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Green Synthesis of Silver Nanoparticles using Red Marine Algae and Evaluation of Its Antibacterial Activity

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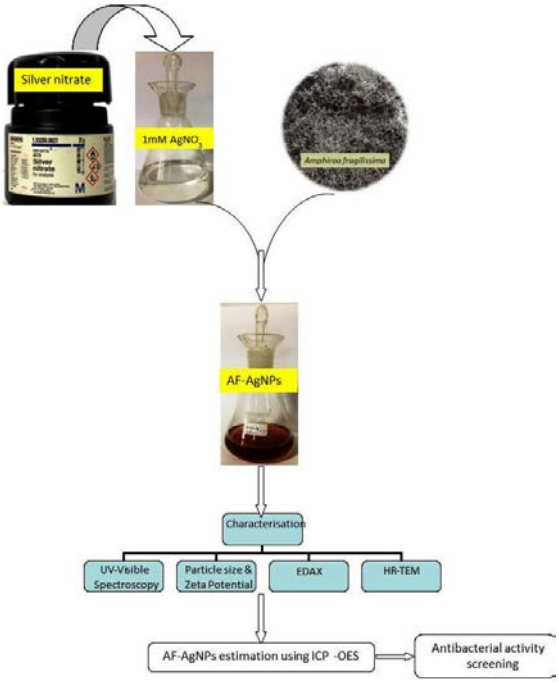
Abstract



Background:
In the biosynthesis of silver nanoparticles, terrestrial plants and various parts of the plants were almost fully utilized. In this area except very few studies, marine algae are not fully explored, so we have selected *Amphiroa fragilissima* for the synthesis of silver nanoparticles.
Objectives:
This study designed to synthesise silver nanoparticles using *Amphiroa fragilissima*, its characterization and evaluation of antibacterial activity against both gram positive and gram negative bacterias.
Materials and methods:
An aqueous extract of *Amphiroa fragilissima* was prepared and treated with 1 mM silver nitrate solution. Initially, the formation of nanoparticles was assessed by color change; later on it was monitored by UV-Visible spectrophotometer. The synthesized nanoparticles were characterized by DLS, EDAX and HR-TEM. The concentration of AgNPs was estimated by ICP-OES. The antibacterial potential of AgNPs was evaluated by an agar well diffusion method.
Results:
The formation of reddish brown colour indicates the formation of silver nanoparticles. It is confirmed by UV-Visible spectra, showed λmax at 429.5 nm which is characteristic for silver nanoparticles. The mean particle size and mean zeta potential value of AgNPs observed by DLS study was 104.6 nm and -38.1 mV respectively. EDAX results confirmed the presence of silver. HR-TEM results revealed that the shape of the particles were triangular, hexagonal and predominantly spherical in nature. The antibacterial activity of the AgNPs was tested and the outcome of the result revealed that AgNPs were showing excellent antibacterial activity against gram positive organisms.
Conclusion:
Amphiroa fragilissima can be used in the extracellular biosynthesis of silver nanoparticles with antibacterial potential.

Key words: Biosynthesis, Silver nanoparticles, *Amphiroa fragilissima*, Characterization, Antibacterial activity

INTRODUCTION

Biosynthesis of metal nanoparticles has gained enormous attention in the recent years, because of its wide range of applications in various fields.[1] The biosynthesized metal nanoparticles were reported to possess various activities like mosquito larvicidal,[2] anti HIV,[3] catalytic,[4] antimicrobial,[5] analgesic, anti-inflammatory,[6] antioxidant and anticancer[7] activities etc. Furthermore, it involves simple synthetic strategies, use of eco-friendly chemicals, solvents and cost-effective. There are other methods also available in the synthesis of metal nanoparticles such as physical and chemical methods. This biosynthetic method outperformed the other two methods due to its advantages.[8] In this biosynthetic strategy various resources like bacteria, fungi, viruses, plants, etc. were exploited.[9] Marine natural products are not fully explored in this area which aroused our interest in the current research. *Amphiroa fragilissima* belonging to the class of Rhodophyceae was selected for the preparation of silver nanoparticles (AgNPs) and the formed

AgNPs were characterized using various techniques. The concentration of AgNPs was estimated using ICP-OES and screened for antibacterial activity.

