodon* against bacterial stains.

Sample concentration (µg)	Absorbance	%SCV
50	0.73	80.2
100	0.69	85.45
150	0.63	89.3
200	0.50	99.5

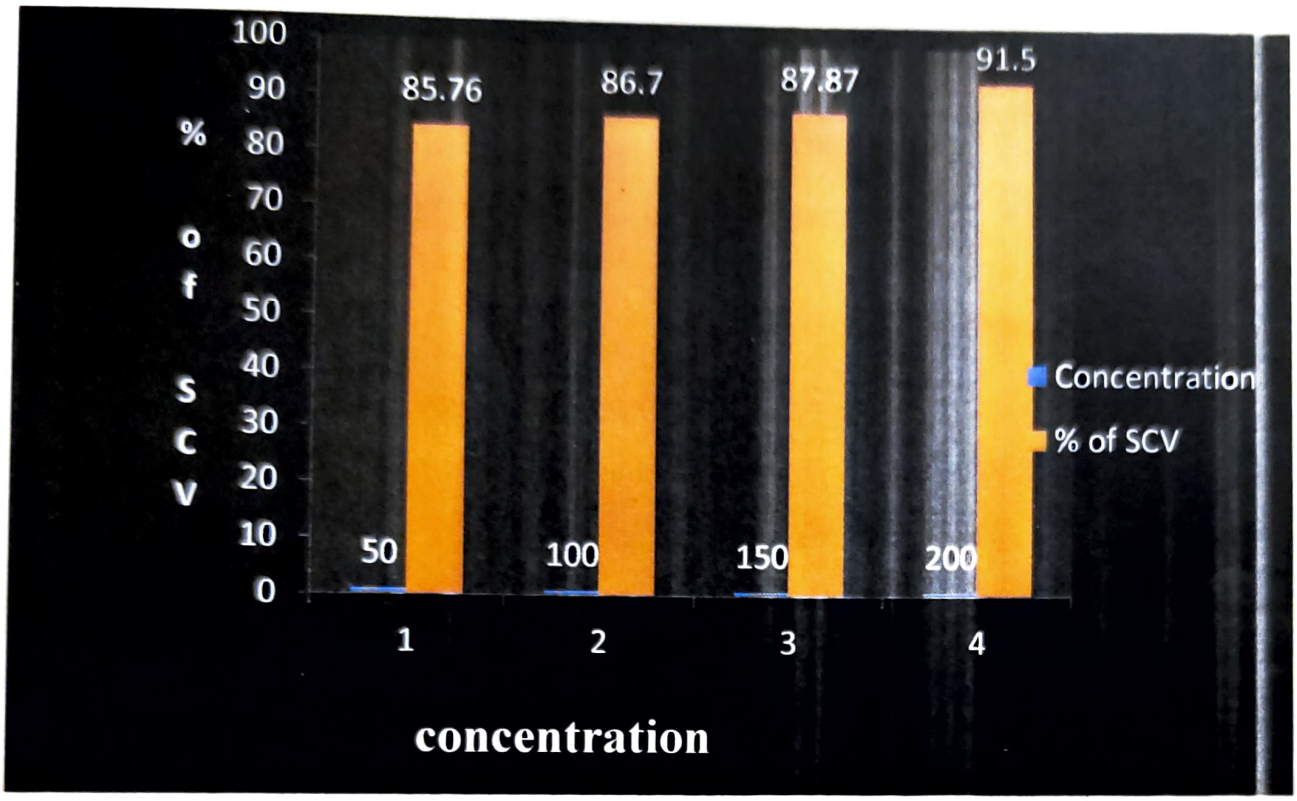


Fig: 8 Antioxidant activity of methanolic extract of *Palaemon adspereus*.

Table - 8

Antioxidant activity of methanolic extract of *Palaemon adsperus* against bacterial stains.

Sample concentration (µg)	Absorbance	%SCV
50	0.78	85.76
100	0.70	86.7
150	0.62	87.87
200	0.46	91.5

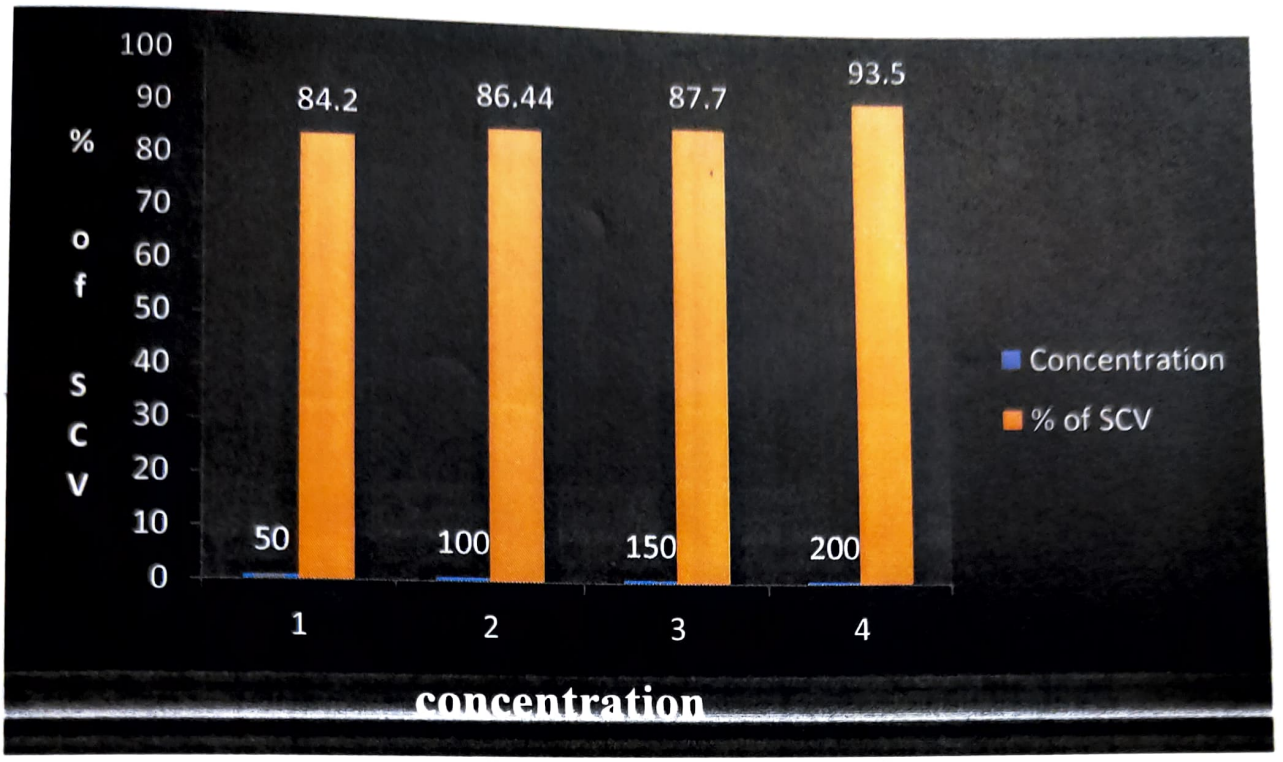


Fig: 9 Antioxidant activity of methanolic extract of *Azadirachta indica*.

Table - 9

Antioxidant activity of methanolic extract activity of *Azadirachta indica* against bacterial stains

Sample concentration (µg)	Absorbance	%SCV
50	0.68	84.2
100	0.61	86.44
150	0.53	87.7
200	0.47	93.5

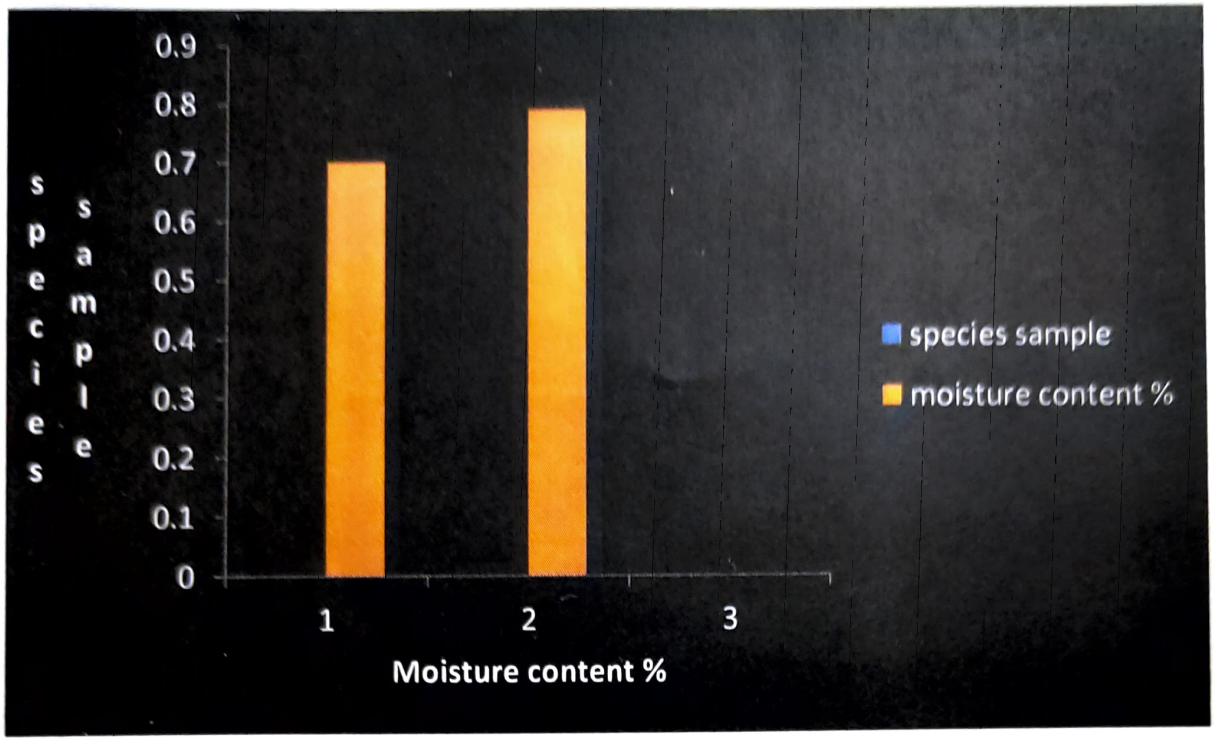


Fig: 10 Moisture content of *Penaeus monodon* and *Palaemon adspereus*

Table - 10

Moisture content

Species sample	Mositure content (%)
Penaeus monodon	77%
Paleamon adsperesus	79%

FTIR:

P.monodon, P. adspersus, A. indica evaluated for Fourier Transform Infrared Spectroscopy .The major absorption band for *P.monodon* is observed between 600/cm and 3100/cm. The major absorption band for *P.adspersus* is observed between 1100/cm and 3250/cm. The major absorption band for *A.indica* is observed between 1100/cm and 3120/cm(Fig 11,12 and 13).

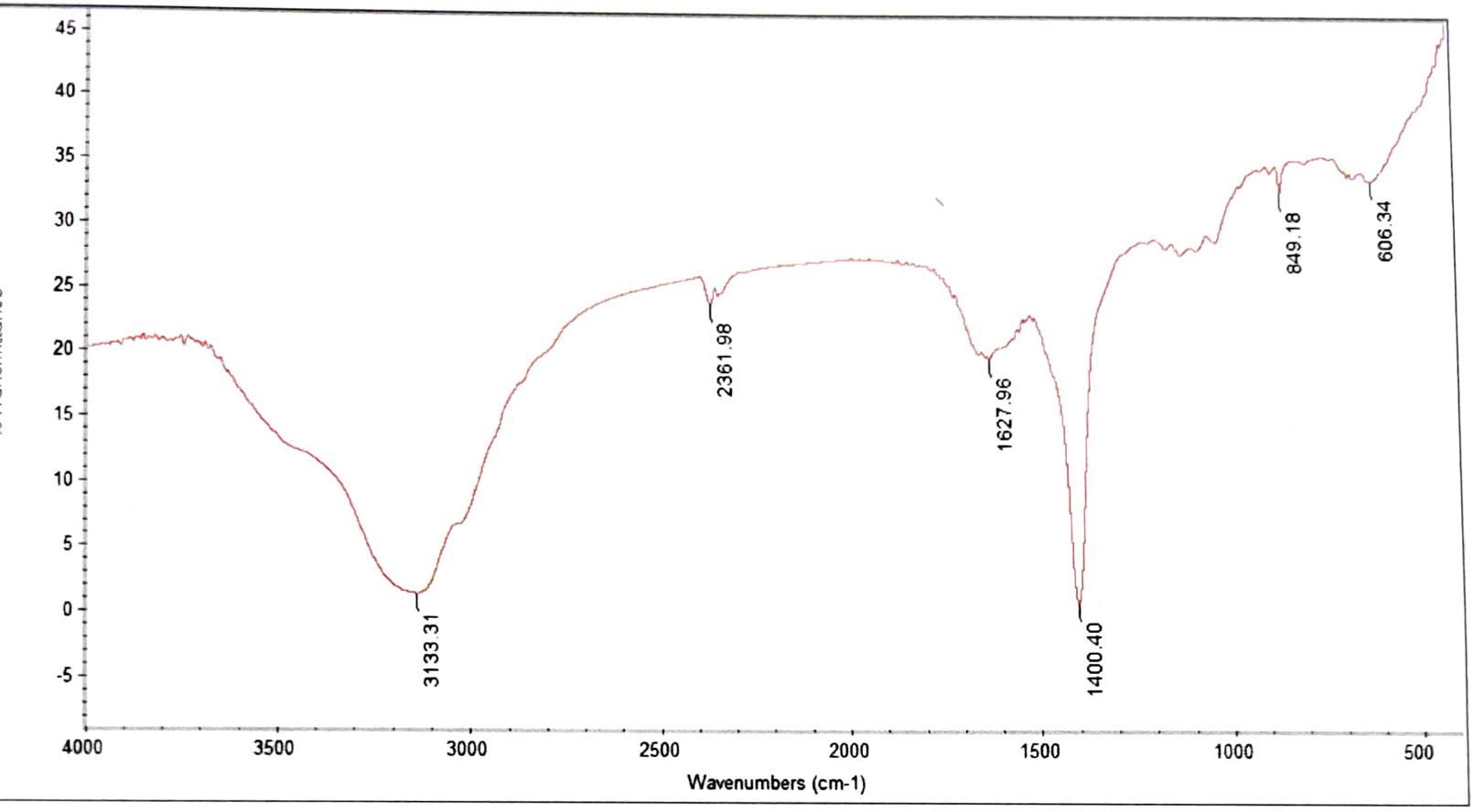


Fig: 11 FT-IR plot for methanolic extract of *Penaeus monodon*

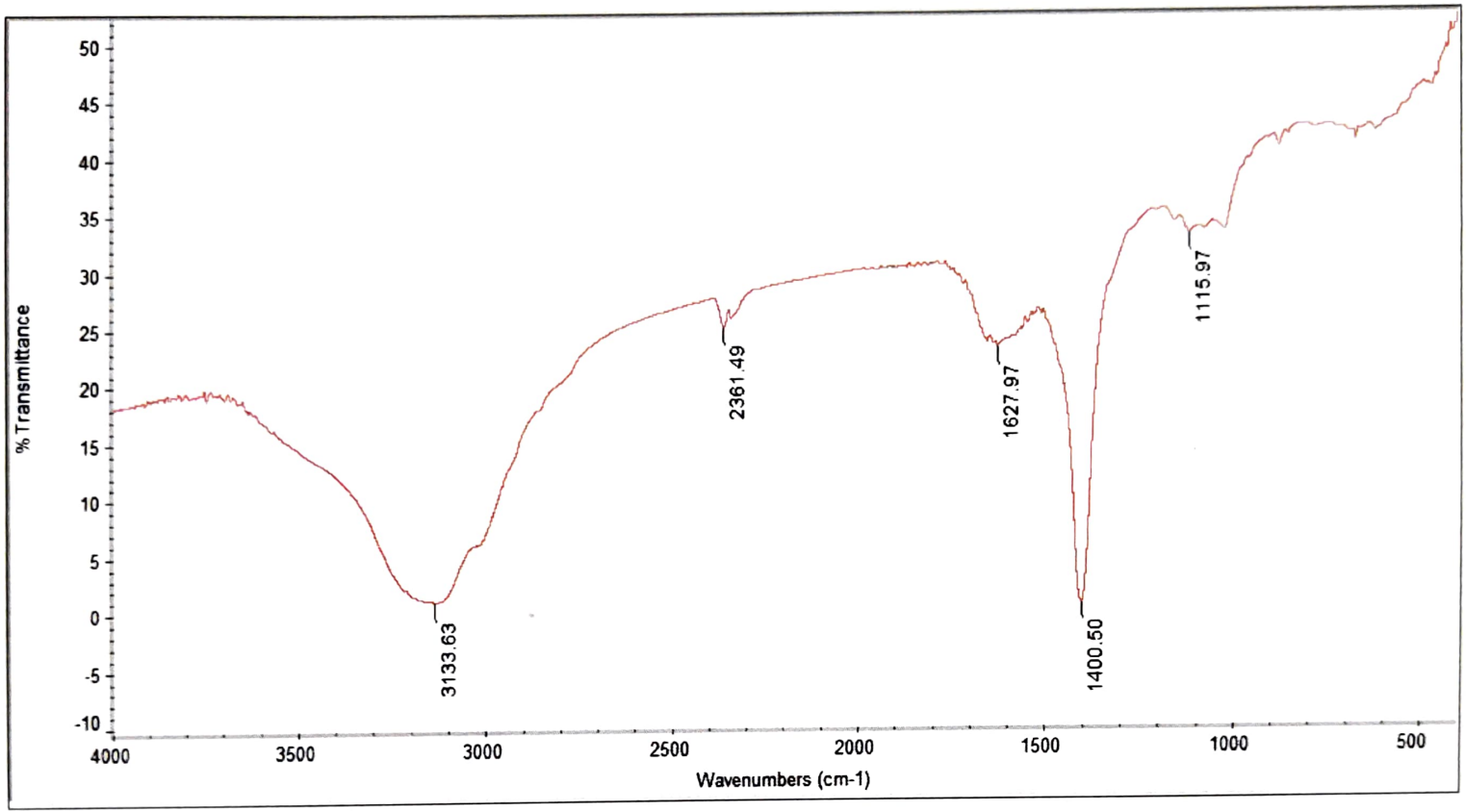


Fig: 12 FT-IR plot for methanolic extract of *Penaeus monodon*

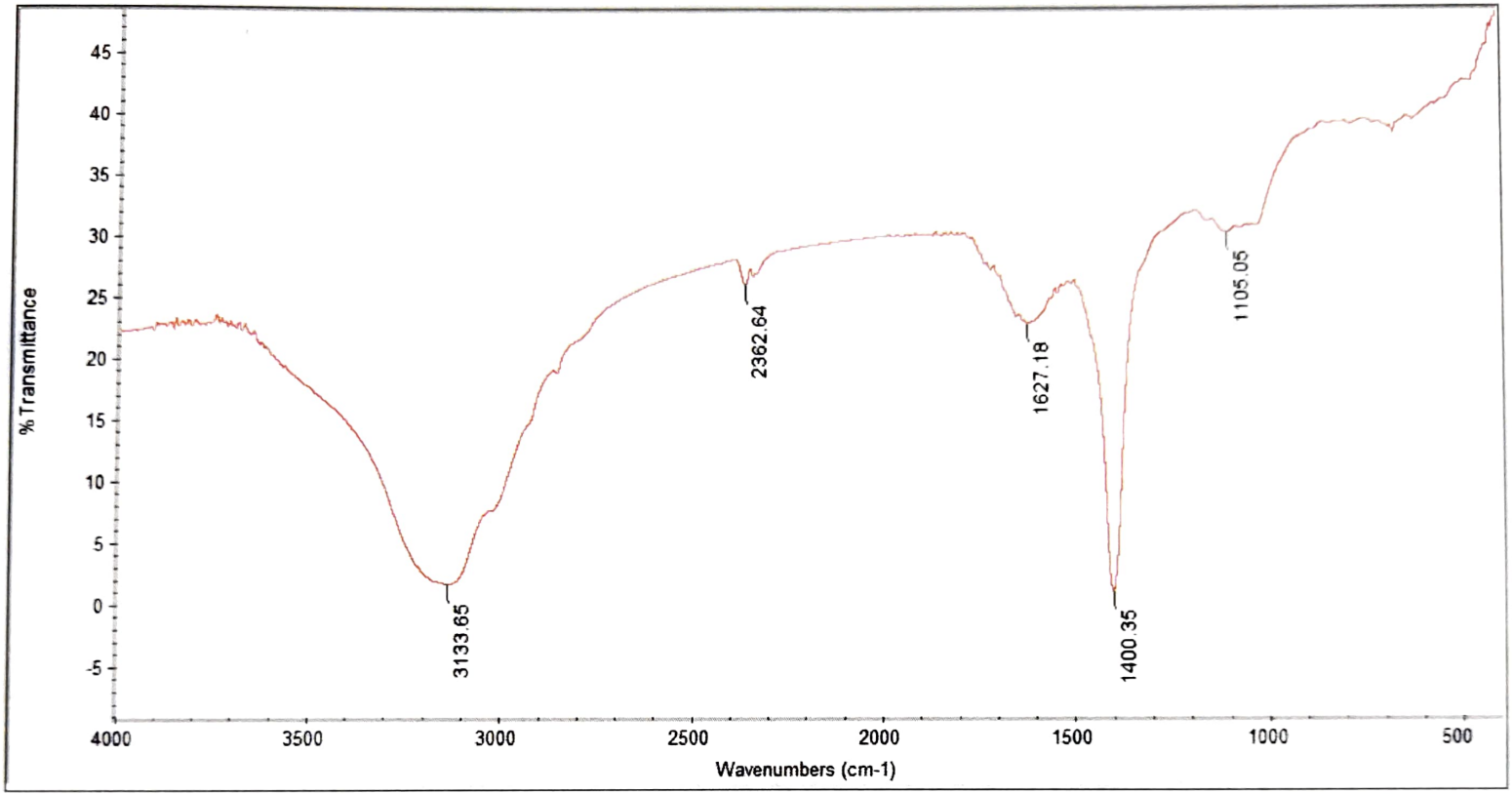


Fig: 13 FT-IR plot for methanolic extract of *Azadirchta indica*

5.DISCUSSION

Chitosan is a cheap and non-toxic, environmentally friendly material, which has the advantage that in the basic structure there are active amino and hydroxyl groups. These groups are easy to be functionalized, resulting in new materials, which often have increased antimicrobial activities with non-functionalized support material.

(Meng *et al.*, 2020); (Kutawa *et al.*, 2021) reported that chitosan is a natural antimicrobial agent and found effective against a variety of bacteria antifungus. (Murugesan *et al.*, 2020) studied that antibacterial activity of chitosan nanoparticles. The mechanism of antibacterial action is an intricate process that varies between G^- and G^+ bacteria as a result of the differences in cell wall and cell membrane chemistry. In previous studies, greater antibacterial activity was more evident against G^- bacteria than G^+ bacteria, whereas in some studies G^+ bacteria were more sensitive.

In the present study, antibacterial property is tested against *B .cereus* (BC), *V. cholera* (VC 0139) and *P. aeruginosa* (PA), the antibacterial property is high in *P. aeruginosa* (PA) in the methanolic extract of *P. monodon.*

Yin *et al.*, (2002) reported that the antioxidant activity of chitosan and its derivatives also hasattracted the most attention. Antioxidant property is animportant characteristic and greatly significant for natural life.

Numerous biological roles of antioxidants are documented like anti-mutagenicity, anti-carcinogenicity and anti-aging among others. (Sumathi *et al.*, 2017) reported that the extraction, characterization and applications of chitosan from prawn shells.

