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Green synthesis of silver nanoparticles using aqueous leaf extract of *Geranium nepalense* and their antimicrobial activities

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Abstract: The present study highlighted the green synthesis of silver nanoparticles (Ag NPs) using *Geranium nepalense* leaf extract. The formation of Ag NPs was monitored through UV-Visible spectroscopy, which exhibited a characteristic surface plasmon resonance (SPR) band at 410 nm. X-ray diffraction (XRD) analysis revealed a face-centred cubic (FCC) crystalline structure, while transmission electron microscopy (TEM) showed that the nanoparticles were predominantly spherical with an average size of 12 nm. Fourier-transform infrared (FTIR) spectroscopy confirmed the presence of phytochemicals involved in the reduction and stabilization of the nanoparticles. The biosynthesized Ag NPs demonstrated significant antibacterial activity against various bacterial strains and moderate antifungal activity against selected fungal pathogens. These findings suggested that *G. nepalense* mediated synthesized silver nanoparticles possess promising antimicrobial properties and hold potential for application in biomedical and pharmaceutical fields.

Keyword: Silver nanoparticles • Green synthesis • Antimicrobial activity.

Introduction

Geranium nepalense (family: Geraniaceae), commonly known as Phori or Syunli, is a perennial, hairy herb characterized by creeping rhizomatous rootstocks. Its leaves are sub-orbicular, palmately 5–7 lobed with irregularly toothed segments; the lower leaves are petiolate, while the upper ones are sessile. The plant bears pink to purple flowers and is typically found in montane regions at elevations above 1400 meters. It is widely distributed across the Himalayas, China, Japan, and Myanmar. Traditionally, the plant has been used for its medicinal properties infusions are employed to treat fever and renal disorders, while root paste is applied to relieve itching and has also found use in the tanning industry (Gaur 1999). Scientific studies have confirmed the anti-inflammatory potential of G. nepalense, particularly in a model of TPA-induced ear edema in mice. The ethyl acetate fraction of its water extract exhibited significant anti-inflammatory activity at a dose of 2.5 g/kg, with aspirin (0.6 g/kg) serving as a positive control. Phytochemical analysis led to the isolation of six polyphenolic compounds from this fraction, including three flavonoids—kaempferol, kaempferol-7-O-β-D-glucopyranoside, and quercetin-7-O-α-rhamnopyranoside—two tannins, pyrogallol and gallic acid, and one lignan, epipinoresinol. The presence of these bioactive polyphenols suggests a scientific basis for the plant's traditional use in treating inflammatory conditions. Additionally, *G. nepalense* is reputed in folk medicine for its effectiveness against influenza, dysentery, and is used as an antiphlogistic, analgesic, haemostatic, stomachic, and antidiabetic remedy (Lu *et al* 2012).

In recent years, the effectiveness of antimicrobial treatments has come under scrutiny because of growing concerns. One major issue is that many microbial pathogens are developing resistance, making standard drugs less effective. Since plant-based natural products are known to have antimicrobial properties due to the presence of wide range of phytochemicals, there's been a rising interest in exploring them further through research. Scientists are increasingly focusing on these natural alternatives as potential solutions to combat microbial infections, especially considering the challenges posed by synthetic drugs (Coutinho *et al* 2009; Gandhimathi *et al* 2021; Srikar *et al* 2016).