Fig.1 Image of *Amphiroa fragilissima* (Linn.) Lamour

MATERIALS AND METHODS

Preparation of *Amphiroa fragilissima* (AF) extract and synthesis of AgNPs

Amphiroa fragilissima was collected in the coastal area of Mandapam, Tamilnadu, India and it was authenticated by J.R.Ramalingam, Retired Technical Officer, Mandapam Regional Centre of Central Marine Fisheries Research Institute, Indian Council of Agricultural Research. Freshly collected AF brought into the field and first it was washed with tap water. Then it was rinsed with MilliQ water to remove extraneous and earthy matter present. It was shade dried for 10-15 days to get a constant weight. Then the marine algae was cut into small pieces and ground into a coarse powder using a mixer. The coarse powder of AF was boiled with MilliQ water, the supernatant liquid was decanted. It was filtered using tea filter and centrifuged at 10,000 rpm to get a clear solution. Then the resultant solution was filtered using Whatman filter paper. 1mM silver nitrate (Merck, Mumbai) solution was prepared and it was mixed with the aqueous extract of AF.

Characterization of AgNPs synthesized using *Amphiroa fragilissima* (AF-AgNPs)

A spectrum was recorded using UV-Visible spectrophotometer (Shimadzu UV-2450, Japan) at the wavelength ranging between 300-800 nm. DLS (Differential light scattering) (Horiba scientific, SZ-100) measurement was done to determine the particle size distribution and zeta potential value of the synthesized AgNPs solution. HR-TEM (High resolution transmission electron microscopy) (JEOL 3010) analysis was performed to investigate the particle size and morphology of AgNPs. EDAX (Energy dispersive X-ray spectroscopy) (Oxford Instruments) analysis was also done to determine the elemental composition of the solution. The concentration of AgNPs was determined by ICP-OES (Inductively coupled plasma optical emission spectrometry, Perkin Elmer Optima 5300 DV)

Antibacterial activity

The antibacterial potential of AgNPs was evaluated by agar well diffusion method[10] against both gram positive (*Bacillus subtilis, Bacillus cereus, Staphylococcus aureus*) and gram negative (*Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Shigella dysentriae, Proteus mirabilis, Pseudomonas aeruginosa*) bacterial strains. The nutrient agar medium was prepared in sterile plates and the bacterial cultures were grown in it. Four wells of suitable size were made and AgNPs of different concentrations 25 µl, 50 µl, 75 µl and 100 µl were placed in a well. The plates were incubated at 37°C for 24 h. The formation of zone around the well measured in mm.

RESULTS AND DISCUSSION

Coarsely powdered *Amphiroa fragilissima* (Figure 1) was used in the preparation of aqueous extract and it was mixed with 1mM silver nitrate solution. Reddish brown colour indicates the formation of silver nanoparticles (Figure 2). An aliquot was taken from the sample at regular time intervals and UV-visible spectra (Figure 3) was recorded which showed λmax at 429.5 nm. This could be due to the surface plasmon resonance (SPR) of AgNPs which is well correlated with the previously published results. At regular time intervals UV-Visible spectra were recorded, it was overlaid and given in the (Figure 4). There was not much shift in the λmax towards longer wavelengths, indicated that particles were highly stable even after 3 months.

Fig. 2 Image of a) Control b) AgNPs synthesised using *Amphiroa fragilissima*

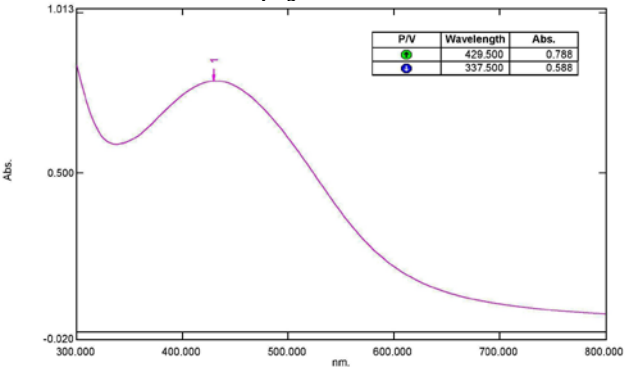


Fig.3 UV-Visible spectra of AgNPs synthesized using *Amphiroa fragilissima*

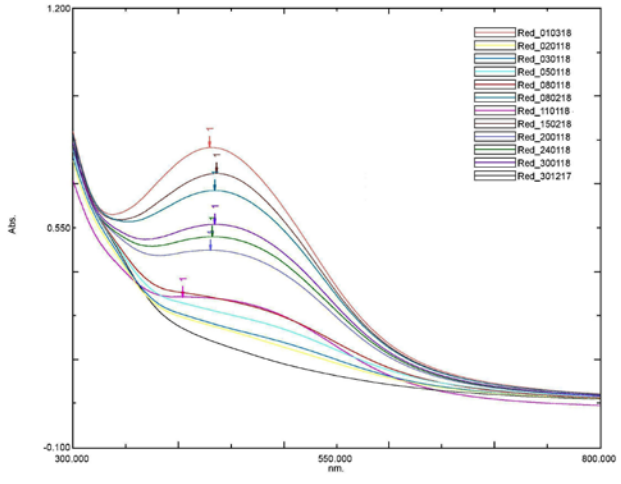


Fig. 4 UV-Visible spectra of AgNPs synthesized using *Amphiroa fragilissima* at various time intervals.

