REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 2 (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

Green Synthesis Of Silver Nanoparticles From *Stylosanthes Hamata* (L.) Taub. Characterization And Its Antimicrobial Potential

Mukeshbabu. N[1], Mary Kensa V[2]

[1]Research Scholar, Department of Botany, S.T. Hindu College, Nagercoil, Affiliated to MS University, Tirunelveli, Tamil Nadu, India-629002. *Email: mukeshbabu17598@gmail.com
[2*]Associate Professor, Department of Botany, S.T. Hindu College, Nagercoil, Affiliated to MS University, Tirunelveli, Tamil Nadu, India-629002. *Email: surejkensa@gmail.com

***Corresponding Author:** Dr. V. Mary Kensa
*Email: surejkensa@gmail.com

ABSTRACT
Among various noble metal nanoparticles, silver nanoparticles (AgNPs) have garnered significant attention due to their unique characteristics, including excellent electrical conductivity, chemical stability, as well as catalytic and antibacterial properties. The green synthesis of silver nanoparticles utilizing plant extracts rich in phytochemical compounds has gained widespread interest. This eco-friendly method is not only more biocompatible and cost-effective but also holds potential for large-scale production. The present study approved the ability of *Stylosanthes hamata* (L.) Taub. Plant extract for the synthesis of silver nanoparticles for the first time. The green synthesis silver nanoparticles were characterized using a UV-visible spectrophotometer, Fourier-transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and energy-dispersive X-ray analysis (EDX). The synthesized silver nanoparticles exhibited a crystalline structure with spherical shaped along with some irregular shapes with an average size of 5 to 100 nm. and a maximum absorbance at 422 nm. The synthesized silver nanoparticles were evaluated for antimicrobial activity. The result showed that the silver nanoparticles of *S. hamata* effectively inhibited *Klebsiella pneumonia*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, and *Pencillium notatum* growth with a maximum inhibition zone of 16 mm, 16mm, 14 mm, and 13 mm, respectively. The study demonstrates the effectiveness of *S. hamata* in the green synthesis of silver nanoparticles, which exhibited notable antimicrobial activity. This research underscores the potential of plant-mediated synthesis for developing eco-friendly antimicrobial agents.

Keywords: Green synthesis of silver nanoparticles; *Stylosanthes hamata* (L.) Taub., antimicrobial activity;

1. Introduction

Nanotechnology has emerged as a groundbreaking scientific advancement with broad applications across multiple domains, including medical diagnostics, pharmaceuticals, biomedicine, cosmetics, agriculture, as well as the food and feed industries (Kathiravan *et al*., 2023). Nanoscience focuses on manipulating and analyzing materials at the nanometer scale (ranging from 1 to 100 nm in diameter), where their properties differ significantly from those of bulk materials (Asif *et al*., 2022). Nanobiotechnology is an emerging field in medicine that utilizes nano-sized materials for targeted medicinal applications at the cellular or tissue level. The primary aim of nanotechnology is to develop and apply techniques for producing nanoparticles that can interact with molecular structures with high specificity, thereby maximizing therapeutic efficacy while minimizing side effects (Hashmi *et al*., 2024). Nanoparticles are distinguished by their minuscule size, large surface-area-to-volume ratio, and unique mechanical, optical, and magnetic properties, making them valuable in anticancer, antimicrobial, and antioxidant applications. Among various types of nanoparticles, noble metal nanoparticles—comprising materials such as silver and gold—have gained significant attention in biological research due to their diverse diagnostic applications (Soliman *et al*., 2024). Nanoparticles can be broadly classified into metallic and non-metallic types based on their composition. Metallic nanoparticles predominantly include materials like gold, silver, copper, cobalt, nickel, and semiconducting elements, whereas non-metallic nanoparticles are mainly carbon-based. Metallic nanoparticles have been extensively studied due to their distinctive electrical, optical, and catalytic properties (Alharbi *et al*., 2022). Among these, silver nanoparticles (AgNPs) have attracted particular interest because of their unique physical and biological characteristics (Sagar *et al*., 2024).

Nanoparticles can be synthesized using physical, chemical, or biological methods (Ejaz *et al*., 2024). However, physical and chemical synthesis techniques are often associated with environmental concerns, low efficiency, extreme reaction conditions, and the generation of hazardous byproducts. These drawbacks limit their use in medical applications (Soliman *et al*., 2024). As a result, biological synthesis methods have emerged as a more sustainable alternative. Biomolecules possess the ability to undergo controlled and hierarchical self-assembly, making them highly suitable for eco-friendly nanoparticles (Dipankar and Murugan, 2012). This green synthesis approach utilizes biological agents such as plant extracts, bacteria, fungi, or enzymes as reducing and capping agents during nanoparticle formation. Among these biological options, plant extracts are particularly promising due to their abundance, safety, and diverse range of bioactive

compounds that facilitate nanoparticle synthesis (Singh *et al*., 2024). Although the green synthesis of silver nanoparticles using plant extracts has been widely studied in numerous plant species, such as *Vernonia amygdalina* Delile (Tesfaye *et al*., 2023), *Azadirachta indica* A. Juss. (Prathibha *et al*., 2024), *Aloe fleurentiniorum* Lavranos & L.E. Newton (Jamil *et al*., 2024), *Ocimum tenuiflorum* L. (Alex *et al*., 2024), and *Mangifera indica* L. (Rana *et al*., 2023) this research field continues to gain interest due to the vast diversity of plant species and their ability to yield nanoparticles with distinct morphological characteristics. The present study is the first to report the green synthesis of silver nanoparticles using *Stylosanthes hamata*, a species belonging to the Fabaceae family. The synthesized nanoparticles were subsequently characterized using various analytical techniques, and their antimicrobial potential was assessed.