Fig. 1: *Geranium nepalense*

In the field of nanotechnology, silver nanoparticles are among the most widely studied and important. These are tiny particles of silver, typically ranging in size from 1 to 100 nanometers. Because of their unique properties and wide range of applications, silver nanoparticles have become a major focus of scientific research. Silver nanoparticles are used in various fields such as catalysis, biosensing, biochemical detection, photography, and especially in medicine, thanks to their unique and beneficial properties. Their small size and special characteristics make them highly effective in these applications (Srikar *et al* 2016; Gohil *et al* 2024; Rauwel *et al* 2015). The biosynthesis of silver nanoparticles using plant extracts has gained attention as a simple, eco-friendly, and practical alternative to traditional chemical and physical methods. This process involves the natural reduction of silver ions into nanoparticles using extracts of medicinal plants such as *Mentha arvensis* (Thiyagarajan and Kanchana 2022), *Achyranthes aspera* (Praveena and Kumar 2014), *Azhadirachta indica* (Lalitha *et al* 2013), *Triumfetta rotundifolia* (Ananthi *et al* 2016), *Ruta graveolens* (Varadarajan *et al* 2022), *Moringa oleifera* (Shirisha *et al* 2023), *Anabaena doliolum* (Zabin *et al* 2022) etc. which act as reducing and stabilizing agents. Therefore, to tackle the harmful and resistant pathogens, there's huge need for safe, natural drugs that are produced through eco-friendly and affordable methods. Keeping this in mind, the current study focused on synthesizing silver nanoparticles (Ag NPs) using the leaf extract of *Geranium nepalense*. The research also aimed to characterize these nanoparticles and further to evaluate their effectiveness against microbial infections.

Materials and Method

All the chemicals of analytical grade which are used in this research work were provided by the Department of Chemistry, HNB Garhwal University, Pauri Campus, Uttarakhand, India. The plant *Geranium nepalense* used in this study was collected from the Nagdev Hills located in Pauri Garhwal, Uttarakhand. This region is known for its rich biodiversity and medicinal flora, providing a suitable natural habitat for the growth of *this plant.*

For antibacterial test, Soyabean casein digest agar/broth and Sabouraud's dextrose agar/broth of Hi Media Pvt. Bombay, India was used for antifungal test. Pure cultures of bacterial [*Bacillus subtilis* (NCFT.583.08), *Staphylococcus aureus* (NCFT.576.08), *Lactobacillus plantarum* (NCFT.623.34) and

Pseudomonas aeruginosa (NCFT.645.11)] and fungal [*Aspergillus niger* (NCFT.623.11) and *Candida albicans* (NCFT.1006.11] strains, were used for this research work.

Preparation of Geranium nepalense leaf extract: Plant specimens of *Geranium nepalense* were identified at the Garhwal University Herbarium, where the accession number GUH20756 was assigned. Fresh leaves of the plant were collected, thoroughly washed multiple times with deionized distilled water to eliminate any dust particles or pathogens, and then finely cut into small pieces. These pieces were placed in an oven at 40°C with the fan on to dry, after which they were ground into a fine powder using a mortar and pestle. For the extraction, 05 grams of the dried powder was added to a 250 ml Erlenmeyer flask containing 100 ml of deionized distilled water. The mixture was heated at 70°C for 20–25 minutes. Following this, the leaf extract was filtered into a separate conical flask and stored for further use in this research (Praveena and Kumar 2014; Ananthi *et al* 2016; Varadarajan *et al* 2022).

Preparation of Silver Nitrate ($AgNO_3$) Solution: 1mM silver nitrate solution was prepared using standard method and by dissolving the required amount of $AgNO_3$ in the of deionized distilled water in a 5L flask. The solution was thoroughly mixed to ensure complete dissolution and then filtered to remove any impurities. The freshly prepared and filtered solution was used for subsequent experimental procedures in the research work.

Synthesis of Silver Nanoparticles: At room temperature, a 1:9 mixture of *Geranium nepalense* leaf extract and 1 mM silver nitrate solution was prepared in a conical flask. The solution was kept undisturbed in a dark place for 24 hours to facilitate the biosynthesis of silver nanoparticles. After 24 hours, a visible color change to dark red was observed, indicating the successful formation of silver nanoparticles. The reaction mixture was then centrifuged at 5000 rpm for 10 minutes and washed with deionized water and ethanol to remove any unreacted or uncoordinated plant material and impurities. The collected material was dried in an oven at 70°C, resulting in a grayish-black nanopowder. And to obtain more uniform and finely grounded nanoparticles for analysis, the dried material was further powdered using a mortar and pestle, yielding the final grayish-black silver nanoparticle product (Lalitha *et al* 2013; Varadarajan *et al* 2022; Shirisha *et al* 2023).

