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Green Synthesis of Silver Nanoparticles Using Flower Extract of *Hemigraphis colorata* as Reducing Agent and its Biological Activity

Murugan Elangovan 1, Dhayalan Ramachandran 1, Krishnan Rajesh 2,*

- Department of Chemistry, Indian Arts and Science College, Tiruvannamalai 606502
- Arignar Anna Govt. Arts College, Cheyyar, Tamil Nadu, India 604407
- * Correspondence: krisrajesh1977@gmail.com;

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Abstract: Green synthesis of silver nanoparticles (Ag NPs) was performed using flower extract of *Hemigraphis colorata* (*H. Colorata*). Synthesized nanoparticles were characterized by UV-Visible Spectrophotometer (UV-Vis), Fourier Transform Infrared (FT-IR), Scanning Electron Microscope (SEM), and Transmission Electron Microscope (TEM-SAED). The crystalline nature of the sample was examined by an X-ray diffraction study (XRD). The UV-Visible spectrum showed surface plasma resonance (SPR) at 360 nm revealed the formation of nanoparticles, SEM and TEM exhibited spherical shape particles with an average size between 10-20 nm. The valuation of the antibacterial and antifungal study revealed its efficiency in killing bacteria and fungi.

Keywords: green synthesis; *Hemigraphis colorata*; flower extract; antibacterial activity.

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1. Introduction

The synthesis of nanoparticles has drawn researchers' attention around the globe due to their size, shape, and properties [1]. Controlling the size and shape of particular causes a drastic change in their properties. The properties of raw materials differ from bulk materials due to their size [2]. Among the many nanoparticles, silver and gold are getting scientists' interest due to their properties and applications [3]. Colloidal Ag NPs have distinctive chemical stability, conductivity, catalytic and bacterial activity [4]. Its applications in various fields of science and technology have been explored due to its physicochemical properties [5]. Silver nanoparticles are non-toxic to human beings and also kill bacteria and fungus [6, 7]. Its efficiency has been evaluated in treating burns, wounds, and several bactericidal infections [8-10].

Applications of Ag NPs have been explored in the field of textile [11], cell biology [12], data storage [13], antimicrobial agents [14], drug delivery [15], ointment [16], agriculture, and cosmetics [17], much more application can be found in the literature. The use of toxic and hazardous chemicals to produce Ag NPs produces a risk to the environment, and hence, green methods gained interest to avoid those chemicals to produce the Ag NPs [18, 19]. Common biological materials used in the green synthesis Ag NPs are bacteria, fungi, and plant extracts due to their antioxidant and reducing properties. The water-soluble compounds present in the plant extract are responsible for reducing metal salt into the respective metal nanoparticles [20, 21]. Various parts of the plant extracts have been used as reducing agents for the green synthesis of Ag NPs. *Clerodendrum phlomidis* [22], *Trapa natans* [23], and *Cleome visosa*. *L*

[24], *Tulsi* leaf [25], *Phoenix dactylifera* [26], *Artocarpus intege* [27], *Cauliflower extract* [28], *Gymnema sylvestre* [29], and *Teucrium polium* leaf [30] are some of the plant extracts that recently have been used for the fabrication of Ag NPs for various applications.

The present work aims to utilize the flower extract of *H.Colorata* as green reducing to produce Ag NPs. Plant*H. Colorata* belongs to the Acanthaceae family [31]. The following bimolecular such as flavonoids, saponins, carboxylic acid, carbohydrate, and other phenolic compounds have been reported in *H. colorata*. This plant possesses many medicinal properties such as healing potential of wonder, bleeding, ulcer and inflammation. It is also used as folk medicine to treat anemia and diabetes[32, 33].

2. Materials and Methods

2.1. Reagents and extract preparation.

AR grade silver nitrate was purchased from Lobachemie, Mumbai, India. Distilled water was to prepare the solutions. Fresh flowers of *H. colarata* were collected from the local area of power, Tiruvannamalai District, Tamil Nadu, India. The flowers were thoroughly washed with water. About 10 g of dried flowers was extracted with 100 ml distilled water. The extract obtained was filtered using Whatman filter paper, stored in a refrigerator, and used for green synthesis silver nanoparticles.

2.2. Synthesis of Ag NPs.

In a 250 ml beaker, 100 ml of 0.1 mm silver nitrate solution was taken and placed on a magnetic stirrer. Aqueous flower extract of *H.Colarata* was dropwise added to the silver nitrate solution followed by continuous stirring. The heating process was continued for 30 min at 85°C. The formation of Ag NPs was confirmed from the brownish appearances of the solution. The solution was kept on a hot plate for the slow evaporation of water content. The Ag NPs were carefully collected from the beaker and thoroughly washed with distilled water to remove excess extract and unreacted silver nitrate solution. Finally, Ag NPs were washed with acetone to remove organic impurities and used for further characterization.

