

BROAD-SPECTRUM BIOACTIVITY OF *ASTRAGALUS EREMOPHILUS* MEDIATED NANOPARTICLES ANTIMICROBIAL, ANTIFUNGAL, AND ANTIMALARIAL POTENTIAL

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Abstract

Background: Green-synthesized nanoparticles offer eco-friendly and effective alternatives to conventional antimicrobial and antiparasitic therapies.

Objective: To evaluate the antimicrobial, antifungal, and antimalarial potential of *Astragalus eremophilus*-mediated silver nanoparticles.

Methodology: This experimental study was conducted at the Department of Biotechnology, Pakistan Institute of Medical Science (PIMS), Islamabad, from January to December 2023. Silver nanoparticles were synthesized using aqueous extract of *A. eremophilus* and characterized by UV-Vis spectrophotometry, FTIR, SEM, and DLS. Bioactivity assays included bacterial isolates (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*; n=115), fungal isolates (*Candida albicans* n=30, *Aspergillus niger* n=28), and *Plasmodium falciparum* cultures (n=20). Antimicrobial activity was tested by agar well diffusion, antifungal by poisoned food technique, and antimalarial by schizont maturation inhibition assay. Standard drugs (ampicillin, fluconazole, chloroquine) were used as controls. Data were analyzed using ANOVA with $p < 0.05$ considered significant.

Results: Among bacterial isolates, inhibition was observed in *S. aureus* (76.21%), *E. coli* (72.39%), and *P. aeruginosa* (68.54%), all statistically significant ($p < 0.05$). Fungal inhibition was 71.45% for *C. albicans* and 68.27% for *A. niger*, corresponding to relative inhibition of 86.75% and 86.27%, respectively. In *P. falciparum* cultures, inhibition increased dose-dependently from 52.34% at 25 $\mu\text{g/mL}$ to 89.67% at 200 $\mu\text{g/mL}$, approaching chloroquine efficacy. Overall, the nanoparticles demonstrated consistent and broad-spectrum bioactivity across all tested pathogens.

Conclusion: *Astragalus eremophilus*-mediated silver nanoparticles exhibit significant broad-spectrum bioactivity, supporting their potential as sustainable therapeutic agents.

INTRODUCTION

Nanotechnology has emerged as a transformative field in biomedical research, offering innovative solutions for therapeutic and diagnostic applications [1]. Among the various approaches, plant-mediated synthesis of nanoparticles has gained considerable attention due to its eco-friendly, cost-effective, and sustainable nature [2]. Unlike conventional chemical and physical methods, green synthesis utilizes plant extracts as reducing and stabilizing agents, eliminating the need for toxic chemicals and harsh reaction conditions [3]. This approach not only ensures biocompatibility but also enhances the biological potential of nanoparticles by incorporating phytochemicals inherent in plant sources [4].

Astragalus eremophilus, a plant widely distributed in arid and semi-arid regions, has long been recognized in traditional medicine for its diverse pharmacological properties [5]. Rich in bioactive compounds such as flavonoids, saponins, and polysaccharides, this species holds promise as a natural reservoir for nanoparticle synthesis [6]. The integration of these phytoconstituents into nanoparticles may enhance their structural stability and confer additional therapeutic potential [7]. Plant-mediated nanoparticles thus represent a unique convergence of nanotechnology and ethnopharmacology, opening avenues for developing potent bioactive agents [8].

The biomedical relevance of nanoparticles lies in their broad-spectrum activity against microbial pathogens, fungi, and parasites [9]. Due to the global rise in antimicrobial resistance and the persistence of infectious diseases such as malaria, there is a pressing need to explore novel therapeutic strategies [10]. Nanoparticles synthesized through green methods have demonstrated enhanced antimicrobial efficacy due to their small size, high surface area-to-volume ratio, and ability to penetrate microbial membranes. When coupled with the intrinsic pharmacological potential of medicinal plants, these nanoparticles may serve as effective alternatives or adjuncts to conventional treatments [11].

