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Research Article

BIOSYNTHESIS OF SILVER NANOPARTICLES FROM *HELIOTROPIUM ZEYLANICUM* WHOLE PLANT EXTRACT AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

Objective: To develop a new approach for the green synthesis of silver nanoparticles using aqueous leaves extracts of Heliotropium zeylanicum which has been proven active against clinically important microbial pathogens. **Methods:** Characterization was determined by using UV-vis spectroscopy, scanning electron microscopy (SEM) and Fourier Transform infrared spectroscopy (FTIR). Antimicrobial activity was screened by using disc diffusion method. **Results:** SEM showed the formation of silver nanaoparticles with an average size 4-30 nm. The significant antibacterial activity was showed against Proteus vulgaris. **Conclusion:** It can be concluded that the leaves of can be good source for synthesis of silver nanaoparticle which shows antimicrobial activity.

Key words: Biosynthesis, SEM, FTIR, Crude extract, Antimicrobial activity.

INTRODUCTION

Heliotropium zeylanicum is an important medicinal plant belongs to the family Boranginaceae. It is a wild herbaceous plant is very common in all tropical countries, including India. In Tamil Nadu this plant is widely found in and around Narthamalai, Pudukottai District. The plant is found to possess high valued medicinal properties. It is used as diuretic, astringent, emollient, vulnerary and also used to local application for fever. Root part is used to cure cough and promote menstruation [1, 2]. The present study, the whole plant extract of *H.zeylanicum* was used for biosynthesis of AgNPs. The synthesized AgNPs were characterized using UV-vis spectroscopy, Fourier Transform infrared spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). The antimicrobial efficacy of the synthesized AgNPs was studies against clinically important gram positive (*Staphylococcus aureus*, *Clostritium perfringens* & *Bacillus subtilis*), gram negative (*Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Proteus vulgaris*, *Enterococcus aeroginosa* & *Methiciltris aresistance*) and fungal pathogens (*Aspergillus niger*, *Aspergillus flavus*, *Acmella clavets, Alternaria brassica* & *Fusarium solani*). The present study was conducted to investigate antibacterial activity of silver nanoparticles synthesized from the leaves of *H.zeylanicum* by preliminary screening.

MATERIALS AND METHODS

Collection of plants

The fresh aerial parts were collected from Narthamalai, Pudukkotai district, Tamil Nadu. The gathered samples were cut into small pieces and shade dried until the fracture is identical and even. The dried plant material was crushed or grinded by using a blender and separated to get uniform particles by using sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant material.

Synthesis of silver nanoparticles

Synthesis of silver nanoarticles is done with different concentration of whole plant extract (5, 10, 15, and 20mL) with different concentration of silver nitrate (0.5,1.0,1,5 and 2mM) solution at different range of boiling temperature (40,60,80 and 1000C). The different incubation times (20, 40 and 60m) is also used. The colour change of solution is checked periodically. The colour change of the whole plant extract from yellow to dark brown indicated the silver nanoparticles were synthesized from the whole plant extract. Bioreduction of silver ions in the solution was monitored using Genesys 10UV-VIS spectrophotometer. After cooling the solution was centrifuged at 3000rpm for 5m. The colored supernatant was transferred to new tube and centrifuged at 1200rpm for 10m. Silver nanoparticles settled down as pellets. The colorless supernatant was discarded and the pellet was resuspended in sterile distilled water of same amount (Hareckrishna Bar *et al.,* 2009).

CHARACTERIZATION

UV-Vis Spectroscopy

UV-visible spectroscopy is used for monitoring the signature of silver nanoparticles. As it is a powerful tool for the characterization of colloidal particles. They exhibit strong surface absorption in the visible region and are highly sensitivity surface modification.

Scanning Electron Microscope (SEM) Analysis

Scanning Electron Microscope (SEM) analysis was done using Hitachi S - 4500 SEM. 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 allowed to dry by putting it under a mercury lamp for 5 min.

