Synthesis of Silver nanoparticles using bioactive seaweeds against poultry pathogens

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Research Article

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Abstract

There is an increasing demand to create nanomaterials that are eco-friendly, non-toxic, and less expensive in relation to human health. In order to investigate the effectiveness of the marine macro algae silver nanoparticles against two specific poultry bacterial infections, methanol extracts of *Halimeda macroloba* (green), *Turbinaria conoides* (brown), and *Spyridia filamentosa* (red) were used. The zone of inhibition against the two bacterial pathogens as well as $AgNO_3$ as a control increases with an increase in the concentration of marine macro algae synthesized nanoparticles. The characterization by UV, FTIR, XRD, SEM and EDAX reveals the formation of silver nanoparticle through seaweeds and the LCMS data reveals the presence of bioactive compounds which exhibit antibacterial activity. Thus the chemical compounds such as phyllophenone and phorbasterone present in the marine macro algae may be responsible for the antibacterial action of poultry pathogens such as *Staphylococcus aureus* and *Salmonella typhi* according to LC-MS characterizations.

1. Introduction

Marine macroalgae are regarded as an ecologically and biologically significant component of the modern ocean because they influence biogeochemistry for their breeding and feeding, create vital habitat in the near shore system, support native biodiversity, convert inorganic to organic carbon to fuel food webs, and support native biodiversity. The antibacterial activity of the silver nanoparticles made by some of the researchers utilising *Halimeda macroloba* (green), *Turbinaria conoides* (brown), and *Spyridia filamentosa* (red) methanolic extract was previously studied. By combining materials science, biological science, medical science, and technology in the 21st century, nanotechnology is a fascinating kind of applied research that has created opportunities for unique technological advancements (Arun kumar *et al*., 2012).

The ability to produce nanoparticles is shared by seaweed, big marine benthic, brackish, unicellular algae (macro algae), and thalloid plants. These nanoparticles are essential to several nanomedicine applications, including gene therapy, tissue engineering, diagnostics, screening, drug delivery systems, and nanorobot configuration. Due to its cutting-edge nature and the effectiveness of manufactured nanoparticles in industrial, medicinal, and electronic applications as catalysts, cancer detection, and bioimaging, biological nanoscience has recently attracted increased attention (Braddy *et al*., 2003) and (Cohen et al., 2007). In order to analyse the biosynthesized AgNPs and investigate the in-vitro antibacterial activity of human multi-resistant infections present in poultry pathogens, the primary goal of our work is to synthesize silver nanoparticles from an methanol extract of *Halimeda macroloba* (green), *Turbinaria conoides* (brown), and *Spyridia filamentosa* (red).

Antibiotics are used in the poultry business to control chicken diseases. Because they affect the microflora in the digestive system, antibiotics such avoparcin, bacitracin, lincomycin, penicillin-G, chlortetracycline, and virginiamycin encourage growth (Collier et al., 2003). However, using dietary antibiotics led to issues like the spread of drug-resistant bacteria, drug residues in the bodies of the birds, and an imbalance in the normal microflora. Through the food chain and other sources, drug-resistant

bacteria like Staphylococcus sp., Escherichia coli, and Enterococcus sp. can spread from poultry to people, potentially resulting in therapeutic failures in both humans and animals. Therefore, the development of alternative medications or strategies to lower infections has been impacted by public demand to limit the use of antimicrobials. Therefore, the goal of the current study was to examine the antibacterial ability of silver nanoparticles against Salmonella typhi and Staphylococcus aureus, two poultry diseases (Duran et al., 2007).

The current study's goals were to prepare seaweed extracts using methanol as a solvent, synthesis silver nanoparticles from seaweed extracts, and characterize the silver nanoparticles using visual identification, particle size analysis, and LC-MS characterization. The study also sought to determine the antibacterial effects of the silver nanoparticles on two poultry pathogens.

2. Materials And Methods

2.1. Collection of seaweed samples

Halimeda macroloba, Turbinaria conoides, and *Spyridia filamentosa* fresh live seaweed samples were obtained from the Mandapam coastline region (78.8°E, 90.17°N), in the Gulf of Mannar, Tamilnadu, South India. In order to remove salt and other foreign substances, the gathered seaweed samples were carefully rinsed with fresh water before being transferred to the lab in polythene bags. Algae were cleaned, then dried for a week at room temperature in the shade.

2.2. Collection of bacterial culture

The Microbiology Department of Bharathidasan University in Tamil Nadu provided the pathogenic pure cultures of *Staphylococcus aureus* and *Salmonella typhi*. The pure cultures were afterwards sub cultured on nutrient agar slants and stored for later use under refrigeration.

2.3. 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.

2.4. 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 Whatman No1 filter

paper, the sample was filtered; the filtrate was collected into the conical flask and kept at room temperature for further use (Siva kumar *et al.*, 2014).

2.5. Preparation of reaction mixture

1mM of Silver nitrate was weighed (0.16987g) and added into a mixture containing 900 ml of distilled water and 100 ml 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 (Govindaraju *et al.*, 2002).

2.6. 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 (Lee et al., 2007).

2.7. Characterization of silver nanoparticles from seaweed extract

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, EDX and LCMS analysis (Marcetol *et al.*, 2005).

2.8. Antibacterial activity studies

Silver nanoparticles synthesized from *Turbinaria conoides, Spyridia filamentosa and Halimeda macroloba* were screened for antibacterial activity against poultry pathogenic bacteria namely *S. aureus* and *S. typhi.* By using the disc diffusion method, the antibacterial activity of silver nanoparticles from extracts of Halimeda macroloba, Spyridia filamentosa, and Turbinaria conoides was assessed. Paper from the Whatman No. 1 Filter was pre-treated to create discs with a 6mm diameter. They were then disinfected for an hour at 160°C in the hot air oven. By applying the bacterial inoculum to the media's surface, the nutrient agar plates were created and inoculated with test bacteria. 3 different seaweed species' silver nanoparticles, totaling 5 mg, were combined with 1 ml of methanol. The impregnation of the discs ranged from 25g to 100g in various concentrations. A blind control was made by dissolving 1mM of silver nitrate in 1ml of methanol. 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) (Parashar et al., 2009).

3. Results

3.1. Visual identification of developed silver nanoparticles

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 colors was observed and reported in Fig. 1.

3.2. Characterization of Silver nanoparticles from seaweed extract

3.2.1. UV-Visible Spectrophotometer analysis of silver nanoparticles developed from seaweed extract

3.3. FTIR analysis of silver nanoparticles developed from seaweed extract

Analysis of the FTIR spectrum revealed a variety of absorbance bands. As illustrated in (Figs. 5, 6 and 7) the strong spectral bands were interpreted for the identification of functional moieties in air dried silver nanoparticles. The findings suggested that an aromatic component, alkanes, an amine, a phenolic compound, or an ester could be the cause of the capping ligand of silver nanoparticles (Tables 1, 2 and 3).

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 (medium)	aromatic amines; alcohols, carboxylic acids, esters, ethers& alkyl halides

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
3459	O-H (strong, broad)	H-bonded alcohols, phenols

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 & aliphatic amines

3.4. XRD analysis of silver nanoparticles developed from seaweed extract

The cubic silver's crystalline planes' unique diffraction peaks were revealed by XRD investigation (Ag). The peaks that were detected at (111), (200), and were present in all three samples (222). It may be deduced from (Figs. 8, 9 and 10) that the values of 2 were 32, 46.5, 67.5, and 77.

3.5. SEM analysis of silver nanoparticles developed from seaweed extract

The reduced form of silver nitrate solution underwent scanning electron microscopic investigation, which is plainly visible. The synthesized nanoparticles were larger than the desired size, which was between 1 and 100 nm. This is a result of the proteins that were bound to the nanoparticles' surface. As can be seen

in (Fig. 11, 12, and 13) the outcome revealed that the particles were both spherical and sheet-like in shape.

3.6. EDX analysis of silver nanoparticles developed from seaweed extract

The elemental characterisation of the silver nanoparticles manufactured from seaweed samples was revealed by EDX analysis in the form of an EDX graph, as shown in (Figs. 14, 15 and 16). The elemental such as silver, carbon, nitrogen compound in sea weed were confirmed through EDAX analysis. The elemental makeup of these silver nanoparticles made from seaweeds is revealed by EDX analysis and is displayed in Tables (4, **5, and 6).**

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

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

Table 6
Elemental composition of silver nanoparticles from *Halimeda macroloba*

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

3.7. LCMS analysis of silver nanoparticles developed from seaweed extract

LC-MS has been widely used for the characterization of the phenolic profiles of different plant and marine samples. A qualitative analysis of the phenolic compounds from different seaweed extracts were achieved by LC-ESI-QTOF-MS/MS analysis in negative and positive ionization modes **(Figs. 17, 18 and 19).** The valuable compound such as hexa decanoic acid were found in large quantities in *H. macrolaba* while other bioactive compounds such as dicaffeoylquinic acid, o-acetyl pruning, acetoxy-pinoresinol, mellerin A were present in *T. connoides.*

Antibacterial activity assay of silver nanoparticles developed from seaweed extract

The presence of an inhibitory zone in the plate demonstrated the antibacterial properties of silver nanoparticles made from methanol extracts of seaweed samples against poultry pathogens **(Table No. 7).** The bacterial action was exceeded by the silver nanoparticles. The zone of inhibition of the antibacterial activity is depicted in **(**Figs. 20 and 21**)**. It can be seen that gram positive bacteria (*Staphylococcus aureus*) are more vulnerable to silver nanoparticles than gram negative bacteria (*Salmonella typhi*) by comparing the zones of inhibition between these two bacteria.

