INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

BIOSYNTHESIS, CHARACTERIZATION AND INVITRO ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES FROM SEAWEEDS AGAINST SELECTED POULTRY PATHOGENS

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ARTICLE INFO

Article history

Received 12/04/2017

Available online

30/04/2017

Keywords

Seaweed,
Halimeda Macroloba,
Turbinaria Conoides,
Spyridia Filamentosa Silver
Nanoparticle Synthesis,
Characterization,
Antibacterial Activity,
Selected Poultry Pathogens.

ABSTRACT

In the present study Silver nanoparticles were synthesized from aqueous seaweed extract of seaweeds such as of green *Halimeda macroloba, brown Turbinaria conoides*, and *red Spyridia filamentosa* and characterized by UV-Vis, FTIR, EDX, XRD and SEM. Characterization by the above said instrument analysis confirmed the presence,size and stability of silver nanoparticles.After characterization, the silver nanoparticles were tested at $25\mu g^{-ml}$,$50\mu g^{-ml}$,$75\mu g^{-ml}$ and $100\mu g^{-ml}$ concentrations to check the bactericidal activity against selected two poultry bacterial pathogens. We observed that, if the concentration of seaweed nanoparticle increases, the zone of inhibition also get increased in all the tested two bacterial pathogens against streptomycin as control and result suggested the potential use of seaweed synthesized silver nanoparticles against other pathogens.

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*Please cite this article in press as **Dr. S. R. Sivakumar** et al. Biosynthesis,Characterization and Invitro Antibacterial Activity of Silver Nanoparticles from Seaweeds Against Selected Poultry Pathogens. Indo American Journal of Pharmaceutical Research.2017:7(04).*

INTRODUCTION

Seaweeds are plant-like ocean organisms that are botanically classified as macrophytic marine algae. The structure of seaweeds somehow resemble to the non-arboreal terrestrial plant. Seaweeds do not have true roots, stem or leaves and whole body of the plant is called thallus that consists of the holdfast, stripe and blade. The important functions of the blade are photosynthesis, absorption of nutrient.Three major groups of seaweeds are recognized according to their pigments which give them characteristic colours of green, brown or red. The green algae (chlorophyta) are truly green with no pigments to mask the chlorophyll. Brown seaweeds (phaeophyta) contain a green pigment (chlorophyll) which is masked by a brown pigment called Fucoxanthin. The red algae (Rhodophyta) in addition to chlorophyll contain the pigments phycocyanin and phycocerythrin, which give the red coloration (Ramanigade, 2013).Many seaweed species are used in the industry, principally for the extraction of phyco-colloids and as a source of pharmaceutical substances. Also, they are used as herbal medicine, fertilizer, fungicides, and herbicides and for the direct use in human nutrition too (Cardozo *et al.*, 2007). Seaweeds are known as a highly nutritive food containing vitamin, protein, mineral, fibre contents, and essential fatty acids (Ortiz *et al.*, 2006). Seaweeds are the only source of phytochemicals namely agar-agar, carrageenan and algin, which are extensively used in various industries such as food, confectionary, textiles, pharmaceuticals, dairy and paper industries mostly as gelling, stabilizing and thickening agents.Seaweeds are considered as source of bioactive compounds and produce a greater variety of secondary metabolites characterized by abroad spectrum of biological activities. Compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae (Newman *et al.*,2003; Chanda *et al.*, 2010).

The field of nanotechnology is an immensely developing field as a result of its wide-ranging applications in different areas of science and technology. "Nano" refers to any parameter when it is expressed as a measure of 10^{-9} times of SI units. The word, nanoparticle can be defined in nanotechnology as a small object that acts as a whole unit in terms of its transport and properties

The concept of nanotechnology though considered to be a modern science has its history dating to as back as the 9th century. Nanoparticles of gold and silver were used by the artisans of Mesopotamia to generate a glittering effect to pots. Though the term nanotechnology was introduced by Professor Norio Taniguchi of Tokyo Science University (Taniguchi, 1974), the idea of nanotechnology was coined by physicist Professor Richard Feynman in his historic talk "there's plenty of room at the bottom"

(Feynman, 1959). The potential uses and benefits of nanotechnology are enormous, ranging from the mundane things like better paints, self-cleaning windows to the bizarre tiny submarines that will glide through our veins destroying pathogens and parasites. Nano-systems in biology, the most complex and highly functional nano-scale materials and machines have been invented by nature.

Generally, nanotechnology deals with developing materials, devices, or other structures possessing at least one dimension sized from 1 to 100 nanometres. Meanwhile, Biotechnology deals with metabolic and other physiological processes of biological systems. The intersection of nanotechnology and biotechnology only emerged very recently, giving new branches; bionanotechnology and nanobiotechnology (Ehud Gazit, 2007). These terms are often used interchangeably and are for various related technologies. When a distinction is intended, though, it is based on whether the focus is on applying biological ideas or on studying biology with nanotechnology.

Bionanotechnology generally refers to the study of how the goals of nanotechnology can be guided by studying how biological "machines" work and adapting these biological motifs into improving existing nanotechnologies or creating new ones (Nolting, 2005). Recently, the use of biological systems to synthesize functional nanoparticles has been of great interest. The biological processes have opened up new opportunities to explore novel applications, for example, the biosynthesis of metal nanomaterials. In contrast to chemical and physical methods, biological processes for synthesizing nanomaterials can be achieved in aqueous phase under gentle and environmentally benign conditions.

