ECO-SYNTHESIS OF AG NANOPARTICLES UTILIZING THE XEROPHYTIC PLANT BLEPHARIS SINDICA AND THEIR BIOLOGICAL ACTIVITY

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Abstract

Using the Methanolic leaf extract of Blepharis sindica, the current work examined the green production of silver nanoparticles (Ag NPs) and assessed their structural characteristics and biological activity. By effectively reducing silver ions, the phytochemical-rich extract produced stable, spherical nanoparticles, which were verified by transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray diffraction (XRD), FTIR and UV-visible spectroscopy. The produced Ag NPs had good crystalline properties and were mostly between 10 and 30 nm in size. Significant antibacterial and antifungal activity were found in biological experiments, which showed high percentages of inhibition against Candida albicans, Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus. The results demonstrated the biological potential of the resultant Ag NPs and demonstrated the efficacy of Blepharis sindica as a natural, environmentally acceptable source for nanoparticle synthesis.

Keywords: *Blepharis sindica*, silver nanoparticles, green synthesis, antimicrobial activity, antifungal activity, phytochemicals.

1. INTRODUCTION

Metallic nanoparticles are unique nanoparticles in their own right. They have been found to contain unique features like high catalytical activity, electrical and thermal conductivity, as well as antimicrobial, anticancer, and biosensing activities (1,22). Silver nanoparticles (Ag NPs), one type of noble metal nanoparticle, have garnered a lot of scientific attention due to their distinct physicochemical characteristics and potent antibacterial and antifungal effects (2). Ag NPs are widely employed in clinical treatment, biomedical devices, medicines, dressings, food, coatings, and other industries because of their exceptional antibacterial properties (3,23). Ag nanoparticles have been successfully applied in drug administration and cancer therapy due to their high electrical conductivity, high thermal conductivity, chemical stability, catalytic activity (4,24). The shape and size of silver nanoparticles (Ag-NPs) are two important parameters that affect their potential applications (5).

The environmental and biological application of traditional chemical and physical synthesis approaches was limited since they frequently entailed high-energy processes or hazardous chemicals (6,25). The growing need for sustainable, non-toxic, and economical synthesis techniques has made the development of environmentally friendly nanomaterials a top priority in recent years. Green synthesis methods that use plant extracts have therefore become viable substitutes because they provide ease of use, safety, and natural reducing agents that can stabilize nanoparticles (7). Plant extracts contain biochemicals that can actively contribute to the creation and stability of phyto-nanoparticles(26). phyto-nanoparticles have two main advantages: biocompatibility and bioactivity. Because of their outstanding qualities and environmentally benign production process, phyto-nanoparticles have great promise for a range of medical applications (8).

Native to arid and semi-arid areas, Blepharis sindica is a medicinal plant that contains a variety of bioactive substances, including as flavonoids, alkaloids, phenolics, tannins, and glycosides (9,18,20). The plant **is** appropriate for the environmentally friendly manufacture of silver nanoparticles since these phytochemicals might function as natural reducing and capping agents. Blepharis sindica leaf extract **is** essential in turning silver ions (Ag^+) into metallic silver at the nanoscale and stabilizing the resultant nanoparticles without the use of extra chemical surfactants.

Blepharis sindica plant in one of the earliest pharmacognostic and phytochemical studies, proving the plant's abundance of bioactive components. Flavonoids, alkaloids, tannins, and phenolic chemicals were found in their investigation, indicating the plant's high potential for usage in natural product research (9,10,19,21). The significance of Blepharis sindica for environmentally friendly. Ag NPs production was supported by the fact that these phytochemicals are recognized to function as stabilizing and reducing agents during the synthesis of green nanoparticles.

2. RESEARCH MATERIAL AND METHODOLOGY

2.1. Collection and Preparation of Plant Material

A certified taxonomist verified the authenticity of fresh *Blepharis sindica* leaves that were gathered from their natural environment. After being cleaned of dust and dirt under running water, the leaves were rinsed with distilled water. A sterile grinder was used to grind the clean leaves into a fine powder after they had been shade-dried for a few days. Until it was needed again, the powdered material was kept at room temperature in sealed containers.

2.2. Preparation of Leaf Extract (Methanolic Extract)

10 grams of the dried leaf powder were mixed with 100 mL of **methanol**. The mixture was heated gently for 24 hours at 40–50°C. After cooling, it was filtered through Whatman No. 1 filter paper to obtain a **methanolic extract.** The extract was stored at 4°C for further nanoparticle synthesis.

