

Sustainable Silver Nanoparticle Synthesis Using *Saussurea Lappa* and *Glycyrrhiza Glabra*: Exploring its Role in Antimicrobial Strategies

Devanshi Vaghela^{1*}, Rajendra Pal Singh Rathore²

¹Research Scholar, Faculty of Bhupal -Noble's University, Udaipur, Rajasthan, India

²Associate professor, Faculty of Bhupal Noble's University, Udaipur, Rajasthan, India

*Corresponding Author: Devanshi Vaghela, Faculty of Pharmacy, Bhupal Noble's University, Udaipur, Rajasthan, India. Email: devanshivaghela175@gmail.com;

Cite this paper as: Devanshi Vaghela, Rajendra Pal Singh Rathore (2024). Sustainable Silver Nanoparticle Synthesis Using *Saussurea Lappa* and *Glycyrrhiza Glabra*: Exploring its Role in Antimicrobial Strategies. *Frontiers in Health Informatics*, Vol.13, No.8, 6568-6582

ABSTRACT

The environmentally safe and sustainable development of silver nanoparticles (AgNPs) have garnered increasing attention from scientists and researchers. Green synthesis provides a resilient and cost-effective approach to silver nanoparticle (AgNP) production, making it highly suitable for applications in medicine and pharmaceuticals. This green approach minimizes environmental impact while offering a viable alternative to conventional nanoparticle production methods. This research introduces an efficient approach to develop silver nanoparticles (AgNPs) utilizing silver nitrate (AgNO₃) and extracts from medicinal plants *Saussurea lappa* and *Glycyrrhiza glabra*, both recognized for their bioactive properties. Silver nanoparticles (AgNPs) were generated through a controlled chemical reaction involving 1 mM silver nitrate with extracts of *Saussurea lappa* and *Glycyrrhiza glabra*. The resulting nanoparticles were thoroughly characterized and assessed for their antimicrobial qualities. UV-visible spectrophotometry was used to comprehensively examine the produced AgNPs. Both gram-positive and gram-negative bacteria were considerably more susceptible to the antibacterial action of the generated AgNPs.

Keywords: Silver nanoparticles, green synthesis, bioactive compounds, *Saussurea lappa*, *Glycyrrhiza glabra*, UV-visible spectrophotometry, antimicrobial action

INTRODUCTION

Silver-containing nanoparticles (AgNPs) are extensively recognized for strong antimicrobial activity and ability to inhibit microbial growth effectively. However, the increasing tolerance of pathogenic microorganism to antimicrobial agents has become a significant challenge for the healthcare industry, leading to extensive research on the topic.^[1] Generally, nanoparticles are defined as particles with a size of less than 100 nanometres. Although chemical and physical techniques are capable of yielding highly pure and unerringly established nanoparticles, they are often costly, energy-intensive, and pose environmental risks. To address these concerns, this paper explores an eco-friendly method for synthesizing AgNPs through extracellular biosynthesis. Biosynthesis techniques utilize plant extracts to generate nanoparticles. One particularly promising approach within biosynthetic processes is the application of botanical extracts in nanoparticle production. Green synthesis methods have recently gained attention as an important and evolving sector of nanotechnology.^[2]

Nanobiotechnology as of now is among most expeditiously advancing fields in High-tech material engineering. Within this discipline, plants and plant-based derivatives perform a fundamental role in formulation of Nanomaterials. [3] Selection of plants *Saussurea lappa* and *Glycyrrhiza glabra* in this study is rooted in its well-documented medicinal properties.

Saussurea Lappa from the Asteraceae family is a renowned medicinal plant. commonly known as costus in English, this plant is recognized by various regional names in India, including Kur in Bengali, Kuth in Gujarati, Sepuddy in Malayalam, Postkhai in Punjabi, and Kushta in Sanskrit. [4] It contains a variety of bioactive compounds, including Sesquiterpene lactones, Essential oils, Inulin, Taraxasterol, Beta-sitosterol.