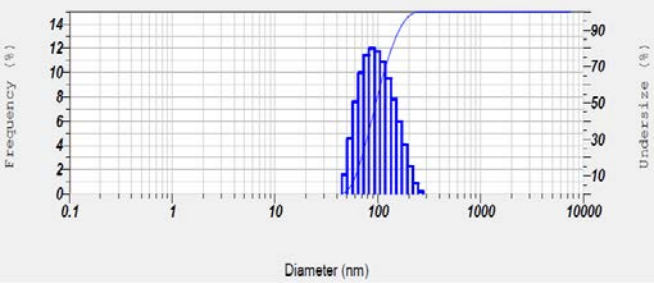


Fig. 5 Particle size distribution of AgNPs by DLS measurement

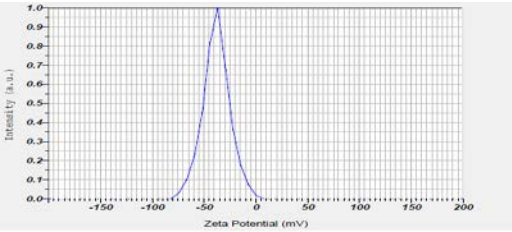


Fig. 6 Zeta potential value of AgNPs by DLS measurement

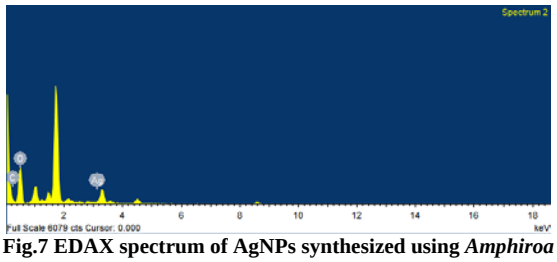


Fig.7 EDAX spectrum of AgNPs synthesized using *Amphiroa fragilissima*

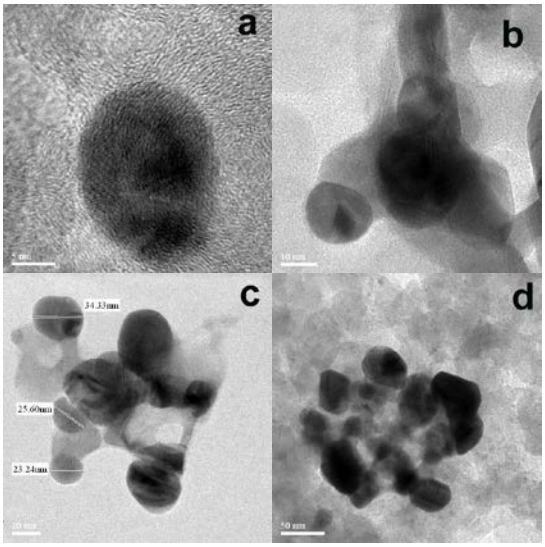


Fig. 8 HR-TEM images of AF-AgNPs at a) 5 nm b) 10 nm c) 20 nm and d) 50 nm scale

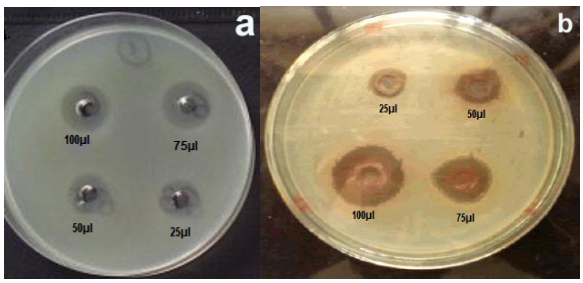


Fig. 9 Antibacterial activity of CA mediated AgNPs against a) *B.subtilis* b) *S.aureus* at 25 µl, 50 µl, 75 µl & 100 µl.