Bionanotechnology is a specific application of nanotechnology which requires the understanding of the material properties of biological systems because those same principles are to be used to create new technologies. This approach has become an attractive focus in current green bionanotechnology research towards sustainable development.

Nanobiotechnology refers to the use of nanotechnology to further the goals of biotechnology by which classical micro-technology can be merged to a molecular biological approach. Through this methodology, atomic or molecular grade machines can be made to study or modulate diverse properties of a biological system on molecular basis. Nanobiotechnology may, therefore, ease many avenues of life sciences by integrating cutting-edge applications of information technology & nanotechnology into contemporary biological issues. This technology has potential to remove obvious boundaries between biology, physics and chemistry to some extent, and shape up our current ideas and understanding.

Nanobiotechnology has multitude of potentials for advancing medical science thereby improving health care practices around the world. Implementation of nanotechnology in medicine and physiology means that mechanisms and devices are so technically designed that they can interact with sub-cellular (i.e. molecular) levels of the body with a high degree of specificity. Thus therapeutic efficacy can be achieved to maximum with minimal side effects by means of the targeted cell or tissue-specific clinical intervention.

Nanobiotechnology is essentially miniaturized biotechnology and takes most of its fundamentals from nanotechnology. Most of the devices designed for nanobiotechnological use are directly based on other existing nanotechnologies. For example, many new medical technologies involving nanoparticles as delivery systems or as sensors would be examples of nanobiotechnology since they involve using nanotechnology to advance the goals of biotechnology.

Silver (atomic symbol: Ag, atomic number: 47) is a Block D, Group 11, Period 5 element with an atomic weight of 107.8682. The number of electrons in each of Silver's shells is 2, 8, 18, 18, and 1. The silver atom has a radius of 144pm and a Van der Waals radius of 203pm. In its elemental form, silver has a brilliant white metallic lustre. Pure silver has the highest electrical and thermal conductivity of all metals and possesses the lowest contact resistance. It is stable in pure air and water. Silver was named after the Anglo-Saxon word "seolfor" or "siolfur," meaning 'silver'.

Silver is a prehistoric metal that has been used in medicine for curing several diseases. The medical properties of silver have been known for over 2,000 years. Since the nineteenth century, silver-based compounds have been used in many antimicrobial applications. However, silver ions have the disadvantage of forming complexes and the effect of the ions remained only for a short time. This disadvantage has been overcome by the use of the silver nanoparticles.

Silver nanoparticles are used as antimicrobial agents in most of the public places such as elevators and railway stations in China. Besides, they are used as antimicrobial agents in surgically implanted catheters in order to reduce the infections caused during surgery and are proposed to possess anti-fungal, anti-inflammatory, anti-angiogenic and antipermeability activities (Kalishwaralal *et al.*, 2009; Gurunathan *et al.*, 2009; Sheikpranbabu *et al.*, 2009).

The potent antibacterial and broad-spectrum activity against morphologically and metabolically different microorganisms seems to be correlated with a multifaceted mechanism by which nanoparticles interact with microbes. Moreover, their particular structure and the different modes of establishing an interaction with bacterial surfaces may offer a unique and under probed antibacterial mechanism to exploit. From a structural point of view, silver nanoparticles have at least one dimension in the range from 1 to 100nm and more importantly, as particle size decreases, the surface area-to-volume ratio greatly increases. As a consequence, the physical, chemical and biological properties are markedly different from those of the bulk material of origin.

Poultry refers to birds that people keep for their use and generally includes the chicken, turkey, duck, goose, quail, pheasant, pigeon, guinea fowl, pea fowl, ostrich, emu, and rhea. Due to modern systems of management, usually with high poultry densities, diseases are able to readily spread. Poultry that have an infection show a variety of symptoms, such as respiratory problems, diarrhoea and paralysis. It should be emphasised at the outset that prevention of infection in a poultry flock through sound management is very important. This is because although some infectious diseases can be treated, for many it is a waste of time and money and infected birds should be disposed of immediately.

Human infection may be caused by direct contact with contaminated animals or animal carcasses. In the case of domesticated animals especially poultry, pathogens can spread via the slaughter process to raw and finished products. In addition, it is important to note that neither of these bacteria is commonly pathogenic to poultry, thus merely serving as a reservoir for these important human pathogens. As European Food Safety Authority (EFSA) reports show, poultry production and poultry products can be a potential source of human salmonellosis. In the poultry industry, poultry pathogens are controlled by antibiotics. Antibiotics such as avoparcin, bacitracin, lincomycin, penicillin-G-procaine, chlortetracycline and virginiamycin promote growth because of an effect on the microflora in the gastrointestinal tract (Coates *et al.*, 1963; deMan, 1975).

However, the use of dietary antibiotics resulted in common problems such as the progress of drug-resistant bacteria, drug residues in the birds' body and imbalance of normal micro flora (Cowan, 1999). Drug resistant bacteria like *Staphylococcus sp*.,

Escherichia coli and *Enterococcus sp.* can be transmitted from poultry to humans through the food chain and other sources, leading to potential therapeutic failures in both humans and animals (Debnam and Jackson, 2005). Therefore, public pressure to reduce the use of antimicrobials has influenced development of alternative medicines or methods to reduce pathogens. Thus, the present study was aimed to investigate the antibacterial potential of silver nanoparticles against poultry pathogens viz., *Staphylococcus aureus* and

Salmonella typhi.

