



Green Synthesis of Silver Nanoparticle Using Aqueous Leaf Extract of *Barleria noctiflora* and its Bioactive Efficacy

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

For millennia, silver has been known to have powerful antibacterial effects. Topical silver dressings are now commonly used to treat infections in burns, open wounds, and persistent ulcers. Because pathogenic organisms evolve on a daily basis as a result of mutation and antibiotic resistance, the manufacture and investigation of nanoparticles for use in antibacterial garments, burn ointments,

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and medical device coatings is an important industrial topic of nanoscience. This is the first study on the synthesis of silver nanoparticles from leaf extract of the medicinal plant *Barleria noctiflora*. Through the use of FT-IR, UV-visible spectrum analysis, XRD analysis, SEM EDAX, and antimicrobial potential, the bioreduced nanoparticles were discovered and verified. The greatest absorption peak in the UV-visible spectrum examination was seen at 480 nm for the silver nanoparticles. Twelve distinct peaks were identified in the FTIR spectra of biogenic AgNPs, located at 3127.21, 1624.74, 1400.35, 1155.96, 1021.11, 906.70, 825.31, 718.73, 669.69, 538.93, 457.21, and 417.49 cm⁻¹. Peaks at 2θ values of 10.545, 19.000, 21.137, 22.033, 23.370, 26.691, 31.507, and 33.747, corresponding heights of 23.40, 235.26, 750.78, 357.58, 335.93, 171.19, 186.87, and 115.38cts, respectively, indicated by XRD analysis that the silver particles generated in our experiments were nanocrystals. The silver nanoparticles were tested with "d" spacing values of 8.38940, 4.67087, 4.20328, 4.03422, 3.80651, 3.33986, 2.83949, and 2.65601. SEM spectral analysis revealed oval AgNPs with a size range of 20 m. Metallic nanoparticles generated from *Barleria noctiflora* were tested for antibacterial activity against gram-positive and gram-negative pathogens such as E. Coli, Staphylococcus aureus, and the fungi Aspergillus niger and Candida albicans using the disc diffusion method. Surprisingly, metallic Ag nanoparticles had strong inhibitory zones against the selected illnesses.

Keywords: *Barleria noctiflora*; green synthesis; AgNPs; microorganisms; characterization.

1. INTRODUCTION

Due to their precise characteristics and wide variety of applications in fields including pharmaceuticals, electronics, chemical units, food and agriculture, mechanics and optics, cosmetics, and so forth, metal nanoparticles (NPs) are intriguing materials in modern research [1–5].

Depending on their size and form, nanoparticles (NPs) are solid, atomic or molecular scale particles that are less than 100 nm and have certain superior physical properties compared to bulk molecules [6-7]. The superior qualities of metal and metal oxide nanoparticles, such as their high surface to volume ratio and good dispersion in solution, have led to a detailed investigation of these NPs employing science and technology [8–9]. As a result, metal and metal oxide nanoparticles have improved antibacterial capabilities [10–11]. These days, NPs are used in molecular imaging to produce highly resolved images for diagnosis. Furthermore, contrast chemicals are infused onto nanoparticles to diagnose tumors and atherosclerosis [12–14]. Additionally, since the FDA approved the first nanotherapeutic in 1990, it has been promoted globally to develop a variety of nano-based medications [15]. Several physical and chemical processes, including chemical reduction and milling, were used to create nanoparticles (NPs) and increase their efficiency around the start of the 20th century [16]. Nevertheless, these traditional methods cannot be regarded as environmentally benign

procedures because they require expensive and hazardous ingredients [17]. Considering this, researchers are currently very interested in the bio-genic production of metal and metal oxide nanoparticles (NPs), which uses microorganisms and aqueous plant extract and is environmentally benign, stable, clinically adaptive, and economical [16,18]. As a result, the production of nanoparticles using bio-inspired technology has grown in importance within the fields of nanoscience and nanotechnology [19–20]. Until now, a variety of plant extracts, microorganisms, and metal oxide nanoparticles have been created [21–22]. In addition to their extensive use in the synthesis of nanoparticles, plant biomass is widely available, renewable, and environmentally acceptable. As such, our group and others have focused on using it as a catalyst for chemical synthesis [23–24] and the generation of biodiesel [25–26]. The scientific world is becoming increasingly interested in silver nanoparticles (NPs) among metal NPs because of their extensive applications in chemistry, food technology, microbiology, cell biology, pharmacology, and parasitology [27–28]. The physical and chemical characteristics of silver nanoparticles are determined by their shape [28]. In general, a number of methods have been used to synthesize silver nanoparticles, including the sol-gel method, the hydrothermal method, chemical vapour deposition, thermal decomposition, microwave-assisted combustion method, etc. [29–31]. The antibacterial activity of silver nanoparticles (AgNPs) produced recently through biogenic synthesis using biomaterials like plant extract and microorganisms as

