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Eco-Friendly Synthesis of Silver Nanoparticles from Trans-Himalayan Herbs: A Comparative Study of Biological Activities

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ABSTRACT

The current study aims to synthesise silver nanoparticles (AgNPs) using three medicinal plants-*Inula racemosa*, *Rhodiola imbricata*, and *Ephedra gerardiana*-native to the Trans-Himalayan region. These plants were specifically selected due to their well-documented ethnomedicinal uses and rich phytochemical profiles, particularly in phenolic compounds. The study aims to harness their bioactive potential for the development of eco-friendly, cost-effective nanomaterials with therapeutic applications. Synthesised AgNPs were characterised via UV–VIS spectrophotometer, SEM, FTIR, DLS, TEM and EDX analysis. The absorption peaks at 410 nm (*I. racemosa*), 440 nm (*R. imbricata*), and 420 nm (*E. gerardiana*), confirming nanoparticle formation via surface plasmon resonance. Phytochemical analysis highlighted phenolics as key agents in nanoparticle reduction and capping. Although the AgNPs showed lower antioxidant activity than the crude extracts but exhibited superior antibacterial efficacy against multidrug-resistant bacterial strains, outperforming the standard antibiotic Ampicillin. Cytotoxicity evaluation against MCF-7 breast cancer cells revealed that *I. racemosa* root extract and its AgNPs had the highest anticancer activity, with IC_{50} values of 64.93 µg/mL and 55.88 µg/mL, respectively. This research proposes a sustainable approach to develop alternative antibacterial and anticancer agents, potentially addressing the rising global challenge of antibiotic resistance and cancer burden through plant-based nanotechnology.

Keywords: Medicinal plants; Green synthesis; Antioxidant; Antibacterial agents; Cytotoxicity

1. INTRODUCTION

The global burden of bacterial infections and cancer poses an ongoing challenge to public health. Although antibiotics are essential for treating bacterial infections, their excessive use has contributed to the concerning emergence of antibiotic-resistant strains such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, among others[1-2]. Concurrently, cancer remains a leading cause of death, with breast cancer ranking as the most diagnosed cancer globally in 2020, affecting 2.3 million women and claiming 6,70,000 lives[3-5]. These realities highlight the urgent need for alternative and effective therapeutic strategies.

In recent years, metal nanoparticles particularly silver nanoparticles (AgNPs) have gained prominence due to their broad-spectrum antimicrobial and anticancer activities. Their small size and large surface-area-to-volume ratio enable enhanced interaction with biological targets, offering superior efficacy over conventional therapies[6-7]. Traditional physical and chemical nanoparticle synthesis methods, however, are often costly, energy-intensive, and environmentally hazardous. In contrast, green synthesis especially plant-mediated biogenic routes offers an eco-friendly, cost-effective alternative that leverages the phytochemical richness of medicinal plants to reduce metal ions and stabilize nanoparticles[8-9].

While several plants have been explored for green nanoparticle synthesis, the therapeutic flora of the trans-Himalayan region of Ladakh remains largely untapped. This ecologically unique region is home to potent medicinal species such as *Inula racemosa*, *Rhodiola imbricata*, and *Ephedra gerardiana*, traditionally used for treating respiratory, cardiovascular, inflammatory, and reproductive disorders. These plants possess a wealth of secondary metabolites, including flavonoids, phenols, and terpenoids, which not only reduce metal ions but also stabilize the nanoparticles by acting as natural capping agents[10-15].

In this context, the present study aims to harness these underexplored Himalayan medicinal plants for the green synthesis of AgNPs and evaluate their antibacterial activity against multidrug-resistant pathogens and cytotoxic effects against MCF-7 breast cancer cells. To our knowledge, no

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previous studies have reported a one-step bio-reduction of silver ions using these three endemic plants, contributing to the development of sustainable nanomedicine for combating antibiotic resistance and cancer.