2. Materials and Methods

2.1 Sample Collection

The collected plant, *Stylosanthes hamata* (L.) Taub. belonging to the family Fabaceae, was collected from an S. T. Hindu college at Nagercoil in Kanyakumari District of Tamil Nadu, India. with an elevation of about 460 meters (Mean Sea Level with 8.11 Latitude and 77.53 Longitude).

2.2 Green Synthesis of Silver Nanoparticles

100 ml of *S. hamata* whole plant extract was mixed with 2.54 g of silver nitrate (0.05 M) in an Erlenmeyer flask and agitated for 24 hours at room temperature. Simultaneously, the positive (Only *S. hamata* whole plant extract) and negative (only silver nitrate aqueous solution) controls were maintained at similar conditions. The reaction solution was observed for colour change. The formed nanoparticles were collected by centrifuging the collected solution at 10,000 rpm for 10 minutes, and the pellets were washed with sterile distilled water and dried at 80 °C for 24 h. The nanoparticles were stored in a refrigerator for future studies (Umoren *et al*., 2014).

2.3 Characterization of silver nanoparticles

2.3.1 UV-visible spectroscopic analysis

The colour change of whole plant extract of *S. hamata* after mixing with silver nitrate was observed using UV-Vis spectroscopy at a wavelength of 300 -800 nm. A double beam UV- Visible Spectrophotometer (Perkin Elmer, Singapore) was used to detect the maximum peak based on the surface plasmon excitation of formed silver nanoparticles. The whole plant extract of *S. hamata* without silver nitrate was used as a blank for the spectrophotometer.

2.3.2 Fourier-transform infrared spectroscopy (FT-IR) analysis

The reduction of silver nanoparticles was investigated using FT-IR. Approximately 0.2 g of green synthesized silver nanoparticles was properly mixed with potassium bromide (KBr) and exposed to intense pressure to form a disc, which was scanned at a wavelength range of 400 to 4000 cm^{-1}.

2.3.3 X-ray diffraction spectroscopy analysis

A powder X-ray diffractometer (Bruker, Germany; model: D8 Advance) was used to examine the crystal structure of the silver nanoparticles fabricated from the whole plant extract of *S. hamata*. The X-ray patterns were obtained in 2 theta configurations with angles ranging from 10° to 100°.

2.3.4 SEM - EDAX analysis

The morphological characteristics of green synthesized silver nanoparticles from. *hamata* whole plant extract was analyzed using SEM. The sample was made into thin films by placing it on carbon-coated copper grids, and the images were documented. Furthermore, the elemental composition of the sample was determined using Energy Dispersive Analysis X-Ray (EDAX).

2.4 Anti-microbial activity of silver nanoparticles

2.4.1 Test Organisms

The test microorganisms used for antibacterial assay such as *Klebsiella pneumoniae*, *Bacillus subtilis, Staphylococcus aureus*, *Escherichia coli* and *Enterococcus sp* were purchased from Microbial Type Culture Collection and Gene Bank (*MTCC*) Chandigarh. The bacterial strains were maintained on Nutrient Agar (NA).

2.4.2 Anti-bacterial Test

The medium was prepared by dissolving 38 g of Mueller-Hinton Agar Medium (Hi Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 Lbs pressure at 121°C for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured into Petri plates (25 ml/plate). The plates were swabbed with pathogenic bacterial culture such as *Klebsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Lactobacillusacidophilus*. Finally, the sample loaded disc was then placed on the surface of Mueller-Hinton Agar medium. The standard drug Streptomycin 100 mg concentration disc was used for positive control and empty sterile disc was used for negative control. The plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones were examined around the disc (including disc) and measured with transparent ruler in millimetres (Kohner *et al.*, 1994; Mathabe *et al*., 2006).

2.4.3 Anti-fungal Test

Antibiotic susceptibility tests were determined by agar disc diffusion (Kirby-Bauer) method. Fungal strains such as *Aspergillus flavus* and *Pencillium notatum* were swabbed using sterile cotton swabs on SDA agar plate. Upto 80 µl of

each concentration of the nanoparticle respectively introduced into to the sterile discs (10 mm) using sterile pipettes. The standard drug Fluconazole 150 mg concentration was used as positive control and empty sterile disc was used as negative control. The disc was then placed on the surface of SDA medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 22°C for 48 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimetres (Bauer *et al*., 1966).

3. Results

3.1 Green Synthesis of Silver Nanoparticles

In the present study, the bioactive metabolites from the dried powder of *S. hamata* were used to synthesised silver nanoparticles initially exciting the reaction and the synthesis of silver nanoparticles was confirmed by the colour swift of the reaction mixture from green to darkish brown (Fig. 3.1). The formation of silver nanoparticles was further confirmed using different Characterization techniques.

