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BIO- SYHTHESIS OF SILVER NANOPARTICLES FROM *LOBOPHORA VARIEGATE.L.* AND *INVITRO* ANTIMICROBIAL ASSAY

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ABSTRACT

In the present study Silver nanoparticles were synthesized from aqueous seaweed extract of Lobophora variegata. and characterized by UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-Ray Diffraction (XRD), Scanning electron microscopy (SEM) and Energy Dispersive X(EDX).Characterization by the above said instrument analysis confirmed the presence, size and stability of silver nanoparticles. After characterization, the silver nanoparticle was tested at 100µg-ml, 200µg-ml, 300µg-ml and 400µg-ml concentrations to check the bactericidal activity against clinical isolates of five bacterial pathogens. We observed that, if the concentration of seaweed nanoparticle increases, the zone of inhibition also get increased in all the test five clinical bacterial pathogens against streptomycin as control and result suggested the potential use of aqueous extract seaweed synthesized silver nanoparticles against other clinical pathogens.

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INTRODUCTION

Anybody who has interest in nanoscience and nanotechnology is not unaware of the name of a great physicist and nobel laureate Richard Feynman;who first introduced concept of nanotechnology in 1959,in a talk entitled "There's plenty of room at the Bottom".Today we all accept nanotechnology as "The design, charactcterization, production, and applications of structures, devices, and systems by controlled manipulation of size and shape at the nanometer scale (atomic,molecular,and macromolecular scale) that produces structures, devices, and systems with at least one novel/superior characteristic or property."

Nanotechnology is the branch of science dealing between nanoscience and biotechnology involving the application of biological system for the production, manipulation and design of new functional nano sized materials with dimension ranging from 1-100nanometer or one billionth. It combines biological methods with physical and chemical procedures to generate nano –sized particles using green technology is advantageous over chemical agents owing to their environmental anxieties. [4,20]. There is significant to produce nanoparticles as they supply greater materials properties with effective resourcefulness.

Owing to their properties and significance over accessible chemical imaging drugs, inorganic nanoparticles have been studies as possible materials for medical imaging along with for treating diseases.Silver nanoparticles have been using in medicine for antibacterial, antifunctional, anti- viral, anti- inflammatory therapy and anticancer therapy [12].

At present biosynthesis of nanoparticles has been projected as a cost effective and eco-friendly alternative to physical and chemical methods [9,10]. Biosynthesis of nanoparticles using plant extracts is the significant methods of green, eco-friendly production of nanoparticles and exploited to a vast extent since the plants with a choice of metabolites, are extensively distributed,easily available,and safe to handle[23].

Among the marine organisms, marine algae (in particular, seaweeds)are important source of phytochemical compounds such as, carbohydrates, alkaloids, steroids,phenols,protein and flavonoids that are used to reduce Ag^+ ions for the synthesis of silver nanoparticles [13,27].

Recently, silver nanoparticles (AgNPs) are widely investigated to owing their broad range of applications as antibacterials [38,41], biosensors [3] and in plant growth metabolism[11]. The green synthesis of inherently AgNPs depends on the adoption of the basic requirements of green chemistry the solvent medium, the benign reducing agents and the non-hazardous stabilizing agents [39].Marine macroalgae production a great variety of secondary metabolites that showed therapeutic potential [35,17]. Chaetomorpha linum (Muller) Kutzing is green seaweed mainly acknowledged for its ecological role as possible regulator of nutrient availability in estuarine habitats [14]. Application of green chemistry to the synthesis of nanomaterials has vital importance in medicinal and technological aspects [19,2]. *Lobophora variegate* seaweed have been used since ancient time as food,fodder,fertilizer and as source of medicine. To characterize the silver nanoparticles by UV,FTIR,EDAX,XRD,SEM and to find out the antimicrobial activity of the synthesized silver nanoparticles against human pathogens.