Table 7
Antibacterial activity of silver nanoparticles from seaweeds

		Zones of inhibition	
Silver nanoparticles	Concentration		
		Staphylococcus	*Salmonella*
	(mg/ml)	*Aureus*	*multocida*
	25	10	11
Turbinaria conoides			
	50	12	12
	75	13	13
	100	14	14
	25	14	11
Spyridia filamentosa			
	50	15	12
	75	17	13
	100	18	14
	25	11	9
Halimeda macroloba			
	50	12	10
	75	13	12
	100	14	14

4. Discussion

Bio-organisms are environmentally beneficial and can be used as a reducing agent and capping agent during this process, synthetic processes utilising them are compatible with the concepts of green chemistry (Li et al., 2007). When biosynthesized nanoparticles are compared to chemically produced nanoparticles, it is discovered that the latter are more environmentally friendly and biocompatible when used in medicinal applications. Plant extracts may be used as a source of nanoparticles because they are non-toxic and include a wide range of metabolites that are necessary for reduction. When the usage of whole plant extracts and plant tissues are compared, it is discovered that using plant extracts for nanoparticle manufacturing is easier, more easily scaled, and may be less expensive (Iravani, 2011).

Recent papers have discussed using plant extracts to make nanoparticles since they are convenient, safe, and include a wide variety of biomolecules that can help with the synthesis of nanoparticles, including alkaloids, terpenoids, phenols, flavonoids, tannins, and quinines. (Zhou et al., 2011). Secondary metabolites from plants have shown to be a great source of novel pharmaceuticals (Park et al., 2009). Water soluble phytochemicals reduce metal ions considerably more quickly than fungus and bacteria, which require a much longer incubation period. Plants are therefore better prospects for the creation of nanoparticles than bacteria and fungi. A number of substances, including primary and secondary metabolites produced by seaweeds, represent a viable source for use in biotechnology and industry (Sonti *et al*., 2007). In order to create colloidal silver with particles that are several nanometers in diameter, silver ions (Ag+) must be reduced in aqueous solution. Metal ions are reduced by a combination of biomolecules present in plant extracts, including enzymes, proteins, amino acids, polysaccharides, and vitamins (Anil Kumar et al., 2007 and Marimuthu *et al*., 2008). When different complexes are reduced by Ag + ions, silver atoms Ag (0) are created, which then aggregate into oligomeric clusters. Colloidal Ag particles eventually form as a result of these clusters. The choice of solvent, reducing agent, and capping agent are the essential factors in the reduction of the metal salts and synthesis of metal nanoparticles utilising plant extracts (sutradhar et al., 2013; bunghez and ion, 2010).

Research on nanotechnology for the creation of silver nanoparticles has shown that using natural plants is safer and more effective. Despite the enormous plant diversity, many more plants have not yet been investigated for their potential in the production of nanoparticles and their use in the pharmaceutical and agricultural industries. As a result, the use of these renewable marine resources has received a lot of interest as a result of the biosynthesis of nanoparticles utilising seaweed (Kumar *et al*., 2012 and Zhu et al., 2009). The physico-chemical characterization of generated nanoparticles is a crucial step in the biosynthesis of nanoparticles. Understanding a nanoparticles size, shape, surface area, homogeneity, and other features will give you important knowledge of nanoscale systems and insight into how to control the synthesis of nanoparticles for commercial applications. In the current study, silver nanoparticles are produced using three different types of seaweed, including the brown seaweed *Turbinaria conoides*, the red seaweed *Spyridia filamentosa*, and the green seaweed *Halimeda macroloba* (Raffi et al., 2008). These samples were extracted using methanol, an organic solvent. The reaction mixture was centrifuged to concentrate it, and it was then dried in an oven. Using the disc diffusion method, the antibacterial activity of the silver nanoparticles from these three seaweed samples was examined. Salmonella typhi, a gram-negative poultry pathogen, and *Staphylococcus aureus*, a gram-positive poultry pathogen, were examined for the antibacterial activity. The alteration in each sample's colour following incubation served as proof that silver nanoparticles had formed (Ramanigade *et al*., 2013 and Tian et al., 2007). As soon as seaweed extract was added to the aqueous silver nitrate solution, the solution's colour changed. There was no colour change after 24 hours, proving that the process of creating silver nanoparticles had finished. Metallic green for *T. conoides* changed to milky brown then to brown milky colour, white *for S. filamentosa* changed to milky white and dark purple for *H. macroloba* changed to lime green then to greenish yellow colour (Shankar et al., 2003).

The characterization reveals that the UV-visible spectrophotometer of sea weed confirms the presence of silver nanoparticle formed. Also the compounds formed like phenolic acid and alcohol were the main constituents present in all the seaweed extracts which exhibit antibacterial activity. By forming a zone of inhibition, silver nanoparticles from seaweed samples proved to have antibacterial activity (Shin et al., 2007 and Singh et al., 2013). The zone of inhibition in the plate demonstrated that silver nanoparticles made from seaweed samples and extracted from methanol have antibacterial effects on chickens. Staphylococcus aureus was found to be more vulnerable to silver nanoparticles than gram negative bacteria by comparing the zones of inhibition between these 2 types of bacteria (*Salmonella typhi*). Compared to those from *T. conoides and H. macroloba*, silver nanoparticles from *S. filamentosa* had a noticeably higher activity against Staphylococcus aureus. Silver nanoparticles from *T. conoides* and *S. filamentosa* have much stronger anti-Salmonella activity than those from *H. macroloba*.

5. Conclusion

Chemical techniques have not been as successful in the therapeutic field as biological approaches in overcoming many of the challenges involved in the production of NPs. We learned through this study that employing seaweeds to make silver nanoparticles has made for a safer and more environmentally friendly approach when compared to other ways. Additionally, the use of marine green resources like macroalgae in the synthesis of AgNPs was thought to be a beneficial alternative to chemical synthesis. Additionally, according to the findings, marine seaweeds are effective at creating extracellular silver nanoparticles, and because biomolecules and secondary metabolites are present, these nanoparticles can be used in solutions for an extended period of time. The produced nanoparticles are quite stable for long periods of time without significant change, making these new biogenic and environmentally benign methods excellent for use in the pharmaceutical and medical fields. As the chosen seaweed-derived nanoparticles demonstrated the best antibacterial activity against multi-resistant poultry pathogens such as *Staphylococcus aureus and Salmonella* along with the conventional commercially prescribed antibiotics, they could be further examined against other contagious and most terrifying disease-causing pathogens in the future as a potential alternative.

Declarations

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Author Contribution

S. R. Sivakumar conceived, designed research, conducted experiments, analysed data and wrote the whole manuscript, He has read and approved the manuscript

Competing Interest

Author declares that there is no potential conflict of interest in this paper.