Glycyrrhiza Glabra belonging to *Fabaceae* family, is a popular medicinal plant and commonly known as Licorice, Yashtimadhu, Mulaithi, Jethimadh. *Glycyrrhiza glabra* exhibits a broad array of therapeutic effects including Anti-inflammatory, Antimicrobial, Anticancer and Antioxidant properties. [5] Licorice is rich in bioactive compounds, including Glycyrrhizin, Flavonoids, Phenolic compound, Polysaccharides, Sterols and Alkaloids. [6]

This paper presents a prompt and streamlined process for synthesizing nanocomposites using botanical extracts of *Saussurea Lappa* and *Glycyrrhiza glabra*. It also interpretate and investigate suppressive activity against bacterial strains of Gram-negative and Gram-positive bacteria. AgNPs were exposed to Gram-positive microscopic organisms such as *Staphylococcus aureus* and *Enterococcus faecalis*, as well as Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*. [7]

Silver nanoparticles are recognized for their antibacterial properties, though their precise mechanism remains under study. Research suggests that when these nanoparticles attach to bacterial cell membranes, they may disrupt respiration and permeability, leading to cellular instability. Additionally, silver ions have been found to interfere with DNA replication in bacteria, preventing their reproduction [8].

MATERIALS AND METHOD

Materials

Dry stems of plant *Saussurea lappa* and dry roots of plant *Glycyrrhiza glabra* were purchased from a wholesale dealer named Batliwala, Dhan Mandi, Udaipur as shown in Figure 1. The specimen copy of samples was kept in the Pharmacognosy Laboratory. The Pharmacognosist authenticated the plant samples. The details of the authentication are presented in Figure 2. The plant samples were kept at room temperature for three days and later transferred in an incubator adjusted at 37°C for a duration of 24 hours. Following completion of the incubation period, dried specimens were separately granulated into finely crushed powders with a sterile mortar and pestle as shown in Figure 3. The powdered samples were then stored in jar at room temperature for subsequent use.



Figure 1: Images of dried sample of (a) *Saussurea Lappa* and (b) *Glycyrrhiza Glabra*

BHUPAL NOBLES' COLLEGE OF PHARMACY
FACULTY OF PHARMACY, BHUPAL NOBLES' UNIVERSITY
(Promoted) by : Vidya Pracharini Sabha, Estd. 1923, Bhupal Nobles' Sansthan, Udaipur
Approved by Pharmacy Council of India, New Delhi

Speed post / Speed Post AD / Registered AD/ Courier / UPC / Ordinary Post / By Hand

BNCP/24/1060

DEPARTMENT OF PHARMACOGNOSY
AUTHENTICATION CERTIFICATE
TO WHOMSOEVER IT MAY CONCERN

This is to certify that the following botanical species selected for Ph.D Research work of Devanshi Vaghela have been identified and authenticated by Professor Dr. Y. S. Sarangdevot, Professor in Pharmacognosy, B.N.College of Pharmacy, Udaipur, Rajasthan.

| Sr. No. | Common Name | Plant part | Botanical Species | Family | Authentication Number |
|---------|-------------|------------|------------------------------|----------------------|-----------------------|
| 1. | Chakwad | Seeds | <i>Cassia tora</i> | <i>Fabaceae</i> | BNCP/24/DV-1 |
| 2. | Kuth | Stem | <i>Saussurea lappa</i> | <i>Asteraceae</i> | BNCP/24/DV-2 |
| 3. | Daruhaldi | Bark | <i>Berberis aristata</i> | <i>Berberidaceae</i> | BNCP/24/DV-3 |
| 4. | Haldi | Rhizome | <i>Curcuma longa</i> | <i>Zingiberaceae</i> | BNCP/24/DV-4 |
| 5. | Mulethi | Roots | <i>Glycyrrhiza glabra</i> | <i>Fabaceae</i> | BNCP/24/DV-5 |
| 6. | Lodh | Bark | <i>Symplocos racemosa</i> | <i>Symplocaceae</i> | BNCP/24/DV-6 |
| 7. | Chameli | Leaves | <i>Jasminum grandiflorum</i> | <i>Oleaceae</i> | BNCP/24/DV-7 |
| 8. | Kaner | Leaves | <i>Nerium oleander</i> | <i>Apocynaceae</i> | BNCP/24/DV-8 |