MATERIALS AND METHODS

SEAWEED COLLECTION

Fresh seaweed *Lobophora variegate J. V. Lamouroux* a brown seaweed was collected from the intertidal regions of the Mandapam coast (Lat. 09 17.417'N; Long. 079˚ 08.558'E) of Rapid island, the Gulf of Mannar coast, Rameswaram, SouthTamil Nadu. India.

PREPARATION OF SEAWEED EXTRACT

The fresh seaweed was washed with running tap water for 20 mints. and dry in shade at room temperature for one week. Then the seaweed are cut into small pieces and make into fine powder.20g of seaweed extract are weighed and dissolved in 200ml distilled water in a 500ml Erlenmeyer flask and boil for 30mins . The extracts was filtered with Whatman No.1filter paper and stored in an airtight container under dark condition until for further use.

PREPARATION OF SILVER NANOPARTICLES

1mM of silver nitrate (AgNO3) was prepared in 1000ml beaker (0.1698 g $AgNO_3$ is added to 1000ml of distilled water). The 100ml seaweed extract were mixed with 900ml silver nitrate solution (1:9) ratio. and kept under dark condition. Color change of the solution from white with pale yellow indicated that the silver nanoparticles get synthesized and then the solution was centrifuged at 7,000rpm, 20^{o}C for 15mints. Then the supernatant was collected from the tube and it was kept for evaporation (to sediment the particles) until it gets fully evaporated. Collect the pellet which is kept in hot air oven at 40^{0}C for twenty four hours. Further, the synthesized sample was used for characterization and antimicrobial activity studies.

CHARACTERIZATION OF SILVER NANOPARTICLES

The characterization of silver nanoparticles was carried out by visual observation, UV- Vis Spectrophotometer, FTIR, XRD, SEM, and EDAX.

UV- VIS Spectrophotometer

The absorption spectra of the samples were taken 300 to 600 nm using a UV–Vis spectrophotometer. and deionised water was used as the blank.

FOURIER TRANSFORM INFRAED SPECTROSCOY (FTIR)

FTIR analysis was carried out to identify the possible functional groups of the bio molecules responsible for reduction of the Ag^{+} ions.

X-Ray diffraction analysis (XRD)

The X-ray diffraction pattern indicated the crystalline nature of silver nanoparticles.The XRD spectrum confirmed the presence of silver nanoparticles at diffracted intensities was recorded in 2Θ angle.

Scanning Electron Microscope (SEM)

The size and shape of biocapped silver nanoparticles are characterized.

Energy- Dispersive X-ray Spectroscopy(EDAX)

The number and energy of the X rays emitted from a specimen can be measured by an energy –dispersive spectrometer.

Antibacterial activity studies

Silver nanoparticles synthesized from *Lobophora variegate* was screened for antibacterial activity against clinical pathogenic bacteria namely *Escherichia coli, Pseudomonas aeruginosa, Rhodococcus rhodnii, Streptococcus aureus* and *Proteus mirabilis*

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 of the medium,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 – $1.^{5g-l}$, agar 15 g^{-l}, pH of 7.2) was prepared, sterilized and poured on to the sterilized petriplates.

Preparation of inoculums:

Bacterial inoculums were prepared by transferring a loop full of bacterial culture from fresh culture plates to tubes containing 10 mL of Nutrient Broth (Hi-media) and incubated for 24 hours at 37^{o}C.These cell suspensions were diluted with sterile Nutrient Broth to provide initial concentration cell counts of about 2 x 10^{3} CFU^{-mL} at 600nm O.D at Spectrophotometer. After the solidification of the media in the petriplate, bacterial cultures were inoculated by swap method.

Disc diffusion method

The antibacterial activity of crystalline bio molecule capped synthesised silver nanoparticles from *Lobophora variegata* was determined by disc diffusion method. Discs of 6mm diameters were prepared from Whatmann No.1 Filter paper and kept 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 inoculums on the surface of the media. The discs were impregnated with different concentrations ranging of $100\mu g^{-ml}$, $200\mu g^{-ml}$, $300\mu g^{-ml}$ and $400\mu g^{-ml}$. A negative control was prepared by taking 1mM Silver nitrate dissolved in 1ml distilled water and positive control Streptomycin used as positive controls (100 μg^{-mL}).

