Research Article

Madiha Iqbal, Saud Bawazeer*, Jehan Bakht, Abdur Rauf*, Muhammad Raza Shah, Anees Ahmed Khalil, and Mohamed A. El-Esawi

Green synthesis of silver nanoparticles from *Valeriana jatamansi* shoots extract and its antimicrobial activity

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Abstract: The present study explores the potential of *Valeriana jatamansi* shoot extract for Ag-metal bioreduction and its antimicrobial activity. Among the different ratios of $AgNO_3$ and extract tested, 1:5 (1 mL $AgNO_3$ and 5 mL extract) gave maximum SPR peak at 411.0 nm during UV-Vis spectrophotometric analysis, indicating the synthesis of maximum amount of AgNPs in solution. XRD analysis reported the crystalline nature of AgNPs with 13.32 nm nanocrystallite size. FTIR studies suggested the involvement of carboxylic acid (–[C═O–O–H]) and methane (═CH–) functional groups of different compounds in AgNPs reduction and fabrication. Average size of synthesized uniform shaped nanospheres was 32 nm by SEM image analysis. The produced AgNPs (1.5 mg/disc) showed growth inhibition of 71.46, 65.97, 61.5, 55.32, and 54.83% against *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, *Xanthomonas campestris*, and *Staphylococcus aureus*. While the least growth inhibition of 48.55% was recorded for *Klebsiella pneumonia*, suggesting it as the least-susceptible microbe among all the tested microbial species. *P. aeruginos*a was found to be most sensitive of all tested microbes, while *E. coli*, *C. albicans*, and *X. campestris* reported moderate susceptibility to AgNPs.

Keywords: AgNPs, *Valeriana jatamansi*, antimicrobial activity, XRD, SEM, FTIR

1 Introduction

Nanobiotechnology is the intersection of biology and nanotechnology. The development and the use of nanotools like nanoparticles in a vast field to achieve numerous goals have attracted scientists and researchers to this field. Drug delivery through nanoparticles (NPs) is an emerging field with promising results. NPs can be synthesized by several ways including chemical, physical, and biological approaches. Biologically synthesized nanoparticles are efficient, sustainable, hazardous chemical-free, and low-cost alternatives of nanoparticles derived from chemical and physical methods [1]. During the past few years, various materials and metals have been used for nanoparticle production [2–4] from bacteria, fungi, algae, viruses, and numerous plants [5–10]. Metallic NPs have shown enhanced antimicrobial, antioxidant, and many other properties due to the increased surface area, smaller particle size, various shapes, and altered characteristics [4,11,12].

Valeriana jatamansi (family Valerianaceae), an indigenous medicinal plant of Himalaya region, is a hairy, perennial dwarf and rhizomatous wild herb. It can be found growing at an altitude of 1,200–3,000 m. Its bitter and acrid thick roots covered with root fibers are used as carminative and laxative. The plant is regarded as nerve tonic, ophthalmic, tranquilizer, aphrodisiac, antispasmodic, expectorant, and sedative. It is also used as tonic in hysteria, cholera, snakebite, scorpion sting, and

* **Corresponding author: Saud Bawazeer,** Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Umm Al-Qura University, P.O. Box 42, Makkah, Saudi Arabia, e-mail: ssbawazeer@uqu.edu.sa
* **Corresponding author: Abdur Rauf,** Department of Chemistry, University of Swabi, Swabi, Anbar, Khyber Pakhtunkhwa, Pakistan, e-mail: mashaljcs@yahoo.com
Madiha Iqbal: Department of Microbiology and Biotechnology, Abasyn University Abasyn University, Peshawar, Khyber Pakhtunkhwa, Pakistan
Jehan Bakht: Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar, Khyber Pakhtunkhwa, Pakistan
Muhammad Raza Shah: Biological Sciences, HEJ Research Institute of Chemistry, University of Karachi, Karachi, Pakistan
Anees Ahmed Khalil: University Institute of Diet and Nutritional Sciences, Faculty of Allied Health Sciences, The University of Lahore, Lahore, Pakistan
Mohamed A. El-Esawi: Botany Department, Faculty of Science, Tanta University, Tanta 31527, Egypt