While antibiotics and antibacterials both attack bacteria, these terms have evolved over the years to mean two different things. An antibiotic is a low molecular substance produced by a microorganism that at a low concentration inhibits or kills other microorganisms. An antibacterial is any substance of natural, semisynthetic or synthetic origin that kills or inhibits the growth of bacteria but causes little or no damage to the host. All antibiotics are antibacterials, but not all antibacterials are antibiotics.

Antibiotics may be used therapeutically in animals for treating bacterial diseases, but they may also be utilized for non-therapeutic purposes such as spectrum antibacterial actions (Sagar and Ashok, 2012).

The present study was carried out with the following objectives, to prepare seaweed extracts using methanol as solvent, to synthesize silver nanoparticles from seaweed extracts and to characterize the silver nanoparticles using,Visual identification,UV-Vis spectrometry,FTIR analysis,XRD analysis,SEM analysis,EXAD analysis.To study antibacterial effect of silver nanoparticles against poultry pathogens.

MATERIALS AND METHODS

Collection of seaweed samples

The fresh live seaweed samples of *Halimeda macroloba, Turbinaria conoides* and *Spyridia filamentosa* were collected from Mandapam coastal region (78.8 E, 90.17 N), in Gulf of Mannar, Tamilnadu, South India. The collected seaweeds samples were brought to laboratory in polythene bags and washed thoroughly with fresh water to remove salt and other extraneous material. After cleaning, algae were dried in shade at room temperature for one week.

H. macroloba

T. conoides

S. filamentosa

Collection of bacterial culture

The pathogenic pure cultures of *Stapylococcus aureus* and *Salmonella typhi* were obtained from Microbiology Department, Bharathidasan University, Tamilnadu. The pure cultures were then subcultured on nutrient agar slants and preserved under refrigeration for further use.

Chemicals

Silver nitrate ($AgNO_3$), peptone, yeast extract, beef extract, sodium chloride and agar were purchased from HiMedia Laboratory Private Limited – Mumbai. All the solutions were freshly made, whereas all the microbiological media were steam sterilized by autoclaving at 15 psi at 121°C for 15 min.

Preparation of seaweed extract

20g of *Spyridia filamentosa, Turbinaria conoides* and *Halimeda macroloba* were weighed and ground into powder using mortar and pestle. The 3 powdered samples were mixed with 200ml of methanol each. The mixtures were boiled at 60°C for 30 minutes, after they were left to cool down. Using Whatmann N°1 filter paper, the sample was filtered, the filtrate was collected into the conical flask and kept at room temperature for further use.

Preparation of reaction mixture

1mM of Silver nitrate was weighed (0.16987g) and added into a mixture containing 900ml of distilled water and 100ml of seaweed extract and was mixed thoroughly. The reaction mixture (metal ion solution + seaweed extract) was kept into the dark room for 24 hours for further use.

Synthesis of silver nanoparticles

The reaction mixture was centrifuged for 15 minutes using 7,000 rpm at the room temperature. The pellet was collected on petri plate and kept to dry overnight in the oven at the temperature below 50°C. The powder was collected for various tests.

Characterization of silver nanoparticles

The characterization of silver nanoparticles from *Halimeda macroloba, Turbinaria conoides and Spyridia filamentosa* were carried out using different instruments and methods, including visual observation, UV-Vis spectrometer, FTIR, XRD, SEM and EDX analysis.

Visual observation

The change in colour of the reaction mixture confirms the synthesis of silver nanoparticles when the seaweed extracts are mixed into a solution of silver nitrate due to the excitation of surface plasmon resonance effect and reduction of silver nitrate during the incubation period.In this study, the first confirmation test was done by visual observation of the incubated reaction mixture. The seaweed extracts from *Halimeda macroloba, Spyridia filamentosa* and *Turbinaria conoides* obtained using methanol as solvent were mixed with 1mM silver nitrate solution and kept into dark at room temperature overnight. The change of colours was confirmed by comparing seaweed extracts with the incubated reaction mixture.

UV-Vis spectrometry

The synthesized silver nanoparticles from *Halimeda macroloba, Spyridia filamentosa* and *Turbinaria conoides* were primarily characterized using UV-Vis spectrometry. Reduction of Ag+ in aqueous solution was monitored by sampling of aliquots (0.2ml) of suspension, then diluting the samples with 2ml of deionized water and subsequently measuring UV-Vis spectra of the resulting diluents.UV-Vis spectral analysis was carried by measuring the spectrum band of the reaction mixture out at room temperature on ultra-violet spectroscopy using SL 210, Double Beam UV-Vis Spectrophotometer ranging from 300nm to 700nm.

FTIR Analysis

Fourier transformation infrared spectroscopy (FTIR) analysis was utilised in the study to identify the possible molecules responsible for the reduction of the Ag+ ions and capping of bioreduced silver nanoparticles synthesized from seaweed samples.Samples of aqueous solution of silver nanoparticles from *Halimeda macroloba, Turbinaria conoides and Spyridia filamentosa* were prepared by centrifugation and the pellet was dried in the oven overnight. The dried powder of silver nanoparticles from seaweeds was mixed with KBr salt and crushed into fine powder before it was mounted into the infrared spectrophotometer and subjected to FTIR analysis and the spectrum was recorded in the range of 4000 – 400cm^{-1} at the resolution of 1cm.

