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Original Research Article

Green Synthesis, Characterization and Antibacterial Potential of Silver Nanoparticles from *Onosma bracteatum* Extract

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ABSTRACT

Antimicrobial resistance is one of the major concerns nowadays. In the present era, green nanotechnology has emerged. Plant materials such as stems, roots, and flowers in the form of an extract have been successfully used in the green synthesis of nanoparticles. The present study was aimed at biologically synthesizing silver nanoparticles (AgNPs) using *Onosma bracteatum* extract and investigating their antibacterial activity against different human pathogenic bacteria. The hydroalcoholic extract was prepared from the leaves and stems of *O. bracteatum.* The extract was used to biologically synthesize AgNPs. The green synthesized AgNPs were characterized using UV-Vis spectroscopy, Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy techniques. The antibacterial potential of different concentrations (0.5 to 1 mg/mL) of the green synthesized AgNPs was tested against *Bacillus subtitis*, *Micrococcus luteus*, *Staphylococcus epidermidis*, *Bacillus pumilus*, *Staphylococcus aureus*, and *Escherichia coli*. The results of the UV-Vis spectroscopy confirmed the synthesis of AgNPs with an absorption peak in the visible range of 380 - 500 nm. According to the FTIR analysis, the extract and AgNPs contained functional groups, which may have the ability to reduce Ag^{+} to Ag^{0} by supplying electrons to silver and therefore stabilizing the AgNPs formed. The AgNPs are crystalline, have face-centered-cubic geometry, and are spherical. Also, the green synthesized AgNPs have a significant dose-dependent bactericidal effect on gram-positive *Staphylococcus aureus* and gram-negative *E. coli*. The findings of the study suggest that plant extract could be used to synthesize metal nanoparticles, with the green nanoparticles having biomedical applications.

***Keywords*:** Antibacterial, Green synthesis, *Onosma bracteatum*, Silver nanoparticles.

Introduction

Nanotechnology is a vital component of modern material research, with several unique applications in fields such as medicine, pharmacology, healthcare, nutrition, and power generation, among others.[1] Due to their nano level size (1–100 nm), some relevant materials exhibit new and enhanced physiochemical and biological properties with distinct functionalities.[2] Metal nanoparticles (NPs) have received a lot of attention recently because of their applications in medicine, pharmacy, biology, material science, physics, and chemistry.[3] The most common NPs used in biomedical applications and the emerging interdisciplinary field of nanobiotechnology are gold and silver nanoparticles. Silver metal NPs have attracted a lot of attention among the various noble metal NPs because of their unique properties, such as strong electrical conductivity, chemical stability, catalytic and antibacterial activity.[4,5] Chemical, physical, photochemical, and biological methods are all being used to synthesize AgNPs at the moment. Each method has its own set of benefits and drawbacks.

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The physical and chemical processes that are used to create NPs are very costly. According to experts, using microorganisms and plant extracts in nanoparticle synthesis is the cheapest way to overcome this limitation.[6,7] Green synthesis is important in biological processes because it is cost-effective, environmentally friendly, and time-saving.[8]

The increased use of antibiotics in medicine, followed by an increase in antibiotic use in agriculture, has resulted in an evolutionary microbial reaction in the form of antimicrobial resistance.[9] Multi-resistant organisms are now responsible for 25,000 deaths each year in both the European Union and the United States. Furthermore, the cost of illnesses caused by resistant species is estimated to reach at least EUR 1.5 billion per year in the European Union alone.[10] Almost 80% of the world's population depends on herbal medications for their health care. These are used due to their medicinal effects, easy accessibility, and also based on generation-to-generation awareness. At present, plant-based industries are growing globally, but they are threatened, unfortunately, due to unregulated population growth and unplanned excess use/misuse of plant materials.[11] *Onosma bracteatum* is a Boraginaceae family member that is frequently known as Gaozaban, Gojivha, or Sedge. It is mostly found in high altitude states of India and Nepal, but it has also expanded to Jammu and Kashmir, Himachal Pradesh, and Uttar Pradesh in the north-western Himalayas.[12] The plant has great medicinal importance due to its antioxidant, analgesic, antimicrobial, antifungal, and antimicrobial properties.[13,14]

The present study was conducted to biologically synthesize and characterize nanoparticles from *Onosma bracteatum*, and investigate their bacteriostatic effect using the disk diffusion technique.