2.3. Green Synthesis of Silver Nanoparticles

1 mM Silver nitrate ($AgNO_3$) solution was prepared. The plant extract was added dropwise in a 1:4 ratio to the silver nitrate solution while being continuously stirred. To stop the silver ions from photoactivating, the reaction mixture was left at room temperature in the dark. The development of silver nanoparticles (AgNPs) was indicated by the color gradually changing from pale yellow to brown. Following a 15-minute centrifugation at 16,000 rpm, the nanoparticles were periodically cleaned with distilled water to get rid of contaminants before being dried for additional analysis.

2.4. Characterization of Eco-synthesized Ag NPs-

UV–Visible Spectroscopic Analysis

The nanoparticle suspension was scanned in the 300–700 nm range using a UV–Visible spectrophotometer. A characteristic Surface Plasmon Resonance (SPR) peak confirmed nanoparticle formation and stability (11).

X-Ray Diffraction (XRD) Analysis

XRD analysis was performed to determine the crystalline nature of the synthesized nanoparticles. Dried nanoparticle samples were mounted on an XRD grid and scanned between $2\theta = 20°–80°$. Distinct diffraction peaks confirmed the crystalline structure (12).

Scanning Electron Microscopy (SEM)

SEM was used to observe surface morphology and approximate size. A thin layer of dried nanoparticles was mounted on a conductive stub before imaging. Aggregation pattern, shape, and texture were evaluated (13).

Transmission Electron Microscopy (TEM)

TEM revealed detailed size distribution and morphology. A drop of nanoparticle suspension was placed on a carbon-coated copper grid and allowed to air dry. TEM micrographs showed spherical or quasi-spherical, well-dispersed nanoparticles (14).

2.5. Antimicrobial Activity Assay

The agar well diffusion method was used to evaluate antibacterial activity against Gram-positive and Gram-negative clinical isolates. Different concentrations of nanoparticles were added to wells in inoculated agar plates and incubated at 37°C for 24 hours (15). Zones of inhibition were measured and compared with standard antibiotics.

2.6. Antifungal Activity Assay

Antifungal activity was tested using the poisoned food technique(17). Potato Dextrose Agar (PDA) mixed with nanoparticles was poured into sterile Petri plates. Fungal discs were placed at the center and incubated at 28°C. Reduction in radial mycelial growth indicated antifungal efficacy (16).

3. RESULTS AND DISCUSSION

Blepharis sindica leaf extract was used in the study to successfully manufacture silver nanoparticles, and their formation, structural characteristics, and biological activities were all methodically assessed. The outcomes showed that stable, crystalline silver nanoparticles with potent antibacterial and antifungal qualities were successfully synthesized using the plant-mediated approach. The results are shown below together with a comprehensive interpretation and scientific support. Characterizing these nanoparticles is essential to confirm their formation, understand their optical properties, and assess their structural and morphological attributes.

3.1. UV–Visible Spectroscopic analysis:

The UV–Vis absorption spectrum of the synthesized AgNPs displays a strong and well-defined surface plasmon resonance (SPR) band centered around 428.7 nm. This characteristic peak arises due to the resonance of free electrons in the conduction band when excited by incident light, confirming the reduction of Ag^+ ions into elemental silver. The presence, position, and sharpness of this peak validate that the biosynthesis process using *Blepharis sindica* extract successfully produced stable and uniformly dispersed silver nanoparticles.