Ethical Approval: Not applicable

Availability of data and materials: Not applicable

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Figures

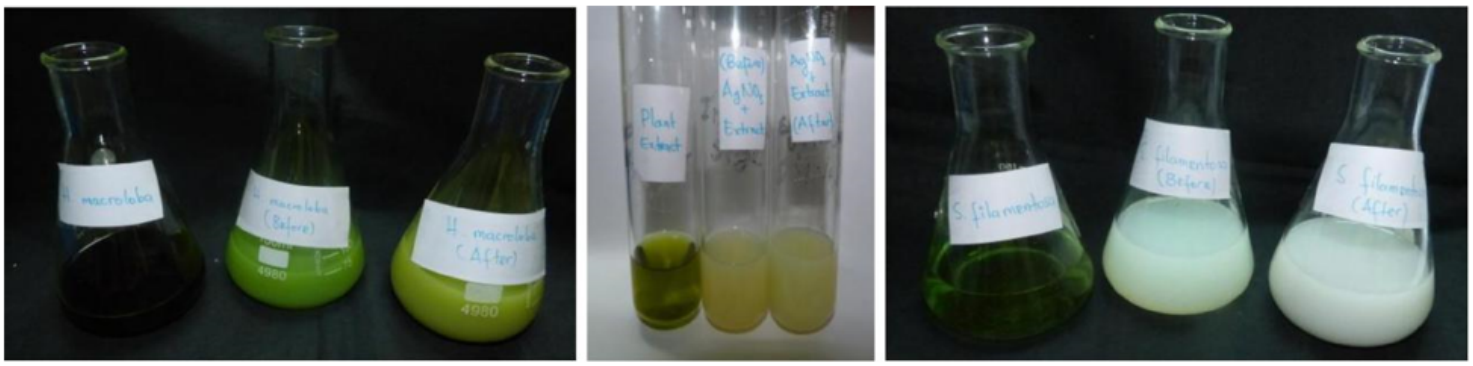


Figure 1

Change in colors of the seaweed extract before and after incubation

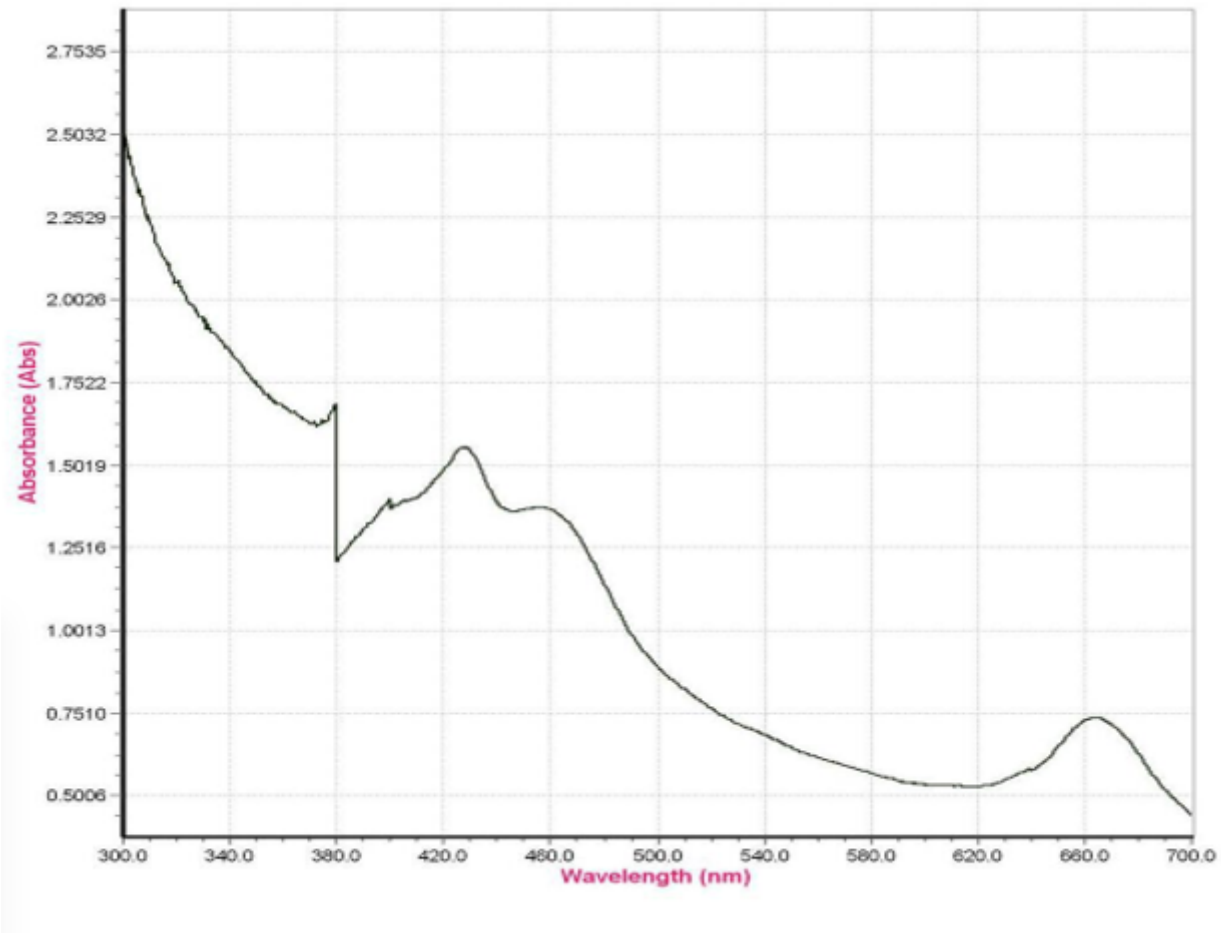


Figure 2

UV-Vis spectrometry of silver nanoparticles from *Halimeda macroloba*

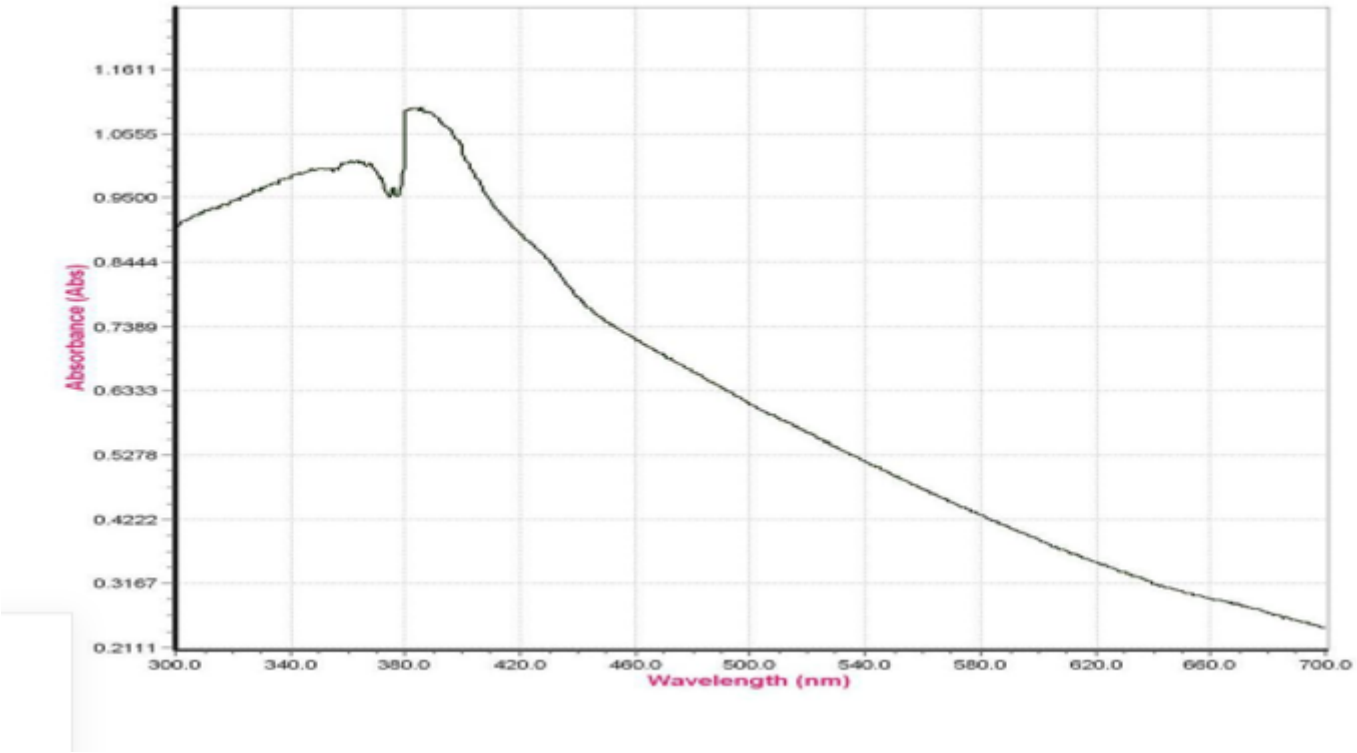