XRD Analysis

X-ray diffraction pattern indicates the crystalline structure of silver nanoparticles. The XRD spectrum was used to confirm the presence of silver nanoparticles.The dried pellet containing silver nanoparticles from *Halimeda macroloba, Spyridia filamentosa* and *Turbinaria conoides* were used for XRD analysis. The spectra were recorded in Philips PW 1830 X-ray generator. The diffracted intensities were recorded from 30◦ to 80◦ at 2 theta angles. The silver metal powder was used as a standard and dry powders of the silver nanoparticles were used for XRD pattern analysis.

SEM Analysis

The silver nanoparticles were also characterized by Scanning Electron Microscopy (SEM). The direct electron microscopic visualization allows measuring of the size and shape of silver nanoparticles formed.

The dried sample of silver nanoparticles from *Halimeda macroloba, Spyridia filamentosa* and *Turbinaria conoides* were used to prepare a solution which was sonicated with distilled water, thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were dried under a mercury lamp for 5 min and was examined under Philips XL 30 SEM at 12 – 15 kV at National College, Tiruchirapalli, Tamilnadu, South India.

EDX Analysis

Energy Dispersive X-ray spectroscopy (EDX) is an analytical technique used for the elemental analysis or chemical characterization of a sample. Its characterization capabilities are due to the fundamental principle that each element has a unique set of peaks on its X-ray spectrum.To stimulate the emission of characteristic X-rays from a specimen, a high-energy beam of charged particles such as electron or protons or a beam of X-ray, is focused into the sample under study. In the X-ray range the energy of a single photon is just sufficient to produce a measurable pulse of X-ray; the output of an ultra-low noise pre-amplifier connected to the lower noise is a statistical measure of the corresponding quantum energy. By digitally recording and counting a great number of such pulses within a so-called multichannel analyser, a complete image of X-ray spectrum is building up almost simultaneously.The number and energy of X-ray emitted from a specimen can be measured by an energy-dispersive spectrometer. A semiconductor material is used to detect the X-ray together with a processing electronics to analyse the spectrum.

Antibacterial activity studies

Silver nanoparticles synthesized from *Turbinaria conoides, Spyridia filamentosa and Halimeda macroloba* were screened for antibacterial activity against poultry pathogenic bacteria namely *Streptococcus. aureus* and *Salmonella typhi.*

Media preparation:

Nutrient broth (Peptone – 5g, Beef extract – 1.5g, Yeast extract – 1.5g, NaCl – 5g and Distilled water – 600ml; pH adjusted to 7.2) was prepared. After sterilization, the bacterial culture was inoculated in the nutrient broth. The inoculated broth has been incubated for 24 hours at 37°C in incubator.

Nutrient agar (Peptone – 5g/l, Yeast extract – 1.5g/l and Beef extract – 1.5g/l, pH of 7.2) was prepared and sterilized before poured on to the sterilized petri plates. After the solidification of the media overnight bacteria culture were prepared and inoculated by swap method.

Disc diffusion method

The antibacterial activity of silver nanoparticles from *Halimeda macroloba, Spyridia filamentosa* and *Turbinaria conoides* extracts were determined by disc diffusion method. Discs of 6mm diameters were prepared from pre-treated Whatmann No.1 Filter paper. Then they were sterilized in the hot air oven at 160°C for 1 hour. The nutrient agar plates were prepared and inoculated with test bacterial organisms by spreading the bacterial inoculum on the surface of the media. 5mg of Silver nanoparticles from 3 species of seaweeds were mixed with 1ml of Methanol. The discs were impregnated with different concentrations ranging of 25µg, 50µg, 75µg and 100µg. A blind control was prepared by taking 1mM Silver nitrate and dissolve into 1ml Methanol.

Evaluation of antibacterial activity

The plates were incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the area in which bacterial growth was inhibited around the well, diameter of the zone of inhibition (in mm).

RESULTS AND DISCUSSION

Visual identification

The seaweed samples of *T. conoides, S. filamentosa* and *H. macroloba* were collected and dried. The extraction was done using methanol as solvent. 1mM of silver nitrate was added in the seaweed extracts and incubated for 24 hours. The change of colours was observed and reported in Fig 1.

UV-Vis spectrometry

UV-Vis spectrometry of the silver nanoparticles from *T. conoides, S. filamentosa* and *H. macroloba* was carried out by measuring the spectrum band of the reaction mixture ranging from 300 to 700nm. The results were shown in Fig 2.

FTIR Analysis

Fourrier Transformation Infrared spectrometry (FTIR) analysis of the silver nanoparticles from *T. conoides, S. filamentosa* and *H. macroloba* was done using dried powder of silver nanoparticles. Various peak values confirmed the presence of various metabolites as illustrated in Fig 3 and Table 1, 2 & 3.

XRD Analysis

X-ray diffraction patterns indicated the crystalline structure of the silver nanoparticles synthesized from *T. conoides, S. filamentosa* and *H. macroloba*. The silver nanoparticles were found to be in nano-sizes as shown in Fig 4.

SEM Analysis

The silver nanoparticles synthesized from *T.conoides, S. Filamentosa* and*H. macroloba* were also characterized by Scanning Electron Microscopy (SEM). The sizes of the silver nanoparticles were found to be above 100nm as shown in Fig 5.