Characterization: UV-Visible spectroscopy plays a crucial role in monitoring the formation and stability of metallic nanoparticles. In this study, the synthesis of silver nanoparticles was monitored using a PEL/Elite UV-Vis spectrophotometer, which detected characteristic absorption peaks indicating nanoparticle formation. To analyze the crystalline structure of the synthesized nanoparticles, X-ray diffraction (XRD) patterns were recorded using an X'PERT-PRO diffractometer. The morphology and size distribution of the biosynthesized silver nanoparticles were examined using a JEOL Transmission Electron Microscope (TEM), providing detailed images of particle shape and dispersion. Additionally, Fourier Transform Infrared Spectroscopy (FTIR) was employed to identify the functional groups present in the plant extract, which were responsible for the reduction and the stabilization of the resulting nanoparticles, highlighting the role of phytochemicals in the green synthesis process.

Determination of Antimicrobial Activity*:* The antimicrobial activity of biosynthesized silver nanoparticles (AgNPs) was assessed using a modified agar well diffusion method (Usman *et al* 2007; Vollekova *et al* 2001). Bacterial cultures were grown in Soyabean Casein Digest Broth, while fungal strains were cultured in Sabouraud's Dextrose Broth. After incubation, agar plates were prepared and wells (8 mm) were filled with AgNPs solution

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(100/200 µl in DMSO), with DMSO as a negative control and standard antibiotics (Erythromycin for bacteria and Fluconazole for fungi) as positive controls. Plates were incubated, and zones of inhibition were measured to evaluate antimicrobial efficacy. To determine MIC and MLC values, nanoparticle solutions were serially diluted and incubated with microbial suspensions. MIC was identified as the lowest concentration showing minimal turbidity, indicating growth inhibition, while MLC was the concentration at which no growth occurred, confirming microbial lethality. All tests were performed in triplicate to ensure accuracy.

Results and Discussion

The UV-Vis absorption spectrum of silver nanoparticles synthesized using *Geranium nepalense* leaf extract exhibited a distinct Surface Plasmon Resonance (SPR) band at 410 nm, characteristic of Ag NPs. This figure (2) is showing the progression of silver nanoparticle synthesis using *Geranium nepalense* leaf extract in a 1:9 mixture over time. The image compares the reaction at two-time intervals: after 12 and 24 hours. The appearance of a sharper and more defined peak in the UV-Vis spectrum at 410 nm after 24 hours suggests the formation of more uniformly sized and increased quantities of silver nanoparticles. The results highlight the impact of reaction time on the efficiency and uniformity of nanoparticle formation (Ananthi *et al* 2016; Varadarajan *et al* 2022; Shirisha *et al* 2023).

Fig. 2: Progression of Formation of Silver Nanoparticles

The XRD pattern shown in Fig. 3 revealed distinct diffraction peaks at 2θ values of 37.98° and 63.32°, which correspond to the (111) and (220) crystallographic planes, respectively. These peaks confirmed the formation of face-centred cubic (FCC) structured silver nanostructures synthesized using *Geranium nepalense* leaf extract. The presence of these characteristic reflections indicates the crystalline nature of the Ag nanoparticles and supports the successful green synthesis of well-defined FCC-phase silver nanoparticles through plant-mediated methods (Singh *et al* 2014, Varadarajan *et al* 2022).