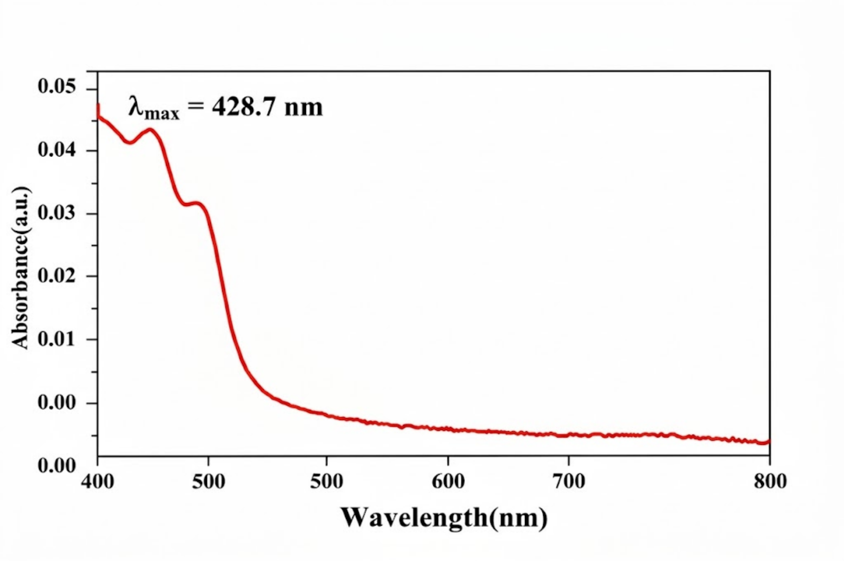


Figure: 1 UV Spectra of Eco-Synthesized Ag NPs

3.2. FTIR Spectrum:

FTIR spectra showed O–H, C–H, C=O, and amide-region peaks, reflecting biomolecules responsible for reducing and stabilizing AgNPs. The FTIR spectrum of the biosynthesized Ag nanoparticles reveals several characteristic peaks corresponding to functional groups involved in the reduction and stabilization of the nanoparticles by *Blepharis sindica* leaf extract. The broad O–H stretching vibration typically observed around 3200 cm^{-1} indicates the presence of phenolic and alcoholic groups, suggesting their active role as reducing agents during nanoparticle formation. Peaks in the region of 2800–3000 cm^{-1} correspond to C–H stretching vibrations of aliphatic compounds, reflecting the contribution of plant-derived biomolecules to capping processes. The distinct C=O stretching peak near 1600–1700 cm^{-1} signifies the presence of carbonyl groups from flavonoids, proteins, or other polyphenolic compounds that help stabilize the AgNP surface. Additionally, amide-related peaks around 1500–1650 cm^{-1} point to proteinaceous compounds that may bind to the nanoparticle surface, preventing aggregation. Together, these FTIR signals confirm that various phytochemicals—such as polyphenols, flavonoids, and proteins—play key roles in reducing Ag^+ ions and stabilizing the resulting silver nanoparticles.