asthma traditionally [13]. It consists of an array of various biologically active components such as terpenoids, flavonoids, sesquiterpenes, and lignans having health-promoting benefits. In Asia, it has been used as an insect repellent, antioxidant, antidepressant, antimicrobial, and cytotoxic agent. Valepotriates and valerenic acid derived from this species is used for the preparation of drugs. Valepotriates/iridoids are predominant bioactive components present in *V. jatamansi* that are used for the treatment of bacterial and fungal infections, cancer, inflammation, oxidation, liver diseases, and neurological-related disorders. Various parts of *V. jatamansi* such as roots (dried), leaves (crushed), and rhizomes (dried) have been used in severe headaches, perfume formulations, asthma, and intermittent fever [14]. Phytochemically this plant is explored very little, and different classes of compounds such as iridoids comprising jatamanins A–M, lignin, and (+)9′-isovaleroxyl lariciresinol have been reported from the whole plant of *Valeriana jatamansi* [15]. Ester iridoids isolated from family Valerianaceae are documented for cytotoxic, sedative, antifungal, and antitumor properties [15]. Therefore, this present study was designed, owing to medicinal potential of *V. jatamansi*, to investigate the green synthesis of silver nanoparticles from *V. jatamansi* shoot extract and its antimicrobial activity.

2 Materials and methods

2.1 Plant collection and extract preparation

Shoots of *V. jatamansi* were collected and shade dried after thorough washing with distilled water. Dried plant material was finely grinded and soaked in methanol for 10 days to get methanolic crude extract. The extract was dried in a rotary evaporator to completely eliminate methanol from the extract.

2.2 Green synthesis of AgNPs

For the production of biologically synthesized AgNPs, methanolic crude extract of *V. jatamansi*, 50 mg extract dissolved in 100 mL deionized water, was employed to reduce 0.1 mM $AgNO_3$ solution. Different ratios of both solutions were mixed to assess the maximum and stable AgNPs yielding ratios. All the solutions were analyzed by UV-visible (UV-Vis) spectrophotometer, and the solution containing maximum amount of AgNPs was further processed for AgNPs characterization.

2.3 AgNPs characterization

UV-Vis spectrophotometric analysis was carried out to monitor the synthesis of AgNPs by observing a characteristic surface plasmon resonance (SPR) peak in the wavelength range of 400–500 nm. X-ray diffraction (XRD) investigation was used to determine nature and nanocrystallite size of AgNPs. Fourier transform infrared (FTIR) spectroscopy was employed to identify the possible functional groups involved in bioreduction of Ag-metal. The size and the shape of AgNPs were determined by scanning electron microscope (SEM) studies.

2.4 AgNPs stability studies

Salt (NaCl – 1 mM, 0.5 M, and 1 M) and temperature (20–40°C and 80–100°C) stress were applied to AgNPs to study their effects, and samples were analyzed by UV-Vis spectrophotometric analysis.

2.5 Antimicrobial potential

Antimicrobial potential of AgNPs was assessed against different clinical isolates of *Klebsiella pneumonia*, *Bacillus subtilis*, and ATCC strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli, Xanthomonas campestris*, and *Candida albicans* by following the protocol described by Bakht et al. [16]. Nutrient broth (NB) media (3.25 g/250 mL) and nutrient agar (NA) media (7 g/250 mL) were prepared as per requirement and autoclaved. After pouring media in plates and solidification of media, fresh cultures of microbes standardized with 0.5 McFarland standards were spread on media. AgNPs (0.5, 1.0, and 1.5 mg $disc^{-1}$) and control ciprofloxacin (50 µg per 6 µL) were applied on 6 mm diameter Whatman filter paper discs. These assay plates were incubated at 37°C temperature for 24 h. The zone of inhibition was measured in millimeters for each sample, and percent (%) inhibition was calculated as follows:

$$\text{Percent inhibition (\%)} = [\text{Zone of inhibition of sample (mm)} / \text{Zone of inhibition of control (mm)}] \times 100. \quad (1)$$

The experiment was repeated in triplicate, and results are reported as mean with standard deviation.