Dr. Y.S. Sarangdevot
Professor (Pharmacognosy)
Professor
Bhupal Nobles' College of Pharmacy
Udaipur (Rajasthan)

Phone : 0294-2413182 (O), Email : bnpharmacy@gmail.com, Website : www.bnuniversity.ac.in

Figure 2: Letter of authentication



Figure 3: Powdered sample of (a) *Saussurea lappa* and (b) *Glycyrrhiza Glabra*

Method

Plant Extract preparations

The powdered samples from each dried plant, weighing 10 grams each, were combined with 100 mL distilled water in a 250ml flask. The samples underwent heat treatment using a water bath regulated at 80°C for a time period of 20 minutes. On reaching environmental temperature, the mixtures were carefully strained using a sterile stainless-steel strainer and subsequently passed through Whatman filter paper.

The obtained extracts were then collected individually and refrigerated at 4°C for preservation. Throughout the entire experiment, strict sterility measures were upheld to ensure precision, reliability, and contamination-free results.

Qualitative Phytochemical Assessment

Phytochemical analysis was conducted on plant extracts to discover existence of various natural metabolites in them. Alkaline reagent test, Froth formation, Liebermann-Burchard reaction, Ferric Chloride test, Quinone identification, Salkowski procedure and Keller Killiani test were conducted to identify the presence of bioflavonoid substance, foaming agents, steroids, botanical tannic compounds, quinones, bioactive terpenes and glycosides respectively. [9] The findings are displayed in Tables 1 and 2.

Flavonoids (Alkaline Reagent Test): A 0.1 ml portion of plant-derived solution when added to 2 ml dilute sodium hydroxide. Intense yellow colour indicates flavonoids.

Saponins (Froth Test): A solution of 0.5 ml natural plant extract and 2.5 ml distilled water was agitated vigorously. Froth indicates presence of saponins.

Steroids (Liebermann-Burchard Test): 0.1 ml natural plant concentrate mixed with 1 ml chloroform, 1mL acetic anhydride and few drops of strong sulfuric acid produce red colour in lower layer indicating existence of steroid compounds.

Qualitative test for tannins with ferric chloride: A solution was formed by mixing 1 ml plant extract with 1 ml distilled water, then incorporating a few drops of 5% ferric chloride. A distinct green precipitate confirms tannin presence in the solution.

Quinones: Herbal extract measuring 0.5 ml was poured to 0.2 ml strong hydrochloric acid produce yellow colour verifying quinones presence.

Terpenoid screening through the Salkowski test: The mixture was formed by combining 1 ml

plant extract with 0.4 mL of chloroform and to it 0.2 ml purified sulfuric acid was introduced. red-brown colour indicates terpenoids.

Glycosides (Keller Killiani Test): 1ml plant extract was blend with 0.4 ethanoic acid and 0.2 ml ferric chloride solution. To it 0.2 ml purified sulfuric acid was added. Reddish brown ring indicates presence of Glycosides.

Silver nanoparticle (Ag NP) preparations

Saussurea lappa:

A silver nitrate (AgNO_3) solution with a 1 mM concentration was made in a 250 ml container. It was placed on magnetic stirrer and agitated for 15 minutes at room temperature under dark condition and plant extract and silver nitrate were added in 1:5 ratio in small aliquots at an interval of every 1 minute for reduction into Ag^+ ions. ^[10] ^[11]

It was further kept on magnetic stirrer for 20 minutes at 60 °C. A shift in color was observed, changing from deep-yellow to greenish-brown. Finally, pH was adjusted to 9 and stored in dark condition for a period of 24 hours. Following centrifugation at 5000 rpm for a total of 10 minutes, the washing process was carried out two times, ensuring purity. The resulting nanoparticles were assessed via UV- Visible Spectrophotometer at 250nm-700nm.