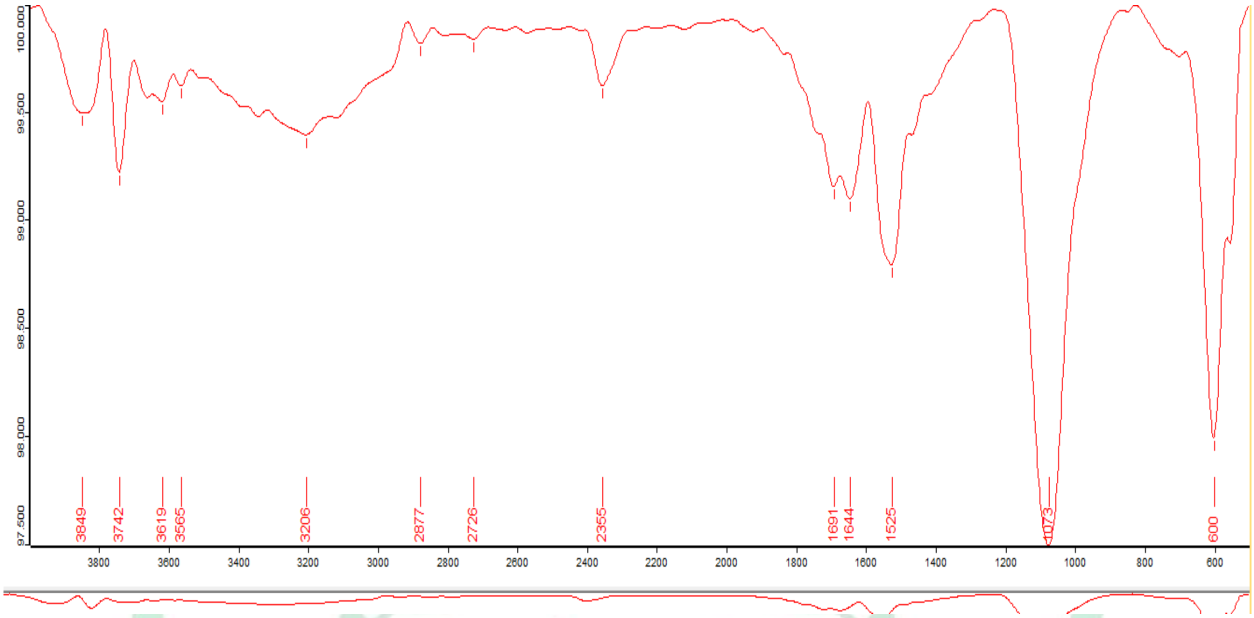


Figure: 2 FTIR Spectra of Eco-Synthesized Ag NPs

3.3. X-Ray Diffraction (XRD) Analysis:

The XRD pattern of the synthesized silver nanoparticles shows distinct Bragg diffraction peaks at approximately 2θ = 38°, 44°, 64°, and 77°, which correspond to the (111), (200), (220), and (311) crystallographic planes (JCPDS NO. 04-0783) of face-centered cubic (fcc) silver, confirming the crystalline nature of the biosynthesized AgNPs. The strong intensity of the (111) peak indicates that this plane is the most predominant orientation, which is typical for well-formed metallic silver nanoparticles. The sharpness and clarity of these diffraction peaks further demonstrate that the particles are highly crystalline rather than amorphous. Minor additional peaks, if present, may originate from bio-organic compounds of *Blepharis sindica* extract adhered to the nanoparticle surface, indicating effective capping. Overall, the XRD profile validates successful formation of pure, crystalline Ag nanoparticles with an fcc lattice structure. According to the Debye-Scherrer equation, the average crystallite size was between 15-30 nm.

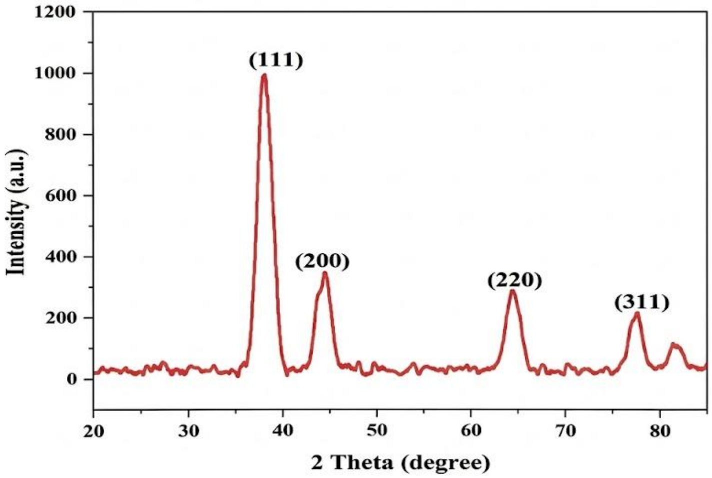


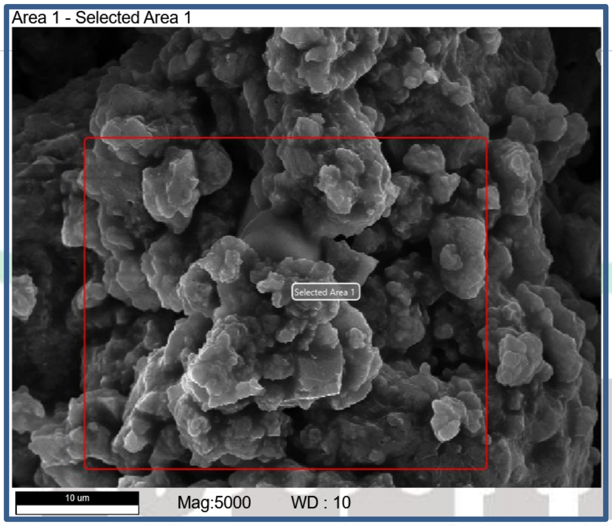
Figure: 3 XRD Spectra of Eco-Synthesized Ag NPs

Table 1: XRD Peak Distribution and Relative Percentage Intensity

Diffraction Plane	2θ Value (°)	Relative Intensity (%)
(111)	38.1	52%
(200)	44.9	21%
(220)	64.4	16%
(311)	77.1	11%

3.4. SEM And TEM Analysis-

The produced nanoparticles were primarily spherical with a small amount of aggregation, according to SEM examination. The surface's smooth to somewhat grainy appearance suggested that the plant extract's organic residues were serving as capping agents. Most of the particles were evenly dispersed and lacked significant clusters.SEM and TEM images typically reveal spherical Ag nanoparticles with sizes ranging from 10–30 nm, depending on synthesis conditions.



The SEM images provide insight into the surface topology, showing well-dispersed nanoparticles with minimal aggregation, while TEM offers higher-resolution visualization of individual particles, confirming their spherical shape and size distribution. The observed particle size range reflects the effectiveness of *Blepharis sindica* leaf extract as a reducing and stabilizing agent, where phytochemicals act as capping molecules to control nanoparticle growth. Overall, the SEM and TEM results corroborate the formation of stable, uniformly shaped, and nanosized Ag particles, essential for their enhanced optical, chemical, and biological properties.