3.2 Characterization of silver nanoparticles

UV-visible spectroscopy results showed that the absorbance of 422 nm was the peak for *S. hamata* mediated silver nanoparticles (Fig. 3.2). The FTIR analysis revealed peak values at wavenumbers 3263.12 cm^{-1}, 2916.28 cm^{-1},1727.36 cm^{-1}, 1535.44 cm^{-1}, 808.22 cm^{-1}, 668.80 cm^{-1}, and 646.38 cm^{-1} for synthesized silver nanoparticle synthesis from *S. hamata* (Fig. 3.3). The peak 3263.12 cm^{-1} corresponds to carboxylic acids, and 2916.28 cm^{-1} corresponds to alkanes. The absorption peak at 1727.36 cm^{-1} indicates the presence of aldehydes. amide (C=O) presence was confirmed with the wavenumber 1535.44 cm^{-1}. The absorption spectra at 808.22 cm^{-1} represent the presence of the Mise. The presence of amines was observed at 668.80 cm^{-1}. The wavenumber 646.38 cm^{-1} denotes the alkyl halides. However, the existence of these functional groups validates the role of diverse reducing and stabilizing agents in the synthesis of silver nanoparticles. XRD analysis was used to investigate the crystalline structure of silver nanoparticles. Fig. 3.4 depicts the XRD pattern of synthesized silver nanoparticles. Results indicated that the seven sharp and intense peaks at 2θ angles of 11.434, 20.727,27.93,28.826, 29.74,30.772, and 46.63, respectively. Applying the Scherrer equation, the determined the crystal size of the fabricated silver nanoparticles was determined to be 5.25 nm. The SEM image of synthesized silver nanoparticles through *S. hamata* was shown in Fig. 3.5. The SEM picture demonstrates that some nanoparticles were spherical-shaped along with some irregular shapes with an average size of 5 to 100 nm. The EDAX analysis of silver nanoparticles of *S. hamata* had the maximum weight element in the sample with a percentage of 55.15. The EDAX chart also showed peaks such as O, Al, and Cl with a weight percentage of 39.05%, 3.02%, and 2.79%, respectively (Fig. 3.6 and Table 3.1).

3.3 Anti-antimicrobial activity for silver nanoparticles

The antimicrobial activity of silver nanoparticle synthesis from the whole plant dried powder of *S. hamata* was studied against the strains (*Escherichia coli, Bacillus subtilis, Klebsiella pneumonia, Staphylococcus aureus* and *Lactobacillus acidophilus*) and three fungal strains (*Aspergillus niger, Aspergillus flavus* and *Pencillium notatum)*. The result showed that the silver nanoparticle of *S. hamata* effectively inhibited *Klebsiella pneumonia*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, and *Pencillium notatum* growth with a maximum inhibition zone of 16 mm, 16mm, 14 mm, and 13 mm, respectively. The zone of inhibition of various extracts against different pathogens was presented in Table 3.2, Fig. 3.7 and Fig. 3.8. the antibiotic (streptomycin and Fluconazole) exhibited maximum inhibition zone against *Bacillus subtilis* (21mm), *Staphylococcus aureus* (23 mm), *Lactobacillus acidophilus* (22 mm) and *Penicillium notatum* (20 mm), respectively.

a b

Figure 3.1: Visible observation; A) silver nitrate with whole plant extract of *S. hamata,* B) Synthesised silver nanoparticles from the whole plant extract of *S. hamata*

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 2 (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

UV-visible spectrum

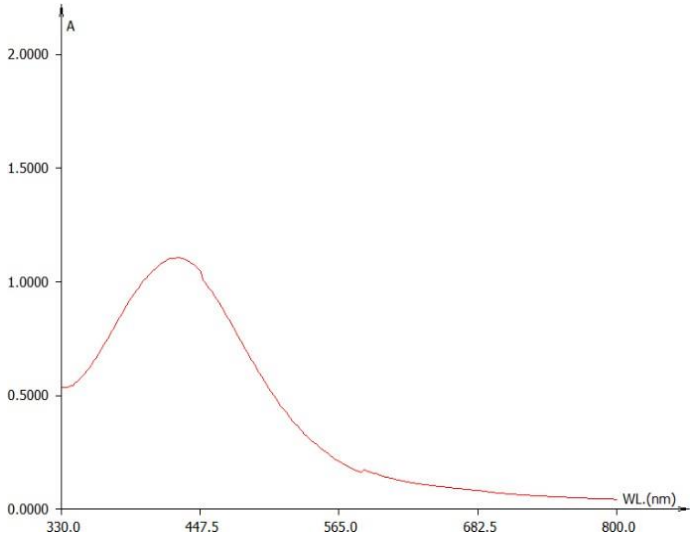

Figure 3.2: UV-visible spectra of synthesised silver nanoparticles from *S. hamata*