The chitosan extracted showed good anti-oxidant activity and also promoted seed germination. (Fernandes *et al.*, 2010) reported that chito-oligosaccharides (COS) are very promising compounds for use as natural antioxidants in biological systems.

In our study, antioxidant activity is tested on methanolic extract of *P.monodon, P.adspersus* and *A. indica*, the antioxidant property is high in the methanolic extract of *P. monodon* (99.5%).

Rajendran (2012) reported that synthesis and characterization of neem chitosan Nanocomposites for development of Antimicrobial Cotton Textiles. The neem chitosan nanocomposites treated fabrics showed an increased antimicrobial activity than the other fabric treatments (neem chitosan composite, neem and chitosan). The scanning electron microscopic results showed that the nanocomposites were essentially spherical in the size range of 50-100nm.The antibacterial activity of the fabrics were assessed using standard AATCC 100 and 147 test methods. (Oyekanmi *et al.*, 2021) reported the study of the films

In our observation, the antibacterial activity is tested in A. indica against *B.cereus* (BC), *V. cholera* (VC 0139) and *P. aeruginosa* (PA), the antibacterial property is high in *V.cholerae* in the methanolic extract of *A.indica* (14%).

Rashmi *et al.*, (2016) reported that chitosan in hygroscopic in nature. Hence, it can be affected by moisture absorption during storage. The moisture content may vary depending on the season, relative humidity and intensity of sunlight.

In our investigation, moisture content is tested on *P.monodon, P.adspersus.* The moisture content is high in *P.adspersus* (79%).

Rashmi *et al.*, (2016) reported that the spectra of the chitosan samples were measured in the spectral range from 400 cm^{-1} to 4000 cm^{-1} using nicolet impact -410, Fourier Transform- Infrared spectrometer in transmittance mode with a resolution of 4 cm^{-1}.

In our study, the *P.monodon, P. adspersus and A. indica* evaluated for Fourier Transform InfraRed Spectroscopy.The absorption band, which is high in P. adsperses(1100/cm and 3250/cm).

SUMMARY

The current study was designed to evaluate the Comparative Study on Bioactive Substances, Antibacterial and Antioxidant Activities of *P.monodon, P.adspersus* and *A.indica.*

Antibacterial activity of ethanolic and methanolic extract of *P.monodon, P.adspersus* and *A. indica* were tested against *B. cereus*(BC), *V. cholera* (VC(0139) and *P.aeruginosa* (PA), *P.monodon* exhibited potent antibacterial activity. Maximum zone of inhibition with 21.0 mm radius was observed in the marine methanolic extract of *P.monodon* on the bacterial strain of *P.aeruginosa* (PA).

Methanolic extract of P. monodon exhibited maximum DPPH free radical scavenging activity with 80.2%, 85.45%, 89.3% and 99.55 at 50 µg, 100 µg, 150 µg and 200 µg respectively.

P.monodon, P. adspersus and *A.indica* were analysed for moisture content.The moisture content of the Chitosan is around 77- 79%.

P. monodon, P. adspersus and *A. indica* were subjected to Fourier Transform – Infrared spectrometer (FT-IR). The absorption band which is high in P. adspersus (1100/cm and 3250/cm).

CONCLUSION AND SUGGESTIONS

Chitin is a macromolecular linear polymer of anhydro N-Acetyl Glucosamine (N –Acetyl ,2-Amino 2-Deoxy D-Glucose) and chitosan is de-acetylated chitin. Both chitin and chitosan have a very wide industrial application in more than 200 different fields like paper, textiles –sizing, dyeing and printing, chromatography, water purification, effluent treatment ,cosmetics ,drugs, pharmaceuticals ,surgery and many others.

Prawn shell wastes our study area were used to obtain chitin and correspondingly for the production of chitosan. This study showed that various products of chitin and chitosan can be generated using prawn shells as starting materials .The chitosan produced by deacetylation of chitin was observed to have many important properties like antibacterial, and radial scavenging activity. These important properties of chitosan are believed to have many commercial applications of high economic interests.

Likewise, the radical scavenging or the anti-oxidant activity of chitosan is of great interest in food industries and its possible use as natural additives has led to a great interest in replacing synthetic additives .The use of antimicrobial activity of chitosan has been used for development of antimicrobial films intended for use in packing materials for foods, medical supplies and so on.

This study reveals that some valuable bioactive substances, antibacterial and antioxidant activities of *P.monodon, P.adspersus* and *A. indica*. This studies demonstrates the great potential of the marine environment to isolate bioactive strains and illustrate the fact that marine bacteria emerge as a significant resources for natural product drug development. Results obtained clearly indicates that, chitin and chitosan possess compounds with many biological activities.

BIBLIOGRAPHY

Acharya, B., Kumar, V., Varadaraj, M. C., Lalitha, R., & Rudrapatnam, N. (2005). "Characterization of chito-oligosaccharides prepared by chitosanolysis with the aid of papain and pronase, and their bactericidal action against *B.cereus* and *E. coli*." Biochemical Journal, 391, pp. 167-175

Anand Srivastav, Singdha Swarupa, Mishra Sukanya, Debnath, Deepshikha Datta, (2018) "Extraction and Characterization of Chitosan Waste", Vol. 5, Issue 6

Antunes Joana, C., Domingues Joana,M., Miranda , Catarina ,S., Silva, G., Francisca ,A., Homem, C., Natalia, Amorim Teresa,P., and Felgueiras,M., Helena,P., (2021) "Bioactivity of Chitosan- Based Particles Loaded With Plant – Derived Extracts For Biomedical Application ; Emphasis On Antimicrobial Fiber- Based Systems", pp.2-40.

Badawy, M.E.I., Lotfy, T.M.R., Shawir, S.M.S., (2020). "Facile synthesis and Characterizations of antibacterial and antioxidant of chitosan monoterpene Nanoparticles and their applications in preserving minced meat". Int. J. Biolog. Macromol. 156, pp. 127–136.

Brine,C.J ., Sandford ,P.A., Zikakis,J.P(2013) ."Advances in chitin and chitosan" .London and New York: Elsevier; 1992 ISSN: pp. 2319-3182,Vol. -2,Issue -3.

Chandrasekaran,M ., Kim,K.D.,Chun.S.C.,(2020) "Antibacterial Activity Of Chitosan Nanoparticles" pp. 2-21

Chen, C.S., Liau, W.Y., and Tsai, G.J.J.,(1998). "Antibacterial effects of N-sulfonated and N-sulfobenzoyl chitosan and application to oyster preservation". Food Prot., 61(9) pp.1124-1128.

Chistiakov, D.A., Hellemans, B., and Volckaert, F.A.M.,(2007). "Review on the immunology of European sea bass *Dicentrarchus labrax*. Vet. Immunol." Immunopathol, 117: pp. 1–16.

Duarte, C. M., Agusti, S., Barbier, E., Britten, G. L., Castilla, J. C., Gattuso, J.-P., et al. (2020). "Rebuilding marine life." *Nature* 580, pp.39–51. doi: 10.1038/s41586-020-2146-7.

Fernandez-Kim, S. O. (2004). "Physicochemical and functional properties of craw fish chïtosan as affected by different processing protocols". A Thesis Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fullfilment of the requirements for the degree of Master of Science in The Department of Food Science, Seoul National University.

Goy, R.C., Britto, D., Assis, O.B.G., (2009). "A review of the antimicrobial activity ofchitosan". Polímeros 19, pp. 241–247.

Jøraholmen, M.W., Bhargava, A., Julin, K., Johannessen, M., Škalko-Basnet, N. (2020) "The Antimicrobial Properties of Chitosan Can Be Tailored by Formulation." Mar. Drugs, vol.no18, pp.96. [CrossRef]

Joana C.Antunes, Joana M.Domingues,catarina S.Miranda,A.Francisca G.Silva,Natalia C.Homem,M.Teresa P. Amorim and Helena P. Felgueiras (2021). "Bioactivity of chitosan-Based particals loaded with plant-derived extracts for Biomedical Applications emphasis on Antimicrobial fiber- Based system" pp:2-40.

Khanafari, A., Marandi, R., & Sanatei, Sh. (2008). "Recovery of chitin and chitosan from shrimp waste by chemical and microbial methods." Iranian Journal of Environmental HealthScience and Engineering, 5(1), pp.19-24

K.Jaganathan,P.Soundarapandian,T.V.Suresh, A.Murali Shankar, S.Arunprabu and S.M.Raffi (2015) "Extraction and characterization of chitin from marine Bycatch crustaceans employing chemical method ',Indo –Asian Journal of Multidisciplinary Research (IAJMR) Vol-1,Issue -1,pp94-102

Kutawa, A. B., Ahmad, K., Ali, A., Hussein, M. Z., AbdulWahab, M. A., Adamu, A., *et al.*, (2021). "Trends in nanotechnology and its potentialities to control plant pathogenic fungi: a review". Biology 10:881. doi: 10.3390/biology10090881

Li, Q., Dunn, E. J., Grandmaison, E. W., & Goosen, M. F. A. (1992). "Applications and properties of chitosan." Journal of Bioactive and Compatible Polymers, 7(4), pp.370-397

Limam, Z., Selmi, S., Sadok, S., & El-abed, A. (2011). "Extraction and characterization of chitin and chitosan from crustacean by-products: biological and physicochemical properties." African Journal of Biotechnology, 10(4), pp.640-647.

Meng, D., Garba, B., Ren, Y., Yao, M., Xia, X., Li, M., et al. (2020). "Antifungal activity of chitosan against Aspergillus ochraceus and its possible mechanisms of action." Int. J. Biol. Macromol. 158, pp.1063–1070. doi: 10.1016/j.ijbiomac.2020.04.213

Mohamad E.1. Badawy,Tesby M.R. Lotfyand Samar M.S.Shawir (2019) "Preparation and antibacterial activity of chitosan- silver nanoparticals for application in preservation of minced meat" pp. 2-14

Murugesan chandrasekaran,kideokkim and sechulchun, .(2020) "Antibacterial Activity of chitosan Nanoparticles" pp.2-21

Niloufar Hajarian Rezazadeh, Foad Buazar and Soheila Matroodi (2020). "Synergistic effects of combinatorial Chitosan and Polyphenol biomolecules on enhanced antibacterial activity of biofuntionalized silver nanoparticles."

Oliveira, D.A.J.; Amaral, J.G.; Garcia, L.B.; dos Santos, M.S.; Silva, L.A.O.; Almeida, M.P.; Gomes, A.F.; Barros, D.R.P.; Lopes, N.P.; Pereira, G.R.; (2021). "Associating chitosan and microemulsion as a topical vehicle for the administration of herbal medicines." Carbohydr. Polym. , 255, pp 117-482.

Oyekanmi,A.A, Seeta uthaya kumar,U., Abdul Khalil, H.P.S, Olaiya,N.G, Amirul, A.A., Rahman,A.A.,Arif Nuryawan, Abdullah, C.K., and Samsul Rizal(2021)."Functional properties of Antimicrobial Neem leaves Extract based macroalgae Biofilms for potential use as active dry packaging Applications." pp. 2-22

Pangestuti R, Kim SK. (2010) " Neuroprotective properties of chitosan and its derivatives". Mar Drugs. ; 8: pp 2117–2128.

Park, B. K., & Kim, M. M. (2010). "Applications of chitin and its derivatives in biological medicine". International Journal of Molecular Science, 11, pp.5152-5164 biological medicine.biological systems:erythrocytes and bacteriophages. Carbohydrate Polymers, 79, pp.1101 -1106.

Pereira, G.R.; (2021). "Associating chitosan and microemulsion as a topical vehicle for the administration of herbal medicines." Carbohydr. Polym, 255, pp. 117-482.

Qin, C., Li, H., Xiao, Q., Liu, Y., Zhu, J., & Du, Y. (2006)." Water-solubility of chitosan and its antimicrobial activity". Carbohydrate Polymers, 63, pp.367-374

Quan, H., Zhu, F., Han, X., Xu, Z., Zhao, Y., & Miao, Z. (2009). "Mechanism of Anti - angiogenic activities of chito oligosaccharides may be through inhibiting hep-aranase activity". Medical Hypotheses, 73, pp. 205-206.

Rabea, E.I., Badawy, M.E.-T., Stevens, C.V., Smagghe, G., Steurbaut, W., (2003). "Chitosanas antimicrobial agent: applications and mode of action." Biomacromolecules 4, pp.1457-1465

Rajendran R. ., Radhai R, Bala kumar C. Hasabo A.Mohammad Ahamad , Vigneswaran C. Vaidekik . (2012). "Synthesis and characterization of Neem chitosan nano composities for development of antimicrobial cotton Textiles" journal of Engineered Fibers and Fabricus vol. 7, issue 1 pp. 137- 141.

Suneeta Kumari, Pradip Kumar Rath "Extraction and characterization of chitin and Chitosan" pp.483-489

Superno and Poernomo.A.1992. "Fish waste utilization in Indonesia." Asean Food Journal .7 (2) pp. 67-72

S.Z. Islam, M.Khan and A.K.M.Nowsad Alam "Production of Chitin and Chitosan from Shrimp Shell Wastes" pp. 254-259

Thakral, J, Borar S, Roopa AN, Kalia N (2010). "Antioxidant potential fraction from methanol extract of aerial parts of Convolvulus arvensis". Linn.IJPSDR. 2:pp. 219-223

Torrecillas, S.; Makol, A.; Caballero, M.J.; Montero, D.; Robaina, L.; Real,F.,(2007). "Immune stimulation and improved infection resistance in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides." Fish Shellfish Immun., 23: pp. 969-981.

Wang, Y., Zhou, P., Yu, J., Pan, X., Wang, P., Lan, W., et al. (2007). "Antimicrobial effect of chito oligosaccharides produced by chitosanase from Pseudomonas CUY8". Asia Pacific Journal of Clinical Nutrition, 16, pp. 174-177.

Yang, E. J., Kim, J. G., Kim, J. Y., Kim, S., and Lee, N. (2010). "Anti-inflammatory effect of chitosan oligosaccharides in RAW264.7 cells." Central European Journal of Biology, 5, pp. 95-102.

AN ECO-FRIENDLY SYNTHESIS OF TELLURIUM NANOPARTICLES FROM THE MARINE BIVALVE SHELL *CUCULLAEA PETITA* (Iredale, 1939)

A Project submitted to

ST.MARY'S COLLEGE (Autonomous), THOOTHUKUDI

affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, TIRUNELVELI

in partial fulfilment for the award of the degree of

Bachelor of Science in Zoology

by

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DEPARTMENT OF ZOOLOGY

ST. MARY'S COLLEGE (AUTONOMOUS), THOOTHUKUDI

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CERTIFICATE

This is to certify that the project entitled **An Eco-Friendly Synthesis of Tellurium Nanoparticles from the Marine Bivalve Shell *Cucullaea petita* (Iredale, 1939)** is submitted to **St. Mary's College (Autonomous), Thoothukudi** in partial fulfillment for the award of the degree of **Bachelor of Science in Zoology** and it is a record of the work done during the year 2021-2022 by the following students.

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CONTENTS

1. INTRODUCTION

The oceans cover more than 70% of the world surface housing 34 living phyla out of 36 and more than 30,000 known species of flora and fauna. The marine environment is known to contain over 80% of world's plant and animal species. In recent years, many bioactive compounds have been extracted from various marine plants, animals and micro organisms. With the terrestrial resources being greatly explored and exploited, researchers turn to the oceans for numerous reasons (Bhimba *et al*., 2010).

Marine organisms have been a rich source of novel chemical compounds which have boosted the development of marine natural products chemistry for about three decades. The phylum Mollusca is one of the most diverse in the animal kingdom, second only to the arthropods in number of species, but with much greater diversity of body plan. There are seven living classes of molluscs and they range from the bivalves (oysters, mussels etc) which are generally immobile with very little central nervous system, to the biggest and most intelligent of invertebrates, the fast moving hunting squids and octopus. The phylum appeared at some time in the early to middle Cambrian period, about 540 million years ago, and because most molluscs have hard calcareous shells they are common and important fossils. There are as many fossil species known as extant ones. Most

molluscs are marine, but two groups, the gastropoda and bivalvia make it into freshwater, and the gastropods are important on land, as snails and slugs (Brooks, 2013).

Molluscs are characterized by the presence of a calcareous shell (in various forms), a sheet of tissue called a mantle, which secretes the shell and encloses a mantle cavity with gills or lungs, division of the body into a ventral muscular foot used for locomotion, and a visceral mass protected by the shell, a special toothed structure called a radula used in feeding. This is a pretty clear set of features, and one could imagine an ancestor of all classes looking a bit like a limpet, living on the sea floor and grazing algae. All of these features are either lost or very substantially changed in one or more of the classes (Dunsten and Hodgson, 2014).

With about 10,000 living and 12,000 fossil species, bivalvia is the second largest group of molluscs. In total contrast to the cephalopods, they have gone in for a quiet, sessile life, burrowed into sediment, or glued onto rocks. Their shell is divided into two plates, left and right over the body, and their very large mantle cavity houses complex gills, used for capturing small particulate food by filter feeding. The foot can be pushed out from the shell allowing burrowing as in cockles, and production of threads to attach the bivalve to the rock as in mussels. They have completely lost the radula, and have done their best to rid themselves of

a central nervous system, although they can react to predators by rapidly closing the shell. In the present study, marine bivalve *Cucullaea petita* have been selected to synthesize the tellurium nanoparticles (Souji *et al*., 2013).

Nanotechnology is significant on account of its pre-eminence upon the comprehension, use, and control of matter at magnitudes of a minute scale, akin to approaching atomic levels, with which to manufacture new substances, instruments, and frameworks. The synthesis of nanocrystals is in the limelight in modern nanotechnology. Biosynthesis of nanoparticles by plant extracts is currently under exploitation. Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences in several ways like imaging, sensing, targeted drug delivery, gene delivery systems, and artificial implants (Huang. J., 2007).

Preparation of nanoparticles using green technologies is advantageous over chemical agents due to their environmental consequences. Green synthetic procedures include mixed valence polyoxometallates, polysaccharides, tollens, irradiation and biological methods. In the biological method, extracts from living organisms may act as both reducing and capping agents in synthesis of nanoparticles. The reduction of metal ions by combination of biomolecules found in these extracts such as enzymes, proteins, amino acids ,polysaccharides and vitamins is environmentally benign, yet chemically complex. Living organisms

have a huge potential for the production of nanoparticles having wide applications. By using the organisms from simple bacteria to highly complex eukaryotes in the reaction mixture, the production of nanoparticles with desired shape and size can be obtained. Rapid and green synthetic methods using biological extracts have shown a great potential in nanoparticle synthesis (Sharma. V., *et al*., 2008).

Tellurium is a chemical element with the symbol Te and atomic number 52. It is a brittle, mildly toxic, rare, silver-white metalloid. Tellurium is chemically related to selenium and sulfur, all three of which are chalcogens. It is occasionally found in native form as elemental crystals. Tellurium is far more common in the Universe as a whole than on Earth. Its extreme rarity in the Earth's crust, comparable to that of platinum, is due partly to its formation of a volatile hydride that caused tellurium to be lost to space as a gas during the hot nebular formation of Earth (Anderson and Don,1983).

Tellurium (Te) Nanoparticles, nanodots or nanopowder are black spherical high surface area particles. Nanoscale tellurium particles are typically 10 - 45 nanometers (nm) with specific surface area (SSA) in the 30 - 50 m^2/g range. Nano tellurium particles are also available in passivated and ultra high purity and high purity and coated and dispersed forms. They are also available as a dispersion through the AE nanofluid production group. Nanofluids are generally defined as suspended nanoparticles in solution either using surfactant or surface charge

technology. Nanofluid dispersion and coating selection technical guidance is also available. Other nanostructures include nanorods, nanowhiskers, nanohorns, nanopyramids, and other nanocomposites. Surface functionalized nanoparticles allow for the particles to be preferentially adsorbed at the surface interface using chemically bound polymers. Green synthesized nanoparticles have been characterized by UV-Vis spectroscopy, FTIR, AFM and SEM(Anderson and Don,1983).

UV-Visible spectroscopy (UV-Vis) measures the extinction (scatter + absorption) of light passing through a sample. Nanoparticles have unique optical properties that are sensitive to the size, shape, concentration, agglomeration state, and refractive index near the nanoparticle surface, which makes UV-Vis a valuable tool for identifying, characterizing, and studying nanomaterials. In its simplest form, a sample is placed between the light source and a photodetector, and the intensity of a beam of light is measured before and after passing through the sample. The measurements are compared at each wavelength to quantify the sample's wavelength dependent extinction spectrum. The data is typically plotted as extinction as a function of wavelength. Each spectrum is background corrected using a "blank"- a cuvette filled with only the dispersing medium- to guarantee that spectral features from the solvent are not included in the sample extinction spectrum (Moudikoudis. S., *et al*.,2018).

Fourier Transform Infra Red Spectroscopy has been developed as a tool for the simultaneous determination of organic components, including chemical bond,as well as organic content (e.g., protein, carbohydrate, and lipid). Fourier Transform Infra Red(FTIR) is one of the important analytical techniques for researchers. This type of analysis can be used for characterizing samples in the forms of liquids , solutions, pastes, powders, films, fibers and gases. Compared to the other types of characterization analysis, FTIR is quite popular. This characterization analysis is quite rapid, good in accuracy and relatively sensitive. In the FTIR analysis procedure,samples are subjected to contact with infrared(IR) radiation. The IR radiations then have the impacts on the atomic vibrations of a moleculein the sample,resulting the specific absorption and transmission of energy. This makes the FTIR useful for determining specific molecular vibrations contained in the sample (Kirk and Othmer, 1953).

The Atomic Force Microscope (AFM) is widely used in materials science and has found many applications in biological sciences but has been limited in use in vision science. The AFM can be used to image the topography of soft biological materials in their native environments. It can also be used to probe the mechanical properties of cells and extracellular matrices, including their intrinsic elastic modulus and receptor-ligand interactions. In this review, the operation of the AFM is described along with a review of how it has been thus far

used in vision science. It is hoped that this review will serve to stimulate vision scientists to consider incorporating AFM as part of their research toolkit (Last. J. A., *et al.*, 2010).

Scanning Electron Microscopy(SEM) is responsible for some of the most detailed nano and microscopic images ever produced. Beyond their aesthetic appeal, this advanced particulate insight has helped researchers to accelerate development in areas as diverse as cement composition to forensic sciences. Numerous microscopy techniques are available, however Scanning Electron Microscopy (SEM) are arguably the most popular for nanoparticles analysis. Scanning Electron Microscopy works by bombarding a sample with a stream of electrons and monitoring the scattering effects. SEM produces an accurate 3D image of particles in the dispersion and SEM enables a large amount of sample to be measured at one time, which can improve both the statistical reliability and efficiency of nanoparticles size and shape distribution measurements. SEM may offer a better performances for surface and shape analysis, particularly in applications such as quality control of colloidal nanoprecipitates or for measuring surfaces and microstructures of nanosized powdered materials. For many nanotechnology developers looking at fundamental size and shape properties, SEM may offer a more productive path to high quality analysis (Jaggi and Vij,2006).

The presence of multidrug resistance pathogens have increased the number of infectious disease and became the main cause of death in the world (WHO, 2000; Tanwar *et al*., 2014). Widely misuse and abuse of antibiotics are the leading cause of antibiotic resistance in the bacteria (Bryan *et al*., 2018). Multidrug resistant bacteria infection may lead to several impacts including increase of mortality and morbidity rates, prolong of hospitalization period, and economic loss (Patel *et al*., 2008). Woh *et al*., (2017) detected multi-drug resistant non-typhoidal *Salmonella* among migrant food handlers, which may cause cross-contamination to the food products. Thus, the development of a new and natural antimicrobial agent is needed as there is a growing concern in multidrug resistant pathogens. The development of antibiotic resistance of bacteria is one of the most pressing problems in world health care. One of the promising ways to overcome microbial resistance to antibiotics is the use of metal nanoparticles and their oxides.