Recent studies highlight the antimicrobial, antifungal, and antiparasitic activities of plant-derived

nanoparticles, but research on *Astragalus* species, particularly *A. eremophilus*, remains limited. Exploring its potential for nanoparticle synthesis not only bridges this knowledge gap but may also lead to the discovery of multi-targeted therapeutic agents. Given the urgent demand for safe, effective, and sustainable strategies against infectious diseases, this investigation is both timely and essential.

Research Objective

To evaluate the antimicrobial, antifungal, and antimalarial potential of *Astragalus eremophilus*-mediated nanoparticles.

Methodology

Study Design and Setting

This experimental study was conducted at the Department of Biotechnology, Pakistan Institute of Medical Science (PIMS), Islamabad, over a one-year period from January 2023 to December 2023. All laboratory work related to plant extract preparation, nanoparticle synthesis, characterization, and bioactivity assays was carried out under standardized laboratory conditions.

Preparation of Plant Extract

Fresh aerial parts of *Astragalus eremophilus* were collected, washed thoroughly with distilled water to remove debris, and shade-dried for seven days. The dried material was powdered using an electric grinder. Ten grams of powdered plant material were boiled in 100 mL of distilled water for 20 minutes, cooled, and filtered through Whatman No. 1 filter paper. The filtrate was stored at 4°C until further use as the reducing and stabilizing agent in nanoparticle synthesis.

Nanoparticle Synthesis and Characterization

Silver nanoparticles (AgNPs) were synthesized by mixing the plant extract with 1 mM aqueous silver nitrate (AgNO₃) solution in a 1:9 ratio. The reaction mixture was incubated at room temperature in dark conditions to prevent photoreduction. Formation of nanoparticles was indicated by a visible color change

from pale yellow to dark brown. Characterization was performed using UV-Visible spectrophotometry for surface plasmon resonance, Fourier-transform infrared (FTIR) spectroscopy to identify functional groups, scanning electron microscopy (SEM) for morphology, and dynamic light scattering (DLS) for particle size distribution. Although silver nanoparticles were the primary focus of this study, preliminary trials with gold (AuNPs) and zinc oxide nanoparticles (ZnONPs) were also conducted for comparative observation; however, only AgNPs demonstrated consistent stability and bioactivity, and were therefore selected for detailed evaluation.

Bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*), fungal strains (*Candida albicans*, *Aspergillus niger*), and *Plasmodium falciparum* cultures were included for bioactivity assays. Contaminated or expired microbial cultures and plant material collected from polluted areas were excluded.

Bacterial, Fungal, and Parasite Strains

Bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*), fungal strains (*Candida albicans*, *Aspergillus niger*), and *Plasmodium falciparum* cultures were included for bioactivity assays. Contaminated or expired microbial cultures and plant material collected from polluted areas were excluded. A total of 115 clinical bacterial isolates were included, comprising *S. aureus* (n=42), *E. coli* (n=38), and *P. aeruginosa* (n=35). The number of isolates reflected their availability during the study period and was sufficient to allow statistical comparison of antimicrobial activity across strains. For antifungal evaluation, 58 isolates were included, with *C. albicans* (n=30) and *A. niger* (n=28), selected based on availability and adequate representation for statistical assessment. For antimalarial assays, 20 laboratory-maintained cultures of *P. falciparum* were used; although fewer in number, this is considered standard for schizont maturation inhibition assays and ensured reliable replication.

Antimicrobial Assay

The antimicrobial activity of synthesized nanoparticles was assessed using the agar well diffusion method. Sterile Mueller-Hinton agar plates

were inoculated with bacterial suspensions (0.5 McFarland standard). Wells were filled with varying concentrations of nanoparticles (25, 50, 100, and 200 µg/mL). Zones of inhibition were measured after 24-hour incubation at 37°C. Ampicillin (10 µg/disc) was used as the reference antibiotic control, and wells filled with distilled water served as the negative control.