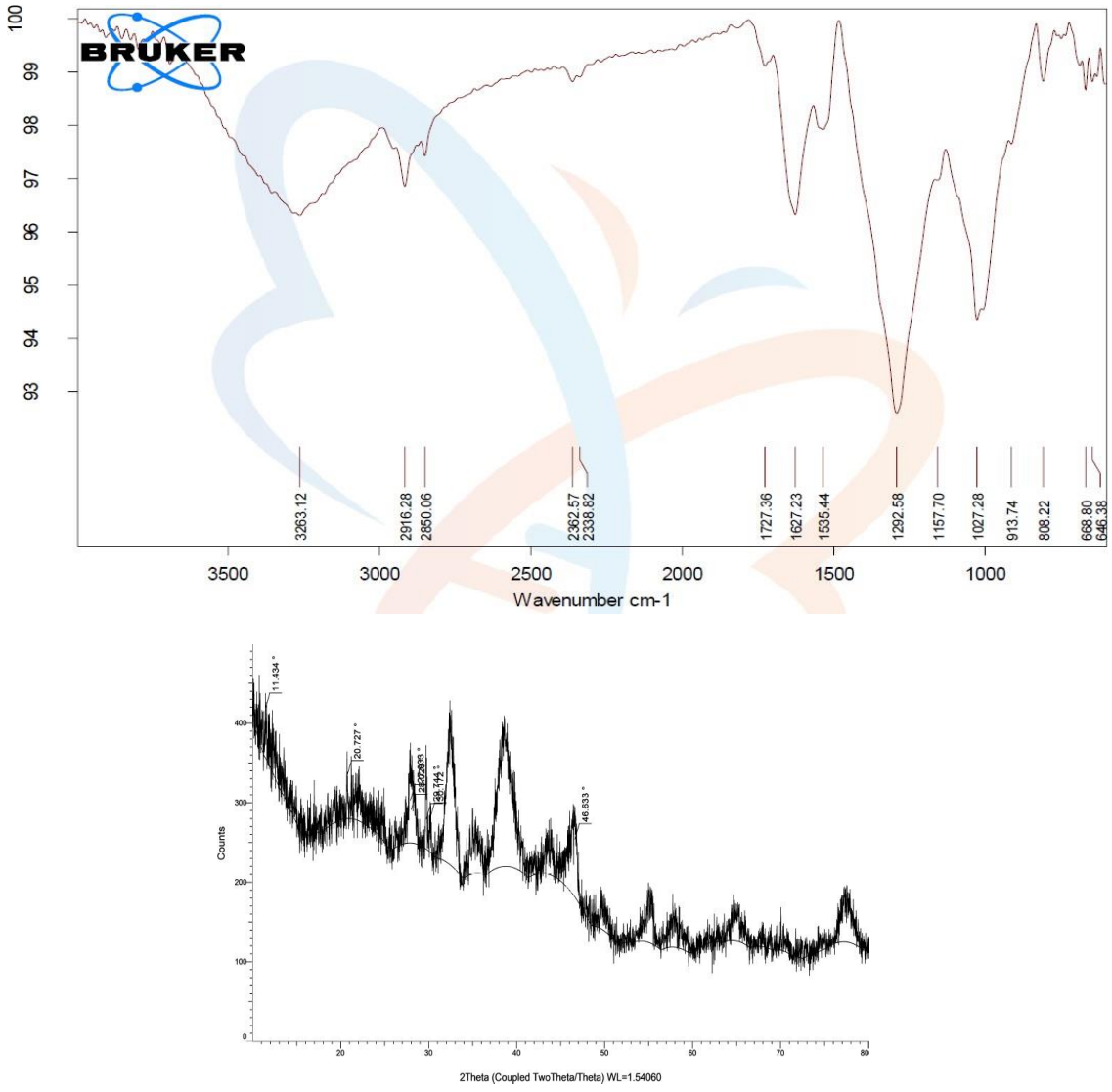

Figure 3.3: FTIR analysis of silver nanoparticles obtained from *S. hamata*

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 2 (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