reducing agents has been extensively studied [32]. This work uses a simple and economical green synthesis methodology. A range of analytical techniques, including as UV-Visible Spectroscopy, Fourier Transforms Infrared Spectroscopy, Scanning Electron Microscopy (SEM), X-ray diffraction (XRD), and Antimicrobial Activity Assessments, will be employed to completely evaluate the resultant nanoparticles.

2. MATERIALS AND METHODS

2.1 Plant Material Collection

Fig. 1 shows the leaves of *Barleria noctiflora* that were chosen for this study. The Acanthaceae plant family was discovered in the Gangaikondan village of Tamil Nadu's Tirunelveli district. Taxonomic characteristics were established by checking sources such as the 'Flora of Tamil Nadu Carnatic' [34] and the 'Flora of the Presidency of Madras' [33].

2.2 Isolation of Nanoparticles

Fresh and healthy *Barleria noctiflora* leaves were harvested with care, and any visible contaminants or dust were removed by properly cleaning them with distilled water and tap water. Next, 100 milliliters of double-distilled water were used to boil 10 grams of fresh leaves. The resultant extract was then filtered through Whatman no. 1 filter paper, with the filtrate being collected in a conical flask. The resulting extract was used to create different kinds of nanoparticles.

2.3 Preparation of Silver Nitrate Solution

A 1mM solution of silver nitrate was prepared by dissolving 0.0169 grams of the compound in 100

milliliters of double distilled water, and subsequently, it was stored.

2.4 Metal-Plant Extracts Interaction

A conical flask was filled with 90 milliliters of silver nitrate solution and 10 milliliters of leaf extract. The Silver Nitrate solution changed color from pale green to brownish green. During the 72-hour incubation period, the conical flask was exposed to light.

2.5 Characterization

UV- Spectrophotometer Analysis: A 1 milliliter sample suspension was put into a quartz tube for examination with a UV spectrophotometer. Following that, the sample was diluted with two milliliters of distilled water for better nanoparticle observation. A Shimadzu UV 1800 spectrophotometer (Germany) was used to scan UV-visible spectra in the wavelength range of 200-900 nm.

FT-IR Analysis: To prepare for FTIR measurements, the solution containing the produced silver nanoparticles was centrifuged for 30 minutes at 10,000 rpm. The resultant pellet was rigorously washed three times with 5 milliliters of deionized water to remove any unbound proteins or enzymes that were not connected with the silver nanoparticles. The pellet was then vacuum dried before being subjected to FTIR analysis.

XRD Analysis: To characterize the pure produced nanoparticles, XRD measurement was performed on freeze-dried powder samples at 40 kV/20 mA with continuous scanning in 2 theta mode [1]. The nanoparticle pellet was re-dispersed in 10 milliliters of deionized water after being centrifuged at 5000 rpm for 20 minutes to filter the nanoparticle solution.



(a- Habit of *Barlerianoctiflora*, b - Silver Nitrate Extract, c- Silver Nitrate with Aqueous Leaf Extract)

Fig.1. Green Synthesis of silver nanoparticle using the aqueous leaf extract of *Barlerianoctiflora*

SEM Analysis of Silver Nanoparticles: A TESCAN VEGA3 SBH instrument was used for the SEM examination. The pellet was the focus of the examination. To produce thin films, a little amount of the material was put onto a carbon-coated copper grid. Excess solution was carefully removed with blotting paper before examining the film on the SEM grid.

Antimicrobial Activity of Nanoparticles: In the clinical laboratory at Scudder Diagnostic Centre, Nagercoil, the Agar well diffusion method was employed to examine the potential inhibitory effects of leaf extracts of *Barleria noctiflora* on a range of human ailments. The antibacterial efficacy of isolated plant extraction pellets was evaluated using the Kirby-Bauer method. The appropriate fungi and bacteria were inoculated overnight on Sabouraud's Dextrose Agar (SDA), Potato Dextrose Agar (PDA), and nutritional agar plates. All of the plates, including the control plates, developed wells. Amikacin, an antibiotic, was used as a control at the same concentration. While *E. Coli* and *Staphylococcus aureus* plates were incubated at 37°C for 24 hours, plates containing *Candida albicans* and *Aspergillus niger* were incubated at 35°C for 48 hours. The diameter of the inhibitory zone was expressed in millimeters [35].