Figure 3

UV-Vis spectrometry of silver nanoparticles from *Turbinaria conoides*

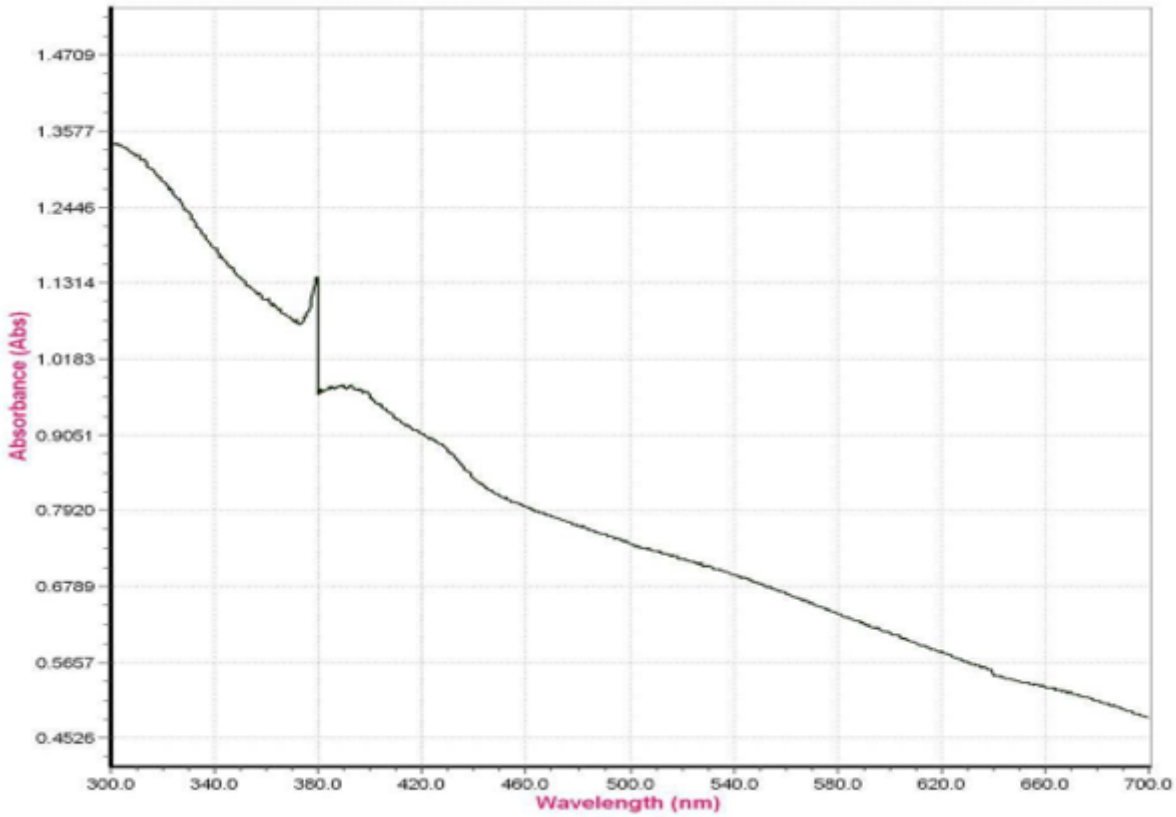


Figure 4

UV-Vis spectrometry of silver nanoparticles from *Spyridia filamentosa*

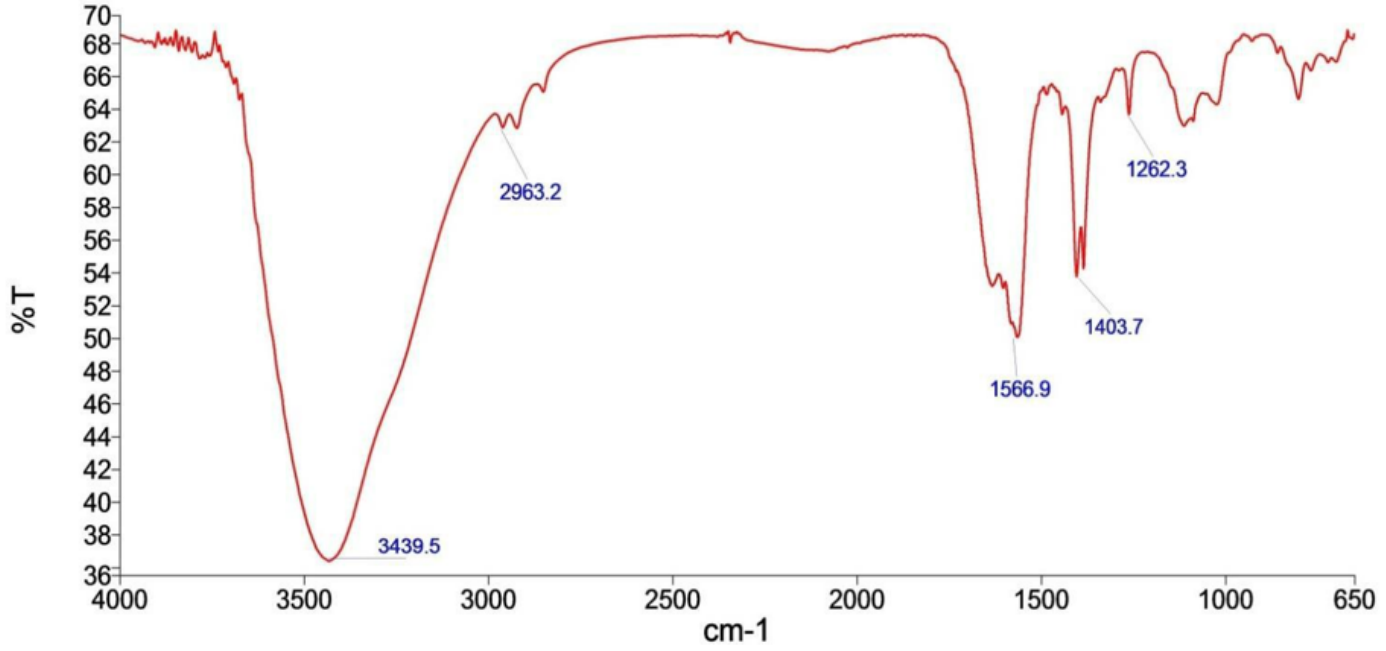


Figure 5

FTIR analysis of silver nanoparticles from *Turbinaria conoides*

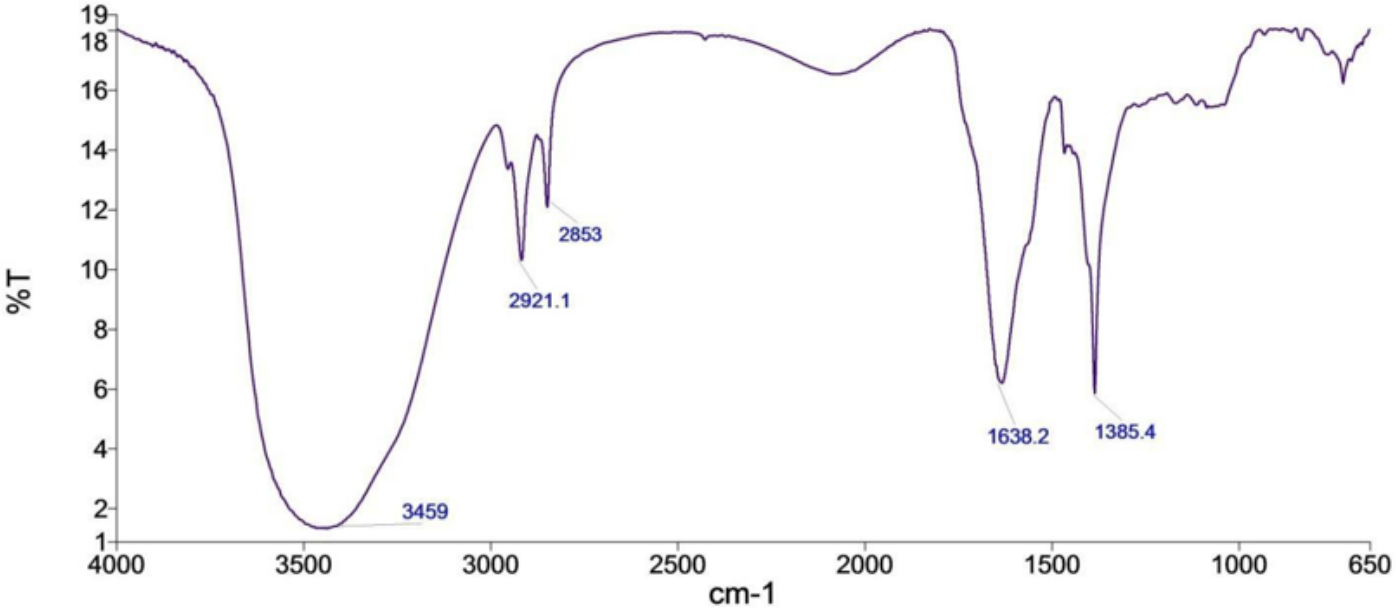


Figure 6