EDX Analysis

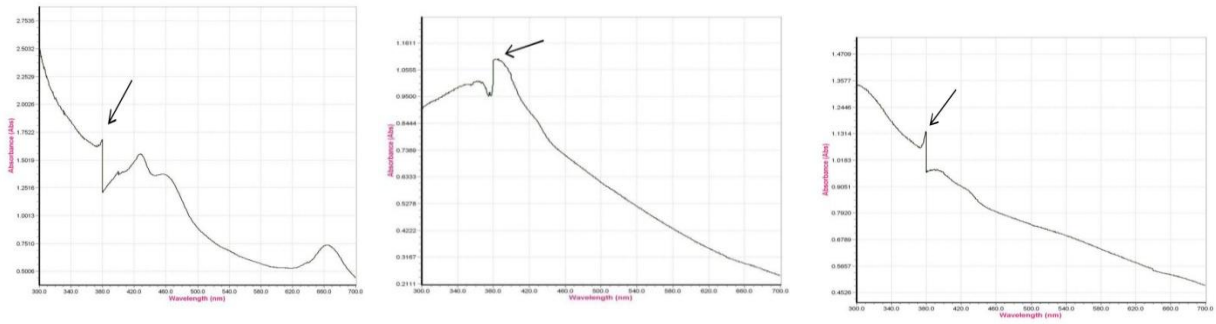
Energy Dispersive X-ray spectroscopy (EDX) analysis of the silver nanoparticles synthesized from *T. conoides, S. filamentosa* and *H. macroloba* resulted in various elemental characterization as reported in Fig 6 and Table 4, 5 & 6.

Antibacterial Activity assay

The antibacterial activities of the silver nanoparticles from *T. conoides, S. filamentosa* and *H. macroloba* against poultry pathogens *S. aureus* and *S. typhi* were confirmed by formation of zones of inhibition. Silver nanoparticles from *S. filamentosa* was found to have a strong antibacterial effect against bacteria compare to other silver nanoparticles studied as illustrated in Fig 7, Table 7 and Graph 1, 2 & 3.

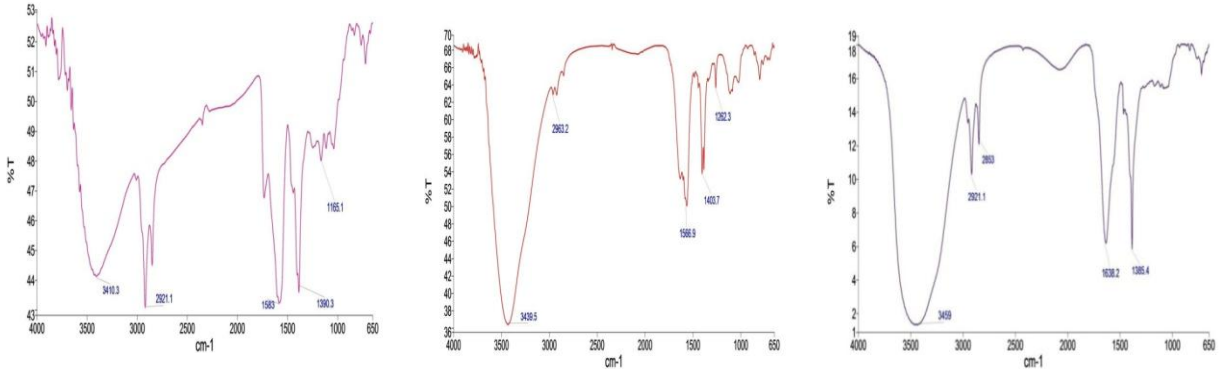
H. macroloba ***T. conoide*** ***S. filamentosa***

Figure 1: Visual identification of silver nanoparticles from seaweeds.



H. macroloba *T. conoides* *S. filamentosa*

Figure 2: UV-Vis spectrometry of silver nanoparticles from seaweeds.



Halimeda macroloba *Turbinaria conoide* *Spyridia filamentosa*

Figure 3: FTIR analysis of silver nanoparticles from seaweeds.

Table 3: FTIR analysis of silver nanoparticles from *Halimeda macroloba.*

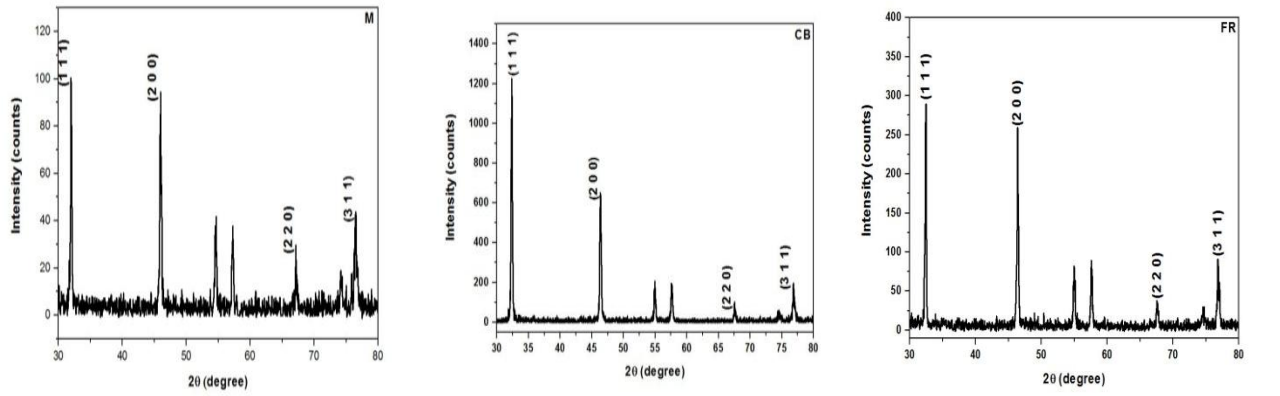
Frequency	Bond	Functional group / Structure
3410.3	O-H (strong, broad)	H-bonded alcohols, phenols
2921.1	O-H & C-H (medium)	carboxylic acids & alkanes
1583	N-H bend (medium)	primary amines
1165.1	C-O (strong), C-H & C-N (medium)	alcohols, carboxylic acids,esters,ethers,alkyl halides and aliphatic amines