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 disc and measured the diameter zone of inhibition (in mm).

RESULT AND DISCUSSION

Green Synthesis of Silver Nanoparticles

The brown seaweed *Lobophora variegata* was used for the synthesis of silver nanoparticles. Reduction of $AgNO_3$ was visually observed the colour change of the extract from pale yellow to brownish color shown in Figure .1and this is may be due to the excitation of surface Plasmon resonance effect and the reduction of silver ions.[22].

Fig.1. ***Lobophora variegate.***

Fig.2.Initial and final visual observation of synthesis Silver nanoparticles from ***Lobophora variegata.***

UV-Visible spectroscopy analysis

The Silver nanoparticles obtained were characterized by UV-Visible spectroscopy and the characteristic absorption peak maximum at 380 nm for the aqueous extract of *Lobophora variegata* confirmed the formation of silver nanoparticles shown in Figure .2.The frequency and width of the surface Plasmon absorption depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium [21].

Fig.2. UV-Vis absorption spectra of silver nanoparticles synthesized by ***Lobophora variegata*** **exposure with 1mM Silver nitrate.**

FTIR spectroscopy analysis

FTIR analysis was used for the characterization of the synthesized silver nanoparticle extract of *Lobophora variegate.* Fig.3.

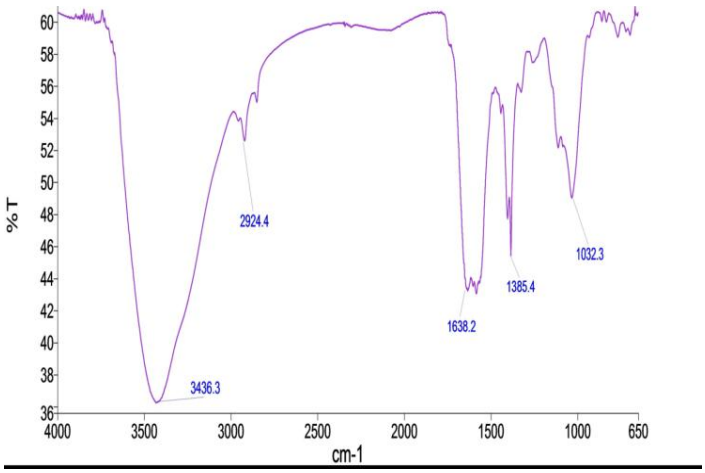


Fig.3.FTIR Spectrum of silver nanoparticles synthesized from *Lobophora variegata.*

The absorbance band was observed at 3436.3 (cm−l) O-H streteh,H -bonded 2924.4 (cm−l) assigned to the C-H stretch alkanes group respectively. The band at 1636.2 (cm−l) corresponding to the N-H bend primary amines group. The band seen at 1385.4 (cm−l) corresponding to the C-H bend alkanes group.The band at 1032.3(cm−l)C-N stretch aliphatic amines.The result revealed that the capping ligand of the silver nanoparticles may be an aromatic compound or alkanes. FTIR analysis was done by using IRPal.2.0 free software and confirned the functional groups found in the biomolecule capped in itTable.1. The biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium [22].

EDX analysis

Result exhibited the EDAX analysis showed strong signal in the Ag region (Figure.4) and confirmed the presence of silver along with carbon, nitrogen and oxygen in the synthesised nanoparticles.