FTIR analysis of silver nanoparticles from *Spyridia filamentosa*

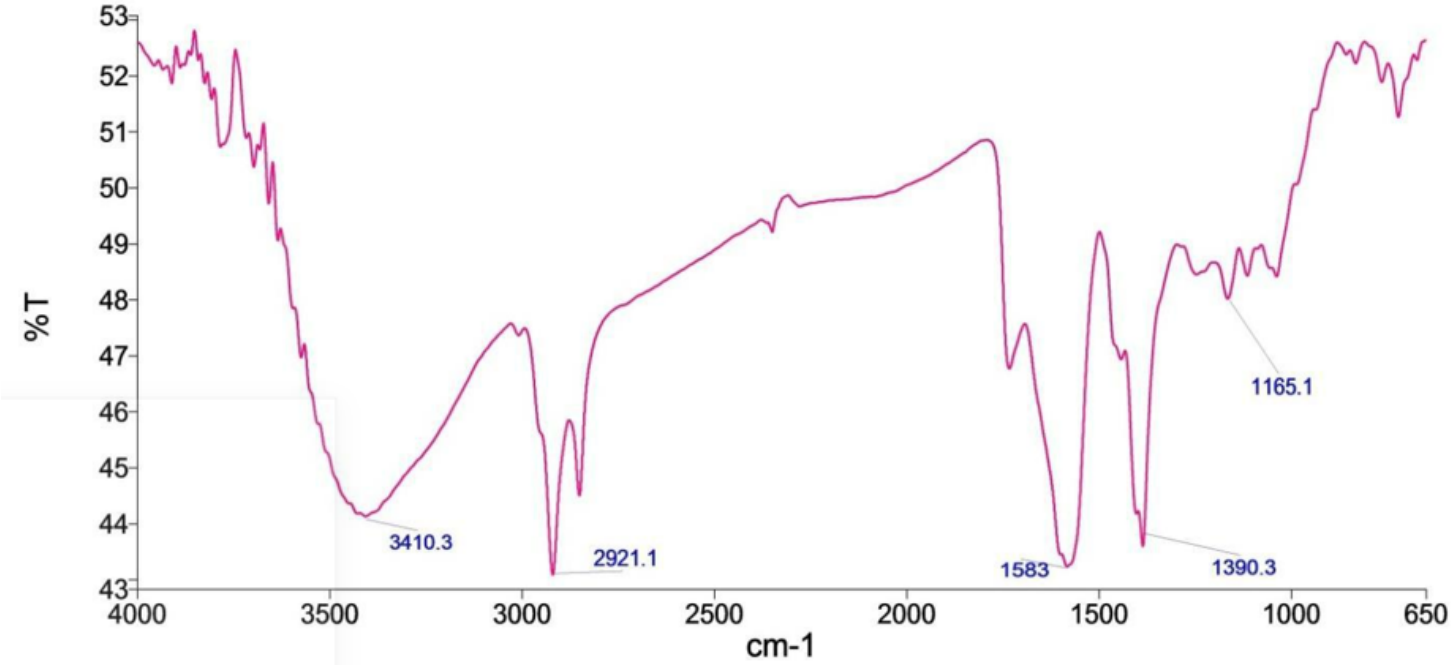


Figure 7

FTIR analysis of silver nanoparticles from *Halimeda macroloba*

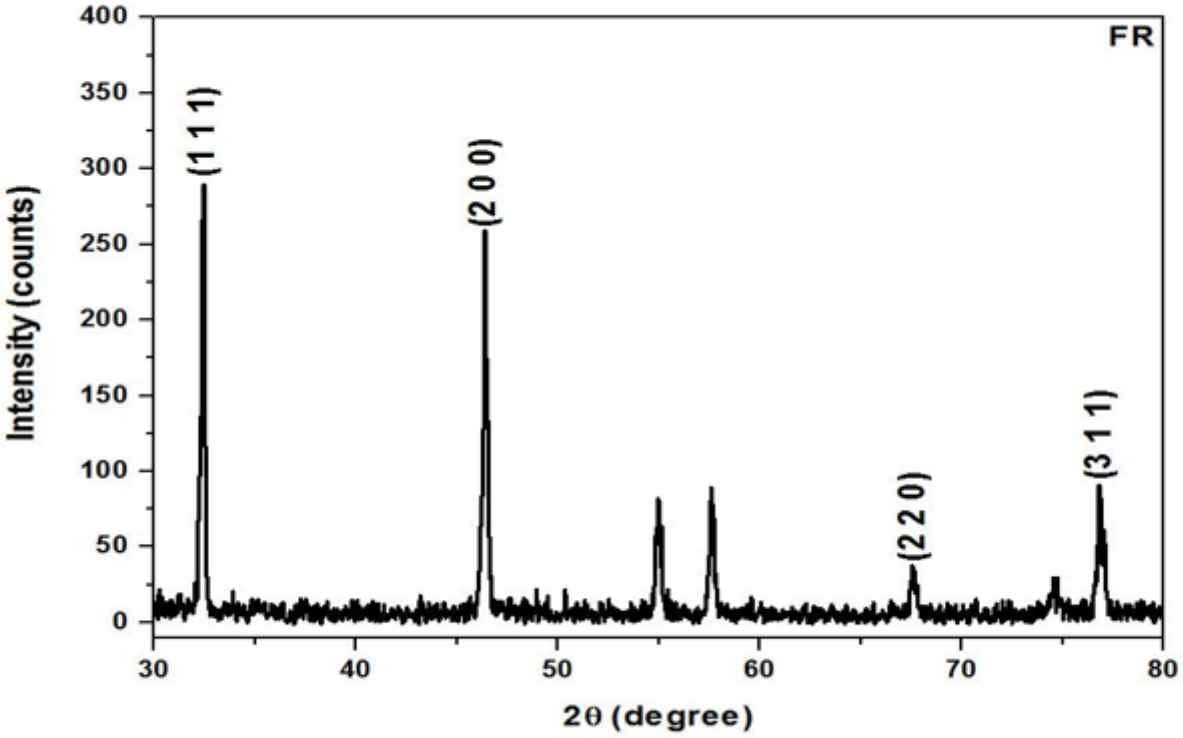


Figure 8

XRD Analysis of silver nanoparticles from *Spyridia filamentosa*

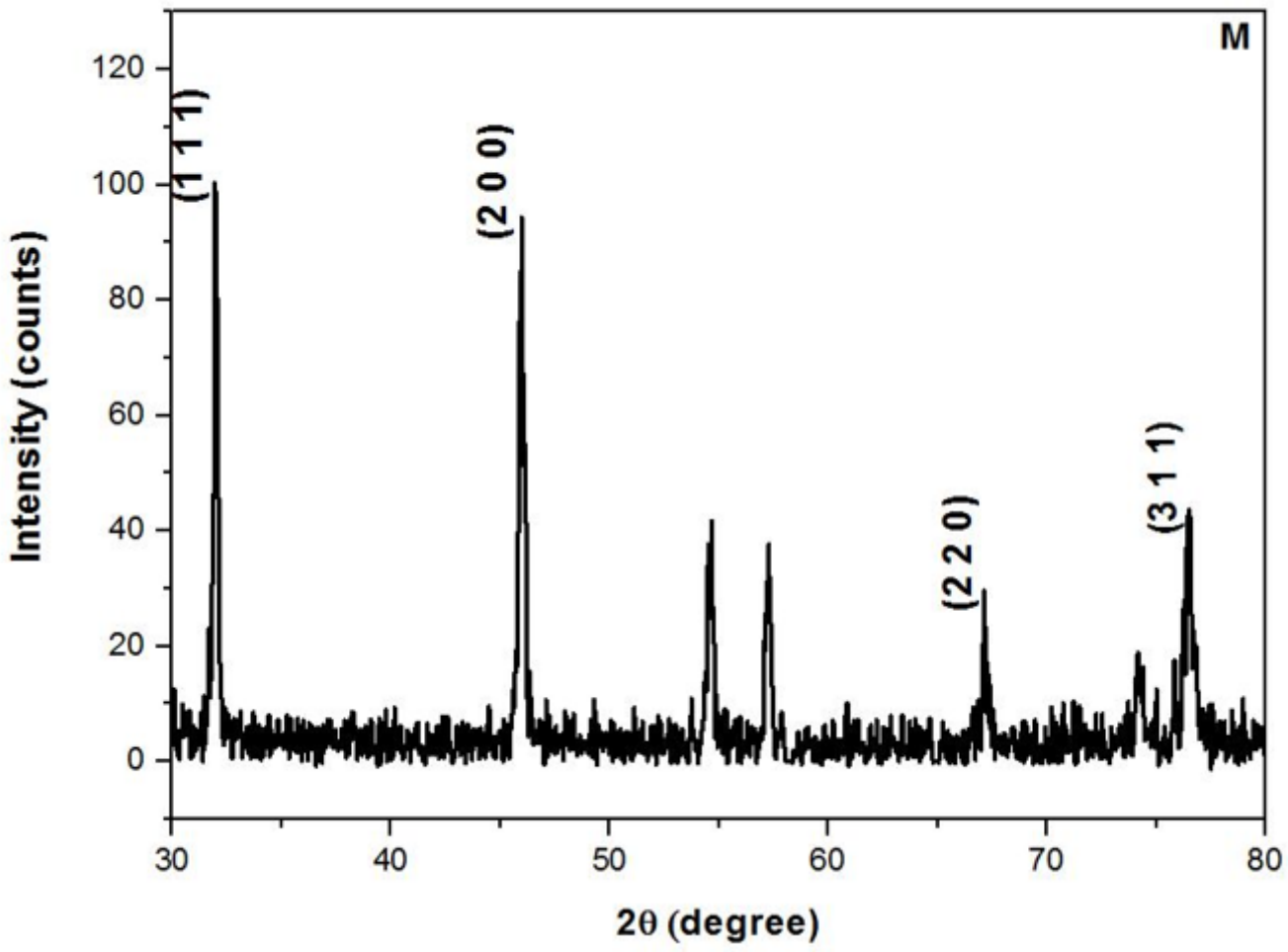


Figure 9

XRD Analysis of silver nanoparticles from *Halimeda macroloba*