Glycyrrhiza glabra:

A silver nitrate (AgNO_3) solution with a 1 mM concentration was made in a 250 ml container. It was kept on magnetic stirrer for 10 minutes at room temperature under continuous stirring. Plant extract and silver nitrate were added in 1:4 ratio in small aliquots at an interval of every 1 minute for reduction into Ag^+ ions.

It was further kept on magnetic stirrer for 20 minutes at 60 °C. The solution gradually darkened, transitioning from yellow to dark-brown. Finally, pH was adjusted to 9 and stored in dark condition for a period of 24 hours. Following centrifugation at 5000 rpm for a time period of 10 minutes, followed by two rounds of washing. ^[12] A UV-Visible Spectrophotometer set to 200–700 nm was then used to analyze the nanoparticles.

UV-Visible spectral analysis

A UV-Vis spectral (Systronics 199 Dual beam spectrophotometer) examination of silver nanoparticles developed was conducted, focusing on wavelengths between 200 and 1000 nm. The green synthesized *Saussurea lappa* silver nanoparticles were added with distilled water as solvent in cuvette (Quartz cuvette 10mm) and the result was recorded. Similarly, the green synthesized *Glycyrrhiza glabra* silver nanoparticles were added with distilled water as solvent in cuvette (Quartz cuvette 10mm) and the result was recorded.

Antimicrobial Action of Nano silver particles

Evaluation of Antimicrobial effect was performed on 1.5% Mueller-Hinton Agar. ^[13] An overnight culture of pathogens *S. aureus*, *P. aeruginosa*, *E. coli*, and *E. faecalis* were prepared at 1:20 dilution. This culture was spread on the plates and 6 mm wells were formed with corkborer. A range of nanoparticle solutions (5 μL , 10 μL , 15 μL , and 20 μL) were dispensed into separate wells, then incubated at 37°C for a period of 12 to 24 hours. The antimicrobial potential of silver nano powders obtained from *Saussurea lappa* and *Glycyrrhiza glabra* was analysed using zone of inhibition against the strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, and *Staphylococcus aureus*. The zone of inhibition was noted in

millimetres and tabulated.

RESULTS AND DISCUSSIONS

Qualitative Phytochemical Assessment

Qualitative phytochemical screening for *Saussurea Lappa* was performed and the observations are noted below and in table 1. Flavanoids, terpenoids and glycosides are absent from *Symplocos racemosa* extract whereas saponins, steroids, tannins, and quinones are present. Similarly, for *Glycyrrhiza glabra* extract presence of all phytochemical turned out to be positive.