Table 1: FTIR analysis of silver nanoparticles from *Turbinaria conoides.*

Frequency	Bond	Functional group /Structure
3439.5	O-H (strong, broad)	alcohols, phenols
2963.5	O-H & C-H (medium)	carboxylic acids & Alkanes
1403.7	C-C (medium) in-ring	aromatics
1262.3	C-N & C-O (strong), C-H	alcohols, carboxylic acids,esters,ethers,alkyl halides and aromatic amines

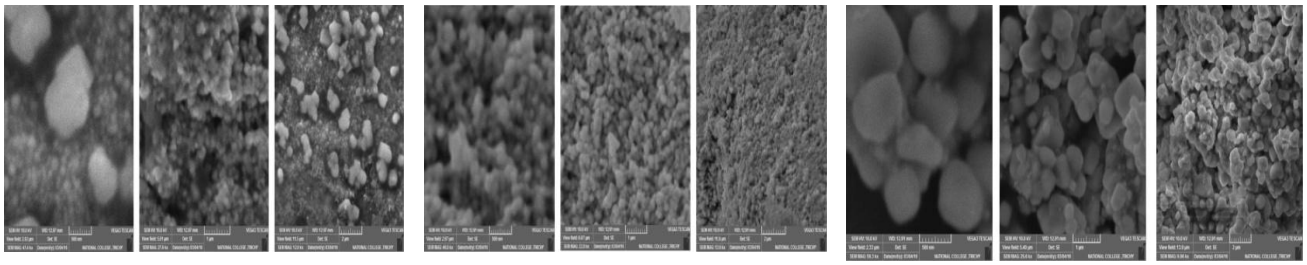
Table 2: FTIR analysis of silver nanoparticles from *Spyridia filamentosa.*

Frequency	Bond	Functional group / Structure
3459	O-H (strong, broad)	H-bonded alcohols, phenols
2921.1	O-H & C-H (medium)	carboxylic acids & alkanes
2853	O-H & C-H (medium)	carboxylic acids & alkanes
1638.2	N-H bend (medium)	primary amines



Halimeda macroloba *Turbinaria conoide* *Spyridia filamentosa*

Fig.4.XRD Analysis of silver nanoparticles from seaweeds.



Halimeda macroloba *Turbinaria conoides* *Spyridia filamentosa*

Figure 5: SEM analysis of silver nanoparticles from seaweeds.

Fig 6. ***Halimed macroloba.***

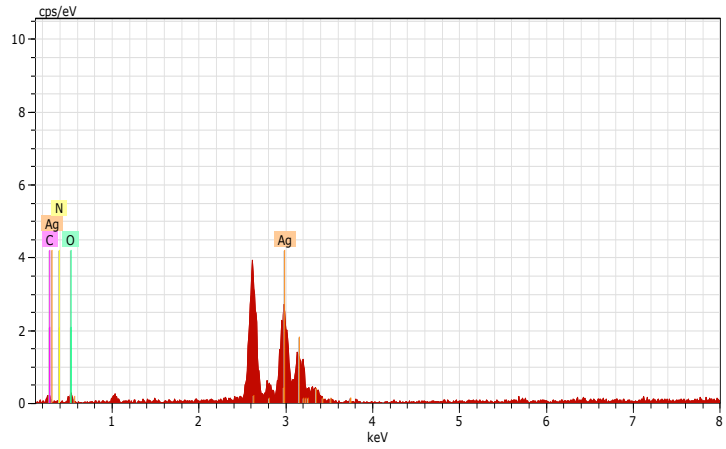


Figure 6: EDX Analysis of silver nanoparticles from seaweeds.

Table 6: Elemental composition of silver nanoparticles from *Halimed macroloba.*

Element	Weight (%)	Atom (%)
Ag	70.75	25.19
C	5.07	16.21
O	22.60	54.26
N	1.58	4.34

(Turbinaria conoides).

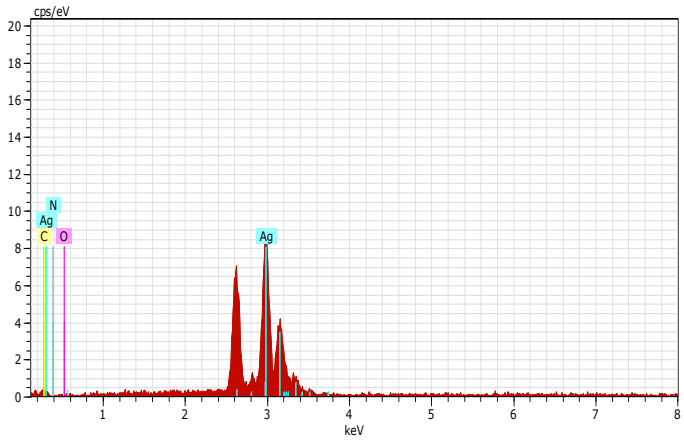


Table 4: Elemental composition of silver nanoparticles from *Turbinaria conoides.*