Antimicrobial activity

The synthesized AgNPs were tested for their antibacterial activity against human bacterial pathogens using the standard disc diffusion method [3,4]. The gram positive (*Staphylococcus aureus*, *Clostritium perfringens* & *Bacillus subtilis*), gram negative (*Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* ,*Proteus vulgaris*, *Enterococcus aeroginosa* & *Methiciltris aresistance*) and fungal pathogens (*Aspergillus niger*, *Aspergillus flavus*, *Acmella clavets, Alternaria brassica* & *Fusarium solani*) were obtained from Department of Microbiology, Kamaraj College, Tuticorin.

The freshly cultured bacterial colonies of tested bacteria were used and 100 ml of inoculum was spread on Mueller-Hinton agar plates. A single colony of each test strain was grown overnight in Mueller-Hinton liquid medium on a rotary shaker at 37^{o}C. The inocula were prepared by diluting the overnight cultures with 0.9% NaCl to a 0.5% Mcfarland standard and applied to plates. After that, different concentrations (25, 50, 75 and 100 mg/ml) of AgNPs were loaded on 6 mm size discs. Finally, inhibition zones were measured after 24 h incubation at 37^{o}C. The experiments

were performed in triplicate and Student's t-test was used to evaluate statistically significant differences.

RESULTS AND DISCUSSION

Synthesis of silver nanoparticles

Reduction of Ag+ into Ag-NPs during exposure to extracts of *H.zeylanicum* could be assessed by the colour change. The fresh suspension of *H.zeylanicum*was yellowish-green in colour. However, after addition of AgNO3 and stirring for 48 h at room temperature, the emulsion turned dark brown in colour in aqueous medium as a result of surface Plasmon vibrations. The study also confirms the completion of the conversion reaction between leaf extract and AgNO3. The UV-vis spectra recorded after time intervals of 15 min, 30 min, 45 min, 60 min and 24 h from the initiation of reaction are seen. Similar results were also reported in *Acalypha indica* leaf extracts [5], *Musa balbisiana* [6], *Azadirachta indica* [7, 8], *Ocimum sanctum* leaf [9]. The colour changes in aqueous solutions synthesizing AgNPs.

UV-vis analysis

The synthesis of well-dispersed, single, structurally flower like Silver nanoparticles (AgNP) was accomplished via one-pot reaction involving the reduction of silver salt using aqueous extract of *H. zeylanicum* (Figure 1). Absorption spectra of AgNPs formed in the reaction media has absorption maxima in the range of 427 to 495 nm due to Surface Plasmon Resonance(SPR) of AgNPs. The UV-vis spectra recorded, implied that most rapid bio-reduction was achieved using *H. zeylanicum.* Absorption spectra of AgNPs formed in the reaction media has absorption maxima in the range of 427 to 495 nm due to SPR of AgNPs (Figure 1). While previous report showed that UV-Vis absorption spectra had broad SPR band contained one peak at 456 nm in *allicarpamaingayi* [10]. This peak illustrates the presence of homogeneous distribution of hydrosol AgNPs after 48 h of stirring times as in *Vitex negundo* [11, 12].