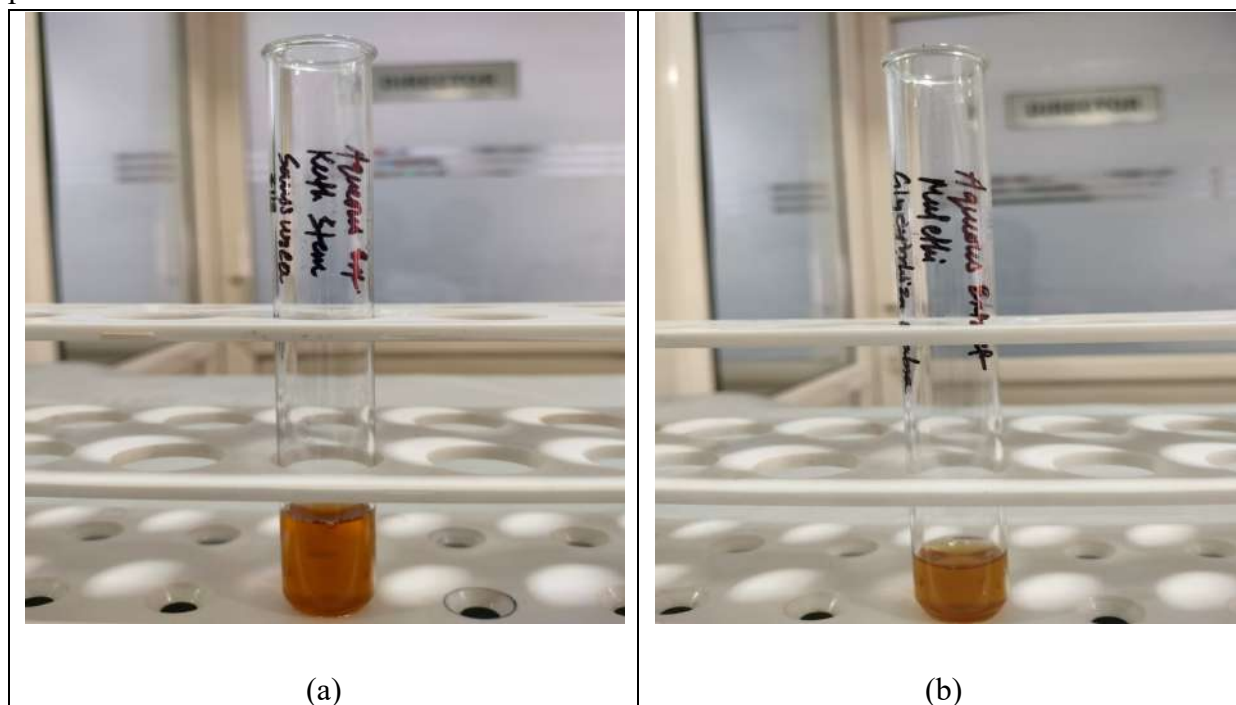


Figure 4: Images showing aqueous extract of (a) *Saussurea Lappa* and (b) *Glycyrrhiza glabra*