2.3. Materials characterization.

Surface Plasmon Resonance (SPR) of the Ag NPs was recorded using a UV-Visible spectrophotometer (Jasco V-670PC). Functional group analysis of the extract and the Ag NPs was assessed by FT-IR spectrometer (Shimadzu, Japan). Crystalline phase analysis was performed by X-ray diffractometer (Bruker, D8 advance). SEM images of the sample were recorded using a scanning electron microscope (Quantachrome, USA).

2.4. Antimicrobial assay.

Antibacterial activity of Ag NPs was carried out by agar well diffusion method according to the method described in the literature [34]. The bacteria used in this study were Escherichia coli, Klebsiella Pneumonia, Salmonella typhi, Pseudomonas aeruginosa, Streptococcus epidermidis, and Staphylococcus aureus. The fungi used were Candida Albicans, Cryptococcus neoformans, Aspergillus niger, Aspergillus flavus, Aspergillus fumigates, and Penicillium notatum. Bacterial isolates were suspended in brain Heart Infusion and diluted to 10⁶ CFC per ml. A sterile core borer was used to cut wells with a five mm

diameter. Various concentrations of Ag NPs were prepared using DMSO and poured into the wells. The plates were incubated at 37°C for 48 h. The zone of inhibition was measured in mm diameter. DMSO solvent was used as a control, and ciprofloxacin was used as a reference substance.

3. Results and Discussion

3.1. UV-Vis study.

Fig. 1 shows the UV-Visible spectrum of green synthesized Ag NPs. The spectrum renewal of the absorption at 439 nm due to the Surface Plasmon Resonance (SPR) color change of the reaction mixture after adding *H. Colorata* extract to the Silver Nitrate solution was observed as dark brown [35]. The collective oscillation of free electrons in particles is responsible for the color change. The SPR peak centered at 439 nm, indicating silver nitrate reduction into metallic silver [28].

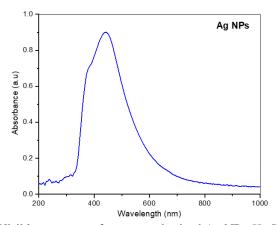


Figure 1. UV-Visible spectrum of green synthesized Ag NPs-H. Colorata extract.

3.2. FTIR studies.

Fig. 2 (a) shows the FTIR spectrum of green synthesized Ag NPs. Broadbands obtained at 3402 cm⁻¹ indicate OH stretching vibration of phenolic compounds. It reveals the presence of phenolic acids or flavonoids. The absorption band noticed at 1635 and 1433 cm⁻¹ arising due to carbonyl (CO) groups [36]. The narrow bands at 1113, 870, 682, and 602 cm⁻¹ attributed to glycoside and substituted phenolic compounds present in the Ag NPs [37].

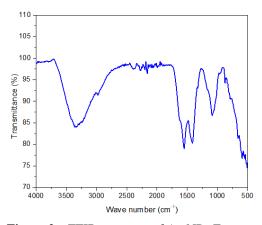


Figure 2a. FTIR spectrum of Ag NPs-Extract.

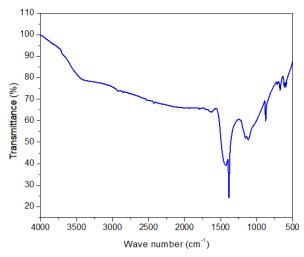


Figure 2b. FTIR spectrum of aqueous extract of *H. colorata*.

FTIR results confirmed the presence of bioactive molecules in the *H. Colorata* flower extract (Fig. 2 (b)). The primary phytochemical screening shows that the flower extract of *H. Colorata* is rich in phenol, carbohydrates, steroids, saponins, coumarins, tannins, proteins, carboxylic acid and flavonoids, xantho proteins and alkaloids [31].

3.3. XRD study.

The crystalline phase of Ag NPs synthesized using flower extract of *H. coralata* was investigated using XRD technique (Fig. 3). The Ag NPs was shown clear peaks of cubic phases (JCPDS No. 87-0720) at 20 values of 38.13, 44.49, 64.68, 77.39 and 81.53 corresponds to (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (222) planes. Thus the XRD pattern proved to be strong evidence favoring the UV–Visible spectra and TEM images for the presence of silver nanocrystals. The average crystallite size of the particle calculated using Debye-Scherrer's equation was 14 nm. The XRD results of Ag NPs synthesized using flower extract of *foenum-graecum Hance extract*, and *Allium ampeloprasum L* leaf extract showed similar confirming the crystalline silver formation [38, 39].

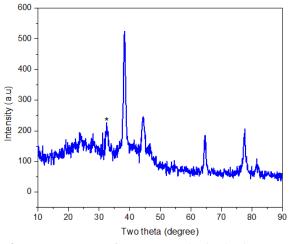


Figure 3. XRD spectrum of Ag NPs synthesized using *H. colorata*.