2. MATERIALS AND METHODS

2.1 Plant Material Collection

The roots of *I. racemosa*, *R. imbricata*, and *E. gerardiana* were collected from Ladakh, India (Latitude: 34°09'54.14"N; Longitude: 77°35'2.47"E; Altitude: 2300–5000 m ASL). The plant was authenticated by a taxonomist, and voucher specimens were deposited at the institutional herbarium (Voucher no. MAP-038, MAP/M-3509, MAP/B-449) respectively. The plants parts were washed under tap water followed by rinsing with deionised water. The roots were shade-dried at room temperature and further stored at 4^0 C till further use.

2.2 Chemicals and Reagents

Qualigens (India) supplied the methyl alcohol (HPLC Grade), sodium carbonate, sodium hydroxide, and sodium nitrite. The following laboratory supplies: Folin-Ciocalteu (FC) reagent, sodium acetate buffer, 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH), aluminium chloride, 2, 4, 6-tripyridyl-s-triazine (TPTZ), Iron(III) chloride ($FeCl_3$) and various standards (Gallic acid, Trolox, Quercetin, Rutin trihydrate) were acquired from Sigma Aldrich Pvt. Ltd. (Switzerland. We procured HCl from Rankem (India). Silver nitrate ($AgNO_3$), the precursor of silver, was acquired from Merck, India. The MCF-7 and L929 cell lines were acquired from NCCS located in Pune, India. The water from Millipore water purifier system (Merck, USA) was utilised for the several analyses.

2.3 Extraction of Plant Material

The plant extracts were prepared via method adopted by Patil *et al.,* with slight modifications[16]. 5 g of each powdered sample (grinded using mechanical grinder) was taken in 250 mL Erlenmeyer flask, and 50 mL of Milli-Q water (1:10 w/v ratio) was added. The mixture was sonicated and heated at 60 °C for 2 hours in an ultrasonic bath (REMI, India), then centrifuged at 8000 rpm for 10 minutes. The supernatant was filtered using Whatman No.1 filter paper (125 mm), and the clear extract was stored at 4 °C for further use.

2.3.1 Biosynthesis of Silver Nanoparticles (AGNPS)

To prepare silver nanoparticles, 5 mL of the respective plant extract was added to 45 mL of a 1 mM silver nitrate solution in a 100 mL conical flask, maintaining a 1:9 (v/v) ratio. The reaction mixture was stirred continuously on a magnetic stirrer at 60 ± 2 °C and protected from light using aluminium foil. The progression of the reaction was preliminarily monitored through colour change and further confirmed using UV-VIS spectrophotometry[16-17].

2.4 Characterisaion

2.4.1 UV-VIS Spectrophotometer Analysis

The formation of AgNPs was analysed using a UV–VIS spectrophotometer (Spectramax i3x Multimode Microplate Reader, Molecular Devices, USA) in the range of 230–800 nm at a resolution of 1 nm.

2.4.2 DLS AND ZETA Potential Analysis

Hydrodynamic diameter and surface charge (ζ-potential) of the synthesised AgNPs were measured using a DLS instrument (NanoBrook Omni, Brookhaven Instruments, UK) after appropriate dilution in deionised water.

2.4.3 SEM, TEM AND Energy-Dispersive X-ray Analysis(EDX)

The morphology and elemental composition of AgNPs were assessed using SEM (EVO MA 15, Carl Zeiss, UK) at an accelerating voltage of 20 kV, integrated with Energy-Dispersive X-ray (EDX) analysis. For higher resolution imaging and particle size confirmation, TEM was performed using a JEM 2100 PLUS instrument (JEOL, Japan). Particle size distribution was further evaluated using ImageJ software.

2.5 Phytochemical Analysis

2.5.1 Total Phenolic Content

Polyphenolic content was estimated with FC reagent test with specific modifications[18].The outcomes were conveyed in micrograms of Gallic Acid Equivalents (GAE) per millilitre of aqueous plant extract.

2.5.2 Total Flavonoids Content

Aluminium chloride method was utilised to determine the flavonoid content with certain modifications.[19] The data was displayed as micrograms of Rutin trihydrate Equivalent (RE) per millilitre of aqueous plant extract.