Figure 5: *Saussurea Lappa* plant extract qualitative phytochemical Results

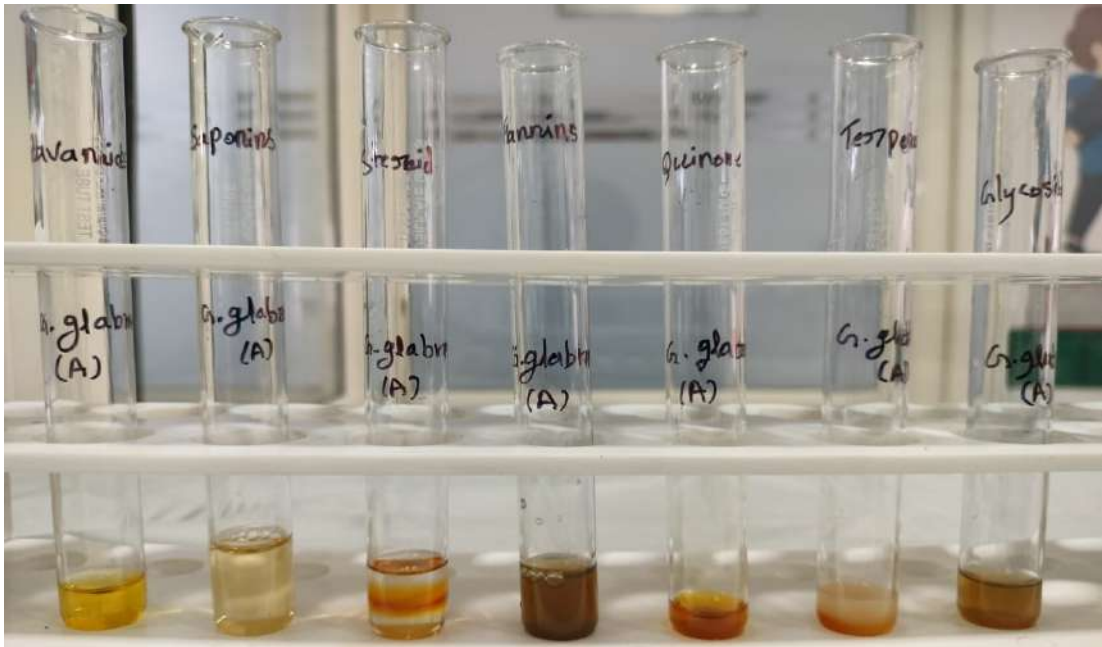


Figure 6: *Glycyrrhiza glabra* plant extract qualitative phytochemical Results

Table 1 Results of secondary metabolite test of *Saussurea Lappa*

| Secondary Metabolites Test Components | Results |
|--|----------|
| Flavanoids | Negative |
| Saponins | Positive |

| | |
|------------|----------|
| Tannins | Positive |
| Steroids | Positive |
| Quinone | Positive |
| Terpenoids | Negative |
| Glycosides | Negative |

Table 2 Results of secondary metabolite test of *Glycyrrhiza glabra*

| Secondary Metabolites Test Components | Results |
|--|----------|
| Flavanoids | Positive |
| Saponins | Positive |
| Tannins | Positive |
| Steroids | Positive |
| Quinone | Positive |
| Terpenoids | Positive |
| Glycosides | Positive |

Silver nanoparticle (Ag NP) synthesis

Saussurea lappa: The reaction led to development of silver nanoparticles (AgNPs), visibly altering color of solution, A noticeable color shift occurred, turning the deep-yellow solution greenish-brown. This change validated the completion of the reaction. ^[14] The nanoparticles underwent characterization via UV-Visible spectrophotometric analysis.

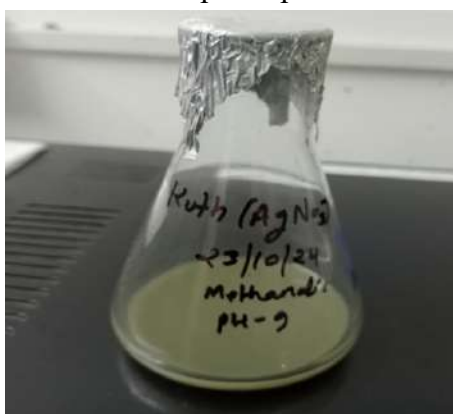


Figure 7 Silver nanoparticles of *Saussurea lappa*

Glycyrrhiza glabra: The image below illustrates the formation of AgNPs, causing the yellow solution to transition into a dark-brown hue. The observed color change confirms the final phase of the reaction between the extract and AgNO₃. A UV-Visible spectrophotometer was employed to characterize the nanoparticles. ^[15]



Figure 8 Silver nanoparticles of *Glycyrrhiza glabra*

UV-Visible spectral analysis

A 10 mm quartz cuvette was used to contain green-synthesized silver nanoparticles suspended in distilled water, with scanning performed within the 240-700 nm wavelength range, as illustrated in Figures 9 and 10. UV-Vis spectrophotometry facilitated the identification of AgNPs within the solution.