Antioxidant properties are currently extensively studied for various materials, including the natural ones, in order to identify new compounds from natural sources. Antioxidants control oxidative reactions by inhibiting, delaying or hindering the oxidation of the biomolecules (Sies *et al*.,1997; Kumar *et al.,* 2011) The key antioxidant enzymes possess certain elements that shield and protect proteins (Harris. R. C., *et al*., 1992). Non enzymatic antioxidants can also neutralize radicals for example water soluble substances such as Vitamin C,

glutathione or fat-soluble substances such as Vitamin E, β-carotene (Sies *et al.*, 1997; Harris. R. C., *et al.,* 1992; Trombino. S., *et al.*,2004). Synthetic antioxidants such as butylated hydroxyl toluene(BHT) and butylated hydroxyl anisole (BHA) have newly been reported to be harmful for human health (Abramovie *et al*., 2006; Kowalski *et al*.,2007). Thus, the search for effective, non-toxic, natural compounds with antioxidative activity has been increased in recent years.

Recently nanomaterials have started playing a fundamental role in human life and health owing to their substantial benefits of biomedical applications (Zolnik and Sadrieh, 2009) like medical imaging, drug delivery, diseases diagnosis (Miyazaki and Islam, 2007),cancer treatment (Garde,2012; Peiris. J., *et al*., 2012), treatment of infectious diseases (Banoee *et al.,* 2010; Radovic-Moreno *et al.*,2012), treatment of neurodegenerative disorder including Parkinson disease (Wong.H ., and Bendayan. R., ,2012) and so on.Moreover, the strong antioxidant property exhibited by some nanomaterials is opening exciting potential to develop new regimens with enhanced and targeted actions. For example,gold, silver and selenium nanoparticles have been shown to possess ability to reduce oxidative stress due to their efficient redox-active radical-scavenging properties (Saad *et al.,* 2017, Sood and Chopia ,2017; Bhakya. S., *et al.*,2016 and Thilagavathi *et al.*, 2016).

Although the synthesis of nanoparticles using biological methodologies has received increasing attention in the last decade, only a few studies reported reliable data on the biological activities of the obtained green-synthesized nanomaterials, highlighting the differences of nanoparticle action in various biological host systems. The resulted green-synthesized nanoparticles were examined by Ultraviolet-Visible spectroscopy (UV-Vis), Fourier Transform InfraRed (FTIR) spectroscopy, Atomic Force Microscopy (AFM) and Scanning Electron microscopy (SEM) to determine their size and charge. In the present study, tellurium nanoparticles were synthesized from the marine bivalve shell *Cucullaea petita* and analyzed for antimicrobial and antioxidant activity.

2.REVIEW OF LITERATURE

Nanoparticle synthesis using biological organisms by green synthesis technology is biologically safe, cost-effective, and environment-friendly. Plants and microorganisms have established the power to devour and accumulate inorganic metal ions from their neighboring niche. The biological entities are known to synthesize nanoparticles both extra and intracellularly. The capability of a living system to utilize its intrinsic organic chemistry processes in remodeling inorganic metal ions into nanoparticles has opened up an undiscovered area of biochemical analysis (Dan Zhang *et al*., 2020).

Inbakandan *et al*., (2010) studied the biosynthesis of gold nanoparticles utilizing marine sponge *Acanthella elongate* (Dendy,1905). James Cookson (2012) has prepared palladium nanomaterials. Preetha Devaraj *et al*., (2013) synthesized and studied the characterization of silver nanoparticles using cannonball leaves and their cytotoxic activity against MCF-7 Cell line. Sri Ramkumar Vijayan *et al*., (2014) studied the synthesis and characterization of silver and gold nanoparticles using aqueous extract of seaweed, *Turbinaria conoides* and their anti microfouling activity. Monaliben Shah *et al*., (2015) synthesized metallic nanoparticles via biological entities.

Safaa Ali *et al*.,(2015) investigated the applications of biosynthesized silver nanoparticles for the control of land snail *Eobania vermiculata* and some plant pathogenic fungi. Suresh Sagadevan and Koteeswari (2015) analysed the structure, surface morphology, optical and electrical properties of copper nanoparticles. Maria Benelmekki (2015) gave an introduction to nanoparticles and nanotechnology.

Hamed Barbadi *et al*., (2015) synthesized and characterized the biogenic tellurium nanoparticles by using *Penicillium chrysogenum.* Aruna Jyothi Kora and Lora Rashtogi (2015) synthesized palladium nanoparticles using gum ghatti (*Anogeissus latifolia*) and studied their applications as an antioxidant and catalyst. Yu Ioni*et al*., (2016) synthesized graphene with noble metals nanoparticles on its surface.

Khwaja Salahuddin Siddiqui and AzamalHusen (2016) studied the green synthesis and investigated the characterization and uses of palladium and platinum nanoparticles. Dmitri Talapin and Elena Shevchenko (2016) gave an introduction to nanoparticle chemistry. Kashinath *et al*., (2017) synthesized and studied the structure of graphene nanoparticles.

Rakhi Majumdar *et al*., (2017) synthesized palladium nanoparticles with green leaf extract of *Chrysophyllum caimito* (star apple) and studied their

applications as efficient catalyst for C-C coupling and reduction reactions. Alpaslan *et al*., (2017) studied the synthesis and characterization of selenium nanoparticles lysozyme nanohybrid system with synergistic antibacterial properties. Results of this efforts highlighted the nanohybrid systems with synergistic antibacterial properties to overcome the emerging antibiotic resistance as well as to define fruitful applications in biomedicine.

Stefanos Mourdikondis *et al*.,(2018) studied the characterization techniques for nanoparticles and compared the complemented the properties of nanoparticles. Mostafa Abo Elsoud *et al*., (2018) synthesized and investigated tellurium nanoparticles. Andras Vladar and Vasile-Dan Hodoroaba (2019) investigated the characterization of nanoparticles by Scanning Electron Microscopy. David Medina Cruz *et al*., (2019) synthesized citric juice mediated tellurium nanoparticles with antimicrobial and anticancer properties.

Williams and Oliver (2018) compared the antimicrobial activity of selenium nanoparticles with different surface chemistry and structure, the microbial screening of the selected systems was determined by the broth microdilution method and inhibitory influence on the production of monomicrobial and dual species biofilm was evaluated.

Andrew Smith *et al*., (2019) investigated the synthesis, properties and applications of graphene oxide or reduced graphene oxide and their nanocomposites. AsepBayu Dani Nandiyanto *et al*., (2019) interpreted the FTIR spectroscopy of organic material. Qaisar Abbas (2019) discussed the UV-Vis spectroscopy technique for nanoparticles.

Sujata Kumari *et al*., (2020) investigated the synthesis of graphene oxide-silver nanocomposite for unique physiochemical applications. KabaliVijai Anand *et al*., (2020) investigated the preparation and characterization of calcium oxide nanoparticles from marine molluscan shell waste as nutrient source for plant growth. Joanna Jagiello *et al*., (2020) studied the synthesis and characterization of graphene oxide and reduced graphene oxide composites with inorganic nanoparticles for biomedical applications.

Tannia Velazquez-Urbina *et al*., (2021) synthesized and characterized the silver nanoparticles supported on bivalve mollusc shell for catalytic degradation of commercial dyes. The green synthesis, physicochemical characterization, and catalytic application of silver nanoparticles (AgNPs) bivalve mollusc shell (MS) composites (AgNPs/ExLG-MS) using a natural aqueous extract of *Gardenia jasminoides* fresh leaves (ExLG) as a reducing-stabilizing agent (RSa) was studied.

Vijai Anand *et al*., (2021) prepared and characterized the calcium oxide nanoparticles from marine molluscan shell waste as nutrient source for plant growth. Somayeh Safat *et al*., (2021) enhanced the sunlight photo catalytic activity and biosafety of marine driven synthesized Cerium oxide nanoparticles. HojatVeisi *et al*., (2021) synthesized palladium nanoparticles fabricated magnetic Fe_3O_4 nanocomposites over *Fritillaria imperialis* flower extract as an efficient recyclable catalyst for the reduction of nitroarenes. Koduru Mallikarjuna *et al*., (2021) synthesized the reduced graphene oxide supported palladium nanoparticles by *Coleus amboinicus* and its enhanced catalytic efficiency and antibacterial activity.

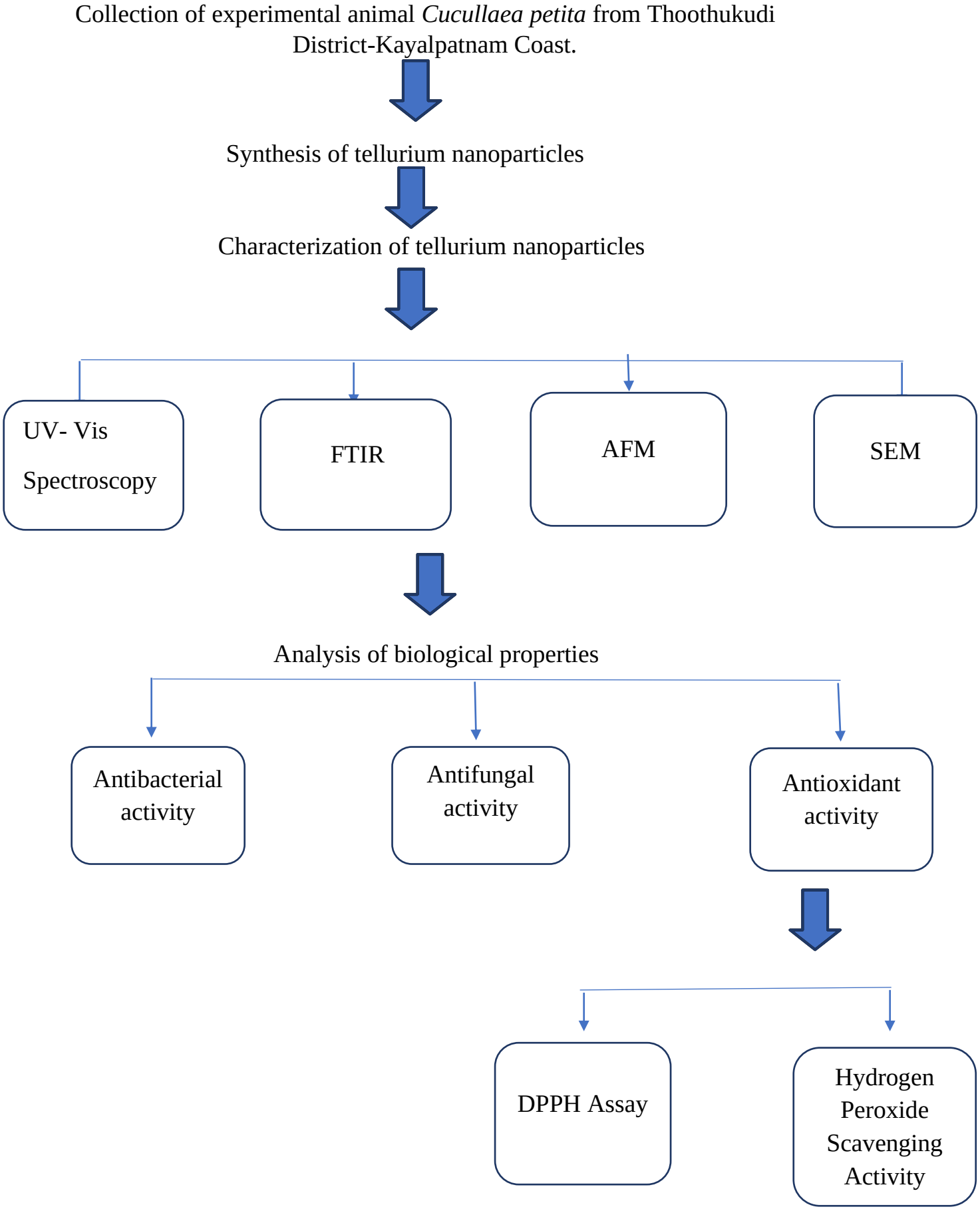
Considering the above importance of nanoparticles in the field of biology, the present study has been carried out with a view to synthesize, characterize and analyze the biological activities of tellurium mediated nanoparticles from the shell of marine bivalve *Cucullaea petita*.

3.OBJECTIVES

Nanotechnology possess a wide range of biological applications in different fields of science. Production of nanoparticles has attained attraction by biological based fabrications as an alternative to physical and chemical approaches due to exceeding need to develop safe, reliable, clean and ecofriendly methods for the preparation of nanoparticles for pharmaceutical and biomedical applications. A number of microorganisms containing bacteria, fungi, yeast, plant extracts and even algae have been reported for bio fabrication of nanoparticles with different shapes and sizes as possible eco friendly alternatives to conventional chemical and physical methods. Eco-friendliness, energy efficiency, cost effectiveness, simplicity and compatibility for biomedical applications are the superiorities of biological approach for the production of nanoparticles over conventional synthesis. So, the present study has been carried out with the following objectives:

- To synthesize the tellurium nanoparticles from the shell of marine bivalve *Cucullaea petita*
- To record the wavelength using UV-Visible spectrophotometer.
- To characterize the nanoparticles using FTIR, AFM and SEM.
- To analyze the antibacterial, antifungal and antioxidant activities of tellurium mediated nanoparticles from the marine bivalve *Cucullaea petita.*

4. EXPERIMENTAL DESIGN



5. MATERIALS AND METHODS

5.1 Description of the Study Area

Kayalpatnam is a coastal town in eastern Tamilnadu, India. It is the seat of the Southern Thoothukudi district and a settlement with the population exceeding 40,000 people. It is believed that in the 13th century BC the town was visited by Marco Polo while he was cruising near the shores of India. The latitude of Kayalpatnam, is 8.568315, and the longitude is 78.123833 with gps coordinates of 8^{o}34’5.9340 N and 78^{o}7’25.7988 E. Kayalpatnam is enriched with rich biota such as corals, seaweeds, sea grasses, sandy, rocky and muddy shore organisms. It is also well pronounced with reference to different molluscan groups(Figure 1).

5.2 Collection of Experimental Animal

In the present study the marine bivalve *Cucullaea petita* was collected from the Kayalpatnam coastal region (Plate 1). The bivalve was collected from the landed by-catch from fishing trawlers operated for crabs and prawns along the Kayalpatnam coastal region. The bivalve was collected during the month of January 2022. The freshly collected samples were brought to the laboratory, cleaned and washed with fresh sea water to remove all impurities. The shells were broken, tissues were removed and the broken shells were dried in hot air oven at 56^{o}C for 48 hours and used for further studies.

5.3 Description of Experimental Organism

5.3.1 Systematic Position of *Cucullaea petita(* Iredale, *1939)*

Kingdom : Animalia

Phylum : Mollusca

Class : Bivalvia

Order : Arcoida

Family : Cucullaeidae

Genus : *Cucullaea*

Species : *petita*

Cucullaea petita is a marine bivalve commonly known as False Ark Shell. The shape of the shell is roughly quadrate.The shell is 3.5 to 4.0cm in length and 2.8 to 3.4 cm in height.The body volume is upto 23.9 cm^3. Individuals can grow upto 73.2 mm. Myophoric flanges are narrower. Periostracum is whitish with brown scattered zig zag lines.They have prominent and dorsally lower umbones. The inner part of the valve is glossy white. Radially arranged hinge teeth are thin.

5.4 Synthesis of Tellurium Nanoparticles

Synthesis of Tellurium nanoparticles is carried out using 0.01M Tellurium chloride in double-distilled water using *Cucullaea petita*. Tellurium chloride and the shell extract of *Cucullaea petita* were mixed together in a ratio of (9:1, 8:2, 7:3, 6:4, and 5:5). In this different ratio concentration,a 5:5 ratio concentration was selected for the bulk preparation because it shows a higher production than other ratios stirred

at 800 rpm using a magnetic stirrer. The mixture turned into milk-white color within 1 hr. The whole reaction was carried out in the dark. The obtained suspension was centrifuged at 15,000 rpm for 15 min. The pellet containing tellurium nanoparticles was washed 3–4 times with deionized water to remove impurities. The precipitated nanoparticles were lyophilized. Lyophilized nanoparticles were stored in a cool, dry and dark place and further characterization was carried out.

5.5 UV – Vis Spectral Analysis

Ultraviolet–visible spectroscopy (UV) refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible spectral regions. Molecules containing bonding and non-bonding electrons (n-electrons) can absorb energy in the form of ultraviolet or visible light to excite these electrons to higher antibonding molecular orbitals. UV-absorption spectra of synthesized tellurium nanoparticles by using *Cucullaea petita* were measured using UV- Visible spectrometer (Shimadzu UV-2700).

5.6 Fourier Transform InfraRed Spectroscopy (FTIR)

An infrared spectrophotometer is an instrument that passes infrared light through an organic molecule and produces a spectrum that contains a plot of the amount of light transmitted on the vertical axis against the wavelength of infrared radiation on

the horizontal axis. The functional group present in the synthesized tellurium nanoparticles was determined using FTIR spectroscopy (Bio-read FTIR 8400S models, USA).

5.7 Atomic Force Microscopy (AFM)

The AFM works much the same way a profilometer works only on a much, smaller scale: a very sharp tip is dragged across a sample surface and the change in the vertical position (denoted the "z" axis) reflects the topography of the surface. By collecting the height data for a succession of lines it is possible to form a three dimensional map of the surface features.

The AFM has three major abilities force measurement, imaging, and manipulation. The Atomic force microscopy analysis using the Nanosurf easy2 scan BT02218 is profilometer.

5.8 Scanning Electron Microscopy (SEM)

A typical SEM instrument, showing the electron column, sample chamber, EDS detector, electronics console, and visual display monitors. The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. Scanning Electron Microscopy (SEM) analaysis of synthesized tellurium nanoparticles was done using a Hitachi S-4500 SEM machine.

5.9 Antibacterial Activity

5.9.1 Agar Well Diffusion Method

Principle

The antimicrobials present in the given sample was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Materials Required

*Propionibacterium acnes and Streptococcus oralis*were purchased from MTCC, Chandigarh, India. Nutrient Agar medium, Nutrient broth, Gentamicin antibiotic solution was purchased from Himedia, India. Test samples, petri-plates, test tubes, beakers conical flasks were from Borosil, India. Spirit lamp, double distilled water.

Nutrient Agar Medium

The medium was prepared by dissolving 2.8 g of the commercially available Nutrient Agar Medium (HiMedia) in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

Nutrient broth

Nutrient broth was prepared by dissolving 2.8 g of commercially available nutrient medium (HiMedia) in 100ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

Procedure

Petri plates containing 20 ml nutrient agar medium were seeded with 24hr culture of bacterial strains *(P. acnes and S. oralis).* Wells were cut and different concentration of sample (500µg/ml, 250µg/ml, 100µg/ml and 50µg/ml) were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Gentamicin antibiotic was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA) (De Magaldi *et al*., 1997).

5.10Anti Fungal Activity

5.10.1 Agar Well Diffusion Method

Principle

The anti- fungal agent present in the given sample was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The

resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Materials Required

Potato dextrose agar medium, Amphotericin B antimycotic solution, test samples, test tubes, beakers, conical flask, spirit lamp, double distilled water and petri-plates.

Potato Dextrose Agar Medium

The potato dextrose agar medium was prepared by dissolving 20 gm of potato influsion, 2 gm of dextrose and 1.5 gm of agar in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100 mm petri plates (25-30 ml/plate) while still molten.

Procedure

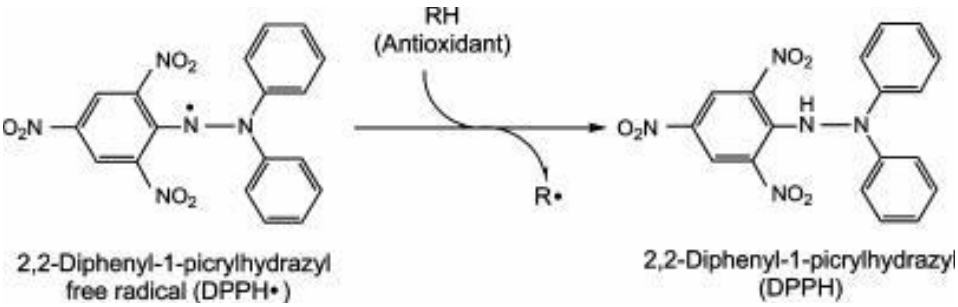
Petri plates containing 20 ml potato dextrose agar medium was seeded with 72 hr culture of fungal strain(*Cryptococcus neoformans and Aspergillus fumigatus*) wells were cut and different concentration of tellurium nanoparticle (500, 250, 100 and 50 μg/ml) was added. The plates were then incubated at 28°C for 72 hours. The anti-fungal activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Amphotericin B was used as a positive control. The

values were calculated using Graph Pad Prism 6.0 software (USA) (De Magaldi *et al*., 1997).

5.11 Antioxidant Activity

5.11.1 DPPH Radical Scavenging Activity

The DPPH assay is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. The figure shows the mechanism by which DPPH accepts hydrogen from an antioxidant. DPPH is one of the few stable and commercially available organic nitrogen radicals. The antioxidant effect is proportional to the disappearance of DPPH in test sample. Monitoring DPPH with a UV spectrometer has become the most commonly used method because of its simplicity and accuracy. DPPH shows a strong absorption maximum at 517 nm (purple). The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant. This reaction is stoichiometric with respect to the number of hydrogen atoms absorbed. Therefore, the antioxidant effect can be easily evaluated by following the decrease of UV absorption at 517 nm..



Materials Required

0.1mM DPPH solution, Ascorbic acid, Methanol

0.1mM DPPH Solution

Dissolve 39 mg of DPPH in 100 ml of methanol and store at -20^{o} C until needed.

Ascorbic acid (Standard)

1mg/ ml of Ascorbic acid

Procedure

1. Prepare 0.1 mM of DPPH solution in methanol and add 100 µl of this solution to 300 µl of the solution of sample at different concentration (500, 250, 100, 50 and 10 µg/mL).
2. The mixtures have to be shaken vigorously and allowed to stand at room temperature for 30 minutes.

3 Then the absorbance has to be measured at 517 nm using a UV-VIS spectrophotometer (Ascorbic acid was used as the reference).

4 Lower absorbance values of reaction mixture indicate higher free radical scavenging activity.

5 The capability of scavenging the DPPH radical can be calculated by using the following formula.

$$\text{DPPH scavenging effect (\% inhibition)} = \frac{\text{absorbance of control - absorbance of reaction mixture}}{\text{Absorbance of control}} \times 100$$

5.12 Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate.

Material Required

Hydrogen Peroxide solution and Sodium Phosphate buffer.

Procedure

Ability of extracts to scavenge hydrogen peroxide was estimated according to the method reported by Ruch *et al*(1989) with minor modification. A solution of hydrogen peroxide (43 mM) is prepared in phosphate buffer (1 M pH 7.4). Different concentration of tellurium nanoparticles (500, 250, 100, 50 and 10 µg/ml) was added to hydrogen peroxide solution (0.6 ml, 43 mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as standard. The free radical scavenging activity was determined by evaluating percentage inhibition (Ruch *et al*., 1989).

Percentage inhibition = [(Control- Test)/Control] ×100.