Element	Weight (%)	Atom (%)
Ag	90.90	56.26
C	3.79	21.04
O	4.43	18.49
N	0.88	4.22

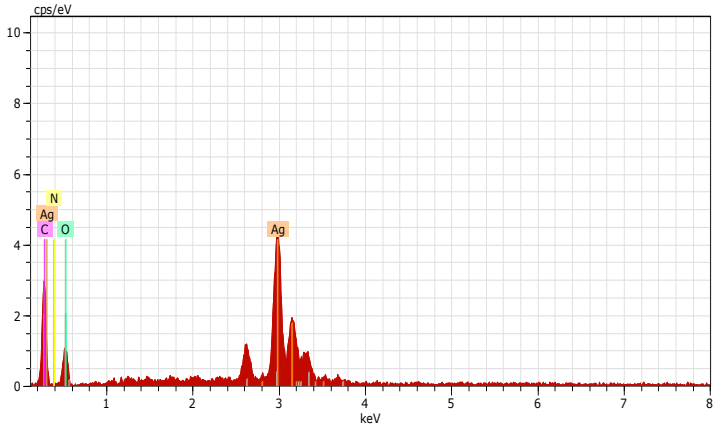
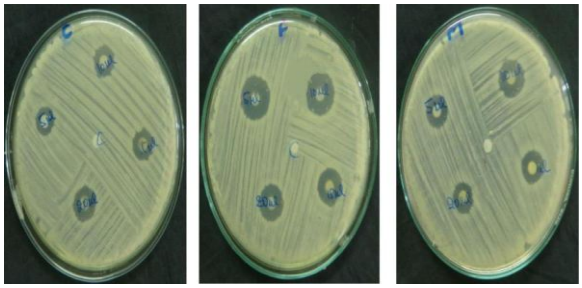


Fig 6. *Spyridia filamentosa.*

Table 5: Elemental composition of silver nanoparticles from *Spyridia filamentosa.*

Element	Weight (%)	Atom (%)
Ag	33.17	5.94
C	32.12	51.63
O	31.60	38.14
N	3.11	4.29

Staphylococcus aureus.



T. conoides ***S. Filamentosa*** ***H. macroloba***

Figure 7: Antibacterial activities of silver nanoparticles against poultry pathogens.

Salmonella typhi

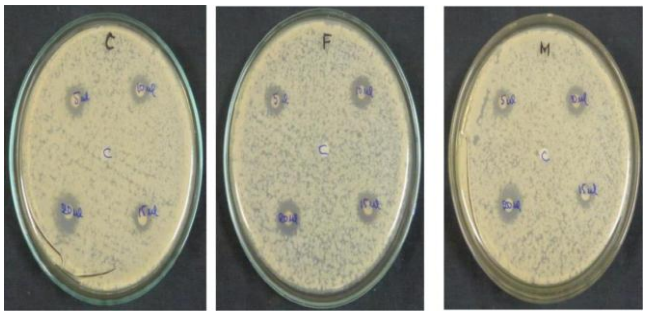
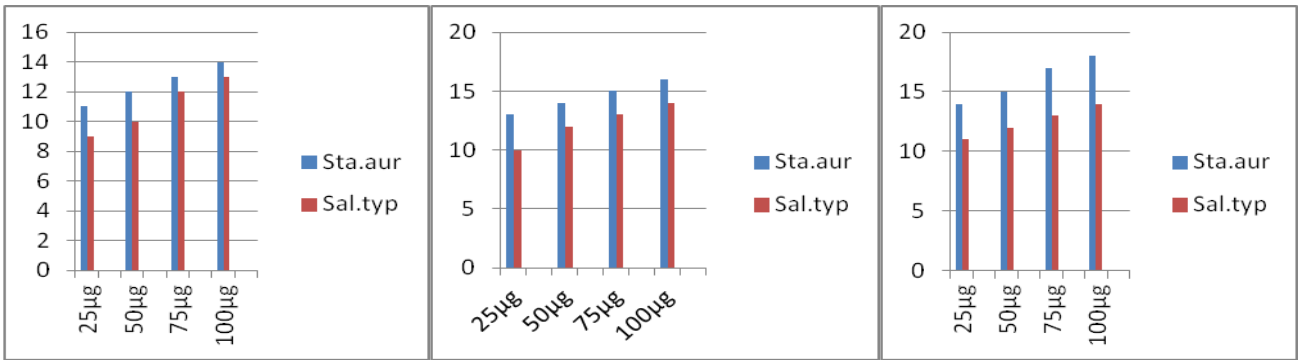


Table 7: Antibacterial activity of silver nanoparticles from seaweeds.

Halimeda macroloba ***Turbinaria conoides*** ***Spyridia filamentosa***



In the present study, 3 species of seaweeds including brown seaweed *Turbinaria conoides*, red seaweed *Spyridia filamentosa* and green seaweed *Halimeda macroloba* are used to synthesise silver nanoparticles. The extraction of these samples involved an organic solvent, methanol. The reaction mixture was concentrated by centrifugation and dried in the oven. The antibacterial effect of the silver nanoparticles from these 3 seaweed samples was investigated using disc diffusion method. The antibacterial activity was tested against poultry pathogens including a gram-negative *Salmonella typhi* and a gram-positive, *Staphylococcus aureus.*The formation of silver nanoparticles was confirmed by the change in the colours of each sample after incubation. The colour of the solution changed immediately after addition of seaweed extract to the aqueous silver nitrate solution. After 24 hours, there was no colour change which indicates that the silver nanoparticles synthesis process was completed. For *T. conoides*, metallic green changed into milky brown then into brown milky colour, for *S. filamentosa*, dark green changed into white then into milky white and for *H. macroloba*, the dark purple changed into lime green then greenish yellow colour.The biosynthesis of silver nanoparticles was confirmed by the UV-Visible spectral analysis at different nm. The SPR vibrations are found between 300-700nm. The λ max of silver nanoparticles synthesized using *T. conoides* was 385nm, while in case of silver nanoparticles synthesized by *S. filamentosa* and *H. macroloba*, it was 380nm.