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Materials and Methods

Source of plant material

In November 2020, *O. bracteatum* was obtained from a commercial market in Bahawalpur, Pakistan. Dr. Ghulam Sarwar of the Department of Botany, Islamia University of Bahawalpur, Pakistan, identified and certified the plant, which was assigned a voucher number (Ref. No.: 05/Botany).

Preparation of plant extract

The leaves and flowers of the *O. bracteatum* plant were washed in distilled water to remove dust and impurities before being dried in the shade. The entire dried plant was ground into a powder. Five grams of powdered plant material were mixed with 100 ml of 30:70 hydro-ethanolic solvent and left for three days. Every day, the mixture was stirred to dissolve the phytochemicals (plant compounds) in the solvent. The mixture was filtered through muslin cloth after three days, and then Whatman filter paper No. 1 was employed for filtering. To extract the phytochemicals in the plant, the filtrate's solvent was evaporated on a rotary evaporator at 35 °C. The final mixture was a sticky extract that was kept in the refrigerator at 4^{o}C.[17]

Green synthesis of silver nanoparticles

The plant extract was mixed with 50 mL of water, and then the $AgNO_3$ solution was added drop by drop. The reaction mixture was stirred for 4 to 5 hours at 400 rpm on a magnetic stirrer while maintaining a temperature of 60^{o}C.[12] The colour of the solution changed from light yellow to dark brown, indicating the synthesis of silver nanoparticles. UV analysis at wavelengths of 195 to 1020 nm was used to confirm the formation of silver nanoparticles. A liquid sample of AgNPs was centrifuged at 12,000 rpm for 30 minutes. The AgNPs pellets were rinsed in water and lyophilized in a lyophilizer. The solid sample of nanoparticles was maintained at 4^{o}C after lyophilization for further characterization and antibacterial testing.[18]

Characterization of silver nanoparticles

UV-vis spectroscopy of silver nanoparticles

The reduction of silver ions and the synthesis of AgNPs were confirmed by measuring the absorbance of the reaction mixture with an ultraviolet-visible spectrometer (Model No. 752N). Spectrometry was used to confirm the synthesis of AgNPs by observing a peak value between 400 and 440 nm. The equipment utilized had wavelengths ranging from 195 to 1,020 nm. The cutoff value of the water used as a reference was measured at 200 nm.[19]

Fourier transform infrared spectroscopy (FTIR) of silver nanoparticles

In this investigation, the FTIR model number FTS-14 (1969) was used to show all of the peaks of the plant extract. Fourier transform infrared (FTIR) spectroscopy is a technique for obtaining high-resolution IR spectra of liquid or solid samples. It gives some insight into the reducing and stabilizing functional groups of *O. bracteatum* AgNPs.[18]

X-ray diffraction of silver nanoparticles

The X-ray diffraction (XRD) technique is used for the detection of crystal shape and size. An X-ray beam is directed towards a sample, and the dispersed intensity is measured as a function of the outgoing direction. The scattered pattern, also known as a diffraction pattern, indicates the sample's crystalline structure once the beam has been split. Using an XRD spectrometer (Model No. DJ-3500), XRD analysis was carried out on samples of four different concentrations (5, 10, 15, and 20 mg/ml) of AgNPs that were documented to determine the nanoparticle size and crystal structure.[20]

Scanning electron microscopy of silver nanoparticles

Scanning electron microscopy (SEM) was used to study the surface morphology of the biologically synthesized AgNPs. Scanning Electron microscopic images of samples were captured by JEOL Model JSM-6390LV. Nanoparticles that were relatively spherical and round were observed.[21]

Antibacterial activity of silver nanoparticles

The minimum inhibitory concentration (MIC) of the nanoparticles was determined using the disc diffusion method with broth medium. *Bacillus subtitis*, *Micrococcus luteus*, *Staphylococcus epidermidis*, *Bacillus pumilus*, *Staphylococcus aureus*, and *Escherichia coli* were all exposed to different nanoparticle concentrations ranging from 0.5 to 1 mg/mL. The various bacterial strains were exposed to different concentrations of AgNPs as well as standard antibiotics (Ciprofloxacin 10 mg/mL). In aerobic conditions, the cultures were incubated at 37^{o}C for 24 hours. Ciprofloxacin was utilized as the standard for determining antibacterial activity. MIC is the concentration that restricts the growth of bacteria.

Statistical analysis

Results for the antibacterial activity were subjected to one way analysis of variance (ANOVA). Significant level was set at $P<0.05$.