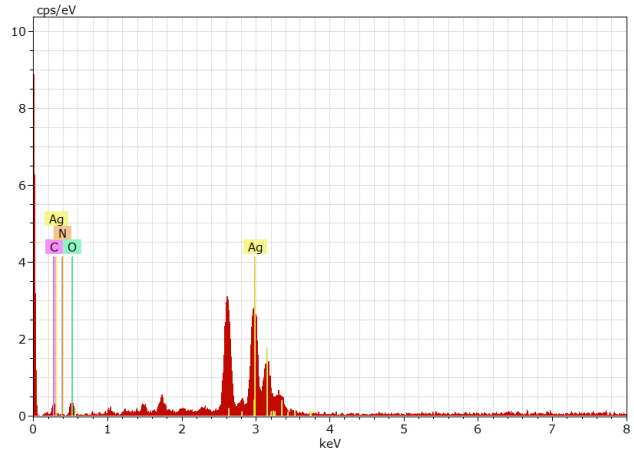


Fig. 4. EDX spectrum of silver nanoparticles synthesized from *Lobophora variegata* with dominant peaks for C,N,O,Cl, and Ag respectively.

The presence of carbon along with Ag suggests the involvement of the bio-molecules in the synthesis of silver nanoparticles and they might have served as stabilizing molecules [33].

X-Ray Diffraction analysis

X-ray diffraction analysis of the synthesized silver nanoparticles was observed at 2 theta angles and the intensive peaks was observed at 32.00, 27.49 and 31.14,with their corresponding lattice plane values at (111), (200),(220) and(311).Thus the synthesized silver nanoparticles was confirmed that it ascrystalline in nature.Figure 5. The typical XRD pattern revealed that the samples contains (crystalline structures) of the silver nanoparticles[16].

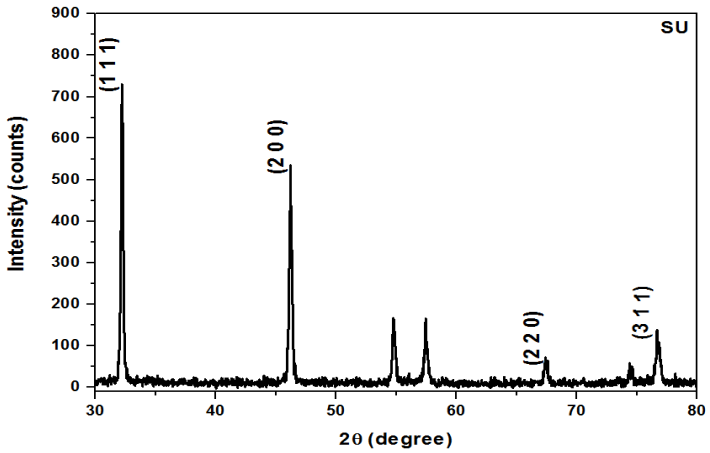


Figure. 5. XRD patterns of capped silver nanoparticles synthesized from *Lobophora variegata.*

Scanning electron microscopy (SEM)

The SEM image showing the high density Ag-NPs synthesized by the *Lobophora variegata* further confirmed the development of silver nanostructures.

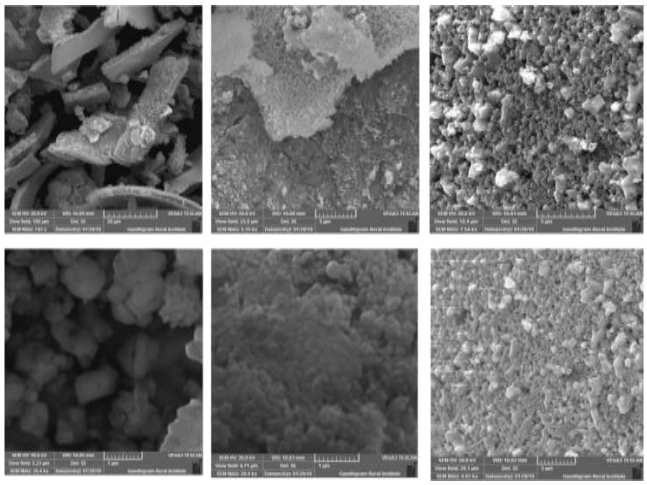


Fig.6. SEM micrograph of silver nanoparticles synthesized from *Lobophora variegata*.