The results of FTIR analysis of silver nanoparticles from *T. conoides* confirmed the presence of alcohols, phenols, carboxylic acids, alkanes, aromatic amines, esters, alkyl halides and ethers with the peak values from 1262.3 to 3439.5. Analysis of silver nanoparticles from *S. filamentosa* confirmed the presence of alcohols, phenols, carboxylic acids, alkanes and primary amines with the peak values from 1638.2 to 3459. Analysis of silver nanoparticles from *H. macroloba* confirmed the presence of alcohols, phenols, carboxylic acids, alkanes and primary amines, aromatic amines, esters, alkyl halides and ethers with the peak values from 1638.2 to 3459.

The exact nature of the silver particles formed can be deduced from the XRD spectrum of the sample. The X-ray diffraction peaks were found to be broad around their bases indicating that the silver particles are in nano-sizes. The peak broadening at half maximum intensity of the X-ray diffraction lines is due to a reduction in crystallite size, flattening and micro-strains within the diffracting domains. XRD analysis showed different distinct diffraction peaks of the crystalline planes of cubic silver (Ag). All three samples had the peaks which were recorded at (111), (200), and (222). It can be indexed that the values of 2$\boldsymbol{\theta}$ were of 32°, 46.5°, 67.5° and 77 for the three samples.

Scanning Electron Microscopic analysis (SEM) has shown the surface morphology and size details of the silver nanoparticles. A representative SEM image recorded from the silver nanoparticles at the magnification of 20,000 X, 10,000 X and 5,000 X. The size of the prepared nanoparticles was more than the desired size i.e. between 1-100nm. This is due to the proteins which were bound in the surface of the nanoparticles.

EDX analysis of the silver nanoparticles synthesized from from *T. conoides* has shown a strong silver signal (90.90%) and weak signals of carbon, oxygen and nitrogen. For silver nanoparticles from *S. filamentosa*, the EDX profile has silver (33.17%), Oxygen (31.60%) and (Carbon 32.12%) and weak signal of nitrogen. For silver nanoparticles from *H. macroloba*, EDX profile has shown a strong silver signal (70.75%) along with Oxygen (22.60%) and weak signals of carbon and nitrogen.

The antibacterial activity of silver nanoparticles from seaweed samples was confirmed by formation of zone of inhibition. The zone of inhibition in the plate showed that silver nanoparticles from seaweed samples synthesized using methanol extract have the antibacterial activities against poultry. By comparing the zones of inhibition between these 2 bacteria, it showed that gram positive (*Staphylococcus aureus*) are more susceptible to silver nanoparticles than gram negative bacteria (*Salmonella typhi*).Silver nanoparticles from *S. filamentosa* showed a significantly stronger activity against *Staphylococcus aureus* compared to those of *T. conoides* and *H. macroloba*. The anti-*Salmonella* activity of silver nanoparticles from *T. conoides* and *S. filamentosa* was significantly higher than those of *H. macroloba*.

SUMMARY

The synthesis, characterization and application of biologically synthesized nanomaterials are an important aspect in nanotechnology. The seaweed samples were extracted using methanol as solvent and silver nanoparticles were synthesized and concentrated into pellet by centrifugation before being dried within oven.

Successful characterization and confirmation of the synthesized silver nanoparticles was done using various techniques including visual identification, UV-visible (UV-Vis) spectroscopy, Fourier transform infrared spectroscopy (FTIR), Energy Dispersive X-ray Spectroscopy (EDX), Scanning Electron Microscopy (SEM), and X-ray diffraction (XRD).The visual identification test consisted of the change in colour of the reaction mixture after incubation period to confirm silver nanoparticles synthesis. The UV-Vis spectral analysis provided the evidence of synthesis of nanoparticles. The FTIR analysis explains the stability of silver nanoparticles that are synthesized by the seaweed. The XRD analysis gives the structural information of nanoparticles. The SEM and EDX analyses confirmed the synthesis of nanoparticles.Antibacterial assays were done using disc diffusion method at different concentrations (25µg, 50µg, 75µg and 100µg) against poultry pathogens namely *Staphylococcus aureus* and *Salmonella typhi*. The formation of zones of inhibition confirmed the silver nanoparticles to be antibacterials and more effective on gram positive bacteria.

It was finally concluded that seaweed extracts using methanol as solvent were effective in the synthesis of silver nanoparticles and that the synthesized silver nanoparticles had antibacterial effect on both gram positive and gram negative bacteria. Out of the three seaweed extracts used to synthesize silver nanoparticles, *S. filamentosa* showed a strong antibacterial activity compared to those of *T. conoides* and *H. macroloba.*

We observed that, if the concentration of seaweed nanoparticle increases, the zone of inhibition also gets increased in all the test five clinical bacterial pathogens against streptomycin.

The current protocol can be further developed to extend the potential use of silver nanoparticles to several fields and further characterization such as ^{1}H and ^{13}C NMR to be done for bio-molecules capped in it.

ACKNOWLEDGEMENT

We would be thankful to Dr. D. Dhanasekaran, Assistant Professor, Department of Microbiology, Bharathidasan University, Trichy for providing lab facilities to carry out the research.

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