Figure 1: Map showing the study area Gulf of Mannar –

Thoothukudi Kayalpatnam Coastal region

Plate 1 Dorsal view of the shell *Cucullaea petita*

6. RESULTS

6.1 Synthesis characterization of Tellurium Nanoparticles

6.1.1 UV-Visible Spectroscopic Analysis

UV-Visible spectroscopic analysis confirmed the formation of the biosynthesized tellurium nanoparticles using the marine molluscan shell extract *Cucullaea petita*. The above solutions were subjected to optical measurements by UV-Visible spectrophotometer. In *C.petita*, the wavelength obtained around 300 nm suggested the presence of tellurium nanoparticles in the solution (Figure 2,3 and 4). This is the specific wavelength which indicates synthesized tellurium nanoparticles. The maximum absorption was obtained around 300 nm. The occurrence peak at absorption intensity between 300 to 1100 nm indicated the presence of surface plasmon resonance.

6.1.2 Fourier Transform InfraRed Spectroscopic Analysis

The IR spectra provided information about the local molecular environment of the organic molecules on the surface of nanoparticle. In the present work, FTIR spectral measurements were carried out to identify the potential biomolecules in *C.petita* shell extract which is responsible for reducing and capping the bioreduced tellurium nanoparticles. FTIR measurements were carried out to identify the

possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized by marine molluscan shell extract.

The results of FTIR analysis of this study show different stretches of bonds shown at different peaks; 3354.98, 2899.20, 1653.45, 1427.79, 1367.64, 1314.72, 1203.81, 1160.33, 1107.07, 1031.65, 896.80, 661.06, 609.00, 559.18, 436.60. The image shows a strong absorption peak around 3354.98cm^{-1} to 1427.79cm^{-1} which shows the presence of C-H stretching vibration. A peak around 800cm^{-1} to 1100 cm^{-1} shows the presence of C-O stretching frequency. A peak around finger print region confirms the presence of tellurium nanoparticles (Figure 5).

6.1.3 Atomic Force Microscopy

AFM technique is the one of the best tools for measuring nano sized materials. This method analysis the particle surface using tip, it is so high-pitched that as it is moved across something, the tip can feel the shape by measuring the forces between the atoms on the tip and the atoms on the object. An AFM topographical image of tellurium nanoparticles is shown in figure 6 which shows the flower like structure. The average length of the rock structure is in 32 nm. It may be due to the metal bindings.

6.1.4 SEM Analysis

Scanning Electron Microscopy is one of the best technique to characterize the nanostructures. Electron–sample interactions reveal surface morphology of the synthesized nanoparticle images of tellurium nanoparticles. SEM analysis shows high-density tellurium nanoparticles synthesized by *C. petita* shell extract (Figure 7). It was shown that relatively spherical and uniform tellurium nanoparticles were formed with diameter of 278.7 nm to 328.8 nm. The SEM image of tellurium nanoparticles was due to interactions of hydrogen bond and electrostatic interactions between the bioorganic capping molecules bound to the tellurium nanopaticles. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent. The larger tellurium particles may be due to the aggregation of the smaller ones, due to the SEM measurements.

6.2 Antibacterial Activity of Tellurium Nanoparticles

The antibacterial efficacy of synthesized tellurium nanoparticles of *C. petita* was investigated against some selected bacterial species *Propionibacterium acnes* and *Streptococcus oralis* by agar well diffusion method. The tellurium nanoparticles synthesized by the shell extract of *C. petita* developed maximum

inhibition zone against all pathogens tested. It has been reported that antibacterial activity was dose dependent.

In *C. petita* the antibacterial activity of tellurium nanoparticles showed maximum zone of inhibition against *Propionibacterium acnes* at the level of 11.5 mm at 500 µg/ml concentration followed by 7.5 mm at 250 µg/ml concentration. In *Streptococcus oralis* at the level of 14.5 mm at 500 µg/ml concentration followed by 13 mm at 250 µg/ml concentration respectively(Table 1) (Figure 8 and 9).

6.3 Antifungal Activity of Tellurium Nanoparticles

The antifungal efficacy of synthesized tellurium nanoparticles of *C. petita* was investigated against some selected fungal species *Aspergillus fumigatus* and *Cryptococcus neoformans* by agar well diffusion method. The tellurium nanoparticles synthesized by the shell extract of *C. petita* developed maximum inhibition zone against all pathogens tested. It has been reported that antifungal effect was dose dependent.

In *C.petita* the antifungal activity of tellurium nanoparticles showed maximum zone of inhibition against *Aspergillus fumigatus* at the level of 16.5 mm at 500 µg/ml concentration followed by 15.25 mm at 250 µg/ml concentration. *Cryptococcus neoformans* at the level of 13.5 mm at 500 µg/ml concentration

followed by 9.25 mm at 250 µg/ml concentration respectively(Table 2) (Figure10 and 11).

6.4 Antioxidant Activity

6.4.1 DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of marine molluscan shell extract of *C. petita* was observed at various concentrations of 500 µg/ml, 250 µg/ml, 100 µg/ml, 50 µg/ml and 10 µg/ml respectively. The highest percentage inhibition of 75.65% was observed at 500 µg/ml followed by 66.95% at 250 µg/ml, 63.1% at 100 µg/ml, 57.26% at 50 µg/ml and 54.98% at 10 µg/ml respectively. The percentage inhibition of 87.73% was found for the standard ascorbic acid. The IC_{50} value of 31.61 µg/ml was noted which shows the good antioxidant activity. It has been found that antioxidant activity was dose dependent and the percentage inhibition was found to increase with increase in the concentration respectively(Figure 12 and 13).

6.4.2 Hydrogen Peroxide Scavenging Assay

The hydrogen peroxide scavenging assay of marine molluscan shell extract of *C. petita* was observed at various concentrations of 500 µg/ml, 250 µg/ml, 100 µg/ml, 50 µg/ml and 10 µg/ml respectively. The highest percentage inhibition of 90.28% was observed at 500 µg/ml followed by 88.63% at 250 µg/ml, 87.14% at

100 µg/ml, 81.96% at 50 µg/ml and 70.31% at 10 µg/ml respectively. The percentage inhibition of 89.95% was found for the standard ascorbic acid. The IC_{50} value of 43.97µg/ml was noted which shows the good antioxidant activity. It has been found that antioxidant activity was dose dependent and the percentage inhibition was found to increase with increase in the concentration respectively (Figure 14 and 15).

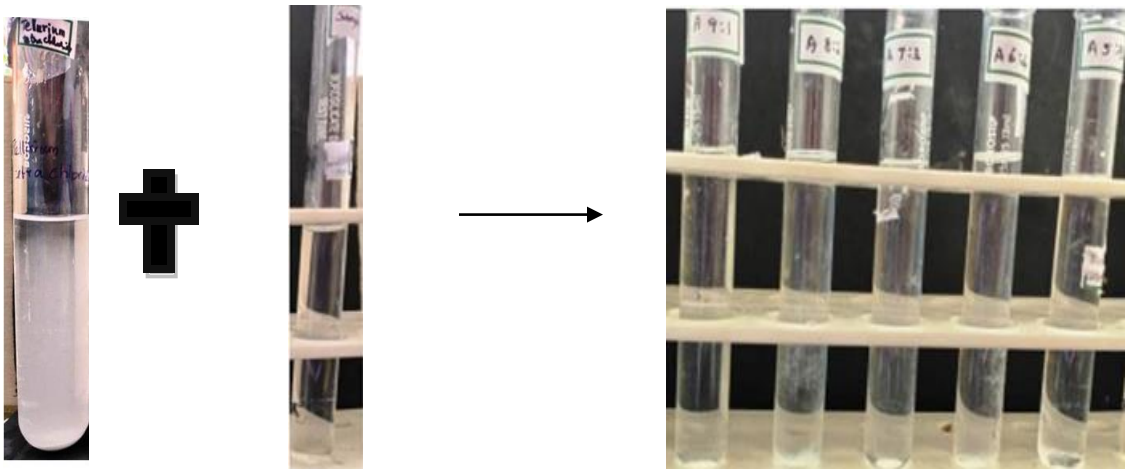


Figure 2. Synthesis of Tellurium Nanoparticles

Figure 3 : Synthesized Tellurium Nanoparticles using the Shell Extract of *C.petita*

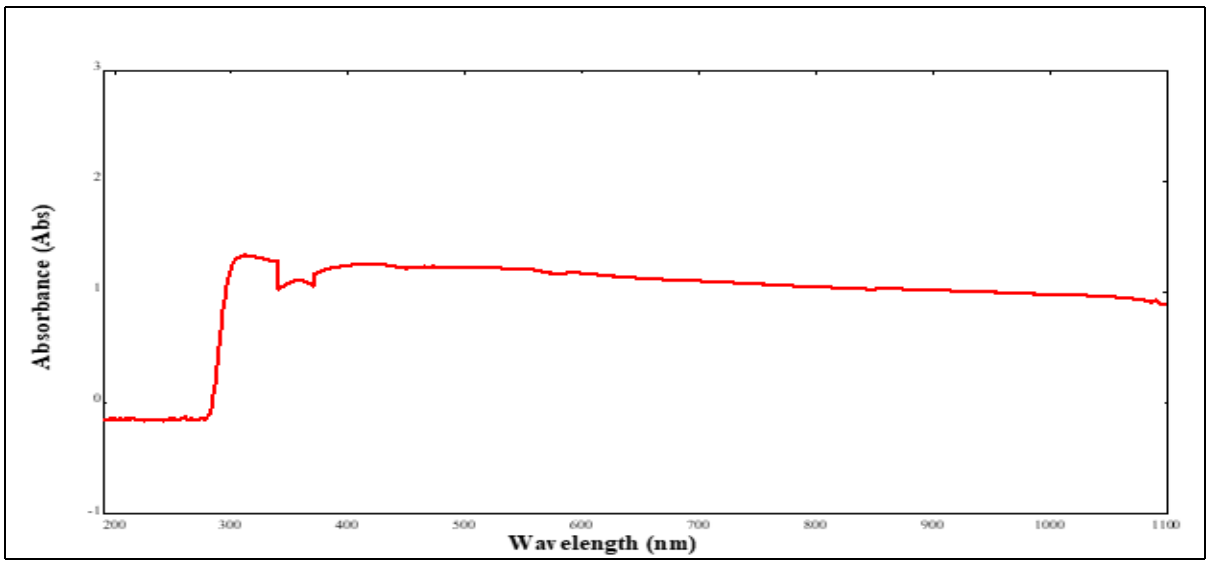


Figure 4 : UV-Visible Spectra of Tellurium Nanoparticles using *Cucullaea petita*

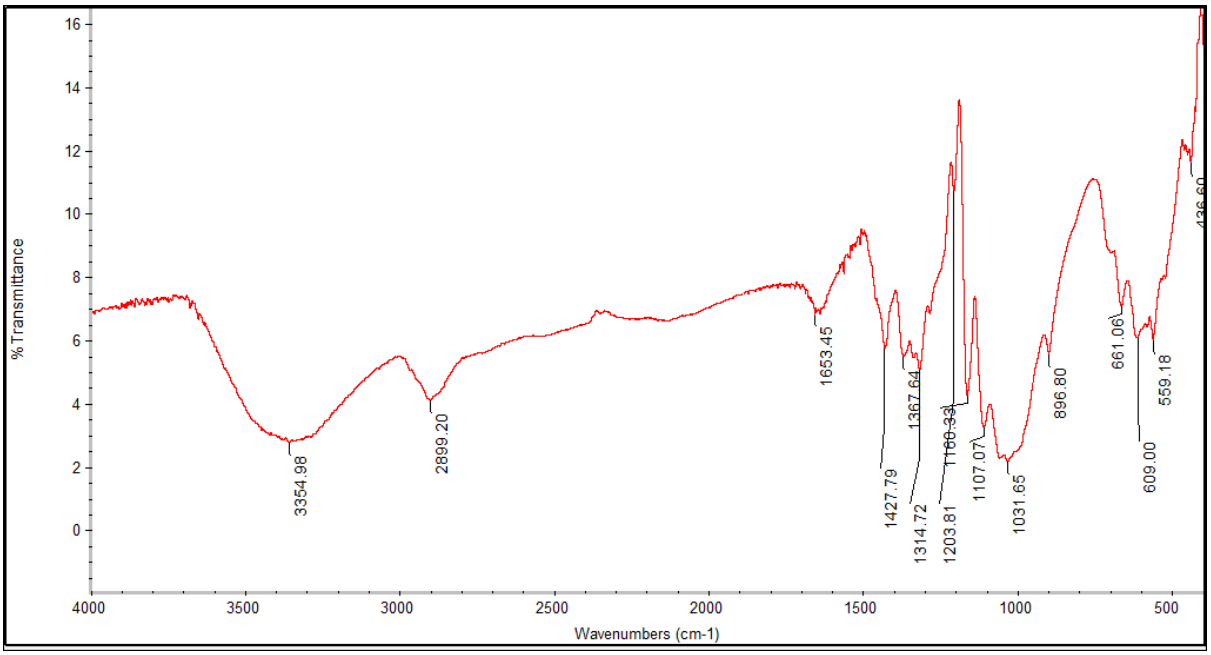


Figure 5: InfraRed Spectra of Tellurium Nanoparticles using *Cucullaea petita*

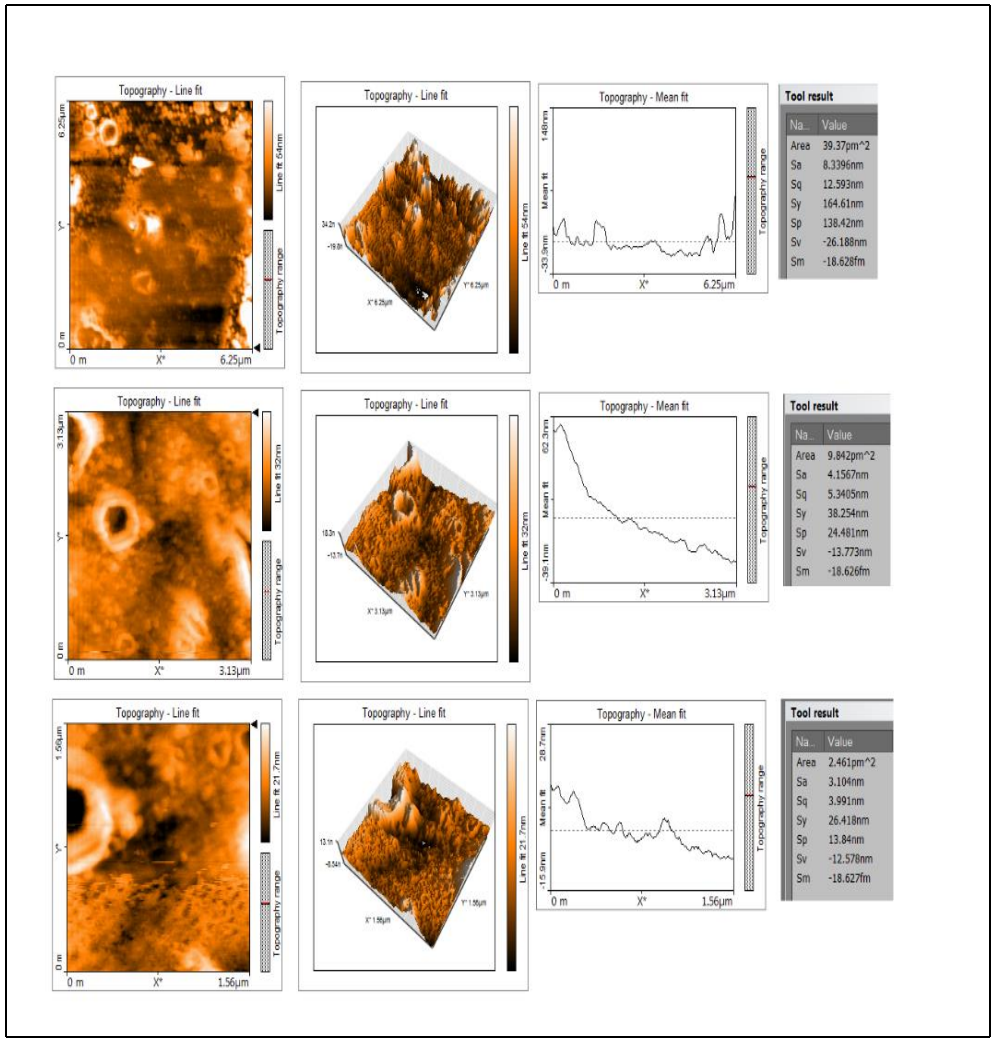


Figure 6:AFM Images of Tellurium Nanoparticles using *Cucullaea petita*

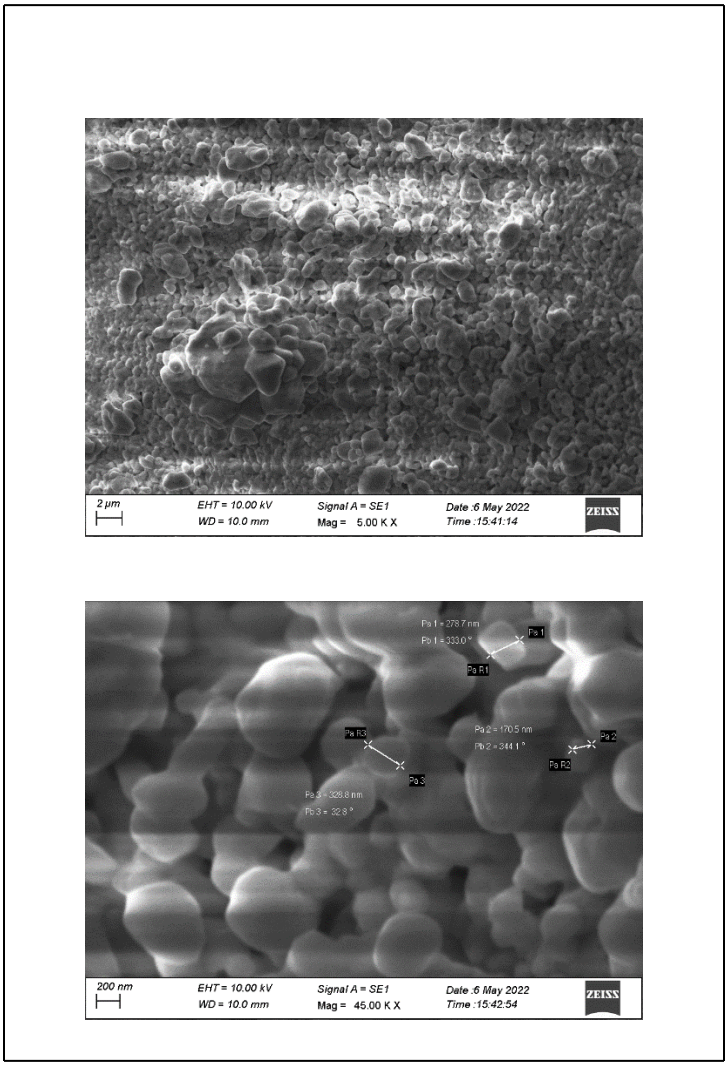


Figure 7: SEM Spectra of Tellurium Nanoparticles using *Cucullaea petita*

Table 1: SD± Means of zone of inhibition obtained by Tellurium nanoparticles against

Propionibacterium acnes and Streptococcus oralis

S.No.	NAME OF THE TEST ORGANISM	ZONE OF INHIBITION (mm) SD ± MEAN				
		PC	500 µg/µl	250 µg/µl	100 µg/µl	50 µg/µl
1.	***Propionibacterium acnes***	24±0	11.5±0.7	7.5±0.7	0	0
2.	***Streptococcus oralis***	12.5±0.7	14.5±0.7	13±0	11±1.4	11.5±0.7

Table 2. SD± Means of zone of inhibition obtained by Tellurium nanoparticles against

Aspergillus fumigatus and Cryptococcus neoformans

S.No.	NAME OF THE TEST ORGANISM	ZONE OF INHIBITION (mm)SD ± MEAN				
		PC	500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml
1.	*Cryptococcus neoformans*	15.25±0.35	13.5±0.7	9.25±0.35	0	0
2.	*Aspergillus fumigatus*	11.5±0.7	16.5±0.7	15.25±0.35	10.25±0.35	0

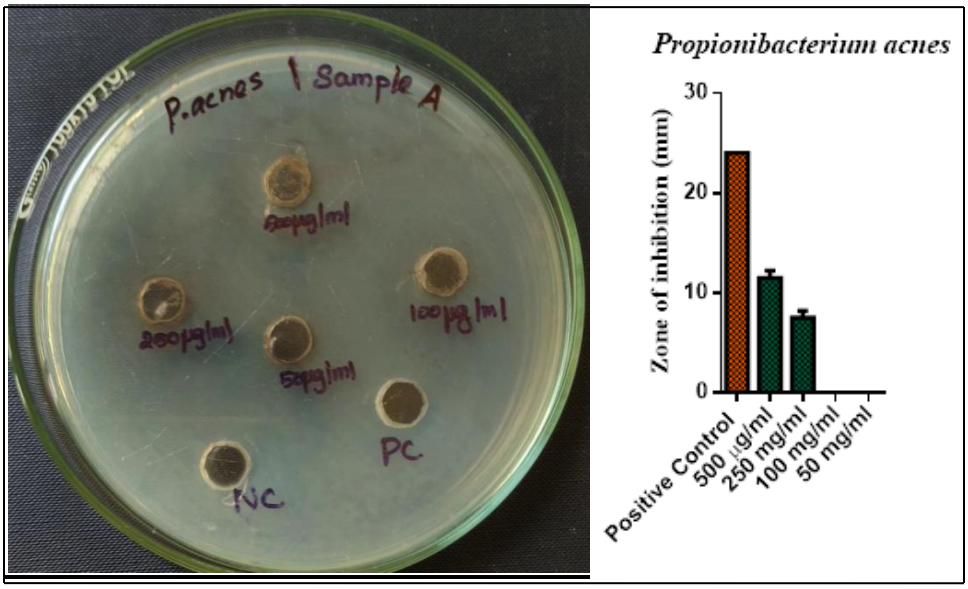


Figure 8: Antibacterial activity of Tellurium Nanoparticles using *C.petita* against *Propionibacterium acnes*

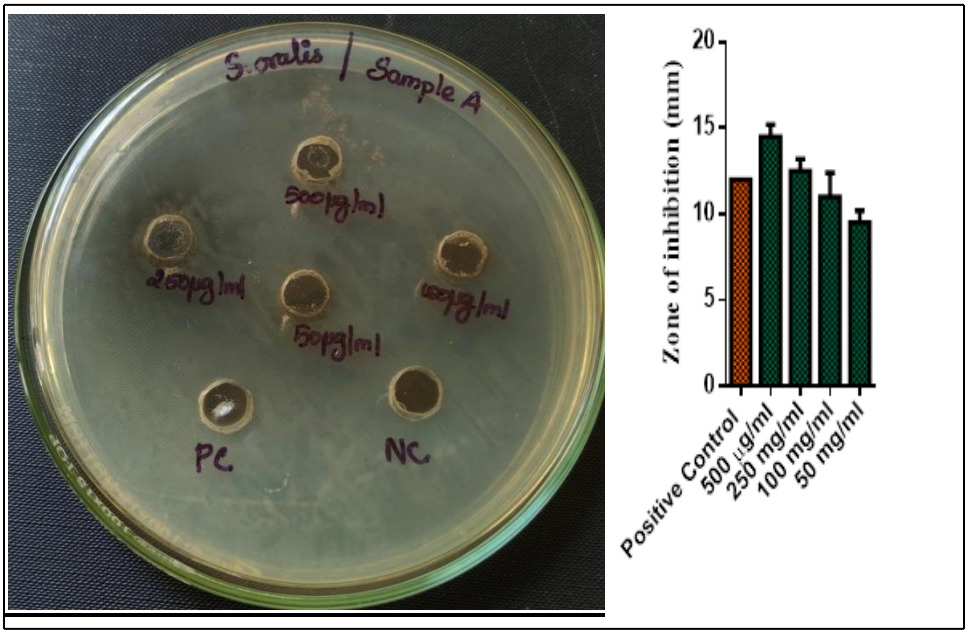


Figure 9:Antibacterial activity of Tellurium Nanoparticles using *C.petita* against *Streptococcus oralis*

