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 AgNO₃ and extract tested, 1:5 (1 mL AgNO₃ 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. aeruginosa 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, tranquillizer, aphrodisiac, antispasmodic, expectorant, and sedative. It is also used as tonic in hysteria, cholera, snakebite, scorpion sting, and
methanol from the extract. Dried in a rotary evaporator to completely eliminate 10 days to get methanolic crude extract. The extract was material was after thorough washing with distilled water. Dried plant fi

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 AgNO₃ 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:

\[
\text{Percent inhibition (\%) } = \left( \frac{\text{Zone of inhibition of sample (mm)}}{\text{Zone of inhibition of control (mm)}} \right) \times 100.
\]
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

AgNO₃ 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 AgNO₃ 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 AgNO₃ 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 AgNO₃. Extract and AgNO₃ solution were mixed in different ratios, and the NP synthesis was monitored during 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 AgNO₃ 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 AgNO₃ 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 1: Comparison of UV-Vis spectra of *V. jatamansi* shoot AgNPs synthesized in different samples.](image)

![Figure 2: UV-Vis spectrum of *V. jatamansi* shoot AgNPs in sample containing 1:5 (1 mL of AgNO₃:5 mL extract) showing AgNPs SPR peak at 411.05 nm.](image)
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 shear 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 3: Comparison of UV-Vis spectra of AgNPs stability at different NaCl salt concentrations.](image3)

![Figure 4: Comparison of UV-Vis spectra of AgNPs stability at different temperatures.](image4)

![Figure 5: XRD patterns of *V. jatamansi* shoot AgNPs.](image5)
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\(_2\)) 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 Valeriana jatamansi shoot on the growth of seven different microbial strains is shown in Figure 8. The results indicate that AgNPs exhibit significant antimicrobial activity against both Gram-positive and Gram-negative bacteria. The zone of inhibition was measured in millimeters and compared with the control.
microbes is shown in Figure 8. Reduction in microbial growth increased with the increase in AgNP concentration. *P. aeruginosa* 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. aeruginosa* 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. aeruginosa* 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 AgNO₃ 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.

References

[1] Rajan R, Chandran K, Harper SL, Yun SI, Kalaichelvan PT. Plant extract synthesized silver nanoparticles: An ongoing source of novel biocompatible materials. Ind Crop Prod. 2015;70:356–73.
[2] Ahmad Z, Sharma S, Khuller GK. Inhalable alginate nanoparticles as anti-tubercular drug carriers against experimental tuberculosis. Int J Antimicrob Agents. 2005;26:298–303.
[3] Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, et al. Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphora leaf. Nanotechnol. 2007;18:105104–15.
[4] Jhaa AK, Prasad K, Kulkarni AR. Synthesis of TiO₂ nanoparticles using microorganisms. Colloids Surf B Biointerfaces. 2009;71:226–9.
[5] Dubey SP, Lahtinen M, Sillanpaa M. Green synthesis and characterizations of silver and gold nanoparticles using leaf extract of Rosa rugosa. Colloids Surf A Physicochem Eng Asp. 2010;364:34–41.
[6] Merin DD, Prakash S, Bhimba BV. Antibacterial screening of silver nanoparticles synthesized by marine micro algae. Asian Pac Trop Med. 2010;3:797–9.
[7] Bankar A, Joshi B, Kumar AR, Zinjarde S. Banana peel extract mediated synthesis of gold nanoparticles. Colloids Surf B Biointerfaces. 2010;80:45–50.
[8] Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N. Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf B Biointerfaces. 2010;76:50–6.
[9] Bai HJ, Yang BS, Chai CF, Yang GE, Jia WL, Yi ZB. Green synthesis of silver nanoparticles using Rhodobacter sphaeroides. World J Microbiol Biotechnol. 2011;27:2723–8.
[10] Santhoshkumar T, Rahuman AA, Rajakumar G, Marimuthu S, Bagavan A, Jayaseelan C, et al. Synthesis of silver nanoparticles using Nelumbo nucifera leaf extract and its larvicidal activity against malaria and filariasis vectors. Parasitol Res. 2011;108:693–702.
[11] Shankar SS, Rai A, Ahmad A, Sastry M. Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. J Colloid Interface Sci. 2004;275:496–502.
[12] Ali DM, Thajuddin N, Jeganathan K, Gunasekaran M. Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. Colloids Surf B Biointerfaces. 2011;85:360–5.
[13] Ahmad M, Khan MA, Rashid U, Zafar M, Arshad M, Sultana S. Quality assurance of herbal drug valerian by chemotaxonomic markers. Afr J Biotechnol. 2009;8:1148–54.
[14] Jugran AK, Rawat S, Bhatt ID, Rawal RS. Valeriana jatamansi: An herbaceous plant with multiple medicinal uses. Phytother Res. 2019;33:482–503.
[15] Lin S, Chen T, Liu X-H, Shen Y-H, Li H-L, Shun L, et al. Iridoids and Lignans from Valeriana jatamansi. J Nat Products. 2010;73(4):632–8.
[16] Bakht J, Ali H, Khan MA, Khan A, Saeed M, Shafi M, et al. Antimicrobial activities of different solvents extracted samples of Linum usitatissimum by disc diffusion method. Afr J Biotechnol. 2011;10:19825–35.
[17] Bharathi D, Josebin MD, Vasantharaj S, Bhuvaneshwari V. Biosynthesis of silver nanoparticles using stem bark extracts of Diospyros montana and their antioxidant and antibacterial activities. J Nanostruct Chem. 2018;8:83–92.
[18] Sreekanth TVM, Ravikumar S, Eom IY. Green synthesized silver nanoparticles using Nelumbo nucifera root extract for efficient protein binding, antioxidant and cytotoxicity activities. J Photochem Photobiol B, Biol. 2014;141:100–5.
[19] Umoren SA, Obot IB, Gasem ZM. Green synthesis and characterization of silver nanoparticles using red apple (Malus domestica) fruit extract at room temperature. J Env Sci. 2014;5:907–14.
[20] Mittal AK, Kaler A, Banerjee UC. Free radical scavenging and antioxidant activity of silver nanoparticles synthesized from flower extract of Rhododendron dauricum. Nano Biomed Eng. 2012;4:118–24.

[21] Singh A, Jain D, Upadhyay MK, Khandelwal N, Verma HN. Green synthesis of silver nanoparticles using Argemone mexicana leaf extract and evaluation of their antimicrobial activities. Dig J Nanomater Biostruct. 2010;5:483–9.

[22] Ganaie SU, Abbasi T, Abbasi SA. Rapid and green synthesis of bimetallic Au-Ag nanoparticles using an otherwise worthless weed Antigonon leptopus. J Exp Nanosci. 2016;11:395–417.

[23] Awwad AM, Salem NM. Green synthesis of silver nanoparticles by Mulberry leaves extract. Nanosci Nanotechnol. 2012;2:125–8.

[24] Jeeva K, Thiyagarajana M, Elangovanb V, Geethac N, Venkatachalama P. Caesalpinia coriaria leaf extracts mediated biosynthesis of metallic silver nanoparticles and their antibacterial activity against clinically isolated pathogens. Ind Crop Prod. 2014;52:714–20.