Results and Discussion

Characteristics of silver nanoparticles as determined by UV-visible spectroscopic analysis

Figure 1 shows peaks in the range of 419 - 432 nm for AgNPs of different concentrations (A-D). Due to the excitation of surface plasmon vibration, AgNPs usually exhibit a plasmon resonance absorption peak in the visible range of 380 - 500 nm. Therefore, the results of the UV-Vis spectroscopy (Figure 1) indicated that AgNPs were formed. The absorbance values were recorded every 24 hours for a week to verify the stability of the biologically synthesized AgNPs.[24] The UV-Vis spectrum (Figure 1) shows that the four different concentrations of AgNP samples had almost the same λmax value confirming the formation of AgNPs. Although, a different absorbance between 0.4 and 0.8, which could indicate the formation of AgNPs of different sizes was recorded. The increase in peak absorbance intensity is linked to an increase in nanoparticle synthesis due to the decrease of Ag^{+} ions.

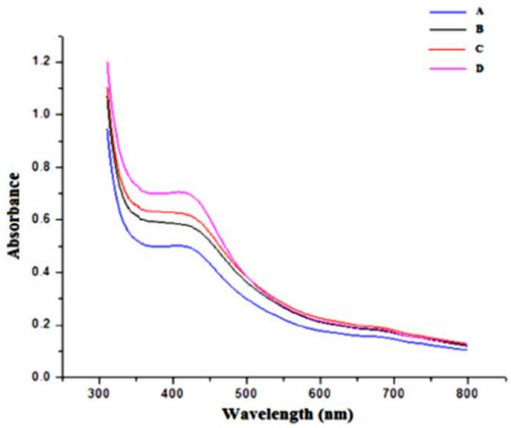

Figure 1: UV-Visible spectra of silver nanoparticles and their plasmon excitations upon interaction with different concentrations.
A: 5 mg\mL; **B:** 10 mg\mL; **C:** 15 mg\mL; **D:** 20 mg\mL

Phytochemical components of Onosma bracteatum extract and biosynthesized silver nanoparticles according to the FTIR analysis

An FTIR spectrum was conducted to determine the essential phytochemical components and bioactive molecules present in *O. bracteatum* extract and AgNPs. *O. bracteatum* extract and silver nanoparticles revealed different functional group peaks (wave numbers 3914.41, 3821.38, and 3300.55 cm^{-1} O-H stretch); C-O stretching of primary alcohol at 1066 cm^{-1}; 2946.73 cm^{-1} stretch for -CH=O 2163.87 cm-1 for stretching; α, β-unsaturated ketone (1902.28 cm^{-1}); 1545.06 cm^{-1} C-O stretch of primary alcohol and 1559.13 cm^{-1} (C-O stretch of primary alcohol), peak at 1354.26 cm^{-1} for alkane gem dimethyl, 1314.98 cm^{-1} C-H bending alkane stretch, 1183.82_alkyl

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aryl ether (C-O-C) stretch, 1272.46_ vinyl ether (H2C=HC-O) stretch (Figure 2A and B). All of the above-mentioned functional groups may have the ability to reduce Ag^{+} to Ag^{0} by supplying electrons to silver and therefore stabilize the AgNPs produced.

Characteristics of silver nanoparticles as determined by X-ray diffraction analysis

At 37.8, 45.9, 64.19, and 77.02^{o}, four prominent diffraction peaks result in 111, 200, 220, and 311 planes, respectively (Figure 3). These planes also represent a face-centered cubic structure of biologically synthesized AgNPs of varying sizes. The peaks at 31.9° were unassigned, however, it was hypothesized that they appeared due to the crystallization of other organic components in the *O. bracteatum* extract. The crystalline nature and face-centered-cubic geometry of the biologically synthesized AgNPs were confirmed by the results. These peaks correspond to the biosynthesized AgNPs reference card (JCPDS Card No. 4-0783).