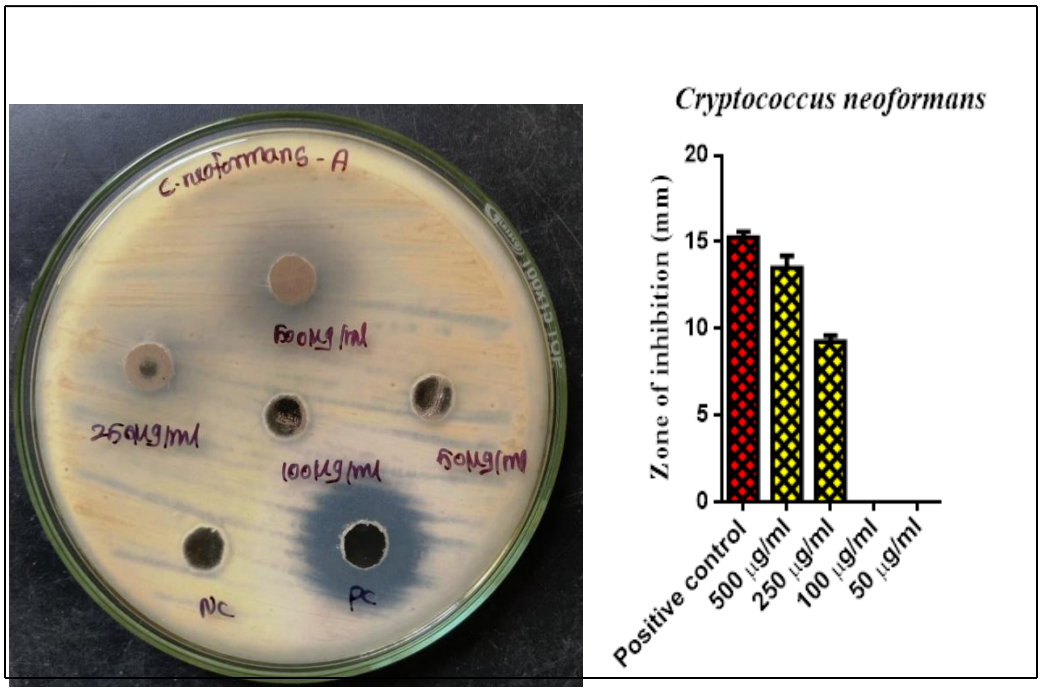


Figure 10: Antifungal activity of Tellurium Nanoparticles using *C.petita* against *Cryptococcus neoformans*

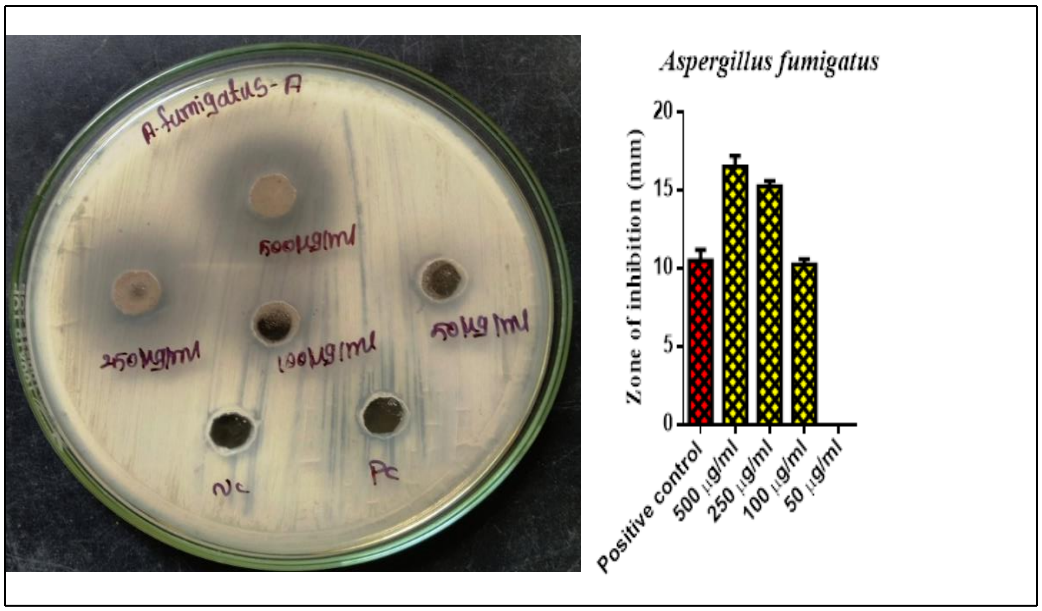


Figure 11.:Antifungal activity of Tellurium Nanoparticles using *C.petita* against *Aspergillus fumigatus*

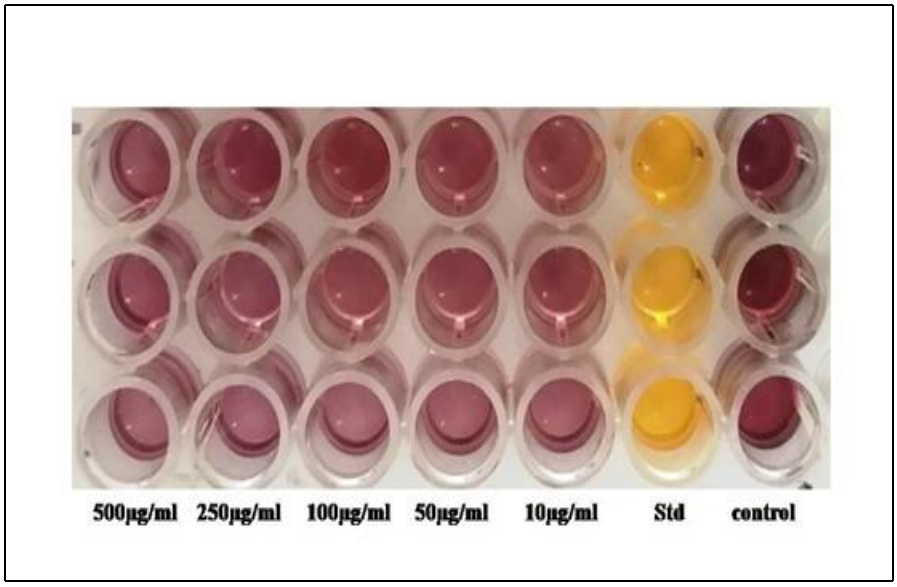


Figure 12:DPPH scavenging assay of Tellurium nanoparticles using *C. petita*

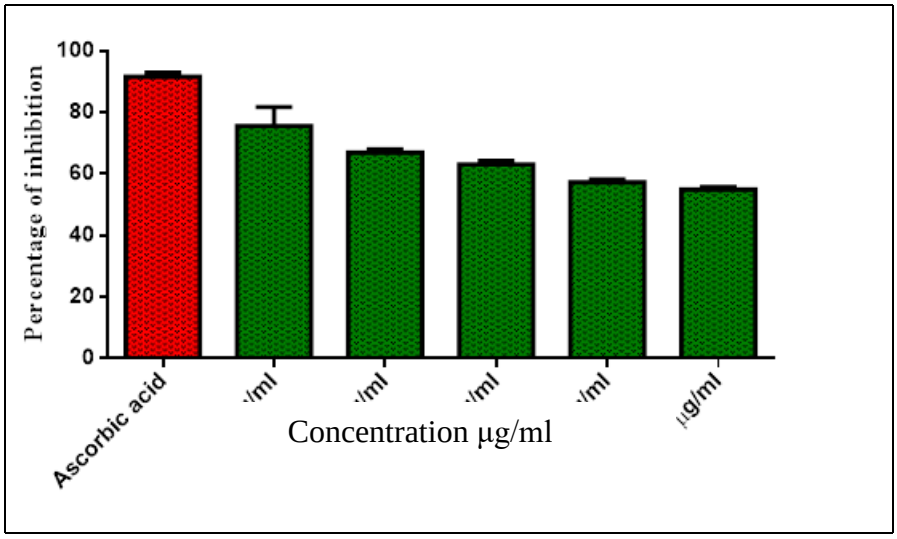


Figure 13:DPPH Scavenging Assay of Tellurium nanoparticles using *C. petita*

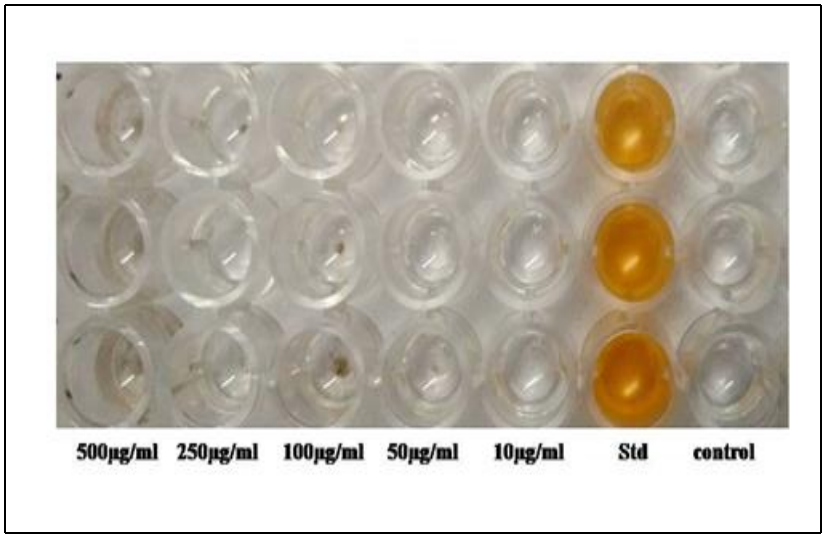


Figure 14: H_2O_2 Scavenging Activity Of Tellurium Nanoparticles using *C. petita*

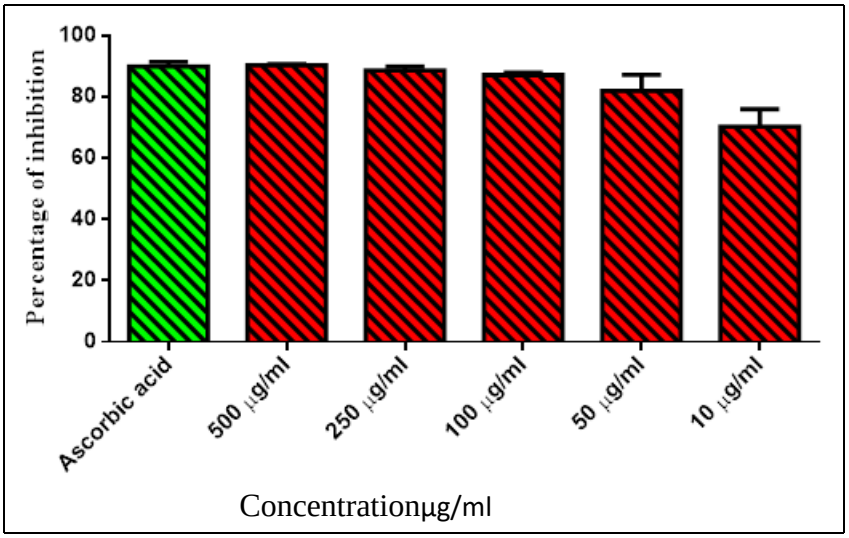


Figure 15:H_2O_2 Scavenging Assay Tellurium nanoparticles using *C. petita*

7.DISCUSSION

Nanotechnology represents a crucial turning point in the history of the universe. The endless desire and intelligence of humans have paved the way for groundbreaking inventions such as the internet, smartphones, rocket science, artificial intelligence and in vitro-fertilization techniques, which also carry many moral issues; for this reason, nanotechnology is a field of science that was previously considered to be controversial. Overcoming these drawbacks, nanotechnology has emerged as an important tool in material science, which involves the fabrication, synthesis and manipulation ofbulk molecules/particles in nanoscale dimensions. The exploitation of metal nanoparticles is beneficial due to their aberrant properties, such as their physical properties, reactivity and probable applications in diagnostics, drug delivery and antioxidant and antimicrobial studies (Deepak Bamal *et al*., 2021).

The formation of tellurium nanoparticles was confirmed by the UV–Vis spectrophotometer (300–600 nm). The absorbance band around 300 nm was recorded due to localized surface plasmon resonance (LSPR) and confirmed the formation of tellurium nanoparticles. Surface plasmon resonance (SPR) is the basis of this observation. Based on this phenomenon (SPR), the free electrons of colloidal metal nanoparticles begin to oscillate with specific energy. Hence, any

wavelength of light are absorbed at that energy resulting in color change compared to the original metal color and/or metallic salt solution (Barabadi *et al*., 2014; Honary, 2013). In *C.petita* the wavelength obtained around 300 nm suggested the presence of tellurium nanoparticles in the solution (Figure 2,3 and 4).

Fourier Transform InfraRed Spectroscopy (FTIR) is a technique which is used to analyze the chemical composition of many organic chemicals, polymers, paints, coatings, adhesives, lubricants, semiconductor materials, coolants, gases, biological samples, inorganics, and minerals. FTIR can be used to analyze a wide range of materials in bulk or thin films, liquids, solids, pastes, powders, fibres, and other forms. FTIR analysis can give not only qualitative (identification) analysis of materials, but, with relevant standards, can be used for quantitative (amount) analysis. FTIR can be used to analyze samples up to ~11 millimetres in diameter and either measure in bulk or the top ~1 micrometer layer. The results of FTIR analysis of this study show different stretches of bonds shown at different peaks; 3354.98, 2899.20, 1653.45, 1427.79, 1367.64, 1314.72, 1203.81, 1160.33, 1107.07, 1031.65, 896.80, 661.06, 609.00, 559.18, 436.60. The image shows a strong absorption peak around 3354.98 cm^{-1} to 1427.79 cm^{-1} which shows the presence of C-H stretching vibration. A peak around 800 cm^{-1} to 1100 cm^{-1} shows the presence of C-O stretching frequency. A peak around finger print region confirms the presence of tellurium nanoparticles (Figure 5).

Atomic Force Microscopy is used to investigate the dispersion and aggregation of nanomaterials, in addition to their size, shape, absorption, and structure; three different scanning modes are available, including contact mode, non-contact mode, and intermittent sample contact mode (Lin *et al*., 2014). AFM can also be used to characterize the interaction of nanomaterials with supported lipid bilayers in real time, which is not achievable with current electron microscopy (EM) techniques (Stephan *et al*., 2006). In the present study, an AFM topographical image of tellurium nanoparticles is shown in figure 6 which shows the flower like structure. The average length of the rock structure is in 32 nm. It may be due to the metal bindings.

Recently, the field of nanoscience and nanotechnology has provided a driving force in the development of various high-resolution microscopy techniques in order to learn more about nanomaterials using a beam of highly energetic electrons to probe objects on a very fine scale (Yao and Kimura, 2007). Among various electron microscopy techniques, SEM is a surface imaging method, fully capable of resolving different particle sizes, size distributions, nanomaterial shapes, and the surface morphology of the synthesized particles at the micro and nanoscales. Using SEM, we can probe the morphology of particles and derive a histogram from the images by either by measuring and counting the particles manually, or by using specific software (Fissan *et al*., 2014). In the current study, it

was shown that relatively spherical and uniform tellurium nanopaticles were formed with diameter of 278.7 nm to 328.8 nm(Figure 7).

The selection of biological methods for synthesis and engineering of nanoparticles is dependent upon several variables. The form of the metal nanoparticle to be synthesized is the most important variable. Resistance developed against a small number of metals by the organisms limit the choice of organisms. Following are a number of the microbial resources (algae, fungi, bacteria, viruses, and yeast) used for most of the frequently studied metal and metal salts nanoparticles consisting of copper, silver, gold, cadmium, platinum, palladium, cadmium sulfide, titanium dioxide, and zinc oxide (Mousavi *et al*., 2018; Gahlawat and Roy Choudhury, 2019).

The biosynthesis of Ag and AuNPs has been a focal point of research because of their antimicrobial attributes. The extensive studies were conducted to synthesize the metallic nanoparticles using Bacillus species due to their metal accumulating abilities (Pugazhenthiran *et al*., 2009). The antibacterial activity of AgNPs was determined against four species of Gram-negative foodborne pathogens: *E. coli* ATCC 25922, *K. pneumoniae* ATCC 13773, *S.* Typhimurium ATCC 14028, and *S.* Enteritidis ATCC 13076. For the disk diffusion test, the presence of clear zone around the AgNPs disk suggesting that the AgNPs possessed antibacterial activity which is able to inhibit the growth of the Gram-

negative foodborne pathogens (Yuet Ying Loo *et al*., 2018). Guzman *et al*., (2012) reported that AgNPs employed antibacterial activity on Gram-negative bacteria.

The inhibition of bacteria growth was reported affected by the concentration of AgNPs and bacteria used in the experiments (Sondi and Salopek-Sondi, 2004). The green synthesized AgNPs in this study are able to inhibit the high concentration of bacteria (approximately 10^6 CFU/mL). This indicated that AgNPs showed an excellent antimicrobial effect as the high CFU concentration of bacteria used in this study are rarely appeared in real-life systems. The antibacterial activity of AgNPs has been reported by many researchers. However, the MIC values from the previous studies showed the range through a large extent of variation. Therefore, the comparison of the results is difficult as there is no standard method for determination of antibacterial activity of AgNPs and different methods have been applied by the researchers (Zarei*et al*., 2014).

In *C. petita* the antibacterial activity of tellurium nanoparticles showed maximum zone of inhibition against *Propionibacterium acnes* at the level of 11.5 mm at 500 µg/ml concentration followed by 7.5 mm at 250 µg/ml concentration. In *Streptococcus oralis* at the level of 14.5 mm at 500 µg/ml concentration followed by 13 mm at 250 µg/ml concentration respectively (Figure 8 and 9).

However, the chemical antimicrobial agents are limited to use especially in medical field as various microorganisms have developed multiple resistance traits

over a period of generations. Thus, the development of tellurium nanoparticles could be an alternative way to overcome the multidrug resistance microorganisms as bacteria are less likely to develop resistance to metal nanoparticles compared to the conventional antibiotics.

There is a trend to perform antifungal tests for different types of silver nanoparticles obtained either by engineering or biological synthesis. The effects observed by different authors depend on the size of the nanoparticles, the stabilizer used, the tested concentrations, and on the species of fungi that was assessed. Different types of silver nanoparticles are known to have antifungal activity against the clinical isolates of human pathogens, for example, against Candida spp. and dermatophytes (Panacek *et al*., 2009). Such activity is manifested by both engineering nanoparticles and Bio-AgNPs. For example, chitosan-conjugated Bio-AgNPs at concentrations of 50 mg/L have an antifungal effect against most clinical Candida isolates tested. The morphological study demonstrates the significant, harmful effects that pathogen cells suffer under the action of these nanoparticles (Vijayan *et al*., 2020). Another type of silver nanoparticles obtained by biosynthesis and stabilized with starch tested on the Candida albicans BWP17 isolate was proven to have significant antifungal activity, with a minimum inhibitory concentration (at 80% inhibition,MIC80) of 280 μg/mL (Prasher *et al*., 2018).

In the present study, *C. petita* the antifungal activity of tellurium nanoparticles showed maximum zone of inhibition against *Aspergillus fumigatus* at the level of 16.5 mm at 500 µg/ml concentration followed by 15.25 mm at 250 µg/ml concentration. *Cryptococcus neoformans* at the level of 13.5 mm at 500 µg/ml concentration followed by 9.25 mm at 250 µg/ml concentration respectively (Figure 10and 11).

Kharat and Mendhulkar (2016) studied the antioxidant activity of synthesized nanoparticles using DPPH assay and observed the antioxidant potentials of photosynthesized nanoparticles. Priya *et al*., (2016) studied *in vitro* antioxidant activity of biosynthesized nanoparticles from *P. pinnata* extract and found significant free radical scavenging potential. Patra and Baek (2016) demonstrated presence of strong antioxidant activity in terms of DPPH radical scavenging (IC_{50} 385.87 µg/mL). The results strongly recommend the application of AgNPs as useful natural antioxidants for health preservation against different oxidative stress associated with degenerative diseases. In fact, antioxidant evaluation is essential for AgNPs before its use *in vivo* models and also human applications.

The DPPH radical scavenging activity of marine molluscan shell extract of *C. petita* was observed at various concentrations of 500 µg/ml, 250 µg/ml, 100 µg/ml, 50 µg/ml and 10 µg/ml respectively. The highest percentage of inhibition of

75.65% was observed at 500 µg/ml followed by 66.95% at 250 µg/ml, 63.1% at 100 µg/ml, 57.26% at 50 µg/ml and 54.98% at 10 µg/ml respectively. The percentage of inhibition of 87.73% was found for the standard ascorbic acid. The IC_{50} value of 31.61 µg/ml was noted which shows the good antioxidant activity (Figure 12 and 13).

The hydrogen peroxide scavenging assay of marine molluscan shell extract of *C. petita* was observed at various concentrations of 500 µg/ml, 250 µg/ml, 100 µg/ml, 50 µg/ml and 10 µg/ml respectively. The highest percentage of inhibition of 90.28% was observed at 500 µg/ml followed by 88.63% at 250 µg/ml, 87.14% at 100 µg/ml, 81.96% at 50 µg/ml and 70.31% at 10 µg/ml respectively. The percentage of inhibition of 89.95% was found for the standard ascorbic acid. The IC_{50} value of 43.97 µg/ml was noted which shows the good antioxidant activity (Figure 14 and 15).

8.SUMMARY

- In the present study,*C.petita* showed the wavelength around 300 nm suggested the presence of tellurium nanoparticles in the solution.
- The results of FTIR analysis of this study show different stretches of bonds shown at different peaks; 3354.98, 2899.20, 1653.45, 1427.79, 1367.64, 1314.72, 1203.81, 1160.33, 1107.07, 1031.65, 896.80, 661.06, 609.00, 559.18, 436.60 respectively.
- An AFM topographical image of tellurium nanoparticles shows the flower like structure. The average length of the flower structure is in 32 nm. It may be due to the metal bindings.
- SEM analysis shows high-density tellurium nanoparticles synthesized by *C. petita* shell extract. It was shown that relatively spherical and uniform tellurium nanoparticles were formed with diameter of 278.7 nm to 328.8 nm.
- In *C. petita* the antibacterial activity of tellurium nanoparticles showed maximum zone of inhibition against *Propionibacterium acnes* at the level of 11.5 mm at 500 µg/ml concentration followed by 7.5 mm at 250 µg/ml concentration. In *Streptococcus oralis* at the level of 14.5 mm at 500 µg/ml concentration followed by 13 mm at 250 µg/ml concentration respectively.

- In *C. petita* the antifungal activity of tellurium nanoparticles showed maximum zone of inhibition against *Aspergillus fumigatus* at the level of 16.5 mm at 500 µg/ml concentration followed by 15.25 mm at 250 µg/ml concentration. *Cryptococcus neoformans* at the level of 13.5 mm at 500 µg/ml concentration followed by 9.25 mm at 250 µg/ml concentration respectively.
- The DPPH radical scavenging activity of marine molluscan shell extract of *C. petita* was observed at various concentrations of 500 µg/ml, 250 µg/ml, 100 µg/ml, 50 µg/ml and 10 µg/ml respectively. The highest percentage of inhibition of 75.65% was observed at 500 µg/ml followed by 66.95% at 250 µg/ml, 63.1% at 100 µg/ml, 57.26% at 50 µg/ml and 54.98% at 10 µg/ml respectively. The percentage of inhibition of 87.73% was found for the standard ascorbic acid. The IC_{50} value of 31.61 µg/ml was noted which shows the good antioxidant activity.
- The hydrogen peroxide scavenging assay of marine molluscan shell extract of *C. petita* was observed at various concentrations of 500 µg/ml, 250 µg/ml, 100 µg/ml, 50 µg/ml and 10 µg/ml respectively. The highest percentage of inhibition of90.28% was observed at 500 µg/ml followed by 88.63% at 250 µg/ml, 87.14% at 100 µg/ml, 81.96% at 50 µg/ml and 70.31% at 10 µg/ml respectively. The percentage of inhibition of 89.95%

was found for the standard ascorbic acid. The IC_{50} value of 43.97 µg/ml was noted which shows the good antioxidant activity.