3 Results and discussion

3.1 Effect of different reaction mixtures on stability of prepared AgNPs

$AgNO_3$ solution and *V. jatamansi* shoot methanolic extract were mixed in different ratios to evaluate the synthesis of AgNPs. AgNPs synthesis in samples was initially traced visually and then confirmed by UV-Vis spectrophotometric analysis. Change in color of the solution from colorless to dark yellow or brown indicated the formation of AgNPs. Figure 1 shows a comparison of UV-Vis spectrums of all the tested ratios. Highest sharp peak was recorded for sample containing 1:5 ratios (1 mL $AgNO_3$ and 5 mL methanolic extract solution), indicating the formation of higher amounts of AgNPs. Samples of other ratios reported very less or no AgNPs synthesis. Furthermore, it was generally observed in some samples that plant extract solution when used in higher ratios than $AgNO_3$ solution resulted in intense colored solution and indicated the synthesis of relatively higher amounts of silver NPs in sample. The highest surface plasmon resonance peak was recorded at 411.0 nm wavelength with 1.783 maximum absorption for the sample containing 1:5 ratios and suggested 1:5 ratios as optimum concentration of reactants to yield AgNPs (Figure 2).

Crude methanolic extract of *V. jatamansi* shoot was used for the bio-reduction of Ag and AgNPs production from $AgNO_3$. Extract and $AgNO_3$ solution were mixed in different ratios, and the NP synthesis was monitored during

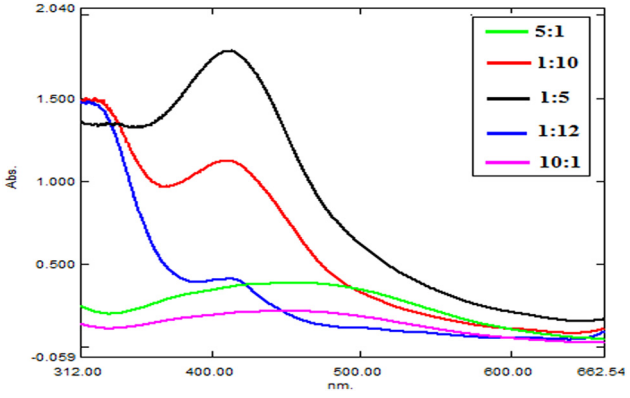

Figure 1: Comparison of UV-Vis spectra of *V. jatamansi* shoot AgNPs synthesized in different samples.

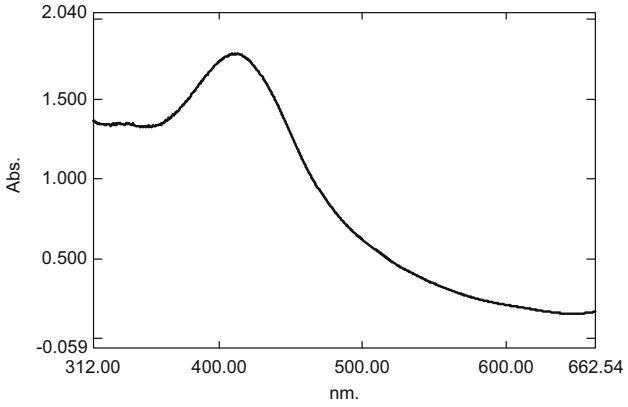

Figure 2: UV-Vis spectrum of *V. jatamansi* shoot AgNPs in sample containing 1:5 (1 mL of $AgNO_3$:5 mL extract) showing AgNPs SPR peak at 411.05 nm.

continuous stirring. Initial observation of AgNP production was made based on the color change of solution. On synthesis of nanoparticles, solution changed its color, and the resultant dense colored solution (dark yellow or brown) indicated the synthesis of AgNPs. This color change agrees with the findings of Bharathi et al. [17]. These researchers reported brown color of solution on AgNPs synthesis from *Diospyros montana* extract. Final confirmation of Ag nanoparticle production was carried out by the UV-Vis spectrophotometric analysis. AgNPs absorb light and give characteristic absorption peak in 400–500 nm wavelength range. Peak intensity refers to AgNPs concentration. Highest SPR peak at 411.0 nm wavelength represented maximum silver NPs synthesis in solution containing 1:5 ratios (1 mL $AgNO_3$ and 5 mL methanolic extract solution) among all samples. Our UV-Vis spectrophotometric data coincide with the study by Sreekanth et al. [18] who reported the synthesis of AgNPs from *Nelumbo nucifera* extract and observed its SPR peak at 412.0 nm. During our studies, an increase in the extract concentration with respect to $AgNO_3$ yields larger amount of AgNPs. Similar observation was also made by Umoren et al. [19] who revealed that higher extract concentration increased the possibility of stable and well-defined AgNPs synthesis.