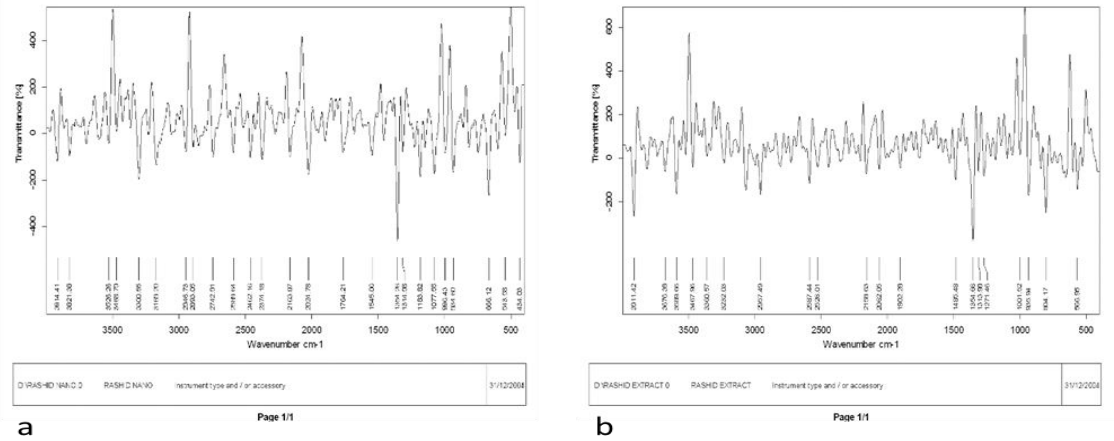

Figure 2: Fourier transform infrared spectroscopy (FTIR) spectra of test solutions.
a: *Onosma bracteatum* extract; **b:** Silver nanoparticles synthesized from *O. bracteatum* extract.

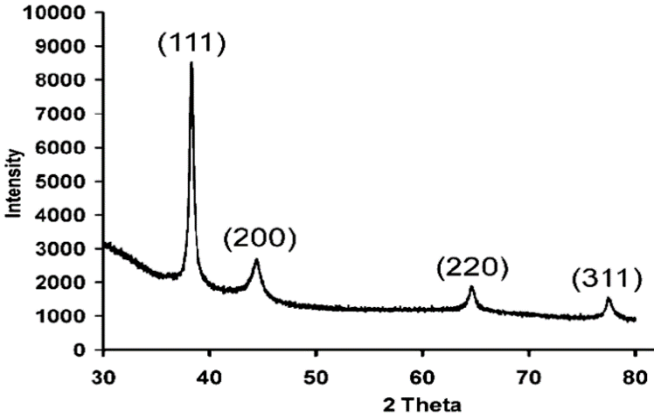

Figure 3: X-ray diffraction spectrum of biosynthesized silver nanoparticles from *Onosma bracteatum* extract.

Characteristics of silver nanoparticles as determined by scanning electron microscopic analysis

Scanning electron microscopy was used to analyze the morphology of biosynthesized AgNPs. It was also utilized to determine the size of the particles. The SEM images of the biologically synthesized AgNPs, as well as their sizes and forms, are shown in Figure 4. The biosynthesized AgNPs had spherical and circular forms, and the majority of them showed up in pairs.

Antibacterial activity of silver nanoparticles

The antibacterial activity of AgNPs was measured using the disc diffusion method. At different concentrations of AgNPs, *Bacillus subtitis*, *Micrococcus luteus*, *Staphylococcus epidermidis*, *Bacillus pumilus*, *Staphylococcus aureus*, and *Escherichia coli* were tested. In comparison to ciprofloxacin (10 mg/mL), AgNPs at 1 mg/mL showed a significant antibacterial effect against the test pathogenic bacteria. This was evident by the diameters of inhibition zones of 28.27, 24.87, and 14.56% in *Staphylococcus aureus*, *Micrococcus luteus*, and gram-negative bacterium, *E. coli*, respectively as presented in Figure 5. When the concentration of AgNPs was reduced to 0.5 mg/mL, bacterial growth was inhibited to some extent. Furthermore, the growth of *Staphylococcus aureus*, *Micrococcus luteus*, and *E. coli* was completely suppressed at higher concentrations of AgNPs. The minimum inhibitory concentration (MIC) value of the biosynthesized AgNPs indicated that they possess significant antibacterial activity (Table 1). The different concentrations were observed against the test bacterial strains which showed that the MIC was higher in the gram-positive bacteria compared to the gram-negative bacteria such as *E. coli*.