9.CONCLUSION AND SUGGESTIONS

Green nanotechnology presented as a feasible solution that is able to produce materials with significant antimicrobial and antioxidant activity, while overcoming the main limitations of traditional synthesis. Nanoparticle synthesis using microorganisms, plants and animals by green synthesis technology is biologically safe, cost-effective, and environment-friendly.

In the present study tellurium nanoparticles have been synthesized from the marine bivalve *Cucullaea petita*. The synthesized tellurium nanoparticles were synthesized and characterized using UV-Vis, FTIR, AFM and SEM. The nanoparticles were also analyzed for biological applications viz., antibacterial, antifungal and antioxidant activities.

Studying the antimicrobial effort of tellurium nanoparticles on bacterial and fungal strains revealed a good activity against *Propionibacterium acnes*, *Streptococcus oralis*, *Aspergillus fumigatus* and *Cryptococcus neoformans*. The synthesized nanoparticles showed good antioxidant activity. It has been found that antioxidant activity was dose dependent and the percentage inhibition was found to increase with increase in the concentration respectively.

These biosynthesized metallic nanoparticles have a range of unlimited pharmaceutical applications including delivery of drugs or genes, detection of pathogens or proteins, and tissue engineering.Improvement of reliable and eco-friendly processes for the synthesis of metallic nanoparticles is a significant step in the field of applied nanotechnology. Further, most of these strategies are still under the developmental stage and challenges need to be taken care of. The separation and purification of nanoparticles is another vital parameter which needs to be explored further.

10. BIBLIOGRAPHY

Ali, S. M., Yousef, N. M. H., & Nafady, N. A., 2015. Application of Biosynthesized Silver Nanoparticles for the Control of Land Snail *Eobania vermiculata* and Some Plant Pathogenic Fungi. *Journal of Nanomaterials*: 1–10.

Anderson, D. L., 1983. Chemical Composition of the Mantle. *Journal of Geophysical Researc.*, 88(S01), B41.

Bamal, D., Singh,A..,Chaudhary,G., Kumar, M.,Singh, M., Rani, N.,Mundlia, P., & Sehrawat, A.. R., 2021. Silver Nanoparticles Biosynthesis, Characterization, Antimicrobial Activities, Applicants, Cytotoxicity and Safety Issues: An Updated Review. *Nanomaterials*,11(8*):*2086.

Banoee, M., Seif, S., Nazari, Z. E., Jafari-Fesharaki, P., Shahverdi, H. R., Moballegh, A., Moghaddam, K. M., & Shahverdi, A. R., 2010. ZnO Nanoparticles Enhanced Antibacterial Activity of Ciprofloxacin Against *Staphylococcus aureus* and *Escherichia coli*. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 93B(2): 557–561.

Barabadi, H., Honary, S., Ebrahimi, P., Mohammadi, M. A., Alizadeh, A., & Naghibi, F., 2014. Microbial Mediated Preparation, Characterization and Optimization Of Gold Nanoparticles. *Brazilian Journal of Microbiology*, *45*(4): 1493–1501.

Bhakya, S., Muthukrishnan, S., Sukumaran, M., &Muthukumar, M., 2015. Biogenic synthesis of silver nanoparticles and their antioxidant and antibacterial activity. *Applied Nanoscience*, 6(5):755–766.

Brooks, R., 2021. A Slow Passion: Snails, My Garden and Me. Bloomsbury Publishing.

Cookson, J., 2012. The Preparation of Palladium Nanoparticles.*Platinum Metals Review*, 56(2) *:*83–98.

David J.Dunstan and David J Hodgson., 2014.SnailsHome.*Phys.Scr*.89:1:10

Devaraj, P., Kumari, P., Aarti, C., &Renganathan, A., 2013. Synthesis and Characterization of Silver Nanoparticles Using Cannonball Leaves and Their Cytotoxic Activity against MCF-7 Cell Line. *Journal of Nanotechnology*:1–5.

Don L. Anderson.,1983. Chemical composition of the mantle. *Journal of Geophysical Research*,Volume 88.

Dunstan, D. J., & Hodgson, D. J., 2014. Snails home. *Physica Scripta*, 89(6): 068002.

Fissan H., Ristig S., Kaminski H., Asbach C., Epple M., 2014. Comparison of different characterization methods for nanoparticle dispersions before and after aerosolization. *Anal. Methods.*6:7324–7334.

Gahlawat, G., and Roy Choudhury, A., 2019. A review on the biosynthesis of metal and metal salt nanoparticles by microbes. *RSC Adv*. 9:12944–12967.

Guzman, M., Dille, J., and Godet, S., 2012. Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. *Nanomedicine* 8: 37–45.

Harris, R. C., Söderlund, K., & Hultman, E., 1992. Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. *Clinical Science,* 83(3): 367–374.

Honary S, Barabadi H, Gharaei-Fathabad E and Naghibi F.,2013. Green synthesis of silver nanoparticles induced by the fungus *Penicillium citrinum. Trop. J. Pharm. Res.* 12: 7-11.

Huang, J., Li, Q., Sun, D., Lu, Y., Su, Y., Yang, X., Wang, H., Wang, Y., Shao, W., He, N., Hong, J., & Chen, C., 2007. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. *Nanotechnology*, *18*(10), 105104.

Inbakandan, D., Venkatesan, R., & Ajmal Khan, S. (2010). Biosynthesis of gold nanoparticles utilizing marine sponge Acanthella elongata (Dendy, 1905). *Colloids and Surfaces B: Biointerfaces*, 81(2): 634–639.

Ioni, Y., Buslaeva, E., & Gubin, S. (2016). Synthesis of Graphene with Noble Metals Nanoparticles on its Surface. *Materials Today: Proceedings*, 3:S209–S213.

Jagiełło, J., Chlanda, A., Baran, M., Gwiazda, M., & Lipińska, L., 2020. Synthesis and Characterization of Graphene Oxide and Reduced Graphene Oxide Composites with Inorganic Nanoparticles for Biomedical Applications. *Nanomaterials*, 10(9), 1846.

Kharat, S. N., and Mendhulkar, V. D., 2016. Synthesis, characterization and studies on antioxidant activity of silver nanoparticles using *Elephantopus scaber* leaf extract. *Mater. Sci. Eng. C* 62: 719–724.

Kora, A. J., & Rastogi, L., 2018. Green synthesis of palladium nanoparticles using gum ghatti (*Anogeissus latifolia*) and its application as an antioxidant and catalyst. *Arabian Journal of Chemistry*, 11(7):1097–1106.

Kumar, A., John, L., Maity, S., Manchanda, M., Sharma, A., Saini, N., Chakraborty, K., & Sengupta, S., 2011. Converging Evidence of Mitochondrial Dysfunction in a Yeast Model of Homocysteine Metabolism Imbalance. *Journal of Biological Chemistry*, 286(24) :21779–21795.

Kumari, S., Sharma, P., Yadav, S., Kumar, J., Vij, A., Rawat, P., Kumar, S., Sinha, C., Bhattacharya, J., Srivastava, C. M., & Majumder, S., 2020. A Novel Synthesis of the Graphene Oxide-Silver (GO-Ag) Nanocomposite for Unique Physiochemical Applications. *ACS Omega*, 5(10):5041–5047.

Last, J. A., Russell, P., Nealey, P. F., & Murphy, C. J., 2010. The Applications of Atomic Force Microscopy to Vision Science. *Investigative Opthalmology & Visual Science*, 51(12), 6083.

Lin P.C., Lin S., Wang P.C., Sridhar R., 2014 . Techniques for physicochemical characterization of nanomaterials. *Biotechnol. Adv*.32:711–726.

Loo,Y. Y., Rukayadi, Y., Nor-Khaizura, M. A. R., Kuan, C. H., Chieng, B. W., Nishibuchi, M.,& Radu, S., 2018. In Vitro Antimicrobial Activity of Green Synthesized Silver Nanoparticles Against Selected Gram-negative Foodborne Pathogens. *Frontiers in Microbiology*,9.

Majumdar, R., Tantayanon, S., & Bag, B. G., 2017. Synthesis of palladium nanoparticles with leaf extract of *Chrysophyllum cainito* (star apple) and

their applications as efficient catalyst for C–C coupling and reduction reactions. *International Nano Letters*, 7(4): 267–274.

Mallikarjuna, K., Reddy, L. V., Al-Rasheed, S., Mohammed, A., Gedi, S., & Kim, W. K., 2021. Green Synthesis of Reduced Graphene Oxide-Supported Palladium Nanoparticles by Coleus amboinicus and Its Enhanced Catalytic Efficiency and Antibacterial Activity. *Crystals*, 11(2): 134.

Medina Cruz, D., Tien-Street, W., Zhang, B., Huang, X., Vernet Crua, A., Nieto-Argüello, A., Cholula-Díaz, J. L., Martínez, L., Huttel, Y., González, M. U., García-Martín, J. M., & Webster, T. J., 2019. Citric juice-mediated synthesis of tellurium nanoparticles with antimicrobial and anticancer properties. *Green Chemistry*, 21(8): 1982–1998.

Merin, D. D., Prakash, S., & Bhimba, B. V., 2010. Antibacterial screening of silver nanoparticles synthesized by marine micro algae. *Asian Pacific Journal of Tropical Medicine*, 3(10):797–799.

Miyazaki, K., & Islam, N., 2007. Nanotechnology systems of innovation-An analysis of industry and academia research activities. *Technovation*, 27(11): 661–675.

Mourdikoudis, S., Pallares, R. M., & Thanh, N. T. K., 2018. Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties. *Nanoscale*, 10(27) : 12871–12934.

Mousavi, S. M., Hashemi, S. A., Younes, G., Atapour, A., Amani, A., Savardashtaki, A., *et al.*,2018. Green synthesis of silver nanoparticles toward bio and medical applications: review study. *Artif. Cells.*,46: S855-72.

O'Bryan, C. J., Holden, M. H., & Watson, J. E., 2019. The mesoscavenger release hypothesis and implications for ecosystem and human well-being. *Ecology Letters*, 22(9):1340–1348.

Panacek, A.; Kolar, M.; Vecerova, R.; Prucek, R.; Soukupova, J.; Krystof, V.; Hamal, P.; Zboril, R.; Kvıtek, L., 2009. Antifungal activity of silver nanoparticles against *Candida spp. Biomaterials:* 6333-6340.

Patra, J. K., and Baek, K. H., 2016. Biosynthesis of silver nanoparticles using aqueous extract of silky hairs of corn and investigation of its antibacterial and anticandidal synergistic activity and antioxidant potential. *IET Nanobiotechnol*. 10:326–333.

Peiris, J., 2012. Coronaviruses. *Medical Microbiology*:587–593.

Prasher, P.; Singh, M.; Mudila, H., 2018. Green Synthesis of Silver Nanoparticles and their Antifungal Properties. *BioNanoScience* , 8:254-263.

Priya, R. S., Geetha, D., and Ramesh, P. S., 2016. Antioxidant activity of chemically synthesized AgNPs and biosynthesized Pongamia pinnata leaf extract mediated AgNPs – A comparative study. *Ecotoxicol. Environ. Saf. 134*: 308–318.

Pugazhenthiran, N., Anandan, S., Kathiravan, G., Udaya Prakash, N. K., Crawford,S., and Ashokkumar, M., 2009. Microbial synthesis of silver nanoparticles by Bacillus sp. J. *Nanoparticle Res*. 11:1811.

Radovic-Moreno, A. F., Lu, T. K., Puscasu, V. A., Yoon, C. J., Langer, R., & Farokhzad, O. C., 2012. Surface Charge-Switching Polymeric Nanoparticles for Bacterial Cell Wall-Targeted Delivery of Antibiotics. *ACS Nano,* 6(5): 4279–4287.

Richard P. Feynman. "There's plenty of Room at the Bottom",Volume 254,no.5036.

Saad, S., Cereghetti, G., Feng, Y., Picotti, P., Peter, M., &Dechant, R., 2017. Reversible protein aggregation is a protective mechanism to ensure cell cycle restart after stress. *Nature Cell Biology*, 19(10):1202–1213.

Safat, S., Buazar, F., Albukhaty, S., & Matroodi, S., 2021. Enhanced sunlight photocatalytic activity and biosafety of marine-driven synthesized Cerium oxide nanoparticles. *Scientific Reports*, 11(1).

Sagadevan, S., 2015. Analysis of Structure, Surface Morphology, Optical and Electrical Properties of Copper Nanoparticles. *Journal of Nanomedicine Research*, 2(5).

Sandeep Kumar., 2012. Antimicrobial activity of SalvadoraoleoidesDecne. against some microorganisms. *Journal of Medicinal Plants Research*, 6(14).

Shah, M., Fawcett, D., Sharma, S., Tripathy, S., & Poinern, G., 2015. Green Synthesis of Metallic Nanoparticles via Biological Entities. *Materials*, 8(11):7278–7308.

Siddiqi, K. S., & Husen, A., 2016. Green Synthesis, Characterization and Uses of Palladium/Platinum Nanoparticles. *Nanoscale Research Letters*, 11(1).

Sies, H., 1997. Oxidative stress: oxidants and antioxidants. *Experimental Physiology*, 82(2):291–295.

Smith, A. T., LaChance, A. M., Zeng, S., Liu, B., & Sun, L., 2019. Synthesis, properties, and applications of graphene oxide/reduced graphene oxide and their nanocomposites. *Nano Materials Science*, 1(1):31–47.

Sondi, I., and Salopek- Sondi, B., 2004. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *J. Colloid Interface Sci.* 275: 177–182.

Stephan T.S., Scott E.M., Anil K.P., Marina A.D.,2006. Preclinical characterization of engineered nanoparticles intended for cancer therapeutics. In: Amiji M.M., editor. *Nanotechnology for Cancer Therapy*. *CRC Press; Boca Raton, FL, USA*: 2006: 105–137.

Talapin, D. V., & Shevchenko, E. V., 2016. Introduction: Nanoparticle Chemistry. *Chemical Reviews*, *116*(18):10343–10345.

Tanwar, J., Das, S., Fatima, Z., & Hameed, S.,2014. Multidrug Resistance: An Emerging Crisis. *Interdisciplinary Perspectives on Infectious Diseases*, :1–7.

Thilagavathi, J., Anandhi, V., &Seshayyan, S., 2016. A study on the variations in the relationship between the recurrent laryngeal nerve and the vascular pedicle of the thyroid gland. *International Journal of Anatomy and Research*, 4(3.2):2689–2691.

Trombino, S., Serini, S., di Nicuolo, F., Celleno, L., Andò, S., Picci, N., Calviello, G., & Palozza, P., 2004. Antioxidant Effect of Ferulic Acid in Isolated Membranes and Intact Cells: Synergistic Interactions with α-Tocopherol, β-Carotene, and Ascorbic Acid. *Journal of Agricultural and Food Chemistry*, 52(8):2411–2420.

Vahdati, M., & Tohidi Moghadam, T., 2020. Synthesis and Characterization of Selenium Nanoparticles-Lysozyme Nanohybrid System with Synergistic Antibacterial Properties. *Scientific Reports*, 10(1).

Veisi, H., Karmakar, B., Tamoradi, T., Tayebee, R., Sajjadifar, S., Lotfi, S., Maleki, B., &Hemmati, S., 2021. Bio-inspired synthesis of palladium nanoparticles fabricated magnetic Fe3O4 nanocomposite over *Fritillaria imperialis* flower extract as an efficient recyclable catalyst for the reduction of nitroarenes. *Scientific Reports*, 11(1).

Velazquez-Urbina, T., Espinoza-Gomez, H., Flores-López, L. Z., & Alonso-Núñez, G., 2021. Synthesis and characterization of silver nanoparticles supported on Bivalve mollusk shell for catalytic degradation of commercial dyes. *Journal of Photochemistry and Photobiology A: Chemistry*, *419*:113481.

Vijai Anand, K., Reshma, M., Kannan, M., Muthamil Selvan, S., Chaturvedi, S., Shalan, A. E., & Govindaraju, K., 2021. Preparation and characterization of calcium oxide nanoparticles from marine molluscan shell waste as nutrient source for plant growth. *Journal of Nanostructure in Chemistry*, 11(3):409–422.

Vijayan, S. R., Santhiyagu, P., Singamuthu, M., Kumari Ahila, N., Jayaraman, R., & Ethiraj, K., 2014. Synthesis and Characterization of Silver and Gold Nanoparticles Using Aqueous Extract of Seaweed ,*Turbinaria conoides* and Their Antimicrofouling Activity. *The Scientific World Journal*, *2014*:1–10.

Vijayan, S; Koilparambil, D; Varghese, S; Shanavas, J.,2020. Antifungal efficacy of chitosan-stabilized biogenic silver nanoparticles against pathogenic Candida spp. isolated from human.. *BioNanoScience*, 10:974–982.

Virender K.Sharma, Ria A. Yngard and Yekatrina Lin., 2008. Silver nanoparticles:Green synthesis and their antimicrobial activities,Volume 145..

Wang, A., & Wang, W., 2009. Superabsorbent Materials. *Kirk-Othmer Encyclopedia of Chemical Technology*.

Wong, H. L., Wu, X. Y., & Bendayan, R ., 2012. Nanotechnological advances for the delivery of CNS therapeutics. *Advanced Drug Delivery Reviews*, 64(7): 686–700.

Yao H., Kimura K., 2007. Field emission scanning electron microscopy for structural characterization of 3D gold nanoparticle super lattices. In: Méndez-Vilas A., Díaz J., editors. Modern Research and Educational Topics in Microscopy.Formatex Research Center; Badajoz, Spain: 2007. pp. 568–575.

Zarei, M., Jamnejad, A., and Khajehali, E., 2014. Antibacterial effect of silver nanoparticles against four foodborne pathogens. *Jundishapur J. Microbiol.*7(1*)*:8720–8723.

Zolnik, B. S., González-FernáNdez, A., Sadrieh, N., &Dobrovolskaia, M. A., 2010. Minireview: Nanoparticles and the Immune System.. *Endocrinology*, 151(2): 458–465.

AN INVESTIGATION OF RHIZOSPHERIC ACTINOBACTERIAL SPECIES

A project submitted to

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In partial fulfilment for the award of the degree of

BACHELOR OF SCIENCE IN ZOOLOGY

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April 2022

DECLARATION

We do hereby declare that this dissertation entitled, "***An Investigation Of Rhizospheric Actinobacterial Species***" submitted by us for the award of the degree of **Bachelor of Science in Zoology** is the result of our original independent research work carried out under the guidance of **Dr. R. Sri Priya M.Sc., Ph.D.,** Assistant Professor, Department of Zoology, St. Mary's College (Autonomous), Thoothukudi and it has not been submitted elsewhere for the award of any other degree.

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CERTIFICATE

This is to certify that the project entitled "***An Investigation Of Rhizospheric Actinobacterial Species***" is submitted to **St.Mary's College (Autonomous), Thoothukudi** in partial fulfilment for the award of the degree of **Bachelor of Science in Zoology** and it is a project work done during the year2021-2022 by the following students.

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CONTENTS

INTRODUCTION

INTRODUCTION

The rhizospheric microbes are a group of organisms associated to plants that are essential to their metabolism. They are found in synergism with plant roots. Rhizosphere bacteria play vital roles in plant nutrition, growth promotion, and disease interactions. Several studies have indicated that bacteria are the most numerous inhabitants of the rhizosphere, although they account for only a small portion of the total biomass due to their small size.

Rhizosphere bacteria have high potential to produce various classes of well-known phytohormones, including auxins, gibberellins, cytokinins, ethylene, and abscisic acid. Plants respond well to these phytohormones in the rhizosphere which can mediate various processes, including plant cell enlargement, division, and extension in roots. A potential tool for growers to increase plant tolerance to abiotic stress is the application of plant growth promoting rhizobacteria (PGPR), a class of microorganisms that can colonize plant roots and produce enzymes and secondary metabolites that positively influence plant stress tolerance. Noteable PGPR include many genera such as Acinetobacter, Agrobacterium, Arthobacter, Azospirillum, Bacillus, Bradyrhizobium, Burkholderia, Pseudomonas, Rhizobium, and Serratia(Goswami et al.,

2016). The endophytic actinomycetes which are associated with plants also play important role in protection of their host from phytopathogenic invasion (Crawford etal., 1993). Several endophytic actinomycetes act as plant growth promotor by producing of phytohormone indole-3-acetic acid, iron chelating moleculed and siderophores (Indananda etal., 2011).

Actinomycetes are the most widely distributed group of microorganisms in nature which primarily inhabit in soil (Oskey et.al 2004). Actinomycetes are Gram-positive mycelial bacteria, known to produce a wide variety of industrially and medically relevant compounds (antibiotics, chemotherapeutics, fungicides, herbicides and immunosuppressants). Actinomycetes are filamentous chemoorganotrophic bacteria that form asexual spores. Populations of these bacteria are usually higher in rhizosphere than in non rhizosphere soil. There are indications that actinomycetesmay comprise higher percentages of the total bacterial numbers than fluorescent pseudomonads in the root zone of some plants. Although some actinomycetes may cause plant diseases, many species have the potential to produce antibiotics that could impact plant–microbe interactions, such as nodulation in legumes or biocontrol efforts against plantpathogens.

Two third of naturally occurring antibiotics, including many of medical importance, have been isolated from actinomycetes. Successful commercialization of enzymes is an important step towards revolutioning “green technology”.

Actinomycetes are good sources of these enzymes . They produce a number of industrially important enzymes like amylase, protease etc. (Divya. et.al, 2013).

The rapid development of resistance to multiple chemotherapeutic drugs and their undesirable side effects with untreatable tumours, and with the greater therapeutic efficiency. Presumably this resemblance result partly from adaptation to the same habitat. The bioactive secondary metabolites produced by microorganisms is reported to be around 23,000 of which 10,000 are produced by actinomycetes, thus representing 45% of all bioactive microbial metabolites. Antibiotics are majorly isolated from the actinomycetes. The first antibiotic, actinomycin for treatment of tuberculosis was isolated from the actinomycete and later the antibiotic Streptomycin was isolated from *Streptomyces* by biochemists Selman Waksman, Albert Schatz, and Elizabeth Bugie in 1943. Selman Wasksoman got the Nobel prize in physiology and medicine for his discovery of Streptomycin from *Streptomyces.*

The bioactive secondary metabolites produced by microorganisms is reported to be around 23,000 of which 10,000 are produced by actinomycetes, thus representing 45% of all bioactive microbial metabolites discovered. Among actinomycetes, approximately 7600 compounds are produced by *Streptomyces* species. As a result of which Streptomyceteshave become the primary antibiotic producing organisms exploited by the pharmaceutical industry. The rapid

development of resistance to multiple chemotherapeutic drugs and their undesirable side effect has increased demand for novel antitumor drugs that are active against fewer side effects with untreatable tumours, and with the greater therapeutic efficiency. Actinomycetes are good source for such new antitunour agents.