2.6 Antioxidant Activity

2.6.1 DPPH Assay

The antioxidant potential of extracts of selected plants and biogenic synthesised AgNPs was assessed using the DPPH assay with slight changes[20] The Scavenging Activity of radical (SA) was determined using the formula:

$$SA(\%) = \frac{(Ab_c - Ab_s)}{Ab_s} \times 100$$

Where, Ab_c: control absorbance(DPPH + methanol) Ab_s: absorbance of sample (DPPH+ standard/sample)

2.6.2 Frap Assay

The ability of antioxidants presents in plant extracts and silver nanoparticles to reduce Fe^{+3} to Fe^{+2} were estimated by FRAP assay[21]. The outcomes were displayed in micrograms of Trolox Equivalent (TE) per millilitre of aqueous plant extract.

2.7 Antibacterial Assay

The antibacterial efficacy of aqueous plant extracts as well as their derived AgNPs, was assessed via well-diffusion method[22] against *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, and *K. pneumoniae* ATCC 700603.

2.8 In-Vitro Cytotoxic Activities of Synthesised AgNPs

The cytotoxicity of plant extracts and their mediated AgNPs on L-929 (Normal human Fibroblast) and MCF-7 cancer cells were assessed through the *in-vitro* MTT (4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay[23]. The percentage cytotoxicity was determined using the mathematical formula:

$$Percentage Inhibition = \frac{\lfloor Ab_c - Ab_s \rfloor}{Ab_c} \times 100$$

Where, Ab_c= absorption of control, Ab_s= absorption of sample

3. RESULTS AND DISCUSSION

3.1 UV–Vis Spectral Analysis

The successful synthesis of silver nanoparticles (AgNPs) was initially confirmed by a distinct colour change in the reaction mixtures containing aqueous extracts of *I. racemosa*, *R. imbricata*, and *E. gerardiana* with 1 mM $AgNO_3$. The transformation from transparent to reddish-brown indicated the formation of AgNPs, attributed to the excitation of Surface Plasmon Resonance (SPR). Such colour change has been consistently reported in earlier works on green-synthesised AgNPs[24].

UV–VIS spectroscopy further validated nanoparticle formation. The characteristic SPR absorption peaks were recorded at 410 nm (*I. racemosa*), 440 nm (*R. imbricata*), and 420 nm (*E. gerardiana*) Fig. 1. These values fall within the expected range (400–450 nm) for spherical AgNPs and correspond well with reported literature[24], confirming successful biosynthesis.

3.2 Particle Analysis and Determination of Zeta Potential

Dynamic light scattering results showed average hydrodynamic diameters of 61.72 nm (*I. racemosa*), 64.11 nm (*R. imbricata*), and 63.97 nm (*E. gerardiana*) with PDI values indicating moderate monodispersity (Fig. 2). Zeta potential analysis showed values of −27.98 mV, −19.74 mV, and −25.05 mV, respectively, suggesting negative surface charge imparted by capping phytoconstituents and confirming colloidal stability. These findings are consistent with previously reported green-synthesised AgNPs[25].

3.3 TEM and EDAX Analysis

The SEM analysis was done at maximum resolution of SEM 1000 kX depicting the dimensions of the particles are very small less than 100 nm. The images obtained were not clear and blurred. Therefore, HR-TEM analysis of the samples was done. Herein, the TEM micrographs depicted the spherical shape of the formulated AgNPs utilising Inula racemosa, Rhodiola imbricata and Ephedera gerardiana plant extracts ranging from 4.34 nm to 9.41 nm with mean dia 6.87±1.13 nm, 7.38 nm to 46.40 nm with mean dia 20.26 ± 10.9 nm and 10.07 nm to 34.57 nm with mean dia 21.91±5.16 nm respectively Fig. 3. Notably, the sizes determined by TEM analysis were smaller than those obtained via DLS analysis. From TEM images it can be clearly seen that root extract of Inula racemosa resulted in uniform monodisperse nanoparticle's having mean dia < 10 nm as compared to AgNPs synthesised utilising Rhodiola imbricata and Ephedera gerardiana plant extract.