XRD spectrum

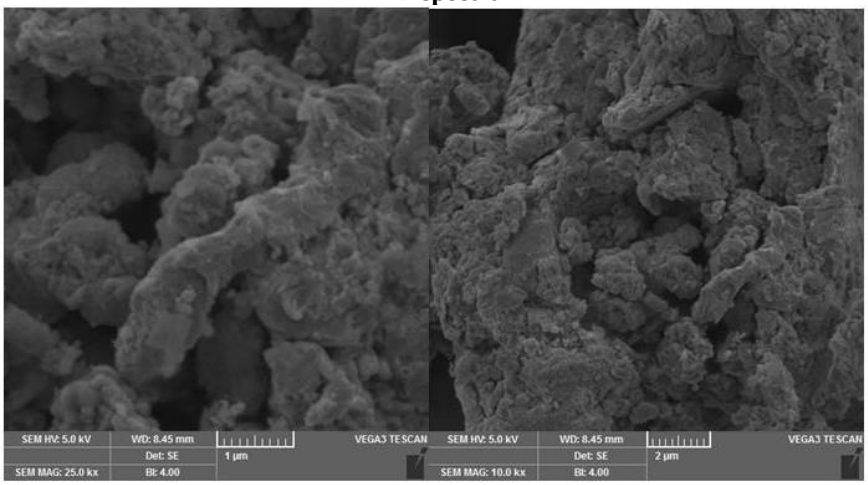

Figure 3.4: XRD pattern of silver nanoparticles of *S. hamata*

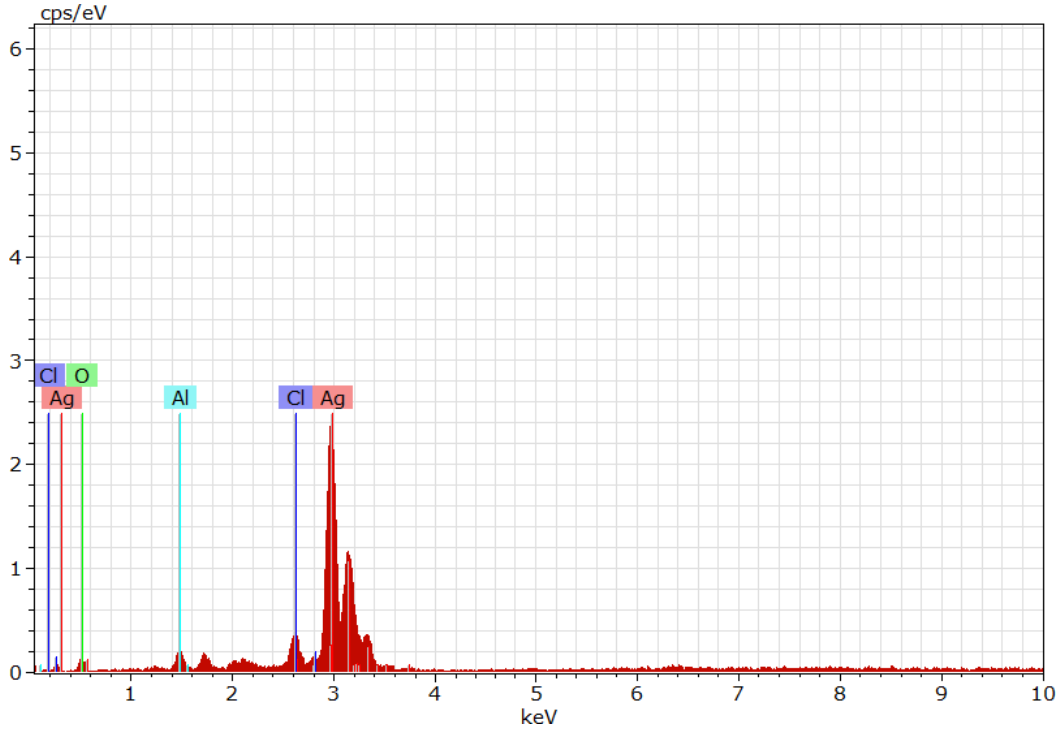

Figure 3.5: SEM image of synthesised silver nanoparticles of *S. hamata*

Figure 3.6: EDAX chart of the synthesised silver nanoparticles of *S. hamata*

Table 3.1: EDAX analysis showing element composition of silver nanoparticles

S. No	Elements	Normal weight (%)	Atom weight (%)
1	Silver (Ag)	88.80	55.15
2	Oxygen (O)	9.25	39.05
3	Aluminum (Al)	1.20	3.02
4	Chlorine (Cl)	1.46	2.79

Table 3.2: Anti-microbial activity of silver nanoparticles of S. *hamata*

Pathogens	**SH-Ag**	**+ ve control**	**- ve control**
Bacteria	**Zone of inhibition (mm)**		
Escherichia coli	12	19	NZ
Bacillus subtilis	13	21	NZ
Klebsiella pneumoniae	16	15	NZ
Staphylococcusaureus	14	23	NZ
Lactobacillusacidophilus	16	22	NZ
Fungus			
Aspergillus niger	10	19	NZ
Aspergillus flavus	NZ	19	NZ
Penicillium notatum	13	20	NZ

NZ = No Zone
SH-Ag = *Stylosanthes hamata* plant extract-coated Ag nanoparticles

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 2 (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

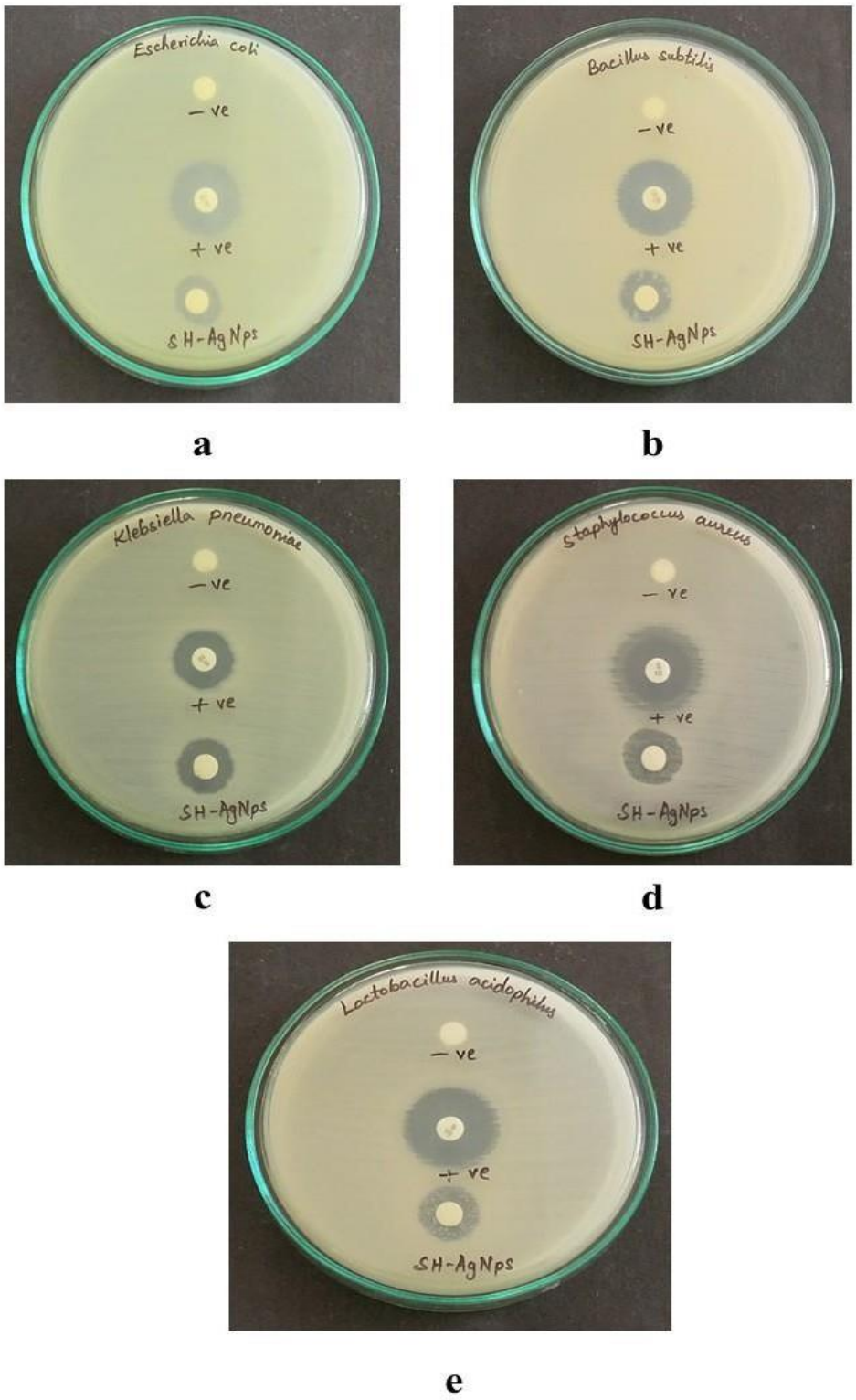

Figure 3.7: Anti-bacterial activity of silver nanoparticles of *S. hamata* A) *E. coli,* B) *Bacillus subtilis,* C) *Klebsiella pneumoniae,* D) *Staphylococcus aureus* and E) *Lactobacillus acidophilus*