364.5 was the particle size of the biomolecule capped crystalline nanoparticles of synthesized seaweed extract by SEM.(Fig.6).The SEM micrographs of nanoparticles obtained in the filtrate showed that Ag-NPs are spherical shaped, well distributed without aggregation in solution [25].

Synthesis of PdNPs was achieved from the seaweed *Sargassum ilicifolium* at room temperature in five days. The synthesized PdNPs are spheri-cal in shape with an approximate size of 60-80 nm. The synthesis of PdNPs can serve as a better alternative for the other conventional methods practiced for the synthesis of Palladium nanoparticle [24].Synthesized silver nanoparticles using *Padina tetrastromatica seaweed* extract of TEM images revealed that the particles are spherical in shape and size varies from 10-100nm. The XRD result also confirms that the particles have a face entered cubic crystalline structure [6]. *Chaetomorpha antennina* also shows synthesis of gold nanoparticle at a faster rate when compared to red seaweeds.[18].The synthesisezed silver nanoparticles obtained in chloroform: methanol (1:1 v/v) from the brown seaweeds *Hedophyllum sessile* and *Spatoglossum asperum* exhibit antimicrobial property related to terpene and phenols against certain plant pathogens. The FTIR, SEM, TEM, XRD and bioactivity data of *Hedopyllum sessile was* more promising than Spatoglossum asperum silver nanoparticles [1].

In Sargassum longifolium the AgNPs showed maximum cytotoxic activity at a least concentration, which revealed AgNPs as novel anticancer drug. [31].Biologically synthesized silver nanoparticles could be of immense use in medical textiles for their efficient antibacterial and antimicrobial properties [30]. The Uv-Vis,FTIR,EDAX SEM confirms the crystalline nature and eize of the synthesised silver nanoparticles(40nm).

Sargassum longifolium extract mediated synthesized silver nanoparticles show high antifungal activity that can be used therapeutically in biomedical applications.[29].The synthesis of silver nanoparticles using seaweed Urospora sps.was confirmed by UV-Vis spectroscopy, FTIR and its spherical shape by HRTEM.(10 to 20 nm) .The antibacterial effect of silver nanoparticles showed greater bactericidal effect against the bacterium *S.aureus* and would be applied in pharmacology. [34].

The seaweed synthesised silver nanoparticles have excellent antifungal activity.[28]. The amines, peptide groups and secondary metabolites flavonoids and terpenoids are present in the silver nanoparticle synthesized green seaweed *Chaetomorpha linum* extract involved in the bioreduction and stabilization of AgNP and also applied in biomedical and agricultural fields. [26].

Recently, the researchers are looking into the development of cost-effective procedures for producing reproducible, stable and biocompatible silver nanoparticles from *Gracillaria dura*.[7]. The algae are also proposed for the biosynthesis of gold and silver nanoparticles as an efficient, eco-friendly and simple process. The gold nanoparticle size and shape could be directly controlled by the initial pH value of the solution from the red seaweed *C. crispus*.In addition; the biosynthesis of gold and silver nanoparticles was also attained with the green alga.[15].It was concluded that plant mediated synthesis of silver nanoparticles possess potential antimicrobial applications and the characterization analysis proved that the particle so produced in nanodimensions would be equally effective as that of antibiotics and other drugs in pharmaceutical applications and in drug delivery systems might be the future thrust in the field of medicine.[5].

Protein extracts of *Padina pavonica*, *Padina tetrastomatica* and *P. gymnospora* have been a good resources for silver nanoparticle synthesis. In addition, the results showed good antibacterial activity besides different bacterial species and might be the capping of silver nanoparticles with proteins,which utilized in medicinal and biomedical applications.[8].

ANTIMICROBIAL ACTIVITY:

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 for the first time at concentration from 100µg^{-ml},200µg^{-ml},300µg^{-ml} and 400µg^{-ml} of this particular brown seaweed *Lobophora variegate*.(Fig.7 and 8.)[36,37].