The presence of silver (Ag) was verified by EDX analysis, which showed an absorption peak at 3 keV. Inula racemosa-mediated synthesised nanoparticles exhibited atomic percentages of 17.5 % C, 12.9 % O, 5.8 % Cl, and 63.7 % Ag. Similarly, Rhodiola imbricata-mediated synthesised AgNPs comprised 30.4 % C, 16.7 % O, 7.6 % Cl, and 45.2 % Ag. Meanwhile, Ephedera gerardiana-mediated synthesised nanoparticles consisted of 32.9 % C, 9.6 % O, 8.9 % Cl, and 48.6 % Ag (Fig. 3). EDX analysis not only confirmed the presence of silver, but also showed the encapsulation of secondary metabolites on the surface of the nanoparticles[26].

3.4 PHYTOCHEMICAL ANALYSIS

3.4.1 Total Phenolic Content(TPC)

The TPC values were notably higher in the crude aqueous plant extracts *I. racemosa* (151.81 ± 1.33 µg GAE/mL), *R. imbricata* (249.08 ± 3.24 µg GAE/mL), and *E. gerardiana* (343.77 ± 1.39 µg GAE/mL) than

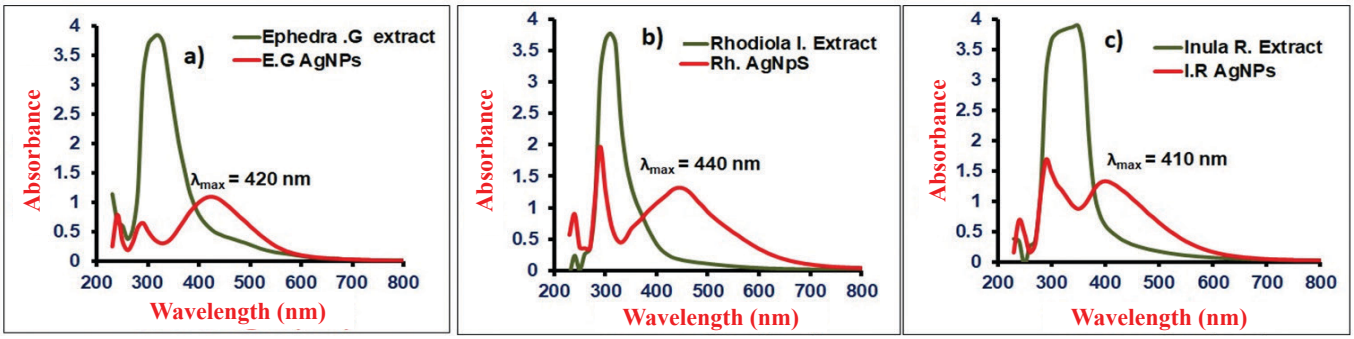

Figure 1. UV-VIS spectra of plant extract I. racemosa (a), R. imbricata (b) and E. gerardiana (c) as well as their derived AgNPs.

in their respective AgNPs as shown in Table 1. These findings align with the observations reported in the literature[27]. A decrease in Total Phenolic Content (TPC) in the AgNP samples implies that phenolic constituents from the extracts actively aid in the reduction of silver ions and contribute to nanoparticle stabilisation and capping.

3.4.2 Total Flavanoids Content(TFC)

Flavonoid content was quantified through the aluminium chloride colorimetric method followed a similar decreasing trend from extract to AgNPs. This supports their key role in electron donation during nanoparticle synthesis. Similar outcomes were reported in studies involving Himalayan herbs[27].