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 2 (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

a **b** **c**

Figure 3.8: Anti-fungal activity of silver nanoparticles of S. hamata
a) Aspergillus niger, b) Aspergillus flavus, c) *Penicillium notatum*

4. Discussion

The development of an appropriate application for microbe-mediated nanoparticle synthesis is an interesting and emerging research area for future sustainable industrial production. Furthermore, fungi have recently been recognized as the most promising source for the fabrication of various nanoparticles (Hussein *et al.,* 2022). Silver nanoparticles (AgNPs) synthesized from plant extracts have sparked considerable interest due to their distinctive properties and wide-ranging applications in medicine, environmental science, and biotechnology. (Pandian *et al*., 2015).

UV spectroscopic characterization of silver nanoparticles exhibited a maximum absorption peak at 422 nm. The obtained findings correspond with Salayová *et al.* (2021), who reported that the maximum surface plasmon response for silver nanoparticles produced by the leaves extract of *Capsella bursa-pastoris* (L.) Medik. was reported at 422 nm. Silver nanoparticles produced from flowers of *Calotropis gigantea* (L.) W.T. Aiton showed a maximum absorption peak at 422 nm (Mathew *et al.,* 2020). Nahar *et al.* (2020) reported a maximum absorption peak of 422 nm for silver nanoparticles from *Clerodendrum infortunatum* L. leaves aqueous extract.The silver nanoparticles formation from *S. hamata* was further confirmed using FTIR studies (Fig. 3.3). The biological activities and high adsorptive capacity of silver nanoparticles can be related to interactions between the nanoparticles and functional groups found in such as carboxylic acid, alkanes, amides and amines (Widatalla *et al.,* 2022). The analysis of crystalline structure of silver nanoparticles fabricated from *Aloe vera* extract was studied using X-ray diffraction (XRD). The XRD analysis shows the face-centred cubic crystalline structure. In another study, the XRD examination of AgNPs produced Bragg reflection peaks at 2 Ɵ angel111,200,200, and 311 were observed in the examination after biological reduction and stabilization with *Aloe vera*, these confirm that there is a cubic lattice structure centred on the nanoparticle face (Kumar *et al*., 2022). According to SEM studies, the size of synthesized silver nanoparticles from *S. hamata* was approximately 5 nm to 100 nm, and the shape of the nanoparticles was found to be spherical. According to Sudha *et al*. (2018), the nanoparticles synthesized from the agglomerated nanoparticles were measured to be between 287.5 and 293.2 nm in size, although the usual particle dimension is predicted to be 70 nm. Moreover, Paul and Sharma (2020), synthesized AgNps that were mostly spheroid with a usual size of 25 nm. EDX analysis of silver nanoparticles from *S. hamata*, Ag had the greatest weight element in the sample, with percentages of 88.80 and atomic percentages of 55.15. The EDX graph showed several weak peaks, such as Ag and Al, with weight percentages of 63.44 % and 21.37%, respectively (Palithya *et al.,* 2022).

The present study demonstrated a significant antimicrobial activity of silver nanoparticles derived from the whole plant extract of *S. hamata*. This activity was observed against various bacterial strains, including *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus* and *Lactobacillus acidophilus*, as well as fungal strains such as *Aspergillus niger, Aspergillus flavus* and *Penicillium notatum.* Shukla and Makwana (2014) conducted a study that investigated the antimicrobial properties of silver nanoparticles synthesized from *Vitex negundo* L. against various bacterial strains, including *Escherichia coli, Bacillus subtilis, Staphylococcus aureus,* and *Bacillus megaterium.* In contrast to our current investigation, the synthesis of silver nanoparticles from *Vitex negundo* exhibited a lower zone of inhibition against *E. coli, B. subtilis,* and *S. aureus.* Murugan *et al*. (2014) conducted a study examining the antimicrobial activity of silver nanoparticles synthesized from *Acacia leucophloea*. The results demonstrated that this extract exhibits significant antimicrobial efficacy against various bacterial strains. In comparison, our current research has yielded similar zones of inhibition against the bacterial strain *Staphylococcus aureus*. In this study, nanoparticles synthesized from *S. hamata* extract have strong antimicrobial properties towards *Escherichia Coli*, (Gram-negative), *Bacillus subtilis* (Gram-positive), with inhibition zones of 12mm and 13mm, respectively. similarly, it was observed that nanoparticles synthesized from *Ocimum sanctum* L. showed the inhibition zone, but the maximum inhibition zone was shown against *Bacillus*

subtilis (14 mm) and *Escherichia Coli* (14 mm) (Banerjee *et al*., 2014). Similarly, plant-based natural products were reported to synthesize MONPs for various biological applications (Govindasamy *et al*., 2018). Nano particles of Ag, Au, Pt, Cu, Zn, Ti, Mg, ZnO, MgO, FeO, and TiO2 have been reported for their numerous medical applications (Bachheti *et al*., 2019; Hemlata *et al*., 2020; Xu *et al*., 2020; Ammulu *et al*., 2021; Montes-Garcia *et al*., 2021).