3.2 Effect of salt concentration and temperature on stability of prepared AgNPs

Synthesized silver NPs were checked for their stability at different temperatures and salt stresses. Different temperature ranges (20–40°C and 80–100°C) and

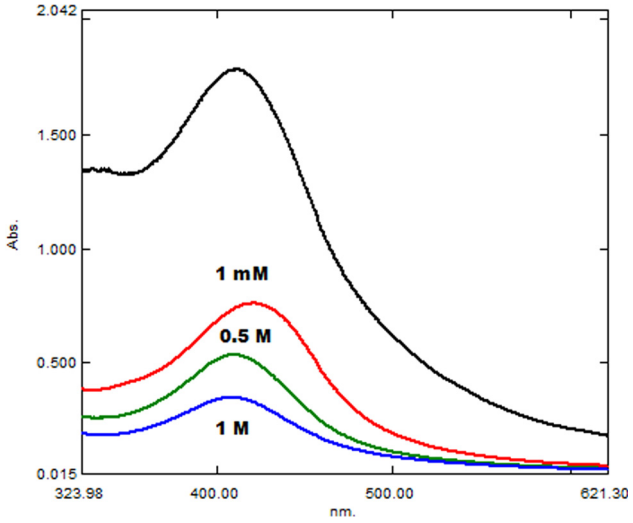

Figure 3: Comparison of UV-Vis spectra of AgNPs stability at different NaCl salt concentrations.

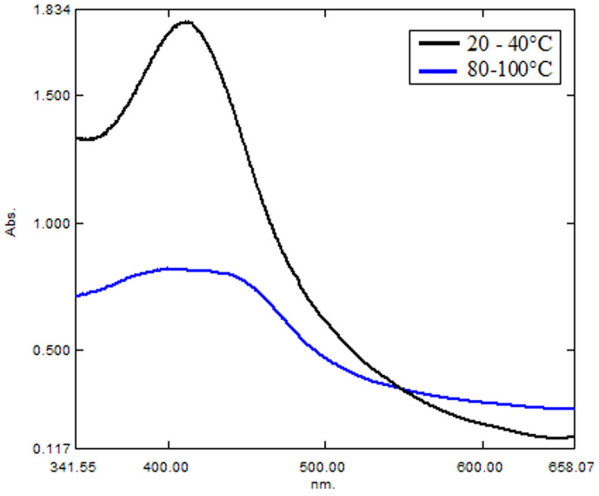

Figure 4: Comparison of UV-Vis spectra of AgNPs stability at different temperatures.

different NaCl (sodium chloride) salt concentration (1 M, 0.5 M, and 1 mM) were applied to the samples separately. On comparison of the UV-Vis spectra of the samples, a decrease in stability of the AgNPs was observed with an increase in the temperature and salt concentration. AgNPs heated at 20–40°C were comparatively stable than the AgNPs heated up to 100°C (Figure 3). Almost complete degradation of the AgNPs was observed at 100°C. Different NaCl stresses affected the synthesized AgNPs (Figure 4). With a gradual increase in salt concentration, stability of AgNPs decreased. AgNPs showed less stability at 1 M NaCl, comparatively moderate stability at 0.5 M, and highest stability at 1 mM NaCl among all the tested samples. A decrease in sharpness and height of the SPR peak suggested degradation of AgNPs in sample in higher saline conditions.

The stability of AgNPs at high temperature and salt conditions was assessed by heating samples at different temperature ranges (20–40°C and 80–100°C) and salt concentrations (1 M, 0.5 M, and 1 mM). AgNPs were comparatively more stable at lower temperatures and salt concentrations. A change in sharpness and height of AgNPs SPR peak represented degradation of NPs in samples isolated at higher salt and temperature levels. Mittal et al. [20] also reported a decrease in UV-Vis light absorbance at higher temperatures referring to AgNPs degradation in solution at higher temperatures [20].