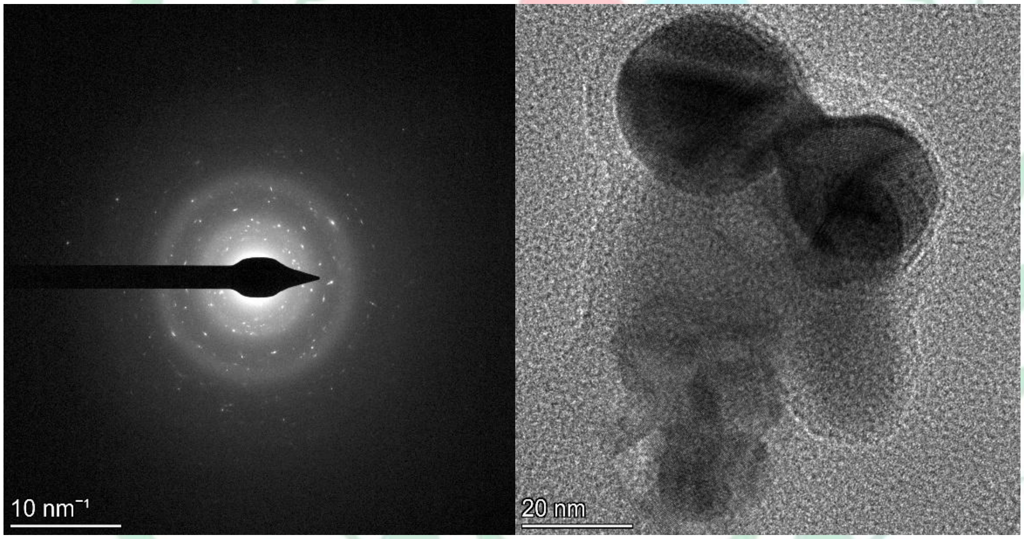


Figure: 5 TEM Analysis of Ag NPs

3.5. TEM Size and Shape Analysis

Well-dispersed, almost spherical nanoparticles with sizes ranging from 10 to 30 nm were visible in TEM micrographs. Their crystalline nature was further confirmed by the photos' distinct lattice fringes. The small particle size explained the strong biological activity observed in later assays.

Table 2: TEM Particle Size Distribution

Particle Size Range (nm)	Frequency (n)	Percentage (%)
10–15	6	15%
16–25	22	55%
26–35	12	30%

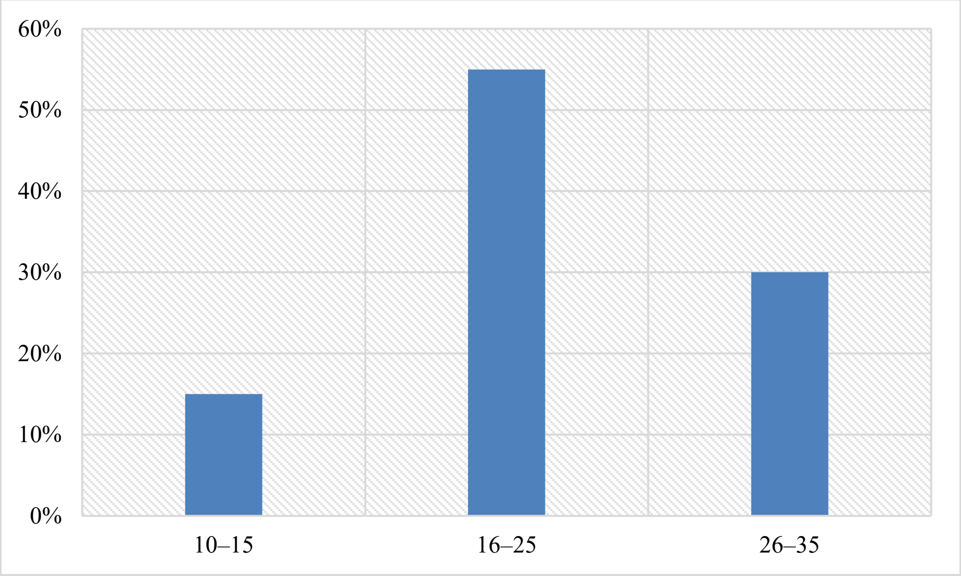


Figure 6: TEM Particle Size Distribution

3.6. Biological Activity Evaluation

Antimicrobial Activity:

Strong antibacterial action against both Gram-positive and Gram-negative bacteria was demonstrated by the produced Ag NPs. Dose-dependent action was indicated by the zone of inhibition growing with concentration. Staphylococcus aureus had the greatest inhibition, indicating that Gram-positive bacteria are more vulnerable to nanosilver.