3. RESULTS AND DISCUSSION

3.1 Synthesis and Characterization of Nanoparticles

When exposed to Silver Nitrate in aqueous solutions, *Barleria noctiflora* leaves changed color in different ways, indicating the creation of three different kinds of nanoparticles. These nanoparticles were then used in a variety of biological processes after being verified by spectral tests including FT-IR, X-Ray Diffraction, UV-Visible Spectroscopy, and SEM EDAX Analysis. Strict control over the raw materials is necessary to guarantee the consistent quality of herbal goods. As a result, the standardization of medicinal plants with therapeutic potential has received more attention in recent years. Even with the availability of contemporary methods, pharmacognostical investigations continue to be a more dependable, accurate, and economical method for the identification and assessment of plant-based medications.

3.2 UV-Vis Spectrum of Silver Nanoparticles Analysis of *Barleria noctiflora* Leaves

The color shift in the reaction solutions indicated the reduction of silver nitrate utilizing the plant

leaf extract (Fig. 2). The reaction solution of reduced silver nitrate containing the leaf extract of *Barleria noctiflora* has its UV-V spectrum recorded (Fig. 1). At 480 nm, the greatest absorbance peak was seen. Similar to this, Prasad and Elumalai [36] found that the absorbance peak of silver nanoparticles generated in the reaction media is located around 430–440 nm [29] in their absorption spectra.

3.3 FT-IR Analysis

Biogenic AgNPs' FTIR spectra, obtained from *B. noctiflora* leaf extract following AgNO₃ reaction. As seen in Fig. 3, the FTIR data shows a little shift in the spectra's peak position. The *B. noctiflora* leaf extract's FTIR spectra (Fig. 2) revealed that the flavonoids' –OH (alcohol), >C=C (alkenes), and =NH (amines) groups are responsible for 12 peaks at 3127.21, 1624.74, 1400.35, 1155.96, 1021.11, 906.70, 825.31, 718.73, 669.69, 538.93, 457.21, and 417.49 cm⁻¹, respectively. The secondary amines and phenolic hydroxyl groups are represented by the peaks at 3127.21 cm⁻¹. The carbonyl groups' C=O stretching vibrations are responsible for the peaks between 1624.74 and 1400.35 cm⁻¹, whereas the C-N amines' peaks were found at 1155.96 cm⁻¹. The presence of the aromatic ring or C=C was verified by the 1372 cm⁻¹ band. An aromatic ether bond is reported to exhibit stretching mode C–O–C from 1021.11 to 417.49 cm⁻¹. Consistent with previous findings [37–38], FTIR spectroscopic examination verified Ag-NPs' significant affinity towards various functional groups in the BL compounds. Therefore, proteins and phenolic agents found in the BL extraction were in charge of the effective conversion of AgNO₃ into Ag-NPs.

3.4 XRD Analysis

Using X-ray diffraction analysis, the biosynthesized nanoparticles from *Barleria noctiflora* leaf extracts were carefully examined and verified (Fig. 4). The goal of the XRD examination was to verify that the nanoparticles were crystalline. When our XRD spectrum was compared to standards, it was confirmed that the nanoparticles that were produced during our research were, in fact, nanocrystals, as the peaks at particular 2θ values showed. Regarding silver nanoparticles, the XRD examination displayed eight unique peaks in the XRD picture, varying in size from 0 to 90 Å (Fig. 4). These peaks matched heights of 23.40, 235.26, 750.78,

357.58, 335.93, 171.19, 186.87, and 115.38 cm, corresponding to 2θ values of 10.545, 19.000, 21.137, 22.033, 23.370, 26.691, 31.507, and 33.747, respectively. The silver nanoparticles with corresponding "d" spacing values of 8.38940Å, 4.67087Å, 4.20328Å, 4.03422Å, 3.80651Å, 3.33986Å, 2.83949Å, and 2.65601Å were measured (Table.1). X-ray diffraction

analysis is utilized to investigate the crystalline nature and phase purity of produced AgNPs, as detailed in. The crystalline peaks that we saw here at 2θ 38.21°, 44.38°, 64.56°, and 77.39° are ascribed to the crystallographic planes (111), (200), (220), and (311) [39].