3.3 AgNPs characterization

XRD patterns of *V. jatamansi* shoot AgNPs suggested the crystalline nature of AgNPs (Figure 5). Bragg's reflection indexing of (311), (232), (220), (202), (200), (141), and (111) corresponding to, respectively, 77.38°, 66.14°, 64.54°, 50.21°, 44.39°, 40.58°, and 38.05° two theta values suggested fcc (face centered cubic) structure of Ag (silver). The average size of the synthesized Ag nanocrystallite was calculated as 13.32 nm by determining the full width half maximum (FWHM) of most intense peaks (202), (141), and (111) and applying sheerer equation. Comparison of FTIR spectrums of *V. jatamansi* shoot extract and its AgNPs indicated vanishing of the same absorption bands at 923.84 and 1264.06 cm^{-1} wave numbers initially present in the extract, thus suggesting the involvement of carboxylic acid (–[C═O–O–H]) and methane (═CH–) functional groups of different compounds in bioreduction of Ag-metal, respectively (Figure 6). A closer insight reported small shift (±1 to ±100) of wave numbers in other absorption bands and confirmed the synthesis of

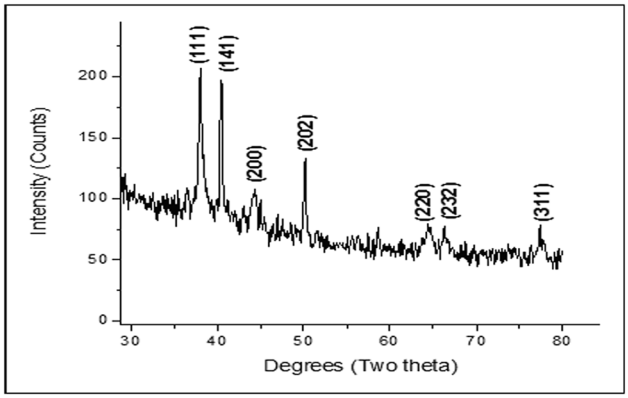

Figure 5: XRD patterns of *V. jatamansi* shoot AgNPs.

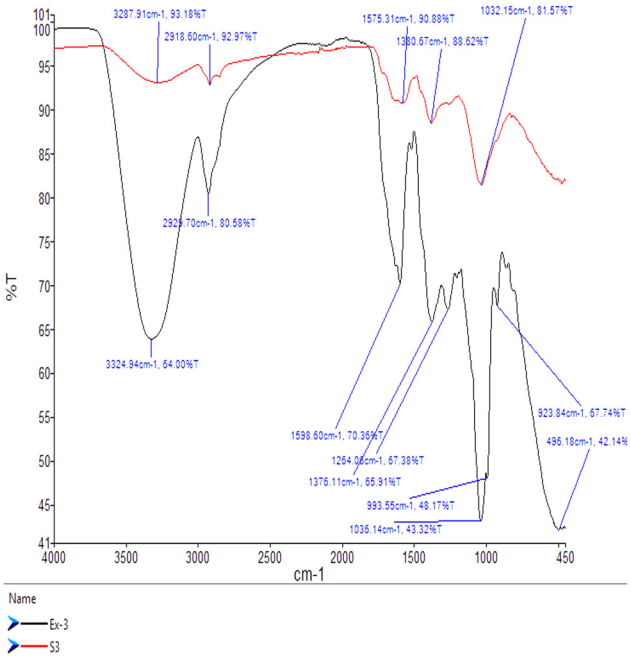

Figure 6: Comparative FTIR spectra of pure extract and AgNPs.

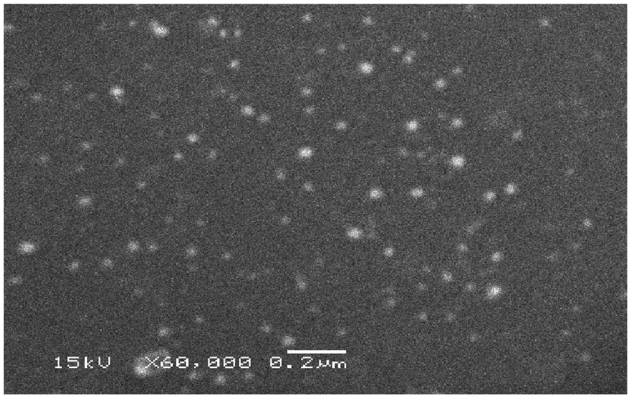

Figure 7: SEM photograph of *V. jatamansi* shoot AgNPs.