Plant growth-promoting rhizobacteria are the rhizosphere bacteria, which can ameliorate plant growth. These microorganisms are able to enhance the recycling of plant nutrients and decrease the use of chemical fertilization (Cakmakci et al., 2007). Rhizosphere microbiome, known as the second genome of plants, collectively containing bacteria, fungi, and oomycetes, are closely related to plant growth and health (Berendsen et al., 2012). They play an important role in phytostimulation, phytoremediation, and biofertilization. Important characteristics of PGPR include production of exopolysaccharides, planthormones, and siderophores; solubilization of P and Ca; and resistance to antibiotics. The PSB facilitate the growth of plants by stimulating the efficiency of nitrogenfixation; accelerating the accessibility of other trace elements; synthesizing important growth-promoting phytohormones like indoleacetic acid (IAA), gibberellic acid (GA), siderophores, and antibiotics; and providing protection to plants against soil borne pathogens.

The rhizosphere accommodate a number of Actinomycete species that are known to play PGPR activity (Sathya et al., 2017). As like other PGPR, actinobacteria also employ both direct and in-direct mechanisms to influence the plant growth and protection. The direct mechanisms involve the production of vital factors for crop growth such as growth hormones and the assistive actions on nitrogen fixation, phosphate solubilization, and iron acquisition. PGP actinobacteria indirectly influence the plant growth by controlling and minimizing the deleterious effects of external stresses of either biotic or abiotic sources through the following modes: competition for nutrients, production of low molecular inhibitory substances such as ammonia, cyanogens, alcohols, aldehydes, sulfides, and ketones, cell-wall degrading enzymes, and secondary metabolites with biocidal properties, in which the latter, two are the key phenomenon deployed by the actinobacterial community (El-Tarabily and Sivasithamparam 2006; Glick 2012; Bouizgarne 2013; Dey et al., 2014).

Hence the current study is focused on isolating Actinomycetes from the rhizosphere of plants and characterize them for PGPR activity.

OBJECTIVES

- To isolate Actinomycetes from the rhizosphere.
- Morphological characterization of the isolated actinomycte strains.
- Biochemical characterization of the isolated actinomycete strains.
- To test their salt tolerance capacity and plant growth promoting activity.

REVIEW OF LITERATURE

The rhizosphere is the zone of soil surrounding the plant root where the biology and chemistry of the soil are influenced by the root. This zone is about 1 mm wide, but has no distinct edge. Rather, it is an area of Intense biological and chemical captivity influenced by compounds exuded by the root, and by Microorganisms feeding on the compounds (Hinsinger et al., 2009; Raaijmakers et al., 2009). The rhizosphere is a centre of intense biological activity due to the food supply provided by the root exudates. Bacteria, actinomycetes, fungi, protozoa, slime moulds, algae, nematodes, enchytraeid worms, earthworms, millipedes, centipedes, insects, mites, snails, small animals and soil viruses compete for water, food and space (Mendes et al., 2013). Soil chemistry and pH can influence the species mix and functions of microbes in the rhizosphere. The soil zone strongly influenced by plant roots, the rhizosphere, plays an important role in regulating soil organic matter decomposition and nutrient cycling. Processes that are largely controlled or directly influenced by roots are often referred to as rhizosphere processes.

A major characteristic feature of the rhizosphere is the release of organic compounds into the soil by plant roots. These compounds, called exudates, make the environment of the rhizosphere very different from the environment in the bulk

soil. The exudes can be used to increase the availability of nutrients in the rhizosphere and they also provide a food source for microorganisms. This causes the number of microorganisms to be far larger in the rhizosphere than in the bulk soil. Their presence attracts larger soil organisms that feed on microorganisms and the concentration of organisms in the rhizosphere can be up to 500 times higher than in the bulk soil.

Another characteristic feature of the rhizosphere is the uptake of water and nutrients by plants. Plants take up water and nutrients into their roots. This draws water from the surrounding soil towards the roots and rhizosphere. The balance between the movement of water and nutrients towards the roots and their removal from the soil by roots means that their concentration in the rhizosphere is usually very different from what it is in the bulk soil.

Numerous life forms inhabit the rhizosphere. Bacteria, including actinomycetes, are the most numerous inhabitants of the rhizosphere, typically numbering 10^6–10^9 organisms g^{-1} of rhizosphere soil, although they account for only a smaller portion of the total biomass because of their small size. The dominant bacterial phyla found in the rhizosphere include Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, and Firmicutes, as well as Chloroflexi, Gemmatimonadetes, and Planctomycetes. In general,

nonsporulatingrods are abundant in the rhizosphere. Pseudomonads and other Gram-negative Proteobacteria are especially competitive in the rhizosphere and typically occupy a large portion of the total bacterial population on the root. Actinobacteria can account for approximately 10%–30% of the total microflora in the rhizosphere, depending on the season or when the nutrients are added. These modifying factors cause difficulty in characterizing populations in the rhizosphere.

The rhizosphere microorganisms stimulate the growth of plants during drought stress by inducing various mechanisms such as production of plant growth regulators (IAA, cytokinins and ABA), production of bacterial exopolysaccharides (EPS), and synthesis of ACC deaminase (Farooq et al., 2009, Porcel et al., 2014). Beneficial or harmful relationships exist between rhizosphere organisms and plants, which ultimately affect root function and plant growth. In addition, the rhizosphere may include organisms that do not directly benefit or harm plants but clearly influence plant growth and productivity.

The bacterial communities play a major roles in the cycling of organic matter; inhibit the growth of several plant pathogens in the rhizosphere and decompose complex mixtures of polymer in dead plant, animal and fungal material

which results in production of many extracellular enzymes which are helpful to the crop t plant growth and yield of commercially important crops.

Actinomycetes are prokaryotic organisms that are classified as bacteria, but are unique enough to be discussed as an individual group.Actinobacteria are typically Gram-positive or Gram-variable and are considered to have a high G and C DNA base ratio. Actinobacteria are often found in soil and water. They are able to change their shape to adapt to their environment by extending out branches, or filaments.

Actinomycete numbers are generally one to two orders of magnitude smaller than the total bacterial population. They are an important component of the bacterial community, especially under conditions of high pH, high temperature or water stress. Morphologically, actinomycetes resemble fungi because of their elongated cells that branch into filaments or hyphae. These hyphae can be distinguished from fungal hyphae on the basis of size with actinomycete hyphae much smaller than fungal hyphae. One distinguishing feature of this group of bacteria is that they are able to utilize a great variety of substrates found in soil, especially some of the less degradable insect and plant polymers such as chitin, cellulose and hemicellulose. Although originally recognized as soil

microorganisms, it is now being recognized that marine actinomycetes are also important. Specifically, marine actinomycetes have been shown to possess novel secondary metabolites that add a new dimension to microbial natural products (Jensen et a*l*., 2005) that have been discovered within soil actinomycetes. The cell walls of the actinomycetes were made up of sugars, amino sugars and amino acids (the latter few in number). The general pattern of components was thus identical with that previously found for Gram-positive bacteria. In the fungi, however, the mycelial walls were composed entirely of carbohydrate.

The Actinomycetes, forming soil micro-flora have gained the greatest importance in recent years as producers of therapeutic substances. Many of the Actinomycetes have the ability to synthesize metabolites which hinder the growth of bacteria; these are called antibiotics, and, although harmful to bacteria are more or less harmless when introduced into the human or animal body. Antibiotics have in modern times great therapeutical and industrial value. The past decade has seen considerable interest in the Actinomycetes as producers of antibiotic substances. The successful use in chemotherapy of streptomycin, chloromphenicol (Chloromycetin is the trade name of this substance), aureomycin and terramycin all metabolites of the Actinomycetes, has stimulated the search for new Actinomycetes and new antibiotics among the Actinomycetes. The genus *Streptomyces* is the largest and the most important one, antibiotically speaking.

Actinomycetes are the economically important organisms that play a fundamental role in many areas like:

Use in Bioremediation :

Actinomycetes digest complex carbohydrates like chitin, cellulose, hemicellulose etc. It also helps in the degradation of toxic compounds from the environment. Thus, it plays an essential role in the bioremediation of organic compounds. Actinomycetes can survive in a harsh environment like high temperature up to 50 degrees Celsius that is crucial for the composting process.

Biomedical Use:

Members of actinomycetes can produce many of the best-known antibiotics like amphotericin, neomycin, novobiocin, chloramphenicol, tetracycline etc. Tetracycline and erythromycin etc. target bacterial ribosomes and cures respiratory infections. Vancomycin mainly attacks the bacterial cell wall of pathogenic bacteria (*Streptococcus aureus*). Rifampicin targets bacterial RNAP (RNA-Polymerase) and cures tuberculosis and leprosy. Adriamycin treats cancer. Amphotericin attacks fungal membranes and shows a few side effects. Rapamycin enables organ transplant.

Use in Regulating Plant growth:

Actinomycetes inhabit the soil and produce phytohormones, extracellular enzymes and bioactive compounds. These compounds promote direct plant growth and protect against phytopathogens and pests by producing indole 3-acetic acid, siderophore and solubilize phosphate.

Industrial Use:

Actinomycetes produce several enzymes, which show a wide range of applications in different fields like industry, agriculture etc. They produce a number of industrially important enzymes. Lipase in detergent and pharmaceuticals industries.Cellulases in the animal feed industry. Catalase in the detergent industry.Amylase in food, textile and paper industries.Chitinase in biochemical industries. In agro based industries Actinomycetes have numerous applications. They produce agro active compounds as they are extensively present in the rhizospheric zone of the plant. Thus, they can actively colonize themselves with the plant roots and protect the plant from pathogenic fungi and other phytopathogens. Frankia is an example of actinomycetes, which acts as a "Symbiont" that promote root nodule formation and thereby in nitrogen fixation.

In attempts to develop commercial Biocontrol and plant growth promoting Products using rhizobacteria, it is Important to recognize the specific Challenges they present. The Interaction between PGPR species and plant symbionts appears

to be Specific, even within a crop or cultivar (Chanway et al., 1988, Glick 1995,KIoepper 1996, Lazarovits and Nowak1997). While a rhizobacterium screened for growth promotion may reveal the Positive effects on one crop, it may have No effect, or even retard growth of another Crop (Gardner et al., 1984, O'Neill et al., 1992).

Actinomycetes are also used as effective biocontrol agents. A prime example of Streptomyces biocontrol agent is *Streptomyces griseoviridis* (Andersen et al., 2007)strain K61. This strain, originally isolated from light Coloured Sphagnum peat (TahvonenAlternari,1982), has been reported to be antagonistic to a variety of plant pathogens together with *Alternariabrassicola* (Schw.) Wiltsh., *Botrytis Cinereapers*.:Fr., *Fusariumavenaceum* . *Streptomyces griseoviridis* strain K61 was used in root dipping or growth nutrient treatment of flowers, potted plants, Greenhouse cucumbers, and varied Alternative vegetables (Mohammadi and Lahdenpera 1992). Mycostop™ (developed by KemiraOy) is a Biofungicide that contains *S. griseoviridis* as the active ingredient.

As the environmental contamination by toxic chemicals increases, different approaches for controlling pest populations have become the need of the hour. These include biological or ecological management strategies for limiting the harmful impacts of pest populations, particularly in agriculture (Nakas and Hagedorn, 1990; Canaday, 1995, Hokkanen and Lynch 1995). Several sorts of

microorganisms including Fungi, bacteria, nematodes and viruses that are antagonistic to insects are reported as methods to biologically control them. Actinomycetes play a significant role in the biological control of insects through the production of insecticidally active compounds against the house fly *Muscadomestica*(Hussain et al., 2002). The mortality of larval and pupal stages, were terribly high reaching up to 90%). Actinomycetes were effectively used against *Culexquinquefasciatus* (Sundarapandian et al., 2002).

Actinomycetes have an extended tradition in the analysis of bioactive compounds. Several species manufacture a large form of secondary metabolites, including anti-Helminthic compounds, anti-tumor agents and majority of identified antibiotics. Free-living actinomycetes have additionally been concerned in the Improvement of plant growth by Production of plant growth-producing Substances like auxins and gibberellin-like Compounds (Persello-Cartieaux et al., 2003, Bloemberg et al., 2001).In recent years the tactic of immobilizing Living cells has gained a large variety of Applications (D'Souza et al. 1999, Baianu Et al. 2004). Encapsulation of microbial Cells for soil application provides a variety Of benefits like application to the soil, Reduced off-site drifting, and protection of Cells from environmental stress (Leung et al., 1997; Bashan 1986)actinomycetes are known to be durable organisms and thus appropriate for soil Applications. The spores of most actinomycetes endure desiccation and show slightly higher resistance to dry

or wet heat than vegetative cells. Actinomycetes will colonize dry soil owing to their filamentous nature and exist in soil for extended periods as resting arthrospore that germinate in the occasional presence of exogenous substrates.

Biocorrosion:

Corrosion is a principal reason of pipe failure and high preservation costs in gas pipelines (Zhu et al., 2003). Biocorrosion is defined as a caustic harm initiated or aggravated by the direct or indirect activities of microorganisms (Zuo et al., 2007). A broad range of bacteria is present in most if not all areas of oil production and have been described from water injection plants, drilling mud, and live reservoir Cores (Feio et al. 2000, Magot et al. 2000,Korenblum et al. 2005, Von Der Weid et al. 2008) . Antimicrobial substance (AMS) formed by A *Streptomycetes* strain having its activity against an aerobic bacterium *B. pumilus* LF-4, and sulfate-reducing bacterium *D. alaskensis* NCIMB 13491 known to be Involved in biofilm formation and Biocorrosion. Strain 235 was identified as belonging to *S. lunalinharesii* species Cluster, was initially isolated from a Brazilian soil. The AMS is a promising anticorrosion molecule that can be widely used in oil making plants, because of its to chemicals and solvents, and over a broad range of Temperature and pH values.

Actinomycetes are organisms which produce a number of useful molecules and hence identifying and characterization of new Actinomycetes species can add value to the current research.

MATERIALS AND METHOD

Collection of sample:

Samples for the experiments were collected from in sterile plastic bags from the rhizospheric of mangroove trees near Punnaikayal beach in Punnaikayal, Thoothukudi district. Soil and root sample were collected from the rhizosphere of mangroove trees. Approximately 35 cm below the surface.

Characterization of culture:

Isolation of rhizospheric bacteria by serial dilution:

1 gram Rhizospheric soil was taken into a test tube and mixed with 10 ml of distilled water. The plant sample was serially diluted till 10^{-6} dilutions and the 200 µl of the sample from each dilution was spread plated to obtain single colony isolates. .

Plating of culture:

The actinomycete isolation agar was prepared and was autoclaved at 121°C for 15 minutes. The medium was poured into petridish and allowed to solidify. The samples were plated on the medium by spread plating. 100 µl of the samples from each dilution (10^{-1} and 10^{-6}) was plated spreading the technique. Plates were

incubated at 37°C till the growth of the colonies. Nalidixic acid was added before pouring the medium into the petri plats.

Selective Isolation of Actinomycete strains:

From the plant samples , microorganism were isolated by serial dilution followed by spreading and restreaking. Actinomycete isolates were obtained by adding 10 mg/l of Nalidixic acid to the medium to inhibit the growth of other bacterial strains. The plates were incubated at 37°C till visible colonies appeared.

Isolation of pure bacterial colonies:

Single colonies were picked and streaked to obtain single pure colonies. Pure colonies were incubated at 37°C for 24 hours and stored at 4^0C for further studies.

Characterization of isolates:

Morphological characteristics of colonies such as shape, size, colour, pigmentation, gram staining were recorded

Gram staining:

Gram staining was performed for isolated colonies according to standard procedure. A smear of bacterial cells was prepared on a clean glass slide by gentle heat fixation. Heat fixed smear filled with crystal violet solution for one minute.

The smear was washed with distilled water and then gram iodine was added. Smears were washed with 95% decolourisation and cleaned with water. Finally safranin was used as counter stains for 60-80 seconds and washed with water. Then observed under a microscope.

Biochemical characterization:

Catalase test:

Slants of bacterial culture in nutrient agar were made and the catalase test was performed. Two drops of hydrogen peroxide was added to the 24 hours bacterial culture. The immediate evolution of the gas bubbles indicates the production of catalase enzyme by the isolates and hence considered catalase positive (Hadioetomo 1990).

Phosphate solubilization:

Bacterial isolates was screened invitro for their phosphate solubilizing activity using potato dextrose rose Bengal agar. The cultures were streaked on the agar. Plates are incubated at 37°C for 24 hours. The growth of the bacterial colony indicates a positive result for phosphate solubilization (Martin, 1950).

Screening for Hydrolytic enzyme production:

Bacterial isolates were screened for their hydrolytic enzyme production like protease and amylase.

Protease production activity:

Bacterial isolates were screened for the ability to produce proteolytic enzymes in skim milk agar (SM medium).The medium was poured into a sterilized petridish and the isolated bacterial strain was streaked on the surface of the medium. Formation of a clear zone around the colonies is indicative of protease production.

Starch hydrolysis activity:

About 20 ml of starch medium was poured into the sterilized petridish. The isolate was streaked and incubated for 24 hours. At the end of the incubation period, two or three drops of Iugol's iodine solution were added on the surface of the medium. A clear zone around the area of growth indicates starch hydrolysis activity of the isolates.

RESULT

ISOLATION OF BACTERIA:

Bacterial strains were isolated from the rizhosphere of the *Avicenia marina* plants growing in the backwaters of Punnaikayal village in Thoothukudi District (Figure 1). The bacterial cultures were isolated by serial dilution. In order to obtain actinomycetes species Actinomycete isolation agar was used. The actinomycete isolation agar was supplemented with Nalidixic acid to inhibit the growth of other bacterial strains and incubated at 37^0C for 7 days. The colonies were named as R1, R2, R3, R4, R5 and R6. Pure culture of the bacterial isolates were maintained as patches and shown in Fig. 3.

MORPHOLOGICAL CHARACTERIZATION:

The bacterial isolates were characterized for the colony morphology such as shape, colour, margin, elevation and opacity and also characterized for cellular morphology using student microscope.

The morphology of the bacterial colonies and their colours were observed and tabulated in Table 1. The colonies had varied morphology. The strains R1, R2, R3, R5, R6 were dull white, R4 was a white colonies. The strains R1, R2, R3, R4, R5, and R6 were circular. The margins of the colony were entire

in R1, R2, R3, R4, R5, R6. The elevations of the colony were raised in R1, R2, R5 and flat in R3, R4, R6. The colonies R1, R2, R3, R4, R5, R6 were opaque (Table - 1).

GRAM STAINING:

Gram staining was done on all the isolates. All the colonies (R1, R2, R3, R4 and R6) were Gram positive except R5 (Fig. 4) (Table 2).

BIOCHEMICAL ANALYSIS OF ISOLATED BACTERIA:

Biochemical tests were performed on the bacterial isolates. Tests like Catalase test, Phosphate Solubilization test, Protease production and Starch hydrolysis tests were performed.

CATALASE TEST:

Catalase test was done to identify strains that produced the catalase enzyme. The isolates R1, R2, R3, R4, R5 and R6 were used for catalase test. To the overnight grown bacterial slants few drops of hydrogen peroxide was added. A brisk effervescence was observed in all the test cultures, showing that all the strains produced catalase enzymes (Fig. 5) (Table 3).

PHOSPHATE SOLUBILIZATION TEST:

Phosphate solubilization ability of bacteria can be detected by culturing the isolates on Potato Dextrose Rose Bengal agar plate method. Growth on this medium confirms their phosphate solubilization activity. The isolates R1, R2, R3, R4, R5 and R6 were checked for phosphate solubilization. The strains R1, R2, R3, R4, R5 and R6 grew well on the Potato Dextrose Rose Bengal agar medium, implicating that these colonies could solubilize phosphate (Fig. 6) (Table 4).

HYDROLYTIC ENZYME PRODUCTION:

PROTEASE PRODUCTION TEST:

To check the ability of the actinobacterial strains to produce protease enzyme, the colonies were patched on Skimmed milk (SM) agar medium. The colonies R1, R2, R3, R4, R5 and R6 were used for analysis. All the colonies grew on the SM agar medium (Fig. 7) (Table 5). This showed that the isolates produce the protease enzyme.

STARCH HYDROLYSIS TEST:

The starch hydrolysis test is performed to confirm the production of the enzyme amylase by the bacterial isolates. The isolates that produced amylase enzyme can hydrolyse the starch present in the Starch agar and show a zone of clearance around their colony when iodine is added. The test was

performed on 6 colonies R1, R2, R3, R4, R5 and R6 (Fig. 8) (Table 6). A zone of clearance was observed around the colonies R1, R2, R3, R4, R5 and R6, indicating these are produce the amylase enzyme.

DISCUSSION

Actinomycetes, which occur in both terrestrial and aquatic habitats, are among the most common groups of gram-positive microorganisms in nature. Actinomycetes decompose organic matter and display antagonism against other bacteria and fungi, with which they compete for nutrients. Actinomycetes have incredible abilities to survive under extreme conditions in their natural environment and have long been the focus of scholarly attention and have been harnessed as valuable sources of natural compounds, such as antibiotics, enzymes, and vitamins. More than 90 percent of chemotherapeutic antibiotics have been isolated from actinomycetes (Newman et al., 2007; Demain, 1999).

PGPR are free living soil bacteria that aggressively colonize the plant roots and when applied to the seeds they enhance the growth and yield of the plants. The use of novel PGP bacteria as biofertilizers, biopesticides and phytostimulator inn agricultural sectors to improve crop yield, quality and maintaining the soil fertility is advisable. The present study is carried out to identify and their activities from Punnakayal, soil and root environment.

Generally, most of the studies that focus on the screening of antibiotic producing actinomyctes are done in a neutral medium (pH 7). In those studies Actinomycetes that are halophilic were not selected and hence, these extremophiles could have been missed out (Trenozhnikova and Azizan, 2018)

The objective of the current study was to isolate Rhizospheric actinobacterial strains. Hence soil and root sample were used to isolate the organisms. Initially the colonies were isolated on Actinomycete selection Agar. On the Actinomycete selection medium, many rhizospheric bacterial strains were identified. For the analysis a few of the colonies which grew on actinomycetes isolation Agar and a few colonies from the medium containing Actinomycetes agar. Morphological and biochemical characterization of the colonies were done. The bacterial colonies showed diverse morphological characteristics as indicated from variation in shape, colour, margin, elevation and opacity. On the basis of their gram reaction R1,R2, R3, R4, R6 were gram positive.

Some of the colonies also produced a wide range of bioactive compounds such as enzymes protease, amylase, catalase etc.,

The exact mechanism by which PGPR stimulate plant growth is not clearly known, although several mechanisms such as production of phytohormones, activation of phosphate solubilization and promotion of the mineral nutrient uptake are usually believed to be involved in plant growth promotion. There are many papaers related to the advantages and screening of PGPR from crop plants but few on Avicenia marina. In this study, about 6 rhizobacterial strains were isolated fromAvicenia marina (Mangrove Plant) rhizosphere samples and screened for different plant growth promoting traits and Biocontrol properties.

In this present study, the isolated bacterial strains solubilised phosphate, produced catalase and showed starch hydrolytic activity. These strains have to the further characterized by 16s rRNA to identify their genus and species. Analyse of more colonies have to be done to identify a strains that could be used efficiently for bioprospecting. Antimicrobial activity of the characterized strains have to be performed.

SUMMARY

- Six putative actinomycete bacterial strains were isolated from rhizosphere soil by selection on actinomycete isolation agar.
- All the isolates were found to be gram positive.
- Morphological characterization of the colonies was done.
- Biochemical characterization of the isolates was done.
- The isolates were found to produce catalase enzyme.
- They produced hydrolytic enzymes, protease and amylase.
- They were able to solubilize phosphate.