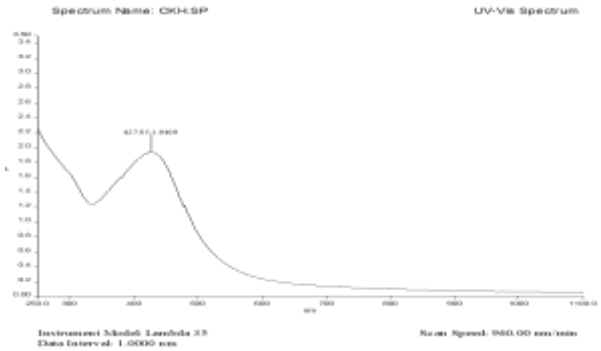

Fig. 1: UV-vis spectroscopy analysis of synthesized silver nanaoparticles

SEM analysis

The SEM image of silver nanoparticle synthesized from the plant extract were observed and found to be assembled on the surface due to the interaction due to hydrogen bonds and electrostatic interaction between the bio-organic capping molecules bound to the AgNPs. The SEM images thus obtained were observed to be of different shapes i.e., spherical, triangular and cuboidal (Figure 2-5). The nanoparticles were not in direct contact within the aggregator. This may be due to the whole plant extracts being used as reducing and capping agents. The SEM images of *Callicarpa maingayi* too had similar characteristics which depicts that AgNP can effectively control the shape and size of the outcome. The SEM image and EDXRF spectrum for the Ag- NPs in *Callicarpa maingayi* 12] and *Moringa oleifera* [13] confirm this notion that they can effectively control the shape and size. The exterior surfaces of Ag due to the presence of small AgNPs become shiny in the sporting spherical shape. Similar reports are seen in *Musa balbisiana*, *Azadirachta indica,* and *Ocimum tenuiflorum* [6]. In contrast flower shaped AgNPs were seen in *Malus domestica* [14].

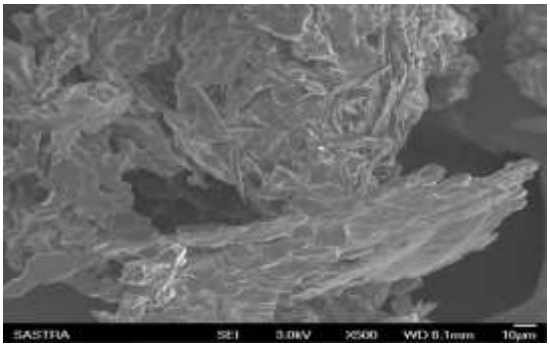

Fig. 2: SEM of *Heliotropium zeylanicum* (Burm.f) Lam

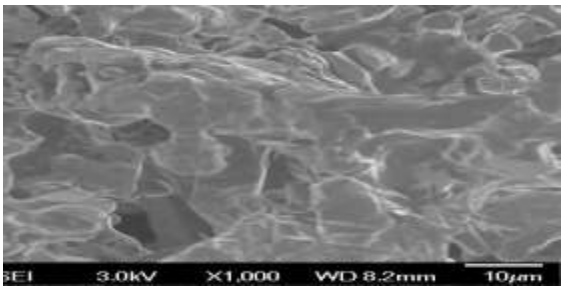

Fig. 3: SEM of *Heliotropium zeylanicum* (Burm.f) Lam

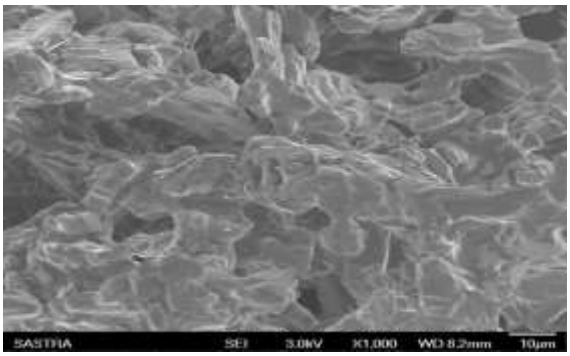

Fig. 4. SEM of *Heliotropium zeylanicum* (Burm.f)