3.4. SEM and TEM morphology study.

Fig. 4 shows the SEM images of *H. colorata* extract stabilized Ag NPs at different magnifications. The results illustrate that the synthesized Ag NPs are spherical. The particles

have well-defined borders around their edges. The boundary between particles in the SEM image areas shows the role of flower extract as a capping agent.

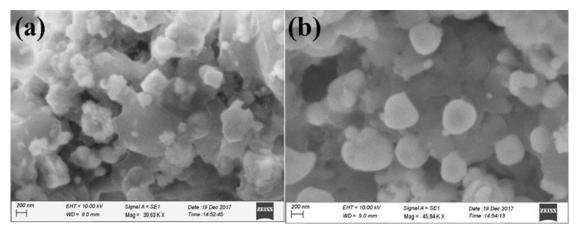


Figure 4. SEM images of Ag NPs synthesized using *H. colorata* flower extract (a) and (b) reveals the spherical shape of the particles at 200 nm magnification.

TEM images of the green synthesized Ag NPs are shown in Fig. 5. As shown in this figure, most of the nanoparticles are found to be spherical. This finding further supports the UV–Vis, XRD, and SEM results. The average size of the particles measured using software (Image J) is in the range of 10–20 nm. The surface morphology of the Ag NPs was studied from the TEM images. Spherical shape particles observed in the TEM images additionally confirmed the results of SEM analysis. The selected area diffraction pattern (SAED) reveals the crystalline nature of the green-synthesized Ag NPs.

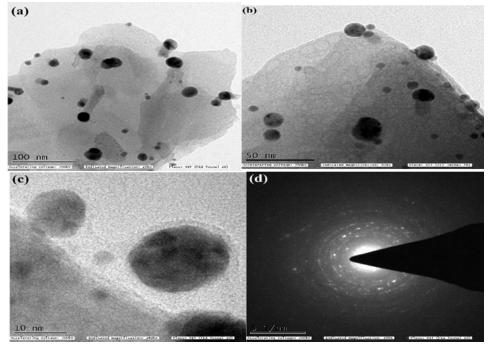


Figure 5. TEM images of Ag NPs synthesized using *H. coralata* flower extract. Images (a), (b), and (c) shows the spherical shape morphology of Ag NPs at 100, 50, and 10 nm magnifications. (d) Shows the selected area diffraction pattern of Ag crystals.

3.5. Antibacterial activity.

Antibacterial and antifungal activity of green-synthesized Ag NPs was tested against pathogenic bacteria and fungi ;the zone of inhibition shown by the bacteria and fungi is

presented in Table 1. Ciprofloxacin was used as a reference substance. The zone of inhibition shown by the synthesized Ag NPs found to be slightly better than the flower extract. However, the zone of inhibition shown by the bacteria is found to be lower than the *ciprofloxacin*. Among the tested bacterial, *S. typhi and S.epidermidis showed* lesser activity. Other bacteria *E. coli, K.pneumoniae*, *P.aeruginosa*, and *S. aureus* showed more or less similar activity. *H. Colorata* flower extract did not show any activity against tested fungi.

 Table 1. Zone of Inhibition of Ag NPs, H. colorata* against bacteria and fungi.

Zone of Inhibition (mm)

Bacteria	H.C. flower extract	Ag NPs	Ciprofloxacin	Fungi	H. C* flower extract	Ag NPs	Ciprofloxacin
Escherichia coli	10±2	13±2	28±4	Candida albicans	-	8±2	12±3
Klebsiella pneumoniae	12±2	14±2	30±5	Cryptococcus neoformans	-	7±2	13±2
Salmonella typhi	6±2	6±2	23±3	Aspergillus niger	-	8±2	12±2
Pseudomonas aeruginosa	14±3	15±3	9±2	Aspergillus flavus	-	6±2	13±3
Streptococcus epidermidis	7±2	7±2	30±4	Aspergillus fumigatus	-	5±2	13±3
Staphylococcus aureus	12±3	14±3	20±4	Penicillium notatum	-	-	13±2

However, Ag NPs exhibit lesser antifungal activity against *C.albicans*, *C. neoformans*, *A. niger*, *A.flavus*, *A. fumigates*, and *P. notatum*. Green-synthesized Ag NPs were found to be less effective against all microorganisms tested, and this activity depends on the magnitude of nanoparticles [40]. In fact, spherical Ag NPs contain a larger surface area. However, all the tested bacteria and fungi showed lesser activity compared with standard substances.

4. Conclusions

Ag NPs were successfully synthesized using the flower extract of *H. colorata* as a green reducing agent. The characterization methods confirmed the formation of Ag NPs. Crystalline of Ag NPs exhibit moderate toxicity against pathogenic bacteria and fungi. It is well known that *H. Colorata* extract is a source of phenolic and flavonoid compounds. It is believed that the water-soluble compounds present in the *H. Colorata* flower extract are played as reducing as well as stabilizing agents. The methodology and extract used to synthesize spherical shape nanoparticles are identified as an appropriate method to control the particles' size and shape.

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Conflicts of Interest

The authors declare no conflict of interest.

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