Figure 9 UV-Visible spectral analysis of *Saussurea lappa* silver nanoparticles

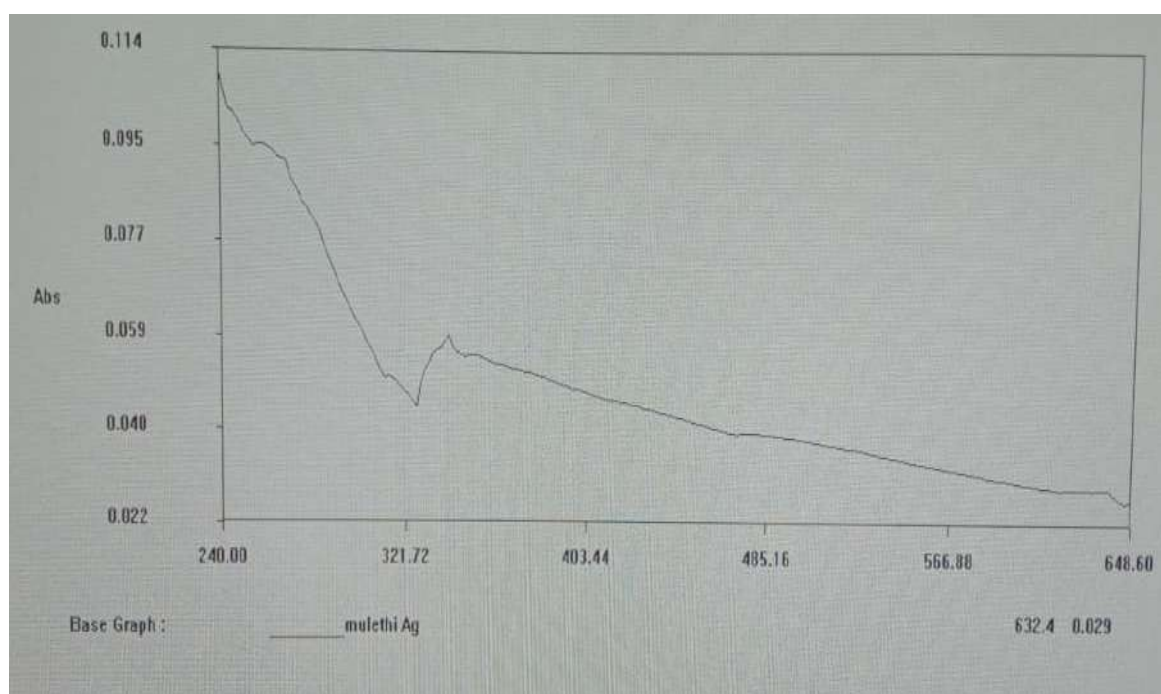


Figure 10 UV-Visible spectral analysis of *Glycyrrhiza glabra* silver nanoparticles

Antimicrobial Action of Nano silver particles

Saussurea lappa

Antibacterial action of Ag NPs derived from the selected plant extract were observed, preventing two Gram-negative and two Gram-positive strains of bacteria, among other bacterial species, from growing. *Saussurea lappa*-derived nano silver particles effectively inhibited the growth of *E. coli* at a low dosage of 5 μ L, with the maximum antibacterial effect occurring at 20 μ L.

Gogoi *et al.* ^[16] explained that *E. coli* cells carry a negative charge, enabling Ag^+ ions to interact readily with their membranes and impair their function. *Saussurea lappa* silver nanoparticles at different concentration exhibited a bacteriostatic effect on *Pseudomonas*. Regarding *Staphylococcus*, antibacterial impact of nano silver particles was found to be good. Compared to *E. coli* a diderm bacteria, the antibacterial action against *Enterococcus* was relatively lower and showed a delayed effect. This slower response is probably attributed to the compact peptidoglycan layer and associated teichoic acids found in the monoderms cell wall. ^[17]