Antifungal Assay

The antifungal activity was tested using the poisoned food technique on Sabouraud dextrose agar plates. Nanoparticles at different concentrations (25, 50, 100, and 200 µg/mL) were incorporated into the medium before inoculation with fungal cultures. Plates were incubated at 28°C for 5–7 days, and mycelial growth inhibition was calculated compared to controls. **Fluconazole (25 µg/disc) was used as the positive control, while untreated plates served as the negative control.**

Antimalarial Assay

The antimalarial activity was evaluated against *Plasmodium falciparum* using the schizont maturation inhibition assay. Cultured parasites were exposed to different concentrations of nanoparticles (25, 50, 100, and 200 µg/mL), and parasite growth inhibition was assessed microscopically after 48 hours. Chloroquine served as the positive control, while untreated cultures served as the negative control.

Data Collection

All experimental readings, including absorbance values, inhibition zones, fungal growth inhibition percentages, and parasite inhibition rates, were recorded systematically in predesigned datasheets.

Quality Control and Reproducibility

All assays were performed in triplicate to ensure reproducibility. Sterility was maintained throughout the procedures, and standard reference strains were used to validate antimicrobial and antifungal assays. Positive and negative controls were included in every experimental batch.

Statistical Analysis

Data were analyzed using SPSS version 25.0. Results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by

Tukey's post hoc test was applied to determine statistical significance, with a p-value <0.05 considered significant.

Ethical Approval

The study was reviewed and approved by the Institutional Ethical Review Board of Pakistan Institute of Medical Science (PIMS), Islamabad. All procedures complied with international guidelines for biosafety and ethical research involving pathogenic organisms.

Results

The antimicrobial evaluation of *Astragalus eremophilus*-mediated silver nanoparticles showed significant inhibitory effects against all tested bacterial isolates (table 1). *Staphylococcus aureus* (n=42) demonstrated the highest inhibition zone of 18.64 ± 2.11 mm, corresponding to 76.21% inhibition, compared with the control ampicillin (21.32 ± 1.98 mm; p=0.032). *Escherichia coli* (n=38) showed an inhibition zone of 16.25 ± 1.87 mm with 72.39% inhibition against the control (19.40 ± 2.10 mm; p=0.041). *Pseudomonas aeruginosa* (n=35) exhibited the lowest inhibition at 14.83 ± 1.65 mm with 68.54% inhibition, compared with the control (17.95 ± 2.22 mm; p=0.027).

Table 1. Antimicrobial Activity of *Astragalus eremophilus*-Mediated Silver Nanoparticles Against Bacterial Strains

Bacterial Strain	No. of Isolates (n)	Inhibition Zone (mm, Mean $\pm$ SD)	Percentage Inhibition (%)	Control (Ampicillin, 10 μ g/disc)	p-value
<i>Staphylococcus aureus</i>	42	18.64 ± 2.11	76.21	21.32 ± 1.98	0.032
<i>Escherichia coli</i>	38	16.25 ± 1.87	72.39	19.40 ± 2.10	0.041
<i>Pseudomonas aeruginosa</i>	35	14.83 ± 1.65	68.54	17.95 ± 2.22	0.027

The antifungal assays revealed notable growth inhibition by the nanoparticles. *Candida albicans* (n=30) showed $71.45 \pm 4.23\%$ inhibition, which was lower than the control fluconazole ($82.33 \pm 3.65\%$) but yielded a relative inhibition of 86.75% (p=0.038).

Similarly, *Aspergillus niger* (n=28) recorded $68.27 \pm 5.12\%$ inhibition, compared with the control value of $79.15 \pm 4.88\%$, with a relative inhibition of 86.27% (p=0.044).