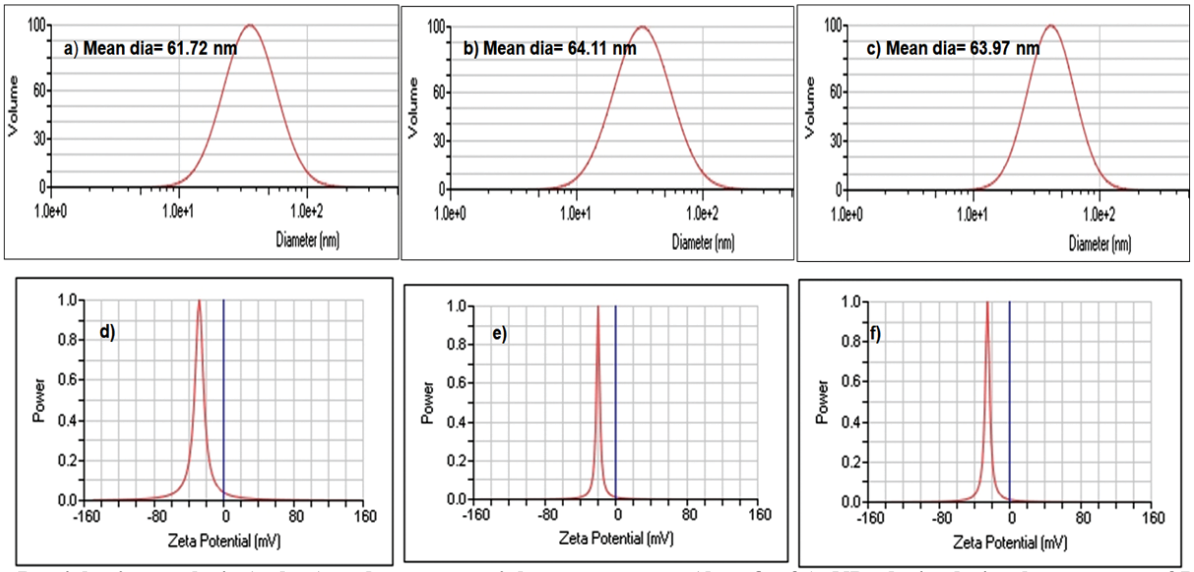

Figure 2. Particle size analysis (a, b, c) and zeta potential measurements (d, e, f) of AgNPs derived via plant extract of I. racemosa, R. imbricata and E. gerardiana respectively.