5. Conclusions

The present study has unveiled that the plant extract of *S. hamata* contains metabolites that offer the potential for synthesizing silver nanoparticles without the requirement for additional chemical reductants. It was found that various biomolecules of the *S. hamata* plant extract were responsible for the production of the silver nanoparticle and their stability. We validated the presence of silver nanoparticles through a variety of characterization techniques, including UV-visible spectroscopy, FTIR, XRD and SEM-EDX. These synthesized silver nanoparticles demonstrated antagonistic activity against the Staphylococcus aureus, Bacillus subtilis, Lactobacillus acidophilus and Penicillium notatum. Our in vitro antimicrobial experiment has demonstrated the significant potential of silver nanoparticles and can be employed in the formulation of antimicrobial drugs. Further, in vivo studies are essential to enhance our understanding of the mechanisms of action and to explore the broader biological applications of these nanoparticles.

References

1. Kathiravan, A., Udayan, E., Rajeshkumar, S., & Gnanadoss, J. J. (2023). Unveiling the Biological Potential of Mycosynthesized Selenium Nanoparticles from Endophytic Fungus Curvularia sp. LCJ413. *BioNanoScience*, *13*(4), 2232-2251.
2. Asif, M., Yasmin, R., Asif, R., Ambreen, A., Mustafa, M., & Umbreen, S. (2022). Green synthesis of silver nanoparticles (AgNPs), structural characterization, and their antibacterial potential. *Dose-Response*, *20*(2), 15593258221088709.
3. Hashmi, S. S., Ibrahim, M., Adnan, M., Ullah, A., Khan, M. N., Kamal, A., ... & Zaman, W. (2024). Green synthesis of silver nanoparticles from Olea europaea L. extracted polysaccharides, characterization, and its assessment as an antimicrobial agent against multiple pathogenic microbes. *Open Chemistry*, *22*(1), 20240016.
4. Soliman, M. K., Hashem, A. H., Al-Askar, A. A., AbdElgayed, G., & Salem, S. S. (2024). Green synthesis of silver nanoparticles from Bauhinia variegata and their biological applications. *Green Processing and Synthesis*, *13*(1), 20240099.
5. Alharbi, N. S., Alsubhi, N. S., & Felimban, A. I. (2022). Green synthesis of silver nanoparticles using medicinal plants: Characterization and application. *Journal of Radiation Research and Applied Sciences*, *15*(3), 109-124.
6. Sagar, P. V., Ramadevi, D., Basavaiah, K., & Botsa, S. M. (2024). Green synthesis of silver nanoparticles using aqueous leaf extract of Saussurea obvallata for efficient catalytic reduction of nitrophenol, antioxidant, and antibacterial activity. *Water Science and Engineering*, *17*(3), 274-282.
7. Ejaz, U., Afzal, M., Mazhar, M., Riaz, M., Ahmed, N., Rizg, W. Y., ... & Yean, C. Y. (2024). Characterization, synthesis, and biological activities of silver nanoparticles produced via green synthesis method using Thymus vulgaris aqueous extract. *International Journal of Nanomedicine*, 453-469.
8. Soliman, M. K., Hashem, A. H., Al-Askar, A. A., AbdElgayed, G., & Salem, S. S. (2024). Green synthesis of silver nanoparticles from Bauhinia variegata and their biological applications. *Green Processing and Synthesis*, *13*(1), 20240099.
9. Dipankar, C., & Murugan, S. (2012). The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from Iresine herbstii leaf aqueous extracts. *Colloids and surfaces B: biointerfaces*, *98*, 112-119.
10. Singh, R. K., Nallaswamy, D., Rajeshkumar, S., & Varghese, S. S. (2024). Green synthesis of silver nanoparticles using neem and turmeric extract and its antimicrobial activity of plant mediated silver nanoparticles. *Journal of Oral Biology and Craniofacial Research*, *15*(2), 395-401.
11. Tesfaye, M., Gonfa, Y., Tadesse, G., Temesgen, T., & Periyasamy, S. (2023). Green synthesis of silver nanoparticles using Vernonia amygdalina plant extract and its antimicrobial activities. *Heliyon*, *9*(6).
12. Prathibha, B. S., Harshitha, N., Neha, D. R., Pranathi, C. N., Kumar, D. V., & Lakshmi, G. C. (2024, April). Green synthesis of silver nanoparticles using Ocimum tenuiflorum and Azadirachta indica leaf extract and their antibacterial activity. In *Journal of Physics: Conference Series* (Vol. 2748, No. 1, p. 012015). IOP Publishing.
13. Jamil, Y. M., Al-Hakimi, A. N., Al-Maydama, H. M., Almahwiti, G. Y., Qasem, A., & Saleh, S. M. (2024). Optimum green synthesis, characterization, and antibacterial activity of silver nanoparticles prepared from an extract of Aloe fleurentinorum. *International Journal of Chemical Engineering*, *2024*(1), 2804165.
14. Alex, A. M., Subburaman, S., Chauhan, S., Ahuja, V., Abdi, G., & Tarighat, M. A. (2024). Green synthesis of silver nanoparticle prepared with Ocimum species and assessment of anticancer potential. *Scientific Reports*, *14*(1), 11707.
15. Rana, A., Kumari, A., Chaudhary, A. K., Srivastava, R., Kamil, D., Vashishtha, P., & Sharma, S. N. (2023). An investigation of antimicrobial activity for plant pathogens by green-synthesized silver nanoparticles using Azadirachta indica and Mangifera indica. *Physchem*, *3*(1), 125-146.