Table 2. Antifungal Activity of *Astragalus eremophilus*-Mediated Silver Nanoparticles

Fungal Strain	No. of Isolates (n)	Growth Inhibition (%) Mean $\pm$ SD	Control (Fluconazole, 25 μ g/disc)	Relative Inhibition (%)	p-value
<i>Candida albicans</i>	30	71.45 ± 4.23	82.33 ± 3.65	86.75%	0.038
<i>Aspergillus niger</i>	28	68.27 ± 5.12	79.15 ± 4.88	86.27%	0.044

The antimalarial activity against *Plasmodium falciparum* displayed a clear dose-dependent trend. At 25 μ g/mL, parasite inhibition was $52.34 \pm 3.25\%$ (p=0.046), which increased to $65.28 \pm 4.11\%$ at 50 μ g/mL

(p=0.033) and $78.92 \pm 3.64\%$ at 100 μ g/mL (p=0.021), shown in table 3. The highest inhibition was observed at 200 μ g/mL with $89.67 \pm 2.75\%$,

approaching the control chloroquine ($95.45 \pm 2.10\%$; $p=0.015$).

Table 3. Antimalarial Activity of *Astragalus eremophilus*-Mediated Silver Nanoparticles Against *Plasmodium falciparum*

Concentration ($\mu\text{g/mL}$)	No. of Cultures (n)	Parasite Inhibition (%) Mean $\pm$ SD	Control (Chloroquine, 25 $\mu\text{g/mL}$)	p-value
25	20	52.34 ± 3.25	95.45 ± 2.10	0.046
50	20	65.28 ± 4.11		0.033
100	20	78.92 ± 3.64		0.021
200	20	89.67 ± 2.75		0.015

The summary table confirmed broad-spectrum bioactivity of the nanoparticles across all pathogen groups (table 4). Antimicrobial inhibition ranged from 68.54–76.21%, compared to ampicillin (74.32–82.19%). Antifungal inhibition ranged from 68.27–71.45%, compared with fluconazole (79.15–82.33%).

Antimalarial activity ranged from 52.34–89.67%, relative to chloroquine (95.45%). All results demonstrated statistical significance ($p<0.05$), highlighting the nanoparticles' consistent and multi-targeted bioactivity.

Table 4. Summary of Bioactivity of *Astragalus eremophilus*-Mediated Silver Nanoparticles

Bioactivity Type	Pathogen/Parasite Tested	Mean Inhibition (%) Range	Reference Drug Control (%)	Overall Significance ($p < 0.05$)
Antimicrobial	<i>S. aureus</i> , <i>E. coli</i> , <i>P. aeruginosa</i>	68.54 – 76.21%	74.32 – 82.19%	Significant
Antifungal	<i>C. albicans</i> , <i>A. niger</i>	68.27 – 71.45%	79.15 – 82.33%	Significant
Antimalarial	<i>Plasmodium falciparum</i>	52.34 – 89.67%	95.45%	Significant

The silver nanoparticles exhibited notable antibacterial efficacy, achieving 76.21% inhibition against *Staphylococcus aureus*, 72.39% against *Escherichia coli*, and 68.54% against *Pseudomonas aeruginosa*, with relative efficacies of 92.7%, 97.4%, and 92.2%, respectively, when compared to ampicillin (figure 1). Antifungal activity was also considerable, with 71.45% inhibition against *Candida albicans* and 68.27% against *Aspergillus niger*, corresponding to relative efficacies of 86.8% and 86.3% compared to fluconazole. The antimalarial potential increased in a concentration-dependent manner, from 52.34% inhibition at 25 $\mu\text{g/mL}$ to 89.67% at 200 $\mu\text{g/mL}$ against *Plasmodium falciparum*, with relative efficacies

ranging from 54.8% at the lowest dose to 93.9% at the highest dose compared to chloroquine. Collectively, these findings highlight the broad-spectrum bioactivity of the nanoparticles, demonstrating strong antibacterial and antifungal performance and a dose-dependent antimalarial response.