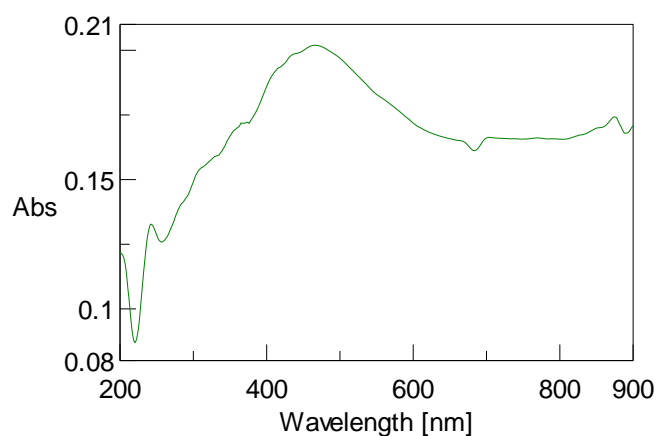


Fig. 2. UV-Visible Spectrum of silver nanoparticles synthesized by using the aqueous leaf extract of leaves of *Barleria noctiflora*

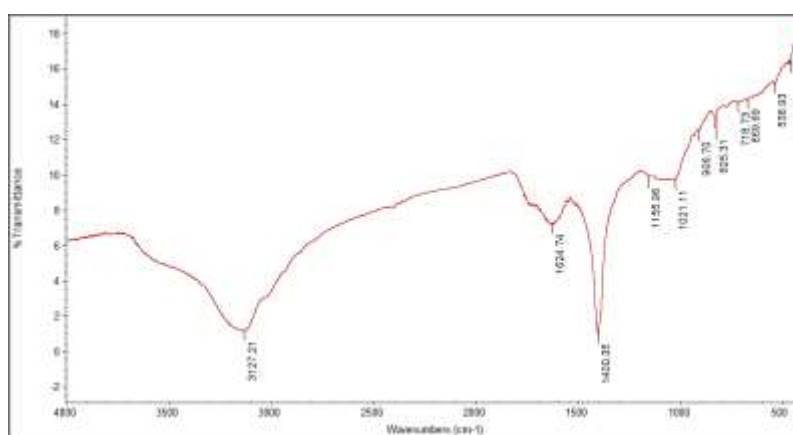


Fig. 3. FTIR Spectrum of silver nanoparticles synthesized by using the aqueous leaf extract of *Barleria noctiflora*

Table 1. XRD Pattern of silver nanoparticles synthesized by using the aqueous leaf extract of *Barleria noctiflora*

| Pos.[°2Th.] | Height [cts] | FWHM Left [°2Th.] | d-spacing [Å] | Rel. Int. [%] |
|-------------|--------------|-------------------|---------------|---------------|
| 10.545 | 23.40 | 0.6927 | 8.38940 | 3.12 |
| 19.000 | 235.26 | 0.8659 | 4.67087 | 31.34 |
| 21.137 | 750.78 | 0.3464 | 4.20328 | 100.00 |
| 22.033 | 357.58 | 0.5196 | 4.03422 | 47.63 |
| 23.370 | 335.93 | 0.5196 | 3.80651 | 44.74 |
| 26.691 | 171.19 | 0.3464 | 3.33986 | 22.80 |
| 31.507 | 186.87 | 0.3464 | 2.83949 | 24.89 |
| 33.747 | 115.38 | 0.8659 | 2.65601 | 15.37 |