CONCULSION

Rhizospheric is an abode of beneficial and economically important microorganisms. Among all the microbes that inhabit the rhizosphere, actinomycetes are a versatile group of economically important bacteria. It is well known that actinomycetes are the most economically and biotechnologically valuable prokaryotes. It is believed that the actinobacteria hold a prominent position due to their biodiversity and potentiality to produce novel compounds. The current study was focused on isolating actinomycetes from the rhizosphere. 2 colonies of putative actinobacterial colonies were isolated. The aim of the study was to identify plant growth promoting actinobacteria from the rhizosphere. Among the different isolates that were screened 6 colonies were found to solubilize phosphate and hence can be considered for their PGP activity. The ability of the isolated colonies were checked for the production of hydrolytic enzymes like protease and amylase. 6 colonies were found to produce protease and 6 colonies were found to produce amylase. 1 Hence these isolates can be further characterized and utilized as biofertilizers or can be used for production of industrially important enzymes.

SUGGESIONS FOR FUTURE

The identification of the bacterial genera has to be done by 16s rDNA sequencing. In future the strain can be taken for future analysis. The identified strains can be made as bioformulation and taken for field application which would be beneficial from crop improvement and crop production. They can be fruitfully used for production of hydrolytic enzymes.

BIBILOGRAPHY

Ahmad, I., Cheng, Z., Meng, H., Liu, T., Wang, M., Ejaz, M., et al. (2013).Effect of pepper-garlic intercropping system on soil microbial andbio-chemical properties. Pak. J. Bot. 45, 695–702.

Albrecht-Gary, A. M., and Crumbliss, A. L. (1998). Coordination chemistry of siderophores: thermodynamics and kinetics of iron chelation and release. Met. Ions Biol. Syst. 35, 239–327.

Andersen, M. K., Hauggaard-Nielsen, H., Høgh-Jensen, H., and Jensen, E.S. (2007).Competition for and utilisation of sulfur in sole and intercrops of pea and barley.Nutr.Cycl.Agroecosyst. 77, 143–153. doi:10.1007/s10705-006-9053-7

Bailey, R. L., West, K. P. W. Jr., and Black, R. E. (2015).The epidemiology of global micronutrient deficiencies. Ann. Nutr. Metab. 66, 22–33. doi:10.1159/000371618PubMed Abstract | CrossRef Full Text

Bais, Harsh P.; Weir, Tiffany L.; Perry, Laura G.; Gilroy, Simon; Vivanco,Jorge M. (2006). "The Role of Root Exudates in Rhizosphere Interactionswith Plants and Other Organisms".Annual Review of Plant Biology. 57 (1):233–266.

Baker, C. J., Mock, N. M., Smith, J. M., and Aver'Yanov, A. A. (2015). The dynamics of apoplastphenolics in tobacco leaves following inoculation with bacteria. Front. Plant. Sci. 6:649. doi: 10.3389/fpls.2015.00649

Bar-Ness, E., Hadar, Y., Chen, Y., Römheld, V., and Marschner, H. (1992).Iron uptake by plants from microbial siderophores: a study with7-nitrobenz-2 oxa-1,3-diazole-desferrioxamine as fluorescent ferrioxamin B analog. Plant Physiol. 99, 1329–1335. doi: 10.1104/pp.99.4.1329

Bashir, K., Ishimaru, Y., Shimo, H., Kakei, Y., Senoura, T., Takahashi, R., (2011). Rice phenolics efflux transporter 2 (PEZ2) plays an important role in solubilizing apoplasmic iron.

Bouizgarne B (2013) Bacteria for plant growth promotion and disease management. In: Maheshwari DK (ed) Bacteria in agrobiology: disease management. Springer, Berlin, pp 15–47

Caris, C., Hördt, W., Hawkins, H. J., Römheld, V., and George, E. (1998).Studies of iron transport by arbuscularmycorrhizal hyphae from soil to peanut and sorghum plants.Mycorrhiza 8, 35–39. doi:10.1007/s005720050208

Chaignon V., and Hinsinger P (2009). "Copper availability and biodiversity are controlled by rhizosphere pH in rape grown in an acidic Cu-contaminated Soil". *Environ pollut*. 157,3363-3369.

ChaigonV., and Hinsinger P (2003). “A biotesr for evaluating copper bioavailability to plants in a contaminated Soil”.*Environ. Qual*. 32,824-833

Chem. 288, 4502–4512.doi: 10.1074/jbc.M112.433300 PubMed Abstract | CrossRef Full Text |

Cheng W., Gershenson A., (2007). “Carbon fluzes in the rhizosphere. In: ZG Carbon. JL Whitebeck, Eds. The rhizosphere”. Burlington, VT, USA: Academic press, 31-56.

Cheng WX., Kuzyakov Y., 2005.Root effects on soil organic matter decomposition. In: RW Zobel. , SF Wright eds. Roots and soil management interaction between roots and the soil. Madison, WI, USA: America, Soil Science Society of America (119-143).

compounds predominantly modulate the soil microbiome. J. Biol.

Crowley, D. E. (2006). “Microbial siderophores in the plant rhizosphere,” in Iron Nutrition in Plants and Rhizospheric Microorganisms, eds L. L. Barton and J. Abadia.

De Vries, F.T.; Griffiths, R.I.; Knight, C.G.; Nicolitch, O.; Williams, A. Harnessing rhizosphere micro biomes for drought-resilient Crop production. Science 2020, 368, 270–274

deficiency responses in dicot plants. Front. Plant Sci. 10:287. Doi:

Delaporte-Quintana, P., Lovaisa, N. C., Rapisarda, V. A., and Pedrza, R. O. (2020). The plant growth promoting bacteria Gluconacetobacterdiazotrophicus and Azospirillumbrasilense contribute to the iron nutrition of strawberry plants through siderophores production.

Devau N., Hinsinger P., jaillard B. "Soil pH control the environmental availability of phosphorus; experimental and mechanistic modelling approaches. Journal of applied" geo-chemistry vol 24.

Dey R, Pal KK, Tilak KVBR (2014) Plant growth promoting rhizobacteria in crop protection and challenges. In: Goyal A, Manoharachary C (eds) Future challenges in crop protection against fungal pathogens. Springer Science, New York, pp 31–58

Dijkstra FA., Carrio Y., Pendall E., Morgan JA., (2013). "Rhizosphere priming: a nutrient perspective, frontiers in Microbiology''4:2016

El-Tarabily KA, Nassar AH, Sivasithamparam K (2008) Promotion of growth of bean (*Phaseolus vulgaris* L.) in a calcareous soil by a phosphate-solubilizing, rhizosphere-competent isolate of *Micromonosporaendolithica*.Appl Soil Ecol 39(2):161–171

Farah Ahmad, Iqbal Ahmad, M.S. Khan (15 March 2008), Screening of free _ living rhizospheric bacteria for their multiple plants growth promoting activities, Volume 163.

Franco-Correa, M., Quintana, A., Duque, C., Suarez, C., Rodríguez, M. X., and Barea, J. M. (2010). Evaluation of actinomycete strains for key traits related with plant growth promotion and mycorrhiza helping activities. Appl. Soil Ecol. 45, 209–217. Doi: 10.1016/j.apsoil.2010.04.007

French, E., Kaplan, I., Iyer-Pascuzzi, A., Nakatsu, C. H., and Enders, L. (2021).Emerging strategies for precision microbiome management in diverse agroecosystems.

Gao, L., Liu, X. M., Du, Y. M., Zong, H., and Shen, G. M (2019). Effects of tobacco-peanut relay intercropping on soil bacteria community structure.

Ghosh, U. D., Saha, C., Maiti, M., Lahiri, S., Ghosh, S., Seal, A.,. (2014). Root associated iron oxidizing bacteria increase phosphate nutrition and influence root to shoot partitioning of iron in tolerant plant Typhaangustifolia.

Hauggaard-Nielsen, H., Gooding, M., Ambus, P., Corre-Hellou, G., Crozat, Y., Dahlmann, C., (2009).Pea–barley intercropping forefficient symbiotic N2-fixation, soil N acquisition and use of other nutrients in European organic cropping systems.

Helms, T. C., Scott, R. A., Schapaugh, W. T., Goos, R. J., Franzen, D. W., and Schlegel, A. J. (2010). Soybean iron-deficiency chlorosis tolerance and yield decrease on calcareous soils.

Hinsinger P, Bengough AG., Veterlein D., (2009) “Rhizosphere, biophysics, biochemistry and ecological relevance”. Plant Soil 321:117-152

Hinsinger P., Gobran GR., Gregory PJ., Wenzel WW (2005) “Rhizosphere geometry and heterogeneity arising from root – mediated physical and chemical process”. New phytol 168:293-303.

Hinsinger P., Marschner P (2006)., “Rhizosphere perspectives and challenges -a tribute to Lorenz Hiltnee” 12-17 September 2004- Munich, Germany. Plant Soil 283

Hinsinger P., Mougel C., Jillard B (2009) “Plant- rhizosphere: and Soil” 321(1) 83-115,.

Hinsinger, P., Plassard, C., Tang, C., and Jaillard, B. (2003). Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review.

Housh, A. B., Powell, G., Scott, S., Anstaett, A., Gerheart, A., Benoit, M., (2021). Functional mutants of Azospirillumbrasilense elicit beneficial physiological and metabolic responses in Zea mays contributing to increased host iron assimilation.

Ingraffia, R., Amato, G., Frenda, A. S., and Giambalvo, D. (2019).Impacts of arbuscularmycorrhizal fungi on nutrient uptake, N2 fixation, N transfer, and growth in a wheat/faba bean intercropping system.

Ingraffia, R., Amato, G., Frenda, A. S., and Giambalvo, D. (2019).Impacts of arbuscularmycorrhizal fungi on nutrient uptake, N2 fixation, N transfer, and growth in a wheat/faba bean intercropping system.PLoS

intercropped wheat through mycorrhizal symbiosis. Appl. Soil Ecol. 107,91–98. doi: 10.1016/j.apsoil.2016.05.008

Jain, A., Singh, A., Chaudhary, A., Singh, S., and Singh, H. B. (2014). Modulation of nutritional and antioxidant potential of seeds and pericarp of pea pods treated with microbial consortium.

Jin, C. W., Li, G. X., Yu, X. H., and Zheng, S. J. (2010). Plant Fe status affects the composition of siderophore-secreting microbes in the rhizosphere.

Kabir, A. H., Debnath, T., Das, U., Prity, S. A., Haque, H., Rahman, M. M., (2020). Arbuscularmycorrhizal fungi alleviate Fe-deficiency symptoms in sunflower by increasing iron uptake and its availability along with antioxidant defense. Plant Physiol.

Kabir, A. H., Debnath, T., Das, U., Prity, S. A., Haque, H., Rahman, M. M., (2020). Arbuscularmycorrhizal fungi alleviate Fe-deficiency symptoms in sunflower by increasing iron uptake and its availability along with antioxidant defense. Plant Physiol. Biochem. 150, 254– 262. doi:10.1016/j.plaphy.2020.03.010

Kazamia, E., Sutak, R., Paz-Yepes, J., Dorrell, R. G., Roch, F., Mach, H., (2018).Endocytosis-mediated siderophore uptake as a strategy for Fe acquisition in diatoms.

Kennedy, A.C. Luna, L.D (2005) in Encyclopaedia of soils in the Environment,

Kobayashi, T., and Nishizawa, N. K. (2012). Iron uptake, translocation, and regulation in higher plants.

Kuiper, I., Bloemberg, G. V., Noreen, S., Thomas-Oates, J., and Lugtengerg, B. (2001). Increased uptake of putrescine in the rhizosphere inhibits competitive root colonization by Pseudomonas fluorescens strain WCS365.

Labidi, S., Ben Jeddi, F., Tisserant, B., Debiane, D., Rezgui, S., Grandmougin-Ferjani, A., (2012).Role of arbuscularmycorrhizal symbiosis in root mineral uptake under CaCO3 stress.

Lasa, A. V., Mašínová, T., Baldrian, P., and Fernández-López, M. (2019). Bacteria from the endosphere and rhizosphere of Quercus spp. use mainly cell wall-associated enzymes to decompose organic matter.

Leake, J. R., Shaw, G., and Read, D. J. (1990).The biology of mycorrhiza in the Ericaceae. XVI. Mycorrhiza and iron uptake in Calluna vulgaris (L.) Hull in the presence of two calcium salts.

Lemanceau, P., Bauer, P., Kraemer, S., and Briat, J.-F.(2009). Iron dynamics in the rhizosphere as a case study for analyzing interactions between soils, plants and microbes.

Lim, H., Y. Kim, and S. Kim. 1991. Enumeration and Identification of rhizosphere bacteria by advance Immunotechniques. Pages 231-237 in C. Keel, B. Koller, and G. Defago (eds.), Plant Growth Promoting Rhizobacteria-Progress and Prospects. IOBC/WPRS Bulletin 14

Lugtenberg, B., Kamilova, F., 2009.Plant-growth-promoting rhizobacteria.Annual review of Microbiology 63, 541e556.

Malhotra, H., Pandey, R., Sharma, S., and Bindraban, P. S. (2019). Foliar fertilization: possible routes of iron transport from leaf surface to cell organelles.

Mary, V., Ramos, M. S., Gillet, C., Socha, A., Giraudat, J., Agorio, A., (2015). Bypassing iron storage in endodermal vacuoles rescues the iron mobilization defect in the natural resistance associated-macrophage protein3natural resistance associated-macrophage protein4 Double Mutant.

Mendes R., Garbeva P, Raaijimakers JM., ‘‘The rhizosphere microbione: Significance of plant beneficial, plant pathogenic, and human pathogenic,

plant pathogenic, and human pathogenic microorganisms." *FEMS MICROBIOLOGY REVIEWS*: Volume 37, Issue5, pp 634-663.

Michel, L., Beyá-Marshall, V., Rombolà, A. D., Pastenes, C., and Covarrubias, J. I. (2019).Evaluation of FE-heme applications or intercropping for preventing iron deficiency in blueberry. J. Soil Sci. Plant. Nutr.

Nagata, T., Oobo, T., and Aozasa, O. (2013).Efficacy of a bacterial siderophore, pyoverdine, to supply iron to Solanumlycopersicum plants.

Neumann, A., Schmidtke, K., and Rauber, R. (2007).Effects of crop density and tillage system on grain yield and N uptake from soil and atmosphere of sole and intercropped pea and oat.

Norton, J., and Ouyang, Y. (2019).Controls and adaptive management of nitrification in agricultural soils.

One 14:e0213672. doi: 10.1371/journal.pone.0213672

Orera, I., Abadía, A., Abadía, J., and Álvarez-Fernández, A. (2009). Determination of o,oEDDHA – a xenobiotic chelating agent used in Fe fertilizers – in plant tissues by liquid chromatography/electrospray mass spectrometry: overcoming matrix effects. Rapid Commun.

Palmer, C. M., Hindt, M. N., Schmidt, H., Clemens, S., and Guerinot, M. L. (2013). MYB10 and MYB72 are required for growth under iron-limiting

conditions. PLoS Genet. 9:e1003953. Doi: 10.1371/journal.pgen.1003953

Pivato, B., Semblat, A., Guégan, T., Jacquiod, S., Martin, J., Deau, F., (2021). Rhizosphere bacterial networks, but not diversity, are impacted by pea-wheat intecropping.

Qiao, Y. J., Li, Z. H., Wang, X., Zhu, B., Hu, Y. G., and Zeng, Z. H. (2012).Effect of legume-cereal mixtures on the diversity of bacterial communities in the rhizosphere.Plant. Soil Environ.

Raaijimakers JM., Paultiz, T. C., Steinberg (2009). "The rhizosphere: A playground and battlefield for soil borne pathogens and Beneficial Microorganisms". Plant Soil 321,341-361.

Raaijimakers JM., Timothy C., Steinberg., C (2009). "The rhizosphere a playground and battlefield for soi borne pathogens and beneficial microorganisms". Plant and soil 321(1), 341-361, 2009

Radzki, W., Gutierrez Mañero, F. J., Algar, E., Lucas Garcia, J. A., Garcia-Villaraco, A., and Ramos Solano, B. (2013). Bacterial siderophores efficiently provide iron to iron-starved tomato plants in hydroponics culture.

Rahman, M. A., Parvin, M., Das, U., Ela, E. J., Lee, S. H., Lee, K. W., x (2020).Arbuscularmycorrhizal symbiosis mitigates iron (Fe)-deficiency

retardation in alfalfa (Medicagosativa L.) through the enhancement of Fe accumulation and sulfur-assisted antioxidant defense.

Rajkumar, M., Ae, N., Prasad, M. N. V., and Freitas, H. (2010).Potential of siderophore-producing bacteria for improving heavy metal phytoextraction.Trends Biotechnol.

Rijavec, T., and Lapanje, A. (2016). Hydrogen cyanide in the rhizosphere: not suppressing plant pathogens, but rather regulating availability of phosphate. Front. Microbiol.

Robin, A., Mazurier, S., Mougel, C., Vansuyt, G., Corberand, T., Meyer, J.-M., (2007). Diversity of root-associated fluorescent pseudomonads as affected by ferritin overexpression in tobacco: diversity of fluorescent pseudomonads in rhizosphere. Environ.

Romera, F. J., García, M. J., Lucena, C., Martínez-Medina, A., Aparicio, M. A., Ramos, J., et al. (2019).Induced systemic resistance (ISR) and Fe deficiency responses in dicot plants.

root exudates of Arabidopsis to the soil reveal that phenolic-related

Sathya, A., Vijayabharathi, R. &Gopalakrishnan, S. Plant growth-promoting actinobacteria: a new strategy for enhancing sustainable production and protection of grain legumes. 3 Biotech 7, 102 (2017). https://doi.org/10.1007/s13205-017-0736-3

Schmid, N. B., Giehl, R. F. H., Döll, S., Mock, H. P., Strehmel, N., Scheel, D.,. (2014). Feruloyl-CoA 6′-Hydroxylase1-dependent coumarins mediate iron acquisition from alkaline substrates in Arabidopsis. Plant Physiol.

Sessitsch, A., Hardoim, P., Döring, J., Weilharter, A., Krause, A., Woyke, T. M., (2012). Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. Mol. Plant Microbe.

Sharma, A., Shankhdhar, D., and Shankhdhar, S. C. (2013).Enhancing grain iron content of rice by the application of plant growth promoting rhizobacteria.

Shenker, M., Chen, Y., Ghirlando, R., Oliver, I., Helmann, M., and Hadar, Y. (1995).Chemical structure and biological activity of a siderophore produced by Rhizopusarrhizus.

Shomer, I., Novacky, A. J., Pike, S. M., Yermiyahu, U., and Kinraide, T. B. (2003). Electrical potentials of plant cell walls in response to the ionic environment. Plant Physiol.

Singh, D., and Prasanna, R. (2020).Potential of microbes in the biofortification of Zn and Fe in dietary food grains.A review Agron.

Slamet-Loedin, I. H., Johnson-Beebout, S. E., Impa, S., and Tsakirpaloglou, N. (2015).Enriching rice with Zn and Fe while minimizing Cd risk.

Spehn, E. M., Joshi, J., Schmid, B., Alphei, J., and Körner, C. (2000). Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems

Strzelczyk E., Leniarska U. Production of B-group vitamins by mycorrhizal fungi and actinomycetes isolated from the root zone of pine (Pinussylvestris L.) Plant Soil.1985; 86:387–394. Doi:10.1007/BF02145459

Tamotsu, F., Yasuhiro, I., Takako, I., S., Noriko, S., Ryuji, Y.2002. Isolation of Actinomycetes From Live Plants and Evaluation of AntiphytopathogenicActivity of antifungal antibiotic pytopathol 50:58-59

Taschen, E., Amenc, L., Tournier, E., Deleporte, P., Malagoli, P., Fustec, J., (2017). Cereal-legume intercropping modifies the dynamics of the active rhizospheric bacterial community.

Theis, K. R., Dheilly, N. M., Klassen, J. L., Brucker, R. M., and Baines, J. F. (2016).Getting the hologenome concept right: an eco-evolutionary framework for hosts and their microbiomes.

Turner, T. R., Ramakrishnan, K., Walshaw, J., Heavens, D., Alston, M., Swarbreck, D., et al. (2013). Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants.

Van Loon, L. C., Bakker, P. A., van der Heijdt, W. H., Wendehenne, D., and Pugin, A. (2008). Early responses of tobacco suspension cells to rhizobacterial elicitors of induced systemic resistance.

Vansuyt, G., Robin, A., Briat, J.-F., Curie, C., and Lemanceau, P. (2007). Iron acquisition from Fe-pyoverdine by Arabidopsis thaliana.

Vasconcelos, M., Eckert, H., Arahana, V., Graef, G., Grusak, M. A., and Clemente, T. (2006).Molecular and phenotypic characterization of transgenic soybean expressing the Arabidopsis ferric chelate reductase gene.

Wahbi, S., Maghraoui, T., Hafidi, M., Sanguin, H., Oufdou, K., Prin, Y., (2016a).Enhanced transfer of biologically fixed N from faba bean to intercropped wheat through mycorrhizal symbiosis.

Wahbi, S., Maghraoui, T., Hafidi, M., Sanguin, H., Oufdou, K., Prin, Y., (2016a). Enhanced transfer of biologically fixed N from faba bean to

wang, y., brown, h. n., crowley, d. e., and szaniszlo, p. j. (1993). evidence for direct utilization of a siderophore, ferrioxamine b, in axenically grown cucumber.

Waters, B. M., and Sankaran, R. P. (2011). Moving micronutrients from the soil to the seeds: genes and physiological processes from a biofortification perspective.

Werra, P., de, Péchy-Tarr, M., Keel, C., and Maurhofer, M. (2009).Role of gluconic acid production in the regulation of biocontrol traits of Pseudomonas fluorescens CHA0.

Winkelmann, G. (2017). A search for glomuferrin; a potential siderophore of arbuscular fungi of the genus Glomus.

Xiong, H., Kakei, Y., Kobayashi, T., Guo, X., Nakazano, M., Takahashi, H., (2013). Molecular evidence for phytosiderophore-induced improvement of iron nutrition of peanut intercropped with maize in calcareous soil.

Xue, Y., Xia, H., Christie, P., Zhang, Z., Li, L., and Tang, C. (2016). Crop acquisition of phosphorus, iron and zinc from soil in cereal/legume intercropping systems: a critical review.

Yadav, R., Ror, P., Rathore, P., Kumar, S., and Ramakrishna, W. (2020). Bacillus subtilis CP4, isolated from native soil in combination with arbuscularmycorrhizal fungi promotes biofortification, yield and metabolite production in wheat under field conditions.

Yuan, J., Chen, Y., Li, H., Lu, J., Zhao, H., Liu, M., et al. (2018).New insights into the cellular responses to iron nanoparticles in Capsicum annuum.

Zaeem, M., Nadeem, M., Pham, T. H., Ashiq, W., Ali, W., Gilani, S. S. M., (2019).The potential of corn-soybean intercropping to improve the soil health status and biomass production in cool climate boreal ecosystems.

Zhang, H., Sun, Y., Xie, X., Kim, M.-S., Dowd, S. E., and Paré, P. W. (2009). A soil bacterium regulates plant acquisition of iron via deficiency-inducible mechanisms.

Zhu, X. F., Wang, B., Song, W. F., Zheng, S. J., and Shen, R. F. (2016).Putrescinealeviates iron deficiency via NO-dependent reutilization of root cell-wall Fe in Arabidopsis.

Zribi, K., and Gharsalli, M. (2002).Effect of bicarbonate on growth and iron nutrition of pea.

Zuo, Y. M., Zhang, F. S., Li, X. L., and Cao, Y. P. (2000).Studies on the improvement in iron nutrition of peanut by intecropping maize on a calcareous soil.

Zviginstev, D. G., K. A. Vinogradova, and L. M Efrementova, 1976.Immediate microscopic detectiok of.