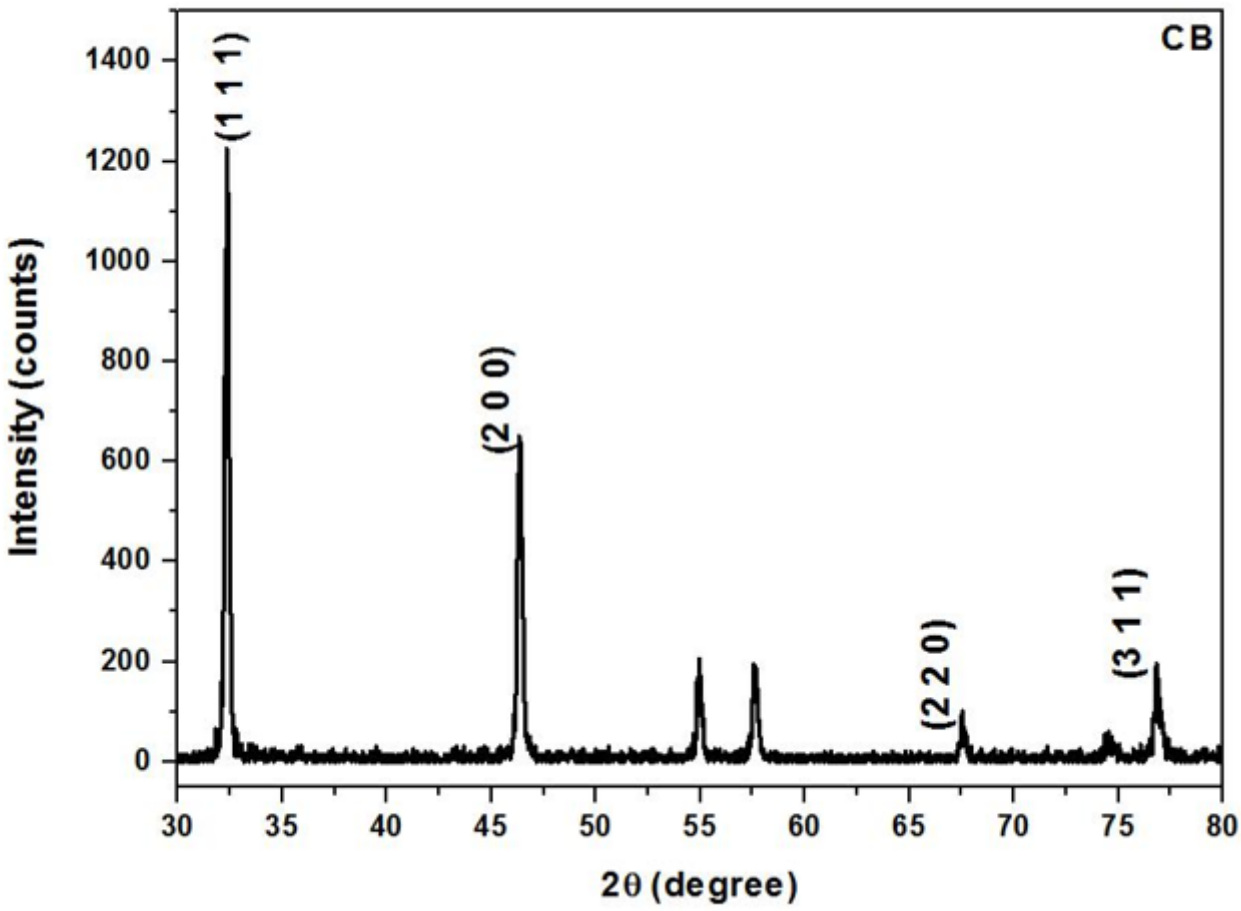


Figure 10

XRD Analysis of silver nanoparticles from *Turbinaria conoides*

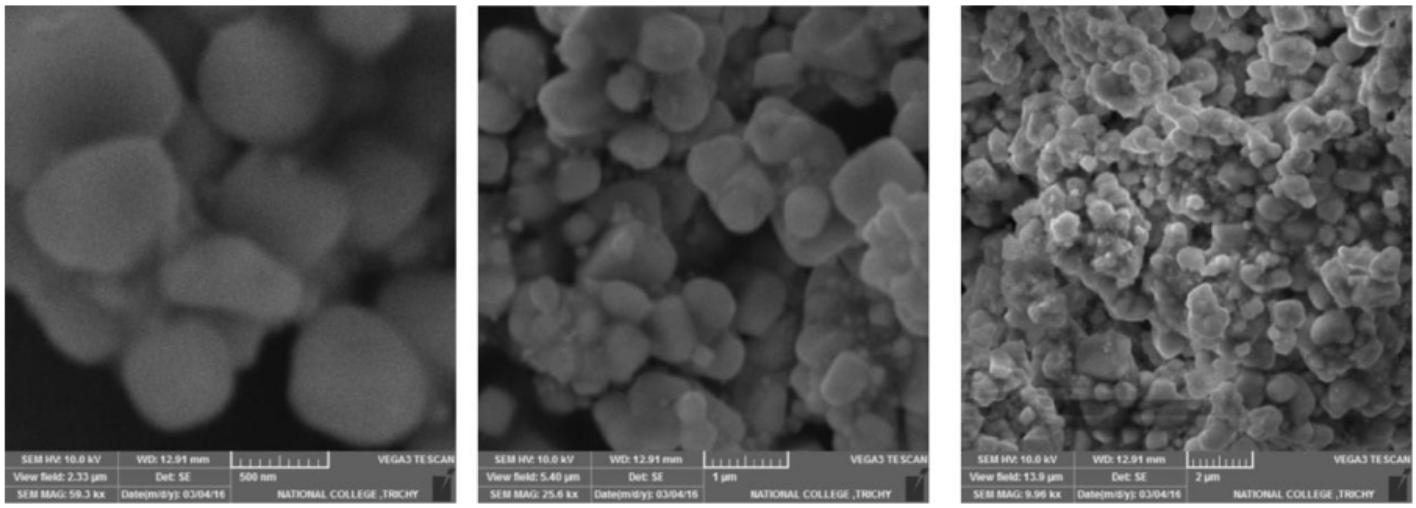


Figure 11

SEM results for *Spyridia filamentosa*

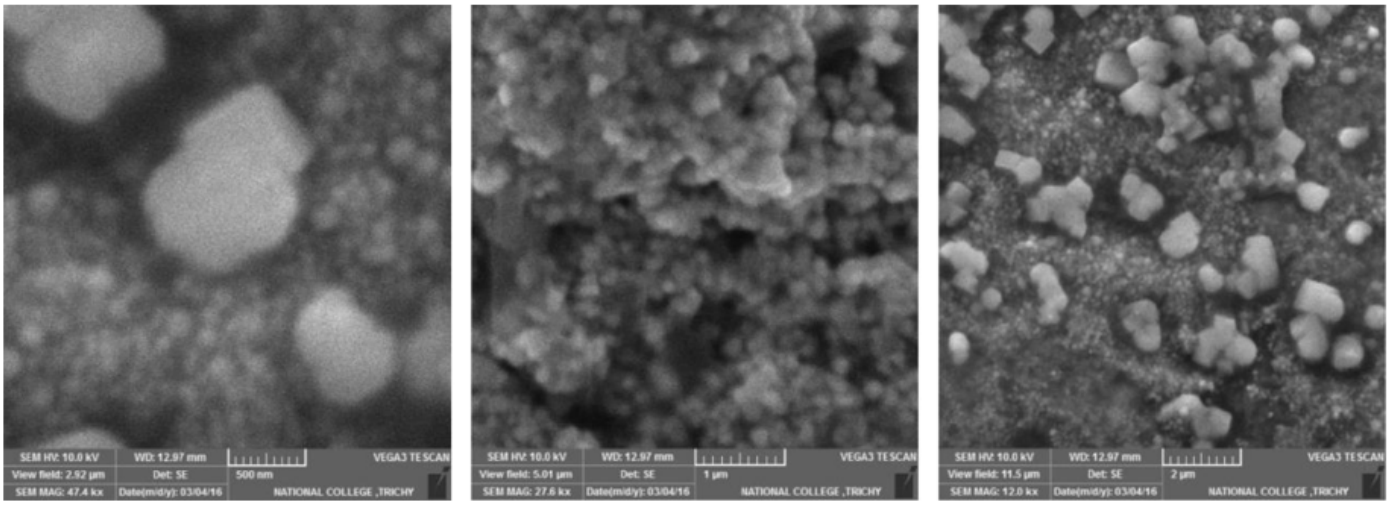


Figure 12

SEM results for *Halimeda macroloba*

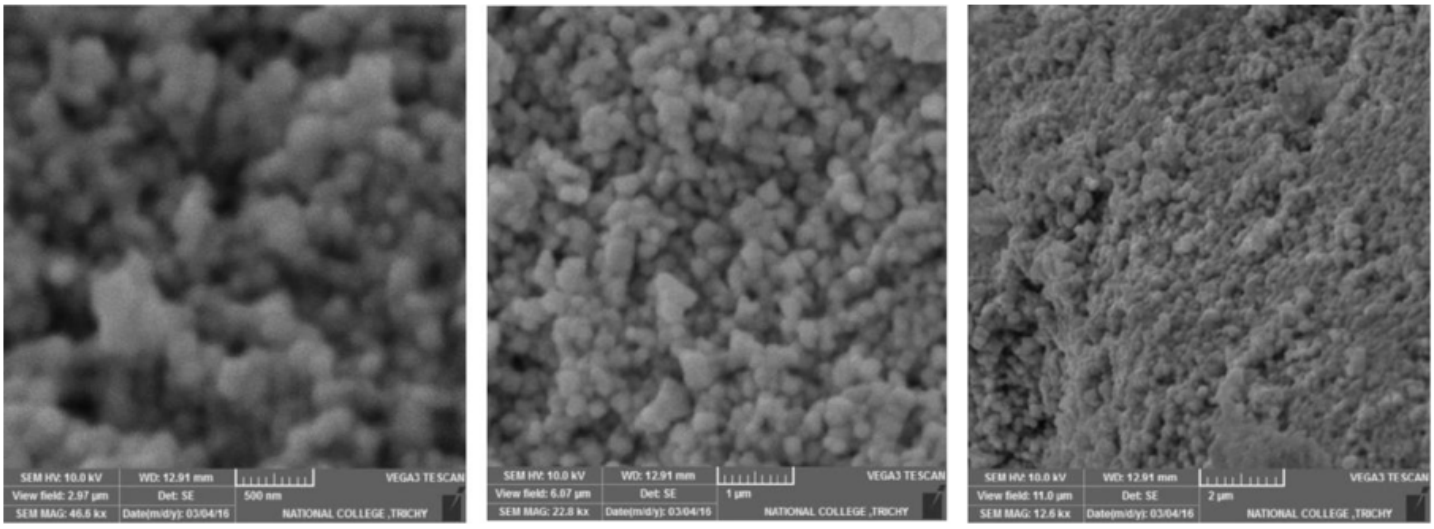


Figure 13

SEM results for *Turbinaria conoides*

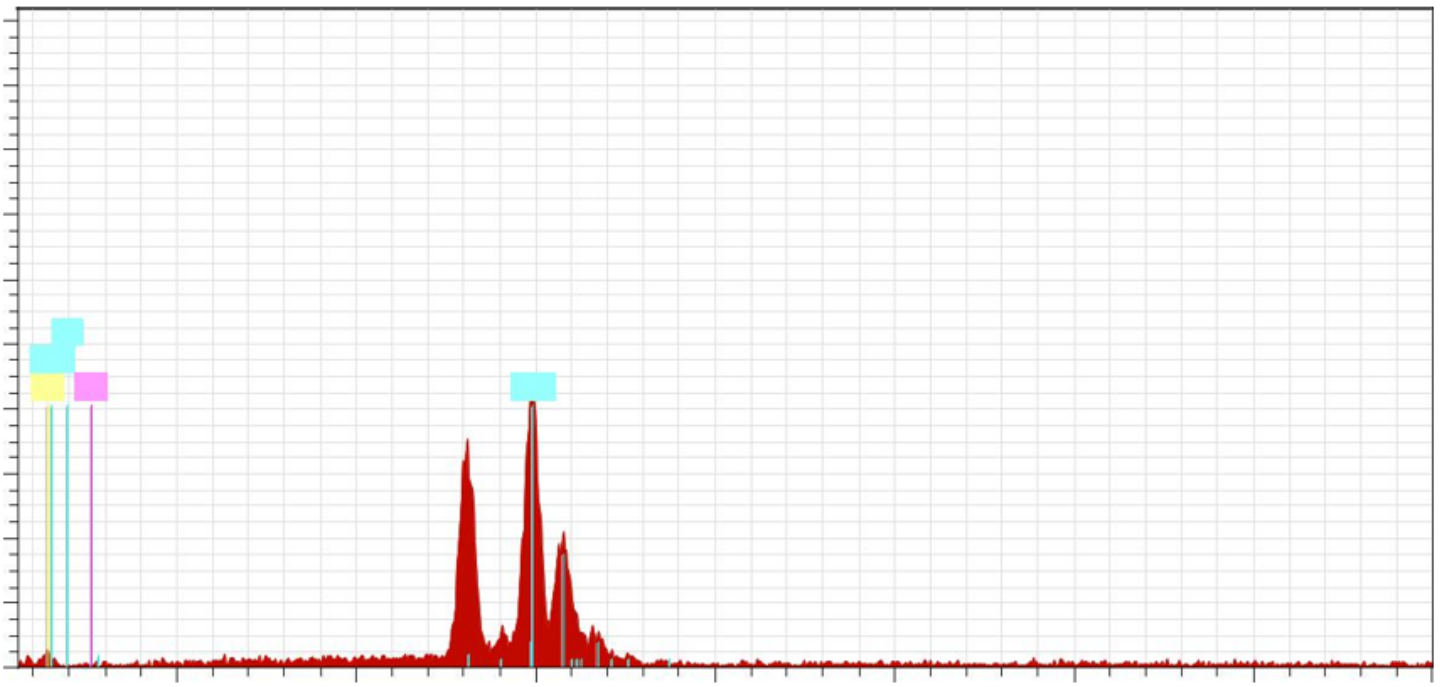