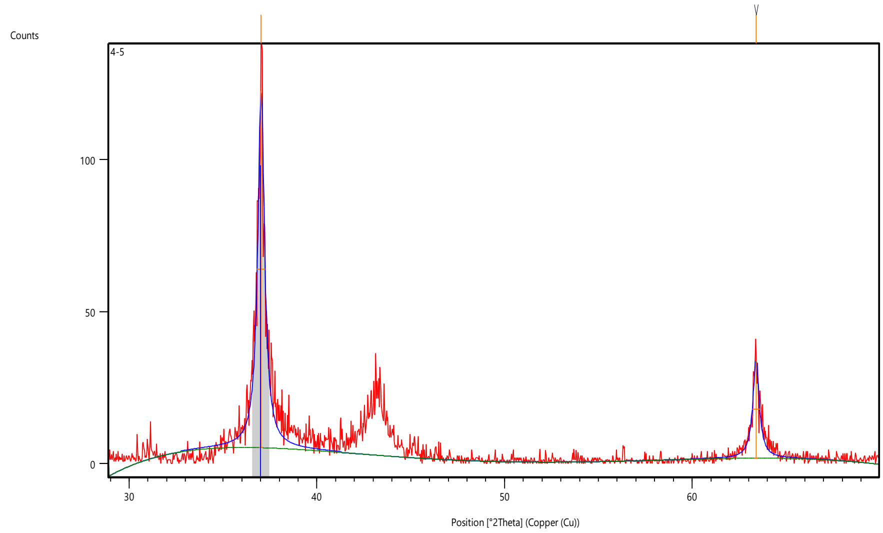

Fig. 3: XRD Spectrum of Biosynthesized Silver Nanoparticles

A TEM micrograph of silver nanoparticles (Ag NPs) synthesized using *Geranium nepalense* leaf extract revealed that the particles were predominantly spherical in shape. Most of these nanoparticles were found to fall within the size range of 08 to 16 nanometres, with an average particle diameter of approximately 12 nanometres. This nearly uniform morphology and narrow size distribution suggested the effectiveness of the plant extract in mediating the controlled synthesis of Ag NPs.

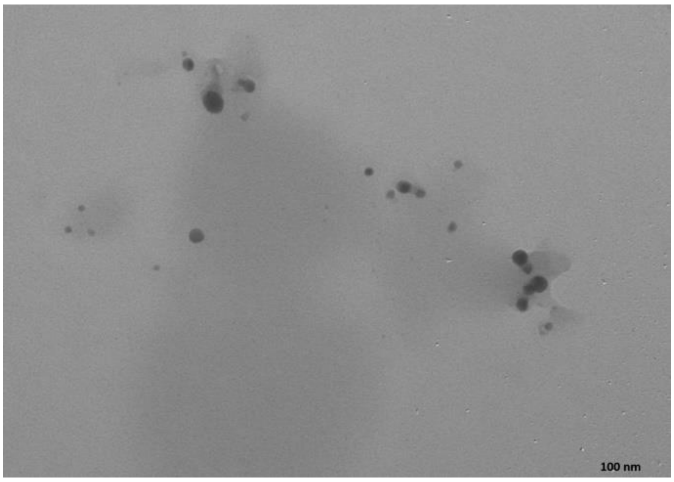

Fig. 4: TEM Micrograph of Biosynthesized Silver Nanoparticles

The FTIR spectrum of silver nanoparticles synthesized using *Geranium nepalense* leaf extract displayed characteristic peaks at 3277.44, 1701.00, 1584.04, and 1349.99 cm^{-1}. These absorption bands correspond to the presence of various functional groups involved in the reduction and stabilization of the nanoparticles. Specifically, the peak at 3277.44 cm^{-1} indicates the presence of hydroxyl (-OH) groups, while the peak at 1701.00 cm^{-1} is attributed to carbonyl (C=O) stretching vibrations. The band at 1584.04 cm^{-1} signifies the presence of C=C stretching, and the peak at 1349.99 cm^{-1} corresponds to aromatic hydroxyl (Ar-OH) groups. These functional groups revealed the involvement of phytochemicals from the leaf extract in the synthesis and capping of the Ag NPs (Gohil et al 2024; Thiyagarajan and Kanchana 2022; Ananthi et al 2016; Varadarajan et al 2022; Singh et al 2014).