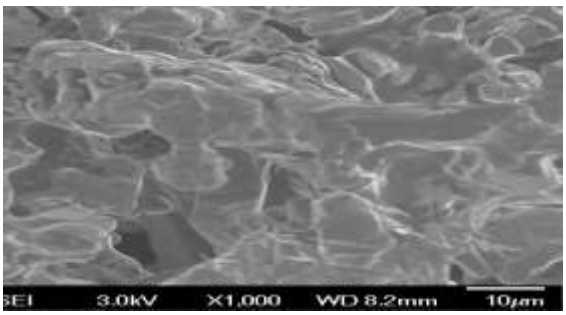

Fig. 5. SEM of *Heliotropium zeylanicum* (Burm.f) Lam

Antimicrobial activity

The antibacterial potential of AgNPs was investigated by microbes like *Staphylococcus aureus*, *Clostritium perfringens, Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Proteus vulgaris*, *Enterococcus aeroginosa* and *Methiciltris aresistance,* (Table 1). The results showed an increase in concentration zone with inhibition diameters ranging from 0.53 cm to 1.6 cm. Among the pathogens studied the better inhibition was seen in *Proteus vulgaris* (1.6 ± 0.115 cm) and *Staphylococcus aureus* (1.23 ± 0.20 cm) followed by *Clostritium perfringens* (1.2 ± 0.057 cm), *Escherichia coli* (1.2 ± 0.16cm), *Salmonella paratyphi* (1.2 ± 0.264cm), *Methiciltris aresistance* (1.16 ± 0.152 cm) and *Pseudomonas aeruginosa* (1.13 ± 0.25 cm).

Table 1: Antibacterial Activity of AgNPs from *Heliotropium zeylanicum*

S.no	Microorganisms	Zone of inhibition (cm) mean SD			
		25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
1	*Escherichia coli*	0.86 ± 0.05	0.96 ± 0.1	1.0 ± 0.128	1.2 ± 0.16
2	*Salmonella typhi*	0.53 ± 0.115	0.66 ± 0.152	0.76 ± 0.05	0.85 ± 0.057
3	*Pseudomonas aeruginosa*	0.93 ± 0.15	1.06 ± 0.152	1.1 ± 0.2	1.13 ± 0.25
4	*Salmonella paratyphi*	1.0 ± 0.1	1.03 ± 0.05	1.13 ± 0.15	1.2 ± 0.264
5	*Proteus vulgaris*	1.0 ± 0.1	1.03 ± 0.11	1.16 ± 0.057	**1.6 ± 0.115**
6	*Enterococcus aeroginosa*	0.91 ± 0.1	1.0 ± 0.15	1.03 ± 0.059	1.03 ± 0.28
7	*Staphylococcus aureus*	0.93 ± 0.5	0.96 ± 0.52	1.0 ± 0.59	**1.23 ± 0.20**
8	*Clostritium perfringens*	0.93 ± 0.152	1.0 ± 0.05	1.0 ± 0.15	1.2 ± 0.057
9	*Methiciltris aresistance*	0.93 ± 0.157	1.03 ± 0.15	1.03 ± 0.15	1.16 ±0.152
10	*Bacillus subtils*	0.66 ± 0.15	0.86 ± 0.58	1.0 ± 0.57	1.2 ± 0.1

Antifungal activity

The antifungal activity of AgNPs against some fungal species like *Aspergillus niger*, *Aspergillus flavus*, *Acmella clavets, Alternaria brassica* & *Fusarium solani* was investigated (Table 2). Silver nanoparticles had significant antifungal activities against the tested fungi.

Table 2: Antifungal activity of AgNPs from *H .zeylanicum*

S.no	Test fungi	Zone of inhibition (cm) mean SD			
		25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
1	*Aspergillus niger*	1.1 ± 0.1	1.3 ± 0.208	1.36 ± 0.152	**1.46 ± 0.15**
2	*Acmella clavets*	1.0 ± 0.57	1.3 ± 0.26	1.36 ± 0.11	1.43 ± 0.1
3	*Aspergillus flavus*	1.1 0.1	1 1.2 ± 0.1	1.36 ± 0.15	1.5 ± 0.1
4	*Alternaria brassica*	0.6 ± 0.15	0.86 ± 0.57	7 0.93 ± 0.5	0.93 ± 0.152
5	*Fusarium solani*	1.0 ± 0.1	1.1 ± 0.1	1.16 ± 0.57	7 1.4 ± 0.1

CONCLUSIONS

In this study, an inexpensive, rapid and a single step technique for the synthesis of silver nanoparticles using medicinal plant is developed. The results of SEM and UV-vis spectroscopy have confirmed that the synthesized silver nanoparticles are mostly spherical in shape with an average size of 4–30 nm. These silver nanoparticles showed antibacterial activity against clinically important microbial pathogens. Therefore, medicinal plant mediated nanoparticles could be used as an excellent source against tested bacteria

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