Fig.7.Silver nanoparticles synthesized seaweed extract and its antimicrobial activity

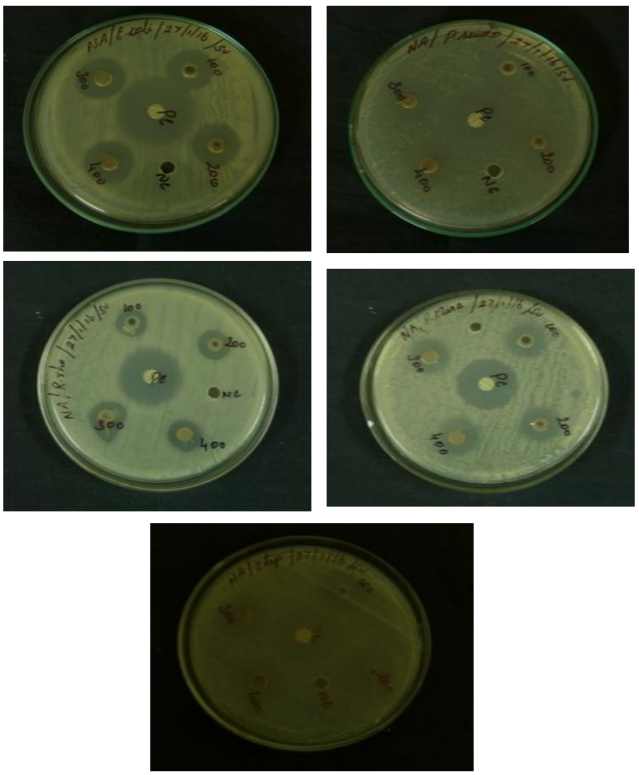
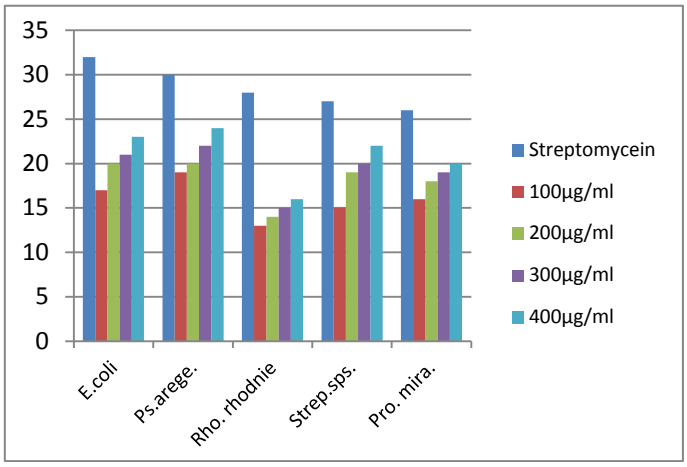


Fig: 8.Antimicrobial activity of *Lobophora variegata* silver nanoparticles synthesis (Diameter zone of inhibition in mm).



Primary conformation of the AgNPs was carried out by UV- Visible spectrophotometric analysis. The nanoparticles show maximum absorbance beak at 380 nm on UV - Vis spectra which is shown in Fig 2. The UV-Vis spectra recorded from the *Lobophora variegata* reaction vessel at different ranges of temperature like 30°C-80°C, but good result was obtained at 70°C and 80°C.

CONCLUSION

In the present study,silver nanoparticles was synthesized using aqueous seaweed extract of *Lobophora variegate* showed brownish colour formation, which indicated the reduction of silver ions by seaweed extract. The particles were analyzed and characterized by UV-Vis spectrophotometer, FTIR, XRD,EDX and SEM. The silver nanoparticles synthesized from seaweed extract of *Lobophora variegata* showed good bactericidal activity against clinical isolates of five bacterial pathogens. The present study also offers the ability of *Lobophora variegata* seaweed extract to reduce silver ions to synthesize silver nanoparticles which can be used for several applications like antifungal, anticancer, antiviral activity and etc.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 have to be done for bio-molecules capped in it.

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