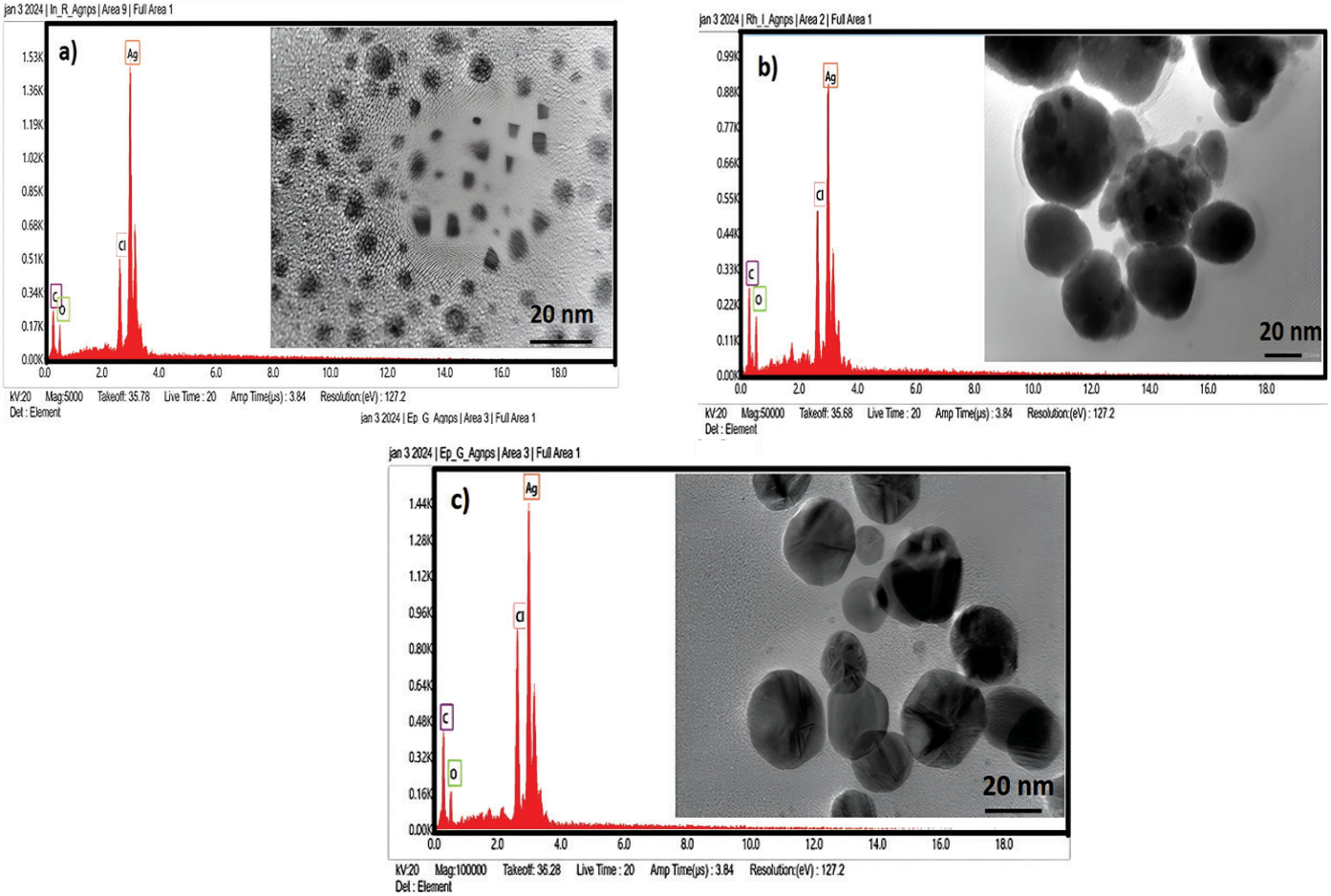

Figure 3. EDX analysis and HR-TEM micrograph of AgNPs synthesised via I. racemosa (a), R. imbricata (b) and E. gerardiana (c) plant extracts.

Table 1. TPC, TFC and antioxidant activity (via DPPH and FRAP assays) of plant extracts and their derived AgNPs

Sample	Plant extract (Mean ± SD)	AgNPs (Mean ± SD)
Total phenolic content (TPC) (µg GAE/mL aq. PE)		
Inula racemosa	151.81 ± 1.33	37.28 ± 0.98
Rhodiola imbricata	249.08 ± 3.24	16.89± 0.31
Ephedera gerardiana	343.77 ± 1.39	76.68 ± 3.15
Total flavanoid content (TFC) (mg RE/mL aq. PE)		
Inula racemosa	357.4 ± 1.65	72.46 ± 1.84
Rhodiola imbricata	193.07 ± 1.65	48.07 ± 0.71
Ephedera gerardiana	774.65 ± 3.89	173.57± 2.83
DPPH (free radical scavenging activity %)		
Inula racemosa	93.47 ± 0.093	41.06 ± 0.31
Rhodiola imbricata	92.62 ± 0.026	7.61± 0.81
Ephedera gerardiana	92.94 ± 0.093	57.91 ± 0.10
FRAP (µg TE /mL aq. plant extract)		
Inula racemosa	502.1 ± 3.31	105.64± 4.19
Rhodiola imbricata	156.25 ± 4.91	70.0 ± 2.72
Ephedera gerardiana	251.39 ± 1.44	210.75± 6.85

3.5 Antioxdant Activity

The antioxidant assay demonstrated that all three plant extracts exhibited significantly higher antioxidant activity than their corresponding AgNPs, as presented in Table 1. This enhanced activity aligns with the higher TPC and TFC observed in the extracts. These findings are consistent with the observations reported in the literature[28], which says that the antioxidant potential of plant-mediated AgNPs is reduced due to the consumption of phenolic compounds during the nanoparticle synthesis process.

3.6 Biological Applications

3.6.1 Antibacterial Activity

Antibacterial testing was conducted using the agar well diffusion method against both Gram-positive and Gram-negative pathogens. While plant extracts showed negligible inhibition, all AgNPs displayed significant antimicrobial zones, surpassing the activity of standard Ampicillin Fig. 4. The enhanced efficacy is likely due to the synergistic effects of silver nanoparticles small size and bioactive surface-bound phytochemicals, as previously demonstrated in the reported studies[29-30].