Figure 14

EDAX Analysis of silver nanoparticles from *Turbinaria conoides*

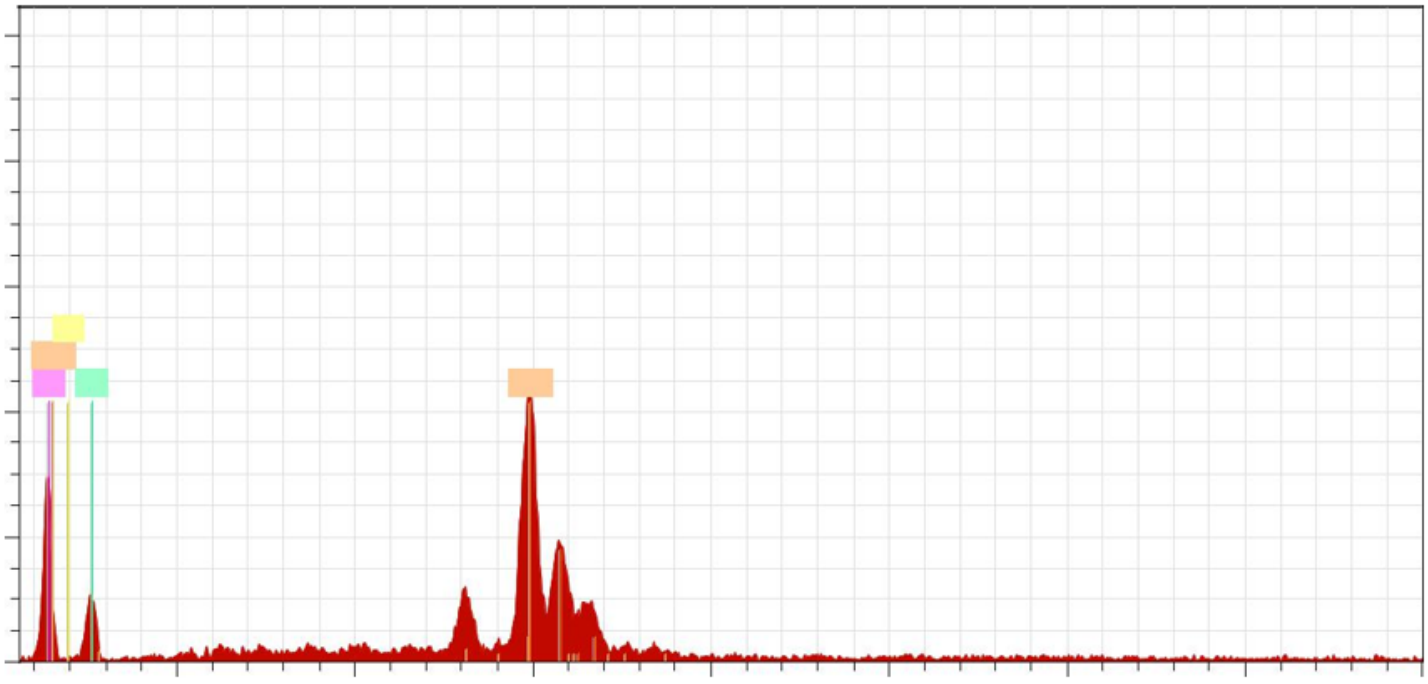


Figure 15

EDAX Analysis of silver nanoparticles from *Spyridia filamentosa*

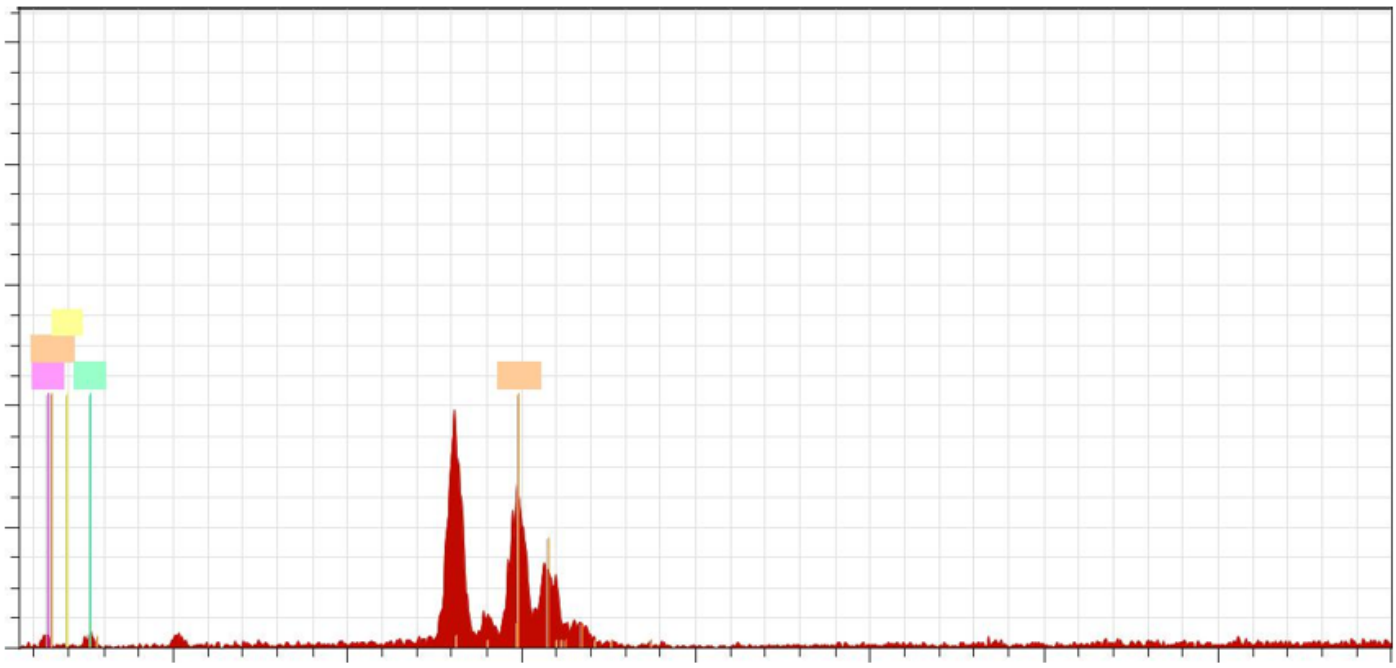
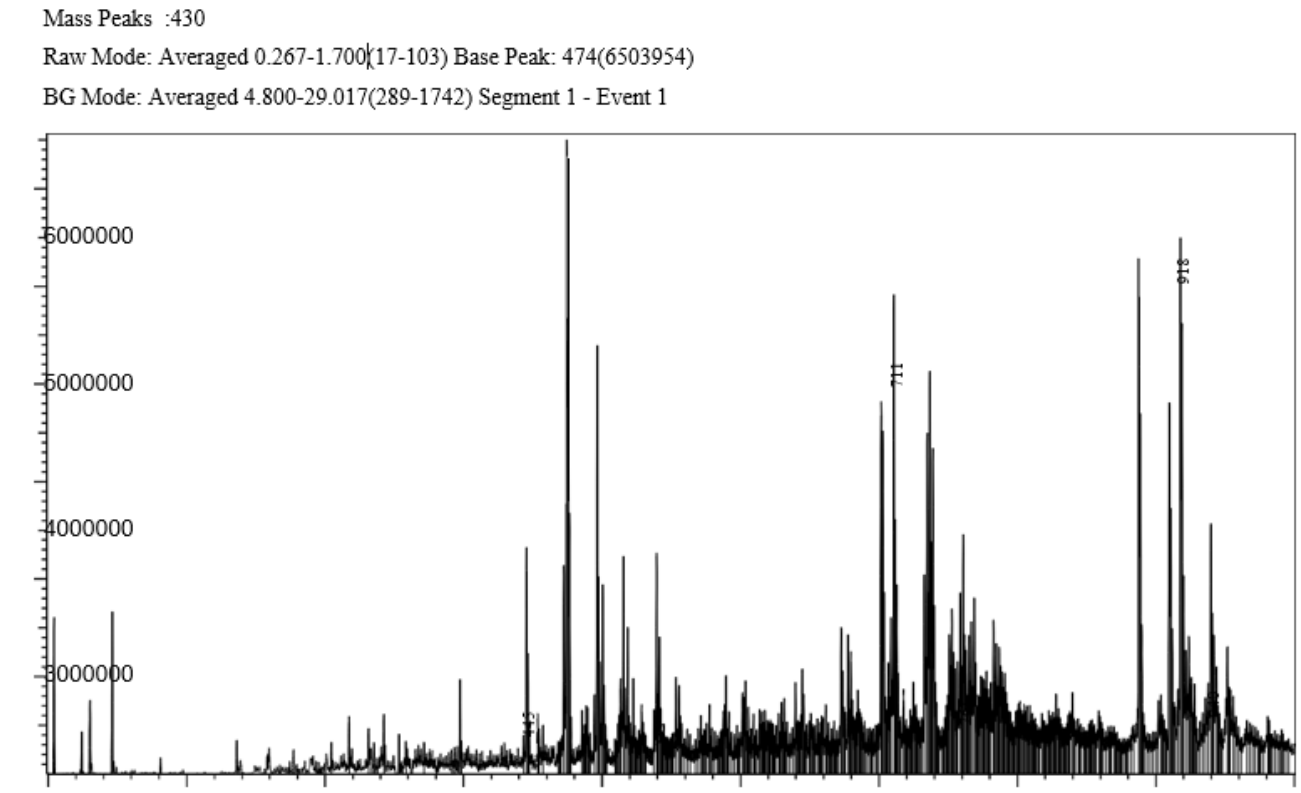


Figure 16

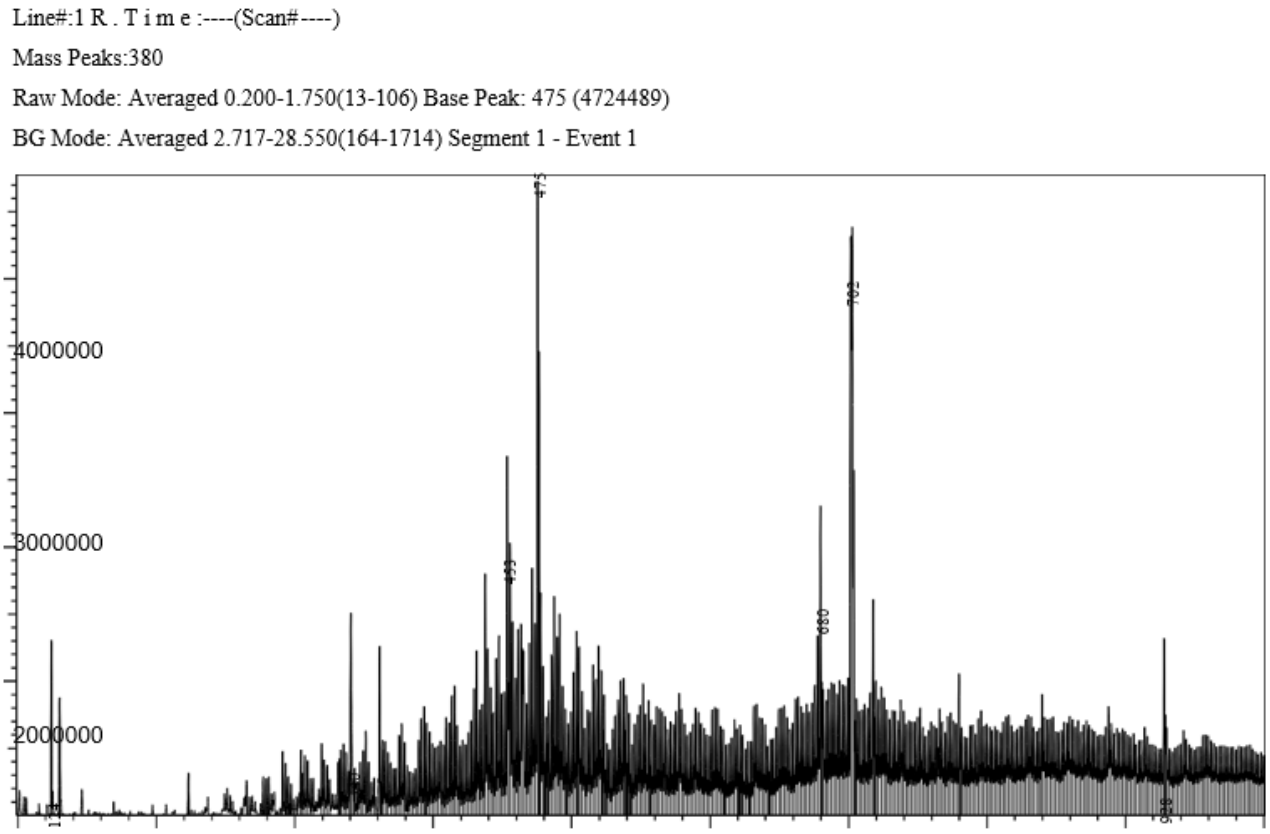
EDAX Analysis of silver nanoparticles from *Halimeda macroloba*