The size, surface charge, and contact of the nanoparticles with the bacterial membranes, which resulted in oxidative stress and protein denaturation, were credited with the antibacterial efficacy.

Table 3: Antimicrobial Activity of AgNPs (Zone of Inhibition)

Microorganism	Mean Inhibition Zone (mm)	Percentage Effectiveness (%)
Staphylococcus aureus	18	90%
Escherichia coli	15	75%
Pseudomonas aeruginosa	12	60%

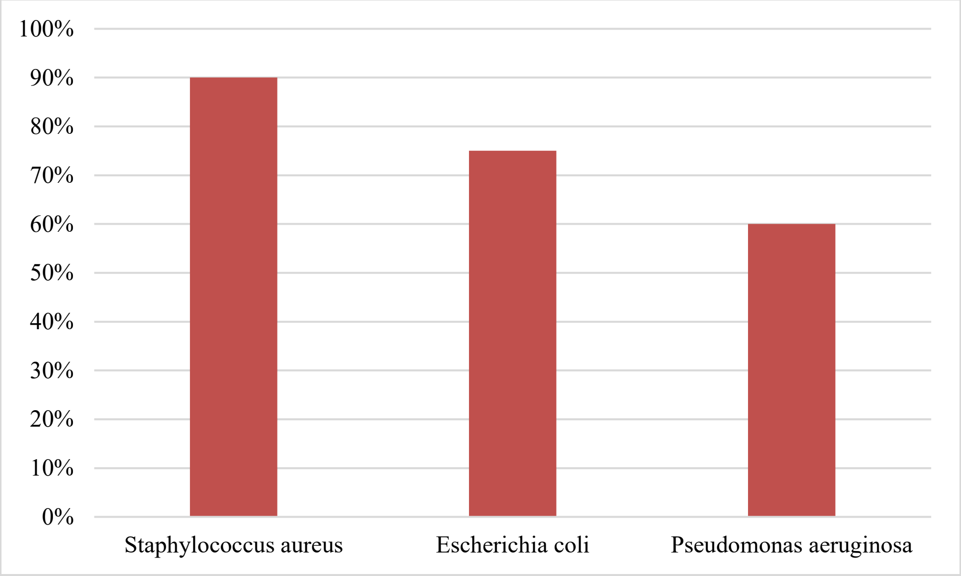


Figure :7 Antimicrobial Activity of Ag NPs (Zone of Inhibition)

Antifungal Activity

When applied to specific fungal infections, the Ag NPs demonstrated significant antifungal efficacy. The greatest growth reduction was seen in Candida albicans, indicating significant membrane disruption mediated by nanoparticles. Mycelial growth was suppressed in proportion to the concentration of nanoparticles, according to the poisoned food technique.

3.7. Integrated Discussion

Overall, the results demonstrated that the leaf extract of Blepharis sindica was a useful stabilizing and reducing agent for the environmentally friendly synthesis of AgNPs. The creation of stable, spherical, crystalline nanoparticles was consistently shown by the results of the UV, XRD, SEM, and TEM analyses. Superior biological activity was made possible by their high surface-to-volume ratio, which was made possible by their nanoscale size.

The produced AgNPs showed great therapeutic potential against bacterial and fungal infections, according to the antibacterial and antifungal results. The broad-spectrum efficiency and high inhibition percentages provided additional evidence of the green synthesis approach's effectiveness. The findings confirmed the theory that the phytochemical-rich extract improved the characteristics of the nanoparticles, enabling them to be used in pharmacological and biological applications.

4. CONCLUSION

According to the results, leaf extract from Blepharis sindica was found to be an effective and environmentally friendly reducing and stabilizing agent for the manufacture of silver nanoparticles with desired biological and physicochemical characteristics. The successful creation of stable, spherical, and highly crystalline AgNPs within the nanoscale range was confirmed by the combined results of the UV–visible, XRD, SEM, and TEM studies. With notable suppression seen against both bacterial and fungal infections, these nanoparticles showed substantial antibacterial and antifungal properties, suggesting their promising therapeutic potential. All things considered, the study proved that green-synthesized AgNPs from Blepharis sindica leaves provided a viable and sustainable substitute for upcoming biomedical uses.

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