16. Sadeghi, B., & Gholamhoseinpoor, F. (2015). A study on the stability and green synthesis of silver nanoparticles using Ziziphora tenuior (Zt) extract at room temperature. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *134*, 310-315.
17. Kohner PC, Rosenblatt JE, Cockerill FR.1994. Comparison of agar dilution, broth dilution and disk diffusion testing of Ampicillin against Haemophilus spp. by using in house and commercially prepared media J. Clin. Microbiol. 32, 1594 -96.
18. Mathabe M.C., Nikolova R.V., Lall N., Nyazema N.Z.Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa, Journal of Ethnopharmacology, 105 (2006), pp. 286-293.
19. Bauer A W, Kirby W M M, Sherris J C & Turck M. Antibiotic susceptibility testing by a standardized single disk method. Amer. I. C/in. Pathol. 45:493-6, 1966. [Depts. Microbiology and Medicine, Univ. Washington, Sch. Med., Seattle. WA]
20. Hussein, H. G., El-Sayed, E. S. R., Younis, N. A., Hamdy, A. E. H. A., and Easa, S. M. (2022). Harnessing endophytic fungi for biosynthesis of selenium nanoparticles and exploring their bioactivities. AMB Express, 12(1), 1-16.
21. Pandian, A. M. K., Karthikeyan, C., Rajasimman, M., & Dinesh, M. G. (2015). Synthesis of silver nanoparticle and its application. *Ecotoxicology and environmental safety*, *121*, 211-217.
22. Salayová, A., Bedlovičová, Z., Daneu, N., Baláž, M., Lukáčová Bujňáková, Z., Balážová, Ľ., & Tkáčiková, Ľ. (2021). Green synthesis of silver nanoparticles with antibacterial activity using various medicinal plant extracts: Morphology and antibacterial efficacy. *Nanomaterials*, *11*(4), 1005.
23. Mathew, S., Victorio, C. P., Sidhi, J., & BH, B. T. (2020). Biosynthesis of silver nanoparticle using flowers of Calotropis gigantea (L.) WT Aiton and activity against pathogenic bacteria. *Arabian Journal of Chemistry*, *13*(12), 9139-9144..
24. Nahar, K., Yang, D. C., Rupa, E. J., Khatun, M., & Al-Reza, S. M. (2020). Eco-friendly synthesis of silver nanoparticles from Clerodendrum viscosum leaf extract and its antibacterial potential. *Nanomedicine Research Journal*, *5*(3), 276-287.
25. Widatalla, H. A., Yassin, L. F., Alrasheid, A. A., Ahmed, S. A. R., Widdatallah, M. O., Eltilib, S. H., & Mohamed, A. A. (2022). Green synthesis of silver nanoparticles using green tea leaf extract, characterization and evaluation of antimicrobial activity. *Nanoscale Advances*, *4*(3), 911-915
26. Kumar S, Kalita S, Das A, Kumar P, Singh S, Katiyar V. 2022. *Aloe vera*: A contemporary overview on scope and prospects in food preservation and packaging. Progress in Organic Coatings. 166:106799.
27. Paul, W, Sharma, CP. 2020. Inorganic nanoparticles for targeted drug delivery. Biointegration of medical implant materials.333-73.
28. Shukla, A., & Makwana, B. A. (2014). Facile synthesis of silver nanoparticle and their potential application. *Am J Nanosci Nanotechnol*, (4), 84-92.
29. Murugan, K., Senthilkumar, B., Senbagam, D., & Al-Sohaibani, S. (2014). Biosynthesis of silver nanoparticles using *Acacia leucophloea* extract and their antibacterial activity. *International Journal of Nanomedicine*. 2431-2438.
30. Banerjee, P., Satapathy, M., Mukhopahayay, A., & Das, P. (2014). Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresources and Bioprocessing*, *1*, 1-10.
31. Govindasamy, R., Muthu, T., Govindarasu, M., Thandapani, G., Ill-Min, C., 2018. Green Approach for Synthesis of Zinc Oxide Nanoparticles from *Andrographis paniculata* Leaf Extract and Evaluation of Their Antioxidant, Anti-Diabetic, and AntiInflammatory Activities. Biopro. Biosyst. Eng. 41, 21–30.
32. Bachheti, R.K., Konwarh, R., Gupta, V., Husen, A., Joshi, A., 2019. Green Synthesis of Iron Oxide Nanoparticles: Cutting Edge Technology and Multifaceted Applications. In In Nanomaterials and Plant Potential; Husen, A., Iqbal, M., Eds.; Springer, Cham, 239–259.
33. Hemlata, M.P.R., Singh, A.P., Tejavath, K.K., 2020. Biosynthesis of Silver Nanoparticles Using *Cucumis prophetarum* Aqueous Leaf Extract and Their Antibacterial and Antiproliferative Activity against Cancer Cell Lines. ACS Omega 5, 5520–5528
34. Xu, L., Wang, Y.Y., Huang, J., Chen, C.Y., Wang, Z.X., Xie, H., 2020. Silver Nanoparticles: Synthesis, Medical Applications and Biosafety. Theranostics 10, 8996–9031.
35. Ammulu, M.A., Vinay, V.K., Giduturi, A.K., Vemuri, P.K., Mangamuri, U., Poda, S., 2021. Phytoassisted Synthesis of Magnesium Oxide Nanoparticles from *Pterocarpus marsupium* Rox.b Heartwood Extract and Its Biomedical Applications. J. Genet. Eng. Biotechnol. 19, 1–18.
36. Montes-Garcia, V., Squillaci, M.A., Diez-Castellnou, M., Ong, Q.K., Stellacci, F., Samorì, P. 2021. Chemical Sensing with Au and Ag Nanoparticles. Chem. Soc. Rev. 50, 1269–1304
37. Palithya, S., Gaddam, S. A., Kotakadi, V. S., Penchalaneni, J., Golla, N., Krishna, S. B. N., & Naidu, C. V. (2022). Green synthesis of silver nanoparticles using flower extracts of Aerva lanata and their biomedical applications. *Particulate Science and Technology*, *40*(1), 84-96.