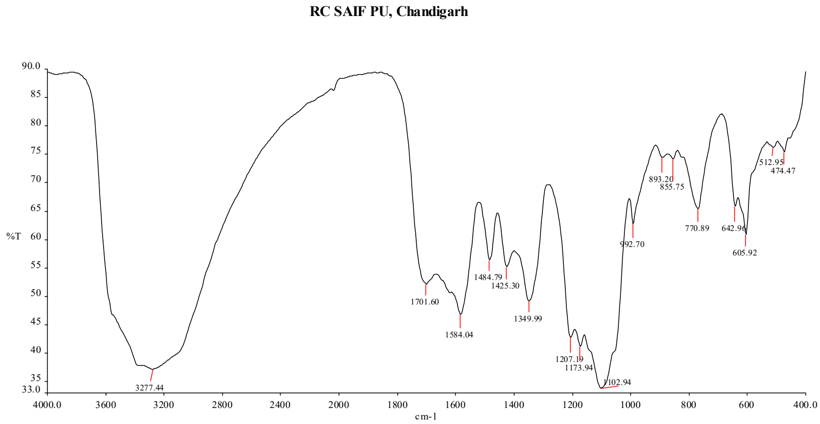

Fig. 5: FTIR Spectrum of Biosynthesized Silver Nanoparticles

Antimicrobial Activity: The antibacterial activity of silver nanoparticles synthesized using *Geranium nepalense* leaf extract was evaluated against various bacterial strains, showing notable zones of inhibition. The diameter of the inhibition zones measured 35 mm for *Bacillus subtilis*, 33 mm for *Staphylococcus aureus*, 38 mm for *Lactobacillus plantarum*, and 28 mm for *Pseudomonas aeruginosa*, indicating good antibacterial effects (Table 1). Furthermore, the Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) values (in µl) were determined to be 15–25 for *B. subtilis*, 30–45 for *S. aureus*, 35–55 for *L. plantarum*, and 38–56 for *P. aeruginosa*. These results highlighted the antibacterial efficacy of the biosynthesized Ag NPs, with varying sensitivity observed among the tested strains.

Table 1: Antibacterial Activity of Biosynthesized Silver nanoparticles

Sample (100 µl)	**Diameter of Zone of Inhibition (mm)**							
	***B. subtilis* (NCFT.583.08)**		***S. aureus* (NCFT.576.08)**		***L. plantarum* (NCFT. 623.34)**		***P. aeruginosa* (NCFT.645.11)**	
Gn-Ag NPs	35		33		38		28	
Erythromycin (1 mg/ml)	45		34		35		38	
	MIC and MLC (µl)							
	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC
Gn-Ag NPs	15	25	30	45	35	55	38	56

The antifungal potential of silver nanoparticles synthesized using *Geranium nepalense* leaf extract was assessed against *Aspergillus niger* and *Candida albicans* strains (Table 2).

Table 2: Antifungal Activity of Biosynthesized Silver nanoparticles

Sample (100 µl)	**Diameter of zone of inhibition (mm)**			
	***A. niger* (NCFT.623.11)**		***C. albicans* (NCFT.1006.11)**	
Gn-Ag NPs	21		25	
Flucanazole (1mg/ml)	34		28	
	MIC and MLC (µl)			
	MIC	MLC	MIC	MLC
Gn-Ag NPs	50	80	62	85

The nanoparticles exhibited zones of inhibition measuring 21 mm for A. niger and 25 mm for C. albicans, indicating moderate antifungal activity. Additionally, the MIC and MLC values for these strains ranged from 50–80 µl for A. niger and 62–85 µl for C. albicans. These findings suggested that while the Ag NPs demonstrate noticeable antifungal effects, higher concentrations are required to inhibit and kill fungal strains compared to bacterial ones.

Conclusion

The study successfully demonstrates the green synthesis of silver nanoparticles using *Geranium nepalense* leaf extract, confirmed by UV-Vis spectroscopy, XRD, TEM, and FTIR analyses. The nanoparticles exhibited a characteristic SPR band at 410 nm and showed a face-centred cubic (FCC) crystalline structure with predominantly spherical shapes averaging 12 nm in size. FTIR analysis confirmed the involvement of phytochemicals in the reduction and stabilization processes. The biosynthesized Ag NPs showed significant antibacterial and moderate antifungal activity. These findings highlighted the potential of *G. nepalense*-mediated Ag NPs as effective antimicrobial agents for future biomedical applications.

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