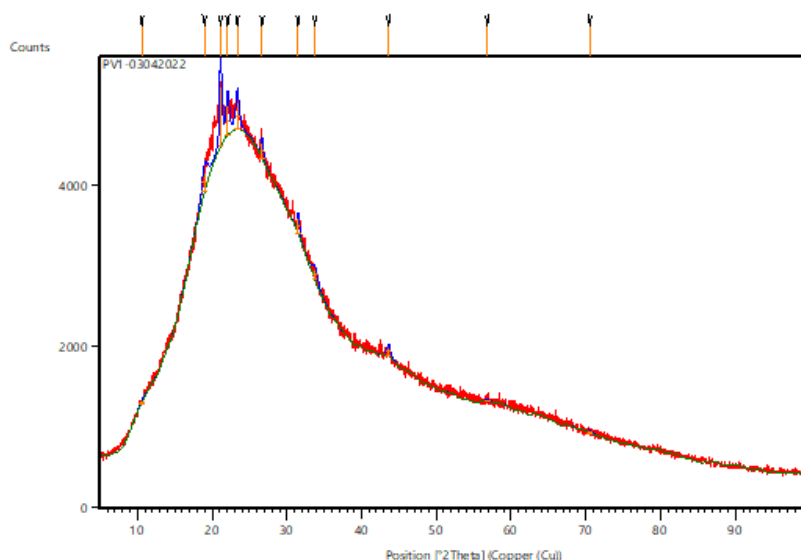


Fig. 4. XRD Spectrum of silver nanoparticles synthesized by using the aqueous leaf extract of *Barleria noctiflora*

3.5 SEM Analysis of Silver Nanoparticles

A closer look at the Scanning Electron Microscopy (SEM) pictures revealed details about the morphology and synthesis of stable silver nanoparticles made from leaf extract from *Barleria noctiflora*. Silver nanoparticles were uniformly distributed on the cell surfaces, as seen by the SEM study (Fig. 5). The silver nanoparticles have a 20µm particle size and a spherical shape. According to Savithramma et al. [40], silver nanoparticles with a roughly spherical shape developed in *Shorea tumbuggaia* and *Boswellia ovalifoliolata*, with diameters ranging from 30 to 40 nm.

3.6 Antimicrobial Activity

The data in Figs. 6 (a-d) show that Ag-NPs have a strong inhibitory effect on human pathogenic bacteria. The zone of inhibition for varied Ag-NP concentrations is shown in Table 2. The findings

suggest that synthesized Ag-NPs can be a powerful antibacterial agent against human illnesses, and that this effect is magnified at greater Ag-NP dosage concentrations. Ag-NPs have a very narrow zone of inhibition and little to no activity against bacterial and fungal strains of *Staphylococcus aureus*, *Aspergillus niger*, *Candida albicans*, and *Escherichia coli*. *Pseudomonas aeruginosa* was moderately toxic to silver nanoparticles, according to Kumar et al. [41], while *P. vulgaris*, *E. coli*, *B. subtilis*, and *P. putida* were moderately toxic. Nanoparticles, on the other hand, were not harmful to *S. Typhi*. [19]

The overall antimicrobial efficacy varied among the different pathogens. Notably *E. coli* exhibited the largest zone of inhibition (19.2 ± 0.4), incontinuity *Staphylococcus aureus* exhibited smaller zone of inhibition (16.5 ± 0.2). While the fungus *Aspergillus niger* exhibited (17 ± 0.2) greater zone of inhibition against *Candida albicans* (16.2 ± 0.3).

Table 2. Antimicrobial activity of silver nanoparticles synthesized by using the aqueous leaf extract of *Barleria noctiflora*

| S. No | Pathogens | Antimicrobial activity - Zone of Inhibition (mm) | |
|-------|------------------------------|--|-------------------|
| | | Silver Nanoparticles (AgNO ₃) | Control(Amikacin) |
| 1. | <i>Escherichia coli</i> | 19.2 ± 0.4 | 22 ± 0.3 |
| 2. | <i>Staphylococcus aureus</i> | 16.5 ± 0.2 | 17 ± 0.2 |
| 3. | <i>Candida albicans</i> | 16.2 ± 0.3 | 15 ± 0.4 |
| 4. | <i>Aspergillus niger</i> | 17 ± 0.2 | 18 ± 0.3 |