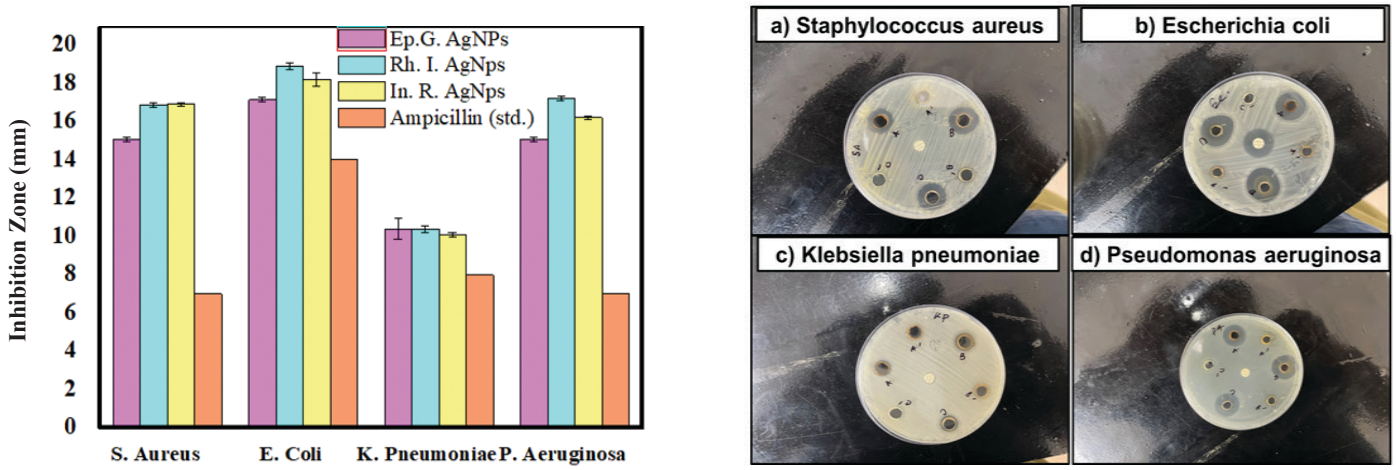

Figure 4. Antimicrobial activity of green synthesised AgNPs against a) S. aureus, b) E. coli, c) K. pneumoniae and d) P. aeruginosa. A-Ephedera AgNPs, A'- ephedera extract, B-rhodiola AgNPs, B'-rhodiola extract, C-inula AgNPs, C'-inula extract.