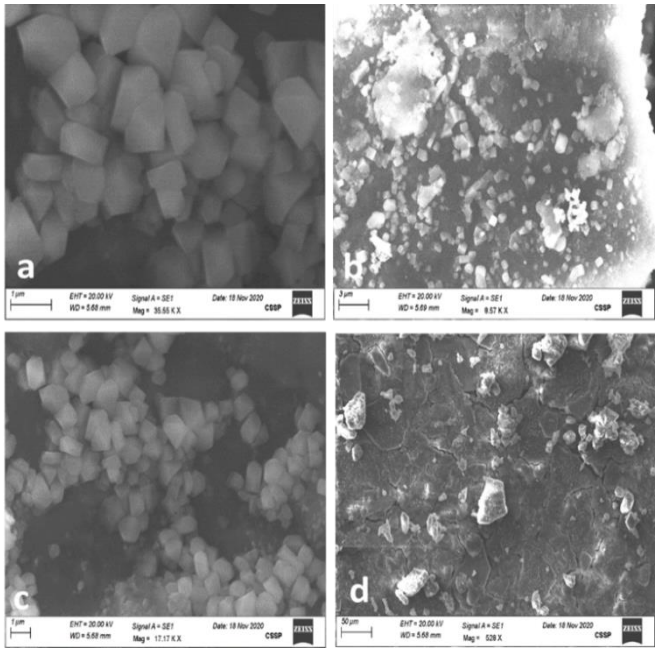

Figure 4: Scanning electron microscopy (SEM) images of variable sizes of silver nanoparticles from different concentrations.
The calculated grain size areas are **A:** 46 nm; **B:** 50 nm; **C:** 71 nm; **D:** 89 nm.

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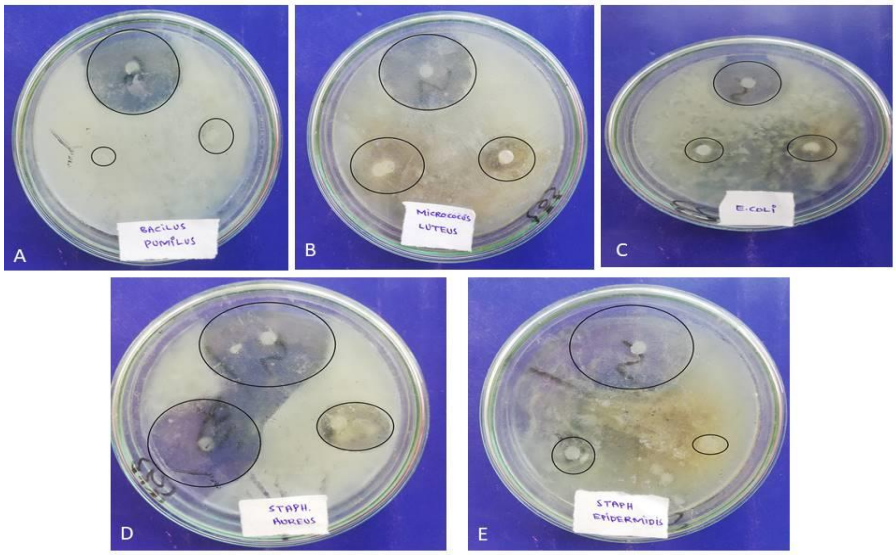

Figure 5: Antibacterial sensitivity testing of human pathogenic bacteria.
A: *Bacilus pumilus;* **B:** *Microcus luteus:* **C:** *E. coli;* **D:** *Staphylococcus aureus;* **E:** *Staphylococcus epidermis*

Table 1: Minimum inhibitory concentrations of silver nanoparticles synthesized with *Onosma bracteatum* extract

Bacterial pathogen	Gram stain (+/-)	MTCC No.	Zone of inhibition of 10 mg/ml standard ciprofloxacin (mm)	Zone of inhibition of 0.5 mg/ml AgNPs (mm)	Zone of inhibition of 1 mg/ml AgNPs (mm)	P-value
Micrococcus luteus	+	435	31.3±4	13.2±3	24.8±7	0.05
Staphylococcus epidermidis	+	732	28.2±5	7.8±4	12.2±6	0.005
Bacillus pumilus	+	411	32.3±3	3.6±2	6.8±3	0.0001
Staphylococcus aureus	+	7405	33.4±5	15.8±4	28.2±7	0.05
Escherichia coli	-	2470	32.4±3	9.3±4	14.5±6	0.005

Conclusion

The findings of this study reveal that AgNPs were successfully synthesized using a hydro-alcoholic extract of *Onosma bracteatum* with Ag^{+} being reduced to Ag^{0} at 37°C by functional groups present in the phytochemicals, as confirmed by UV-Vis, XRD, and SEM analyses. According to FTIR analysis, *O. bracteatum* extract contains phytochemicals that aid in the reduction of silver into nanoparticles as well as the ability to stabilize these particles. SEM analysis indicated that biosynthesized AgNPs of various concentrations are spherical and circular, with sizes ranging from 46 to 89 nm. The biosynthesized AgNPs have high antibacterial activity against gram-positive bacteria such as *Staphylococcus aureus, Micrococcus luteus,* and *Staphylococcus epidermidis.* Moderate antibacterial activity against *E. coli* was observed.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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