± Standard Error, + Present, - Absent

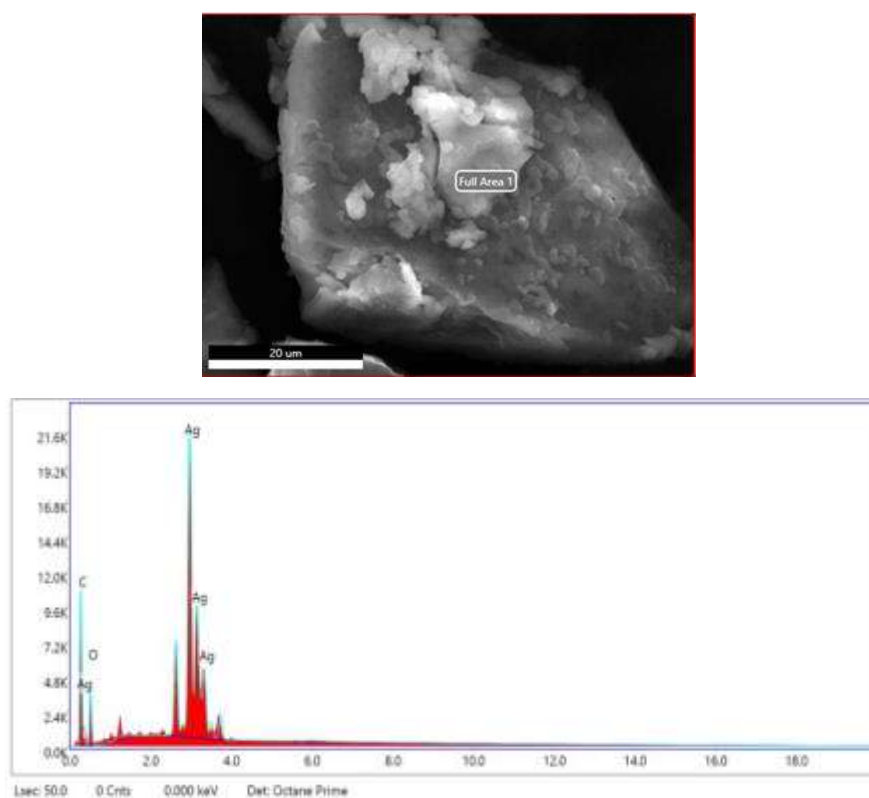


Fig. 5. SEM EDAX image of silver nanoparticles synthesized by using the aqueous leaf extract of *Barleria noctiflora*

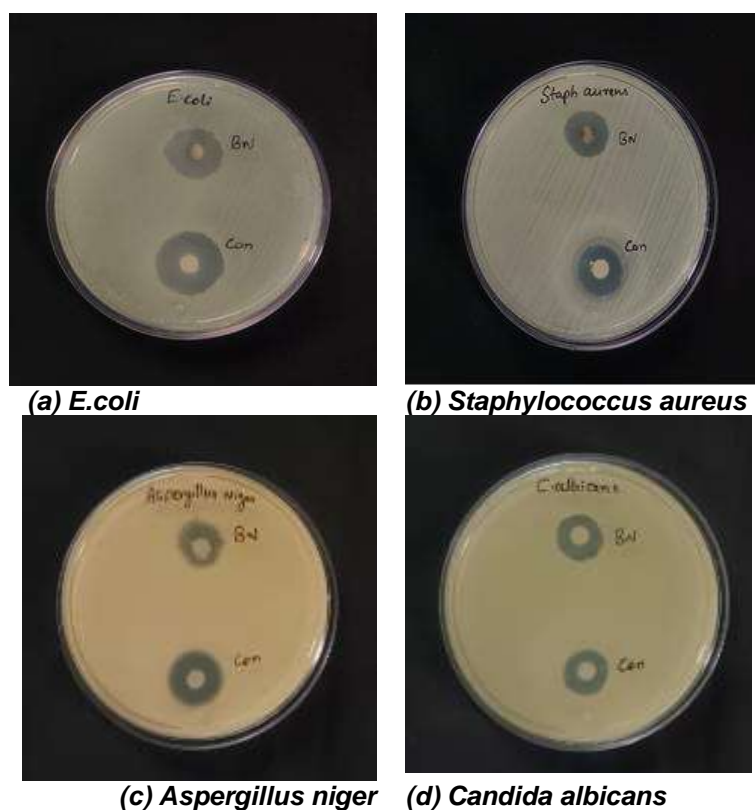


Fig. 6. Antimicrobial activity of silver nanoparticles against selected human pathogens

4. CONCLUSION

Silver nanoparticles' appealing physiochemical characteristics have made them extremely useful in the fields of biology and medicine. In the current work, we have shown that metal nanostructures may be efficiently produced using green nanochemistry using a natural, inexpensive biological reducing agent and extracts from *Barleria noctiflora* leaves, without the need for waste or hazardous solvents. Ag-NPs were made from aqueous leaf extract using an environmentally friendly green synthesis method, which was verified by FTIR spectra and XRD analysis. With the use of EDAX analysis, spherical surface shape was investigated using SEM. Ag-NPs demonstrated reduced antibacterial activity against *Staphylococcus aureus* and *Candida albicans* and increased antimicrobial activity against *E. Coli* and *Aspergillus niger*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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