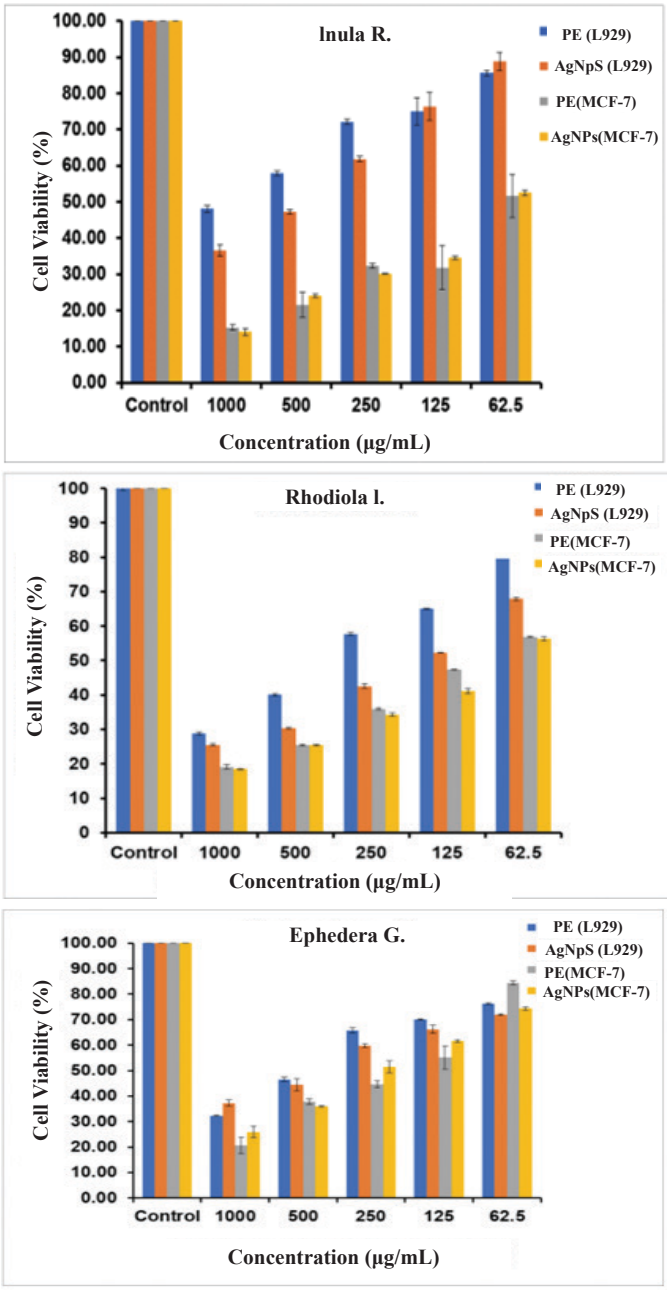

Figure 5. Cytotoxic evaluation of plant extracts and their derived AgNPs against normal L929 and MCF-7 breast cancer cell lines.

One of inhibition data highlighted that AgNPs synthesised using *R. imbricata* were most effective against *E. coli*, while those from *I. racemosa* showed broad-spectrum activity. The antimicrobial activity may be attributed to membrane disruption, ROS generation, and interference with bacterial enzymes mechanisms well-supported in literature[31].

3.6.2 Cytotoxic Studies

The MTT assay results showed that AgNPs derived from *I. racemosa* had the strongest cytotoxic effect on MCF-7 cells (IC_{50} = 55.88 µg/mL) while exhibiting no toxicity toward L929 cells (IC_{50} > 160 µg/mL) Fig. 5. This selectivity is promising and aligns with studies reported in the literature[32] which suggest size-dependent cytotoxicity and enhanced internalisation of smaller AgNPs in cancer cells. The observed bioactivity may result from the synergistic effects of metallic silver and plant-derived bioactives, enhancing ROS-mediated apoptosis. However, further more thorough investigation is necessary to fully understand the underlying mechanism behind the anticancer activity of the synthesised silver nanoparticles.

4. CONCLUSION

In this study, an eco-friendly and sustainable approach was employed to synthesize silver nanoparticles (AgNPs) using extracts of Trans-Himalayan medicinal plants *Inula racemosa*, *Rhodiola imbricata*, and *Ephedra gerardiana*. The resulting AgNPs were spherical, stable, and predominantly below 20 nm in size. Among the three, *I. racemosa* root extract was the most effective in reducing silver ions, yielding highly stable nanoparticles. The observed reduction in TPC and TFC in AgNPs compared to their respective plant extracts highlights the critical role of these phytochemicals in the reduction and stabilisation processes. This was further corroborated by antioxidant assays, where the plant extracts demonstrated stronger radical-scavenging activity than their corresponding AgNPs.

Importantly, the AgNPs produced through biosynthesis showed superior antibacterial potential compared to Ampicillin, effectively targeting both Gram-positive and Gram-negative bacteria. Notably, AgNPs derived from *I. racemosa* also displayed selective cytotoxicity against MCF-7 breast cancer cells while being non-toxic to normal fibroblast (L929) cells, underscoring their potential for targeted anticancer therapy.

Beyond antibacterial and anticancer applications, green-synthesised AgNPs have shown promise in diverse biomedical fields, including wound healing, anti-inflammatory treatment, drug delivery, and diagnostic biosensing. While not directly explored in the current study, these broader applications present compelling avenues for future research. Our findings provide a foundation for advancing the translational potential of plant-mediated AgNPs, particularly those synthesised using *I. racemosa*, in next-generation pharmaceutical and therapeutic developments.

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