Code	Name of the compound	Molecular weight
M	Pregnenolone	316
M	phyllophenone B	396
M	Phorbasterone B	444
M	13-Heptadecyn-1-ol	500
M	9-Hexadecenoic acid	504
M	Methyl docosahexaenoate	342
M	Eicosapentaenoic acid methyl ester	316

Figure 17

LC-MS characterization of M-*Halimeda macroloba* green seaweed synthesized silver nanoparticles



Code	Name of the compound	Molecular weight
C	1-acetoxy-pinoresinol	415
C	Mellerin A	474
C	Medrogestone	340
C	3-Oxoniostigmast-5-ene	415
C	2-Naphthalenemethanol	222
C	l-Saccharopine	276

Figure 18

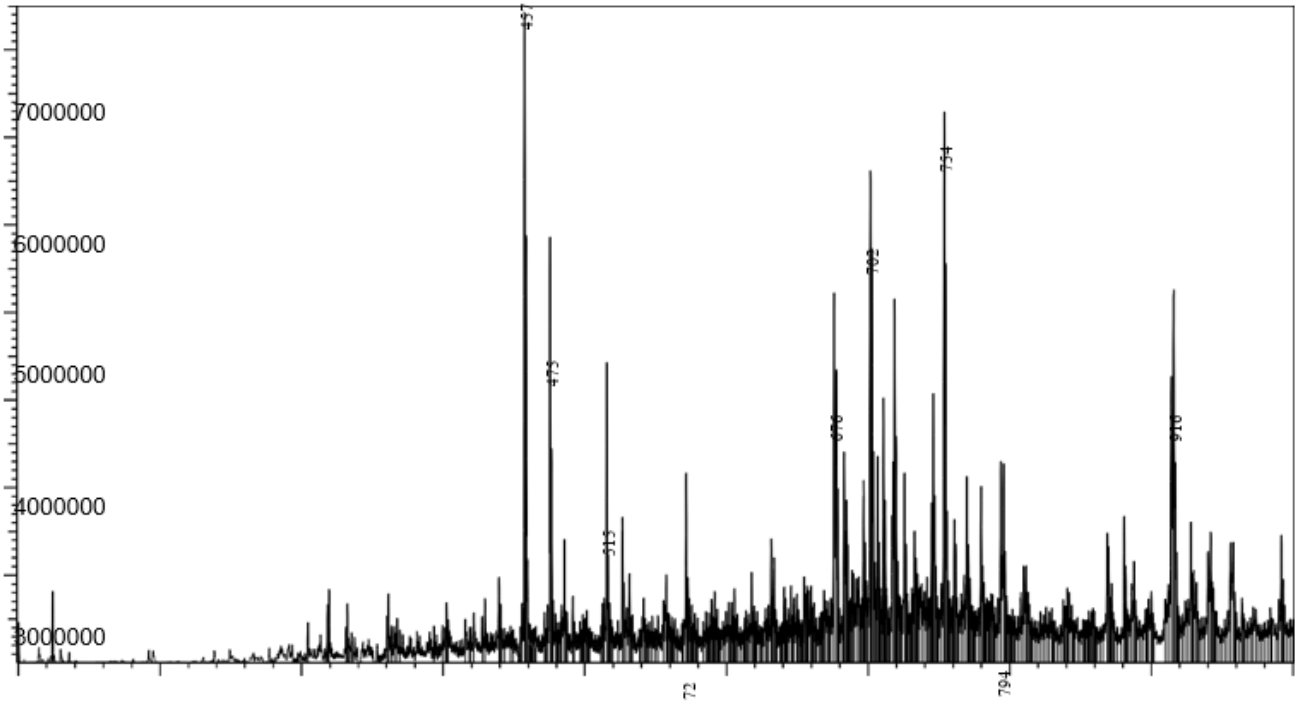
LC-MS characterization of C-*Turbinaria conoides* seaweed synthesized silver nanoparticles

Line#:1 R. Time : (Scan#)

Mass Peaks: 415

Raw Mode : Averaged 0.267-1.833(17-111) BasePeak:457(7428755)

BG Mode: Averaged 4.700-28.717(283-1724) Segment 1 - Event 1



Code	Name of the compound	Molecular weight
F	Dicaffeoylquinic acid	515
F	O-acetylprunin	475

Figure 19

LC-MS characterization of F-*Spiridia filamentosa* red seaweed synthesized silver nanoparticles

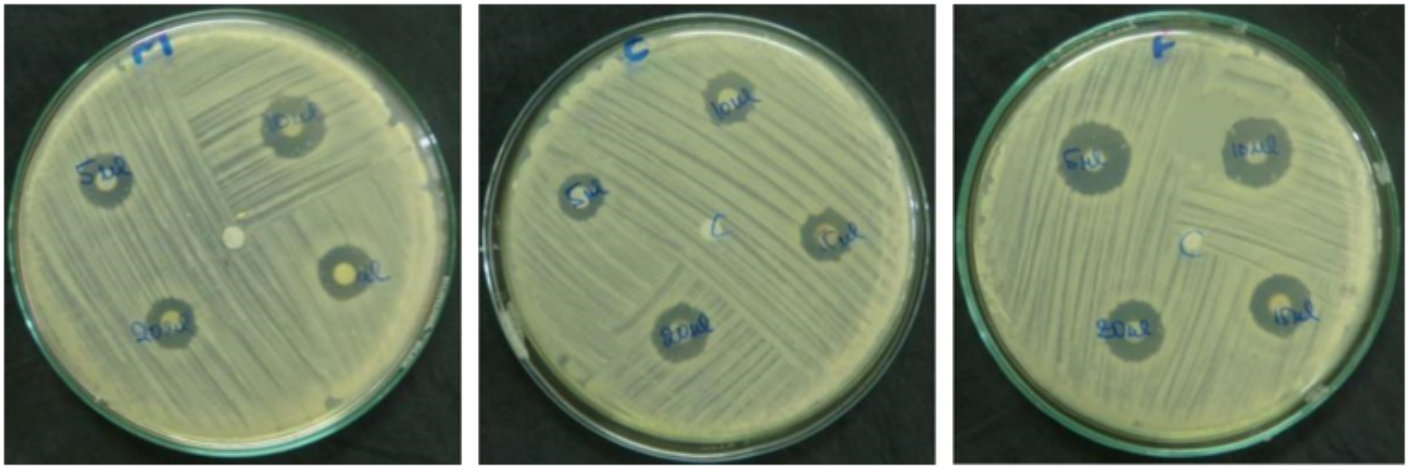


Figure 20

Antibacterial activities of silver nanoparticles against *Staphylococcus aureus*

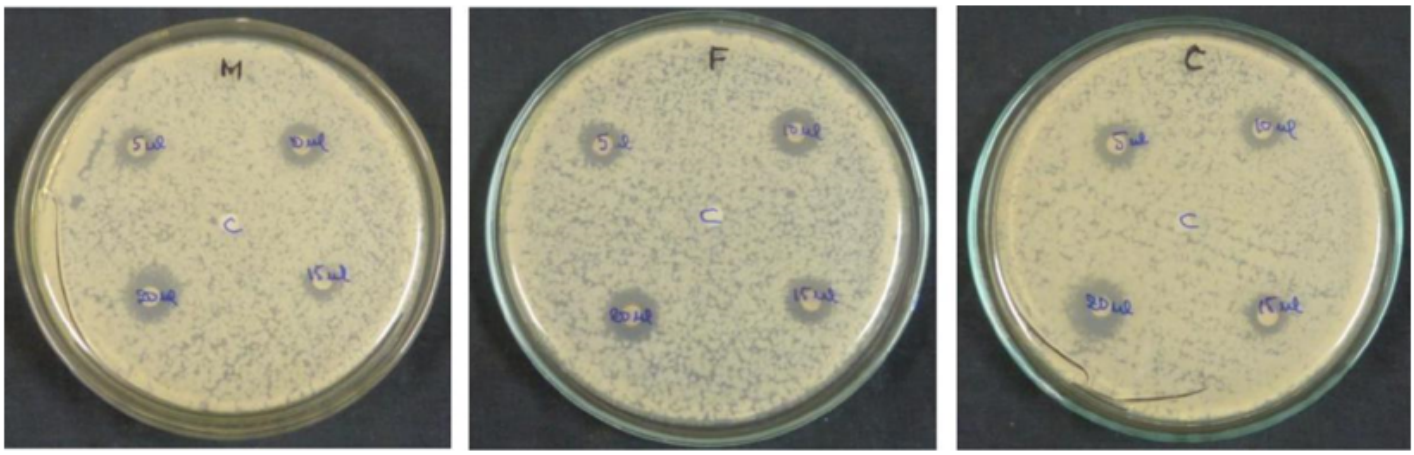


Figure 21

Antibacterial activities of silver nanoparticles against *Salmonella typhi*