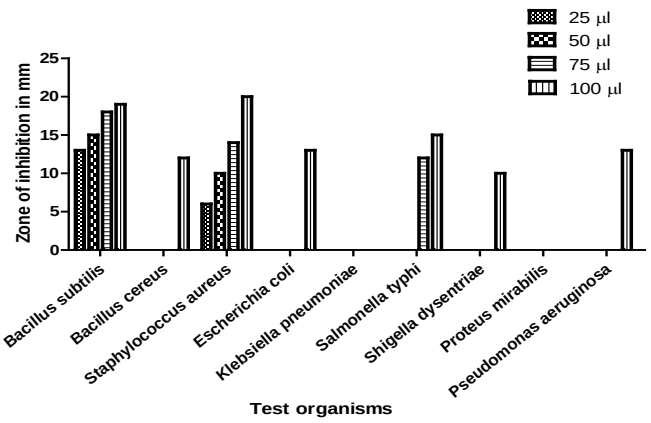


Fig. 10 Antibacterial activity of AgNPs synthesized using *Amphiroa fragilissima* at different concentrations

Table 1: Antibacterial activity of AF-AgNPs

Test organisms	Zone of inhibition in mm at different concentrations			
	25 µl	50 µl	75 µl	100 µl
Bacillus subtilis	13	15	18	19
Bacillus cereus	-	-	-	12
Staphylococcus aureus	6	10	14	20
Escherichia coli	-	-	-	13
Klebsiella pneumoniae	-	-	-	-
Salmonella typhi	-	-	12	15
Shigella dysentriae	-	-	-	10
Proteus mirabilis	-	-	-	-
Pseudomonas aeruginosa	-	-	-	13

The particle size distribution of the synthesized AgNPs solution was recorded. The mean particle size of AgNPs was 104.6 nm and the results were given (Figure 5). The stability of the AgNPs solution was ascertained by its zeta potential value. The mean zeta potential value of AgNPs was -38.1 mV (Figure 6). The high negative zeta potential value suggested that AgNPs were highly stable[11] which results are in good agreement with the SPR band of UV-Visible spectra.

EDAX spectrum (Figure 7) showed a signal for silver, which confirmed the presence of silver. EDAX results also revealed that other than silver, signals for carbon, oxygen and some other insignificant peaks were also found. These elements could be due the presence of active constituents from marine algae. This might have formed a layer over silver nanoparticles.

HR-TEM images (Figure 8a, 8b, 8c and 8d) showed at different scales such as 5 nm, 10 nm, 20 nm and 50 nm. HR-TEM results displayed that the particles were of different sizes and shapes. There were morphological variations such as triangular, hexagonal and spherical shaped particles were found, predominantly the particles were spherical in shape. A layer was found around AF-AgNPs indicated that the phytoconstituents present in marine algae could have capped the silver nanoparticles. This is well correlated with the EDAX results in which other than silver, carbon and oxygen peaks were found.

In the antibacterial activity, AF-AgNPs at different concentration levels (25 µl, 50 µl, 75 µl and 100 µl) were evaluated against three gram positive bacterias such as *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus* and six gram negative bacterias such as *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Shigella dysentriae, Proteus mirabilis, Pseudomonas aeruginosa*. Zone of inhibition of AF-AgNPs against different test organisms were given in the Table -1 and Figure 10. In the case of gram

positive organisms, silver nanoparticles effective against all the three strains tested. Particularly the nanoparticles were highly effective against *B.subtilis* (13 mm, 15 mm, 18 mm and 19 mm) and *S.aureus* (6 mm, 10 mm, 14 mm and 20 mm) at all the four concentration levels tested (Figure 9a and 9b), it is showing a zone of inhibition against *B.cereus* only at the higher concentration (13 mm at 100 µl) level. In the case of antibacterial study of AF-AgNPs against gram negative organisms, except *K.pneumoniae* and *P.mirabilis*, silver nanoparticles showing zone of inhibition against other gram negative strains tested. Only at the higher concentration 100 µl, AF-AgNPs showing antibacterial activity against *E.coli* (13 mm), *S.dysentriae* (10 mm) and *P.aeruginosa* (13 mm). On the other hand, AgNPs were effective against *S.typhi* at two concentration levels (75 µl, 12 mm and 100 µl, 15 mm).

Conclusion

We have achieved a simple, economical, eco-friendly green synthesis of silver nanoparticles using *Amphiroa fragilissima*, red marine algae. In this study, the synthesized silver nanoparticles were characterized by UV-Visible Spectrophotometer, DLS, EDAX, HR-TEM and estimated in solution by ICP-OES technique. It was screened for antibacterial activity against gram positive and gram negative organisms. Mainly, AgNPs were effective against all the gram positive organisms, particularly against *B.subtilis* and *S.aureus* showing excellent antibacterial activity. This method can be scaled up and the test compound could be useful against the infections caused by gram positive bacterias. The test compound can be screened for various *invivo* studies to lead the compound to the next level.

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Abbreviations Used

DLS: Differential light scattering; HR-TEM: High resolution transmission electron microscopy; AgNPs; Silver nanoparticles; ICP-OES: Inductively coupled plasma optical emission spectrometry; AF: *Amphiroa fragilissima*; EDAX: Energy dispersive X-ray spectroscopy; SPR: Surface plasmon resonance; AF-AgNPs: AgNPs synthesized using *Amphiroa fragilissima*;

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