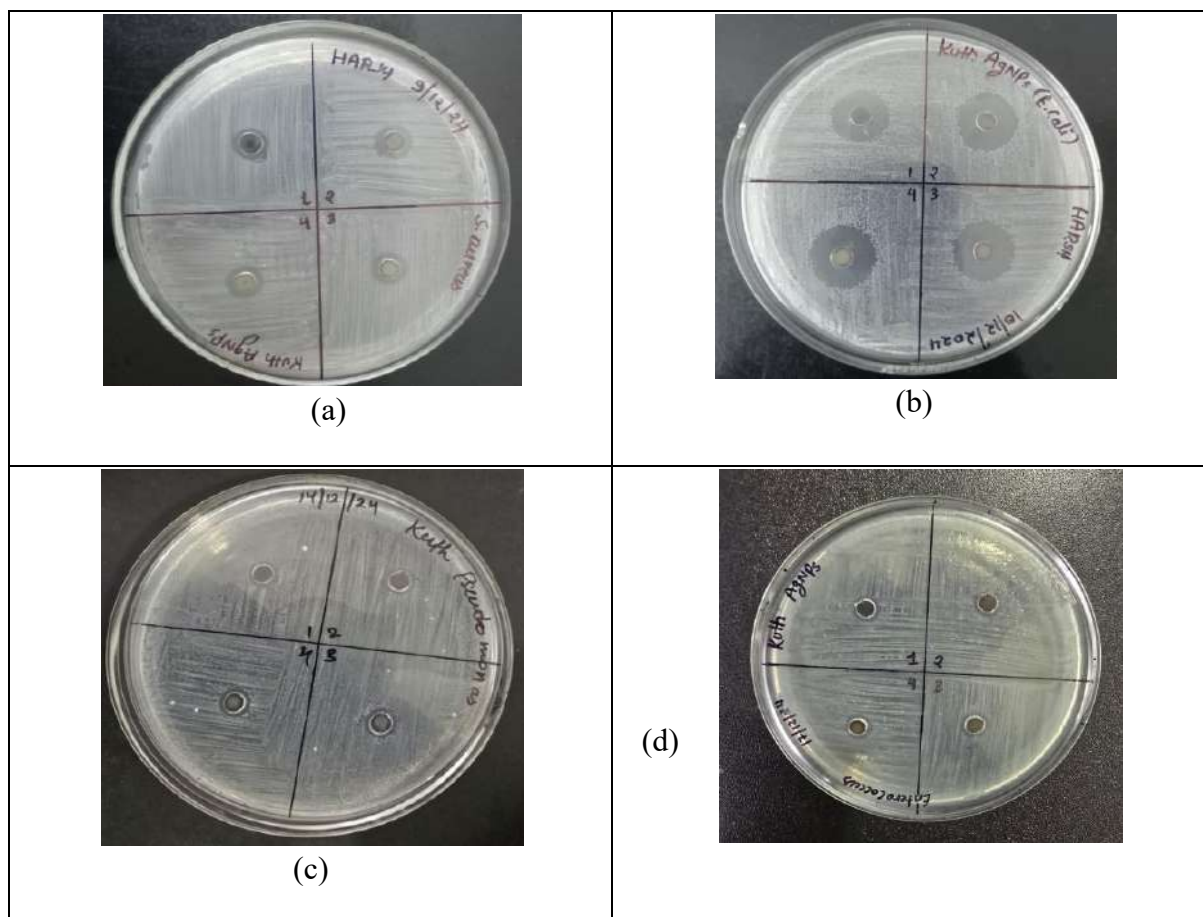


Figure 8 Growth suppression zone for silver nanoparticles of *Saussurea lappa* tested for its impact on (a)*Staphylococcus Aureus*, (b)*Escherichia coli*, (c)*Pseudomonas Aeruginosa* and (d) *Enterococcus*

Glycyrrhiza glabra

The chosen plant extract used to create nano silver particles showed inhibitory effects on bacterial growth, impacting both diderm and monoderm species.^[18] At the lowest concentration of 5 µg/mL, silver nanoparticles obtained from *Glycyrrhiza glabra* successfully suppressed *Pseudomonas* growth, as indicated in Table 4. For *Pseudomonas*, the silver nanoparticles exhibited peak antibacterial effectiveness at a 20 µL concentration.

The bacteriostatic potential of *Glycyrrhiza glabra* silver nanoparticles was observed against *E. coli* at varying concentrations as reported in Table 4. Nano silver particles demonstrated exceptional antibacterial activity against *Staphylococcus*. When *Staphylococcus aureus* cells encountered AgNPs, their cell walls weakened, allowing intracellular materials to escape into the external environment, resulting in cell destruction.^[19] *Enterococcus* experienced a more intense antibacterial impact from *Glycyrrhiza glabra* silver nanoparticles, characterized by a prompt response.