AgNPs in solution. FT-IR profiling shows that peaks at 3,324 and 3,287 cm^{-1} may be attributed to O–H vibrations of hydroxyl functional groups. However, bands at 2,929 and 2,918 cm^{-1} might be assigned to C–H vibrations of alkanes. Peaks in region of 1,598 and 1,575 cm^{-1} correspond to C=O stretching vibrations. Two distinct bands noticed at 1,376 and 1,380 cm^{-1} are characteristic to C–N stretching vibrations aromatic amino groups. Likewise, a peak recorded at 1,264 cm^{-1} may be because of C–O stretching of flavonoids. Two bands observed in the region of 1,035 and 1,032 cm^{-1} may correspond to N–C bond stretching of aliphatic amine groups.

SEM analysis indicated the average size of synthesized uniformed shaped nanospheres as 32 nm (Figure 7). Same XRD patterns are reported by Singh et al. [21] during their studies on AgNPs synthesis from *Argemone mexicana* extracts. Comparison of FTIR spectra of extract and AgNPs indicated the possible involvement of carboxylic acid (–[C=O–O–H]) and methane (=CH–) functional groups of different compounds in Ag metal reduction. Ganaie et al. [22] also reported the involvement of same functional groups in reduction of metal to synthesize NPs. Interpretation of SEM images confirmed the uniform shape of synthesized nanospheres and size as 32 nm. Same size of AgNPs synthesized from Mulberry leaves through SEM in the range of 20–40 nm was investigated by Awwad and Salem [23].

3.4 Antimicrobial properties of prepared AgNPs

Effect of AgNPs synthesized from methanolic crude extract of *V. jatamansi* shoot on the growth of seven different

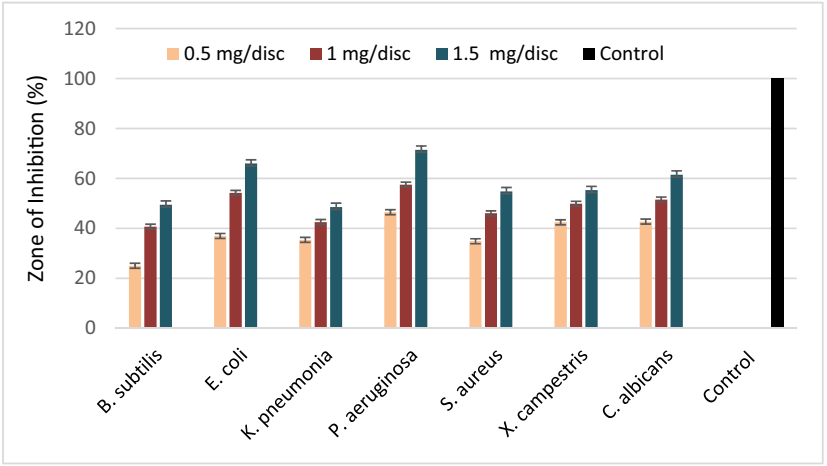

Figure 8: Antimicrobial potential of AgNPs, with standard deviation, against tested microbial strains.

microbes is shown in Figure 8. Reduction in microbial growth increased with the increase in AgNP concentration. *P. aeruginos*a was the most sensitive of all the tested microbes and showed 71.46% growth inhibition at 1.5 mg $disc^{-1}$ concentration. Moderate growth inhibition of 65.97, 61.5, 55.32, and 54.83% was recorded for *E. coli*, *C. albicans*, *X. campestris*, and *S. aureus* at highest tested concentration, respectively. Least growth inhibition of 48.55% recorded for *K. pneumonia* suggested it as the least-susceptible microbe among all the tested microbial species.

AgNPs were found efficient in inhibiting the microbial growth. Highest zone of inhibition by AgNPs was measured for *P. aeruginos*a followed by *E. coli* and *C. albicans*. The least activity of AgNPs was recorded against *K. pneumonia* among all the test microbes. Our findings are supported by the results of Jeeva et al. [24]. During their study, Jeeva et al. [24] found *P. aeruginos*a as the most susceptible, while *K. pneumonia* as less susceptible to AgNPs among all the tested microbes.

4 Conclusion

Synthesis of AgNPs from *V. jatamansi* shoot extract and $AgNO_3$ during the present study supports the efficient reduction of silver and synthesis of stable, spherical, and crystalline AgNPs at this much lower concentration of extract and silver salt. Moreover, prepared AgNPs were active against the tested bacterial and fungal strains and inhibited their growth.

Conflict of interest: One of the authors (Abdur Rauf) is a member of the Editorial Board of Green Processing and Synthesis.

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