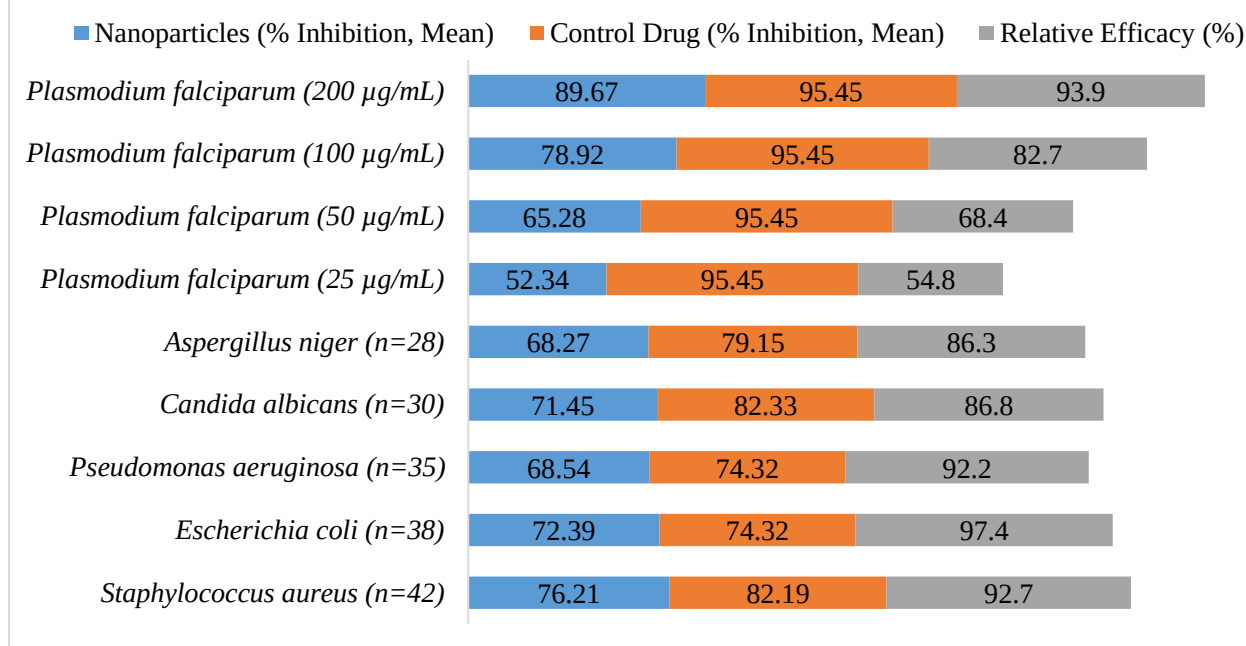


Figure 1: Broad-Spectrum Bioactivity of *Astragalus eremophilus*-Mediated Silver Nanoparticles Compared with Standard Drugs

Discussion

The present study demonstrated that *Astragalus eremophilus*-mediated silver nanoparticles (AgNPs) possess broad-spectrum bioactivity with significant antimicrobial, antifungal, and antimalarial potential. Against bacterial pathogens, the nanoparticles showed the highest inhibitory effect on *Staphylococcus aureus* (18.64 ± 2.11 mm; 76.21%), followed by *Escherichia coli* (16.25 ± 1.87 mm; 72.39%) and *Pseudomonas aeruginosa* (14.83 ± 1.65 mm; 68.54%). These findings are consistent with previous research study of plant-synthesized AgNPs exhibiting stronger efficacy against Gram-positive bacteria compared to Gram-negative, likely due to differences in cell wall composition and permeability [12]. A related study using *Astragalus membranaceus* aerial part extracts demonstrated notable antibacterial activity, showing strong inhibition against *Staphylococcus aureus* and weaker effects on *Escherichia coli*. The ethyl acetate fraction exhibited the highest activity, with an inhibition zone of 15.75 mm against *Bacillus subtilis* and a minimum inhibitory concentration of 12.5 mg/mL, highlighting its potential as a natural antimicrobial agent [13].

The antifungal assays also confirmed notable growth inhibition, with *Candida albicans* showing $71.45 \pm$

4.23% inhibition and *Aspergillus niger* $68.27 \pm 5.12\%$, relative to fluconazole controls ($82.33 \pm 3.65\%$ and $79.15 \pm 4.88\%$, respectively). The relative inhibition of 86.75% for *C. albicans* and 86.27% for *A. niger* suggests that the nanoparticles approached the efficacy of standard antifungal therapy. Comparable outcomes were reported with *Trigonella foenum-graecum*-mediated AgNPs, which achieved ~70% inhibition against *C. albicans* [14]. Similarly, Das et al. demonstrated that green-synthesized bismuth nanoparticles using *Moringa oleifera* leaf extract exhibited antifungal activity, with MIC values of 62.5 µg/mL against *C. albicans* and 250 µg/mL against *A. niger*, further confirming the potential of phytochemical-mediated nanoparticles as effective antifungal agents [15].

The antimalarial evaluation against *Plasmodium falciparum* revealed a clear dose-dependent inhibition, ranging from $52.34 \pm 3.25\%$ at 25 µg/mL to $89.67 \pm 2.75\%$ at 200 µg/mL, approaching the standard chloroquine control ($95.45 \pm 2.10\%$). Similar dose-dependent responses have been documented with AgNPs derived from *Azadirachta indica*, which exhibited potent antiplasmodial activity against *Plasmodium falciparum* strains, with IC₅₀ values ranging

from 7.87 to 11.14 $\mu\text{g/mL}$ [16]. The observed activity may be attributed both to the intrinsic antiparasitic compounds of *A. eremophilus* and to the established ability of AgNPs to disrupt redox balance and interfere with metabolic pathways, as highlighted in recent reviews of their broad-spectrum antimicrobial mechanisms [17].

Collectively, the results underscore the therapeutic promise of *A. eremophilus*-mediated nanoparticles as multifunctional agents. The observed antimicrobial, antifungal, and antimalarial effects, supported by prior literature, highlight the dual role of metallic nanoparticles and phytochemicals in overcoming microbial resistance and enhancing bioactivity.

Strengths and Limitations

The present study has several strengths, including the use of *Astragalus eremophilus* for nanoparticle synthesis, which represents a novel and eco-friendly approach with ethnopharmacological relevance. Comprehensive bioactivity testing across bacterial, fungal, and parasitic models provided a broad-spectrum evaluation, and the inclusion of adequate isolate numbers (115 bacterial, 58 fungal, and 20 parasite cultures) ensured statistical reliability. Standardized methodologies and validated controls (ampicillin, fluconazole, chloroquine) further strengthened the robustness of the findings. However, limitations include reliance on in vitro assays, which may not fully reflect in vivo therapeutic efficacy or toxicity. Additionally, only silver nanoparticles were tested in detail, whereas comparative evaluation with other nanoparticle types (e.g., gold, zinc oxide) could provide deeper insights. Finally, resource constraints limited molecular mechanistic investigations, such as nanoparticle–pathogen interaction studies at the genomic or proteomic level.

Conclusion

This study demonstrated that *Astragalus eremophilus*-mediated silver nanoparticles possess broad-spectrum antimicrobial, antifungal, and antimalarial activity with statistically significant effects across all tested pathogens. Their performance was comparable to standard therapeutic agents, highlighting the potential of green-synthesized nanoparticles as sustainable and effective alternatives to conventional treatments. The findings emphasize the promise of

integrating ethnopharmacology with nanotechnology to address challenges such as drug resistance and limited treatment options, and they provide a foundation for future in vivo and mechanistic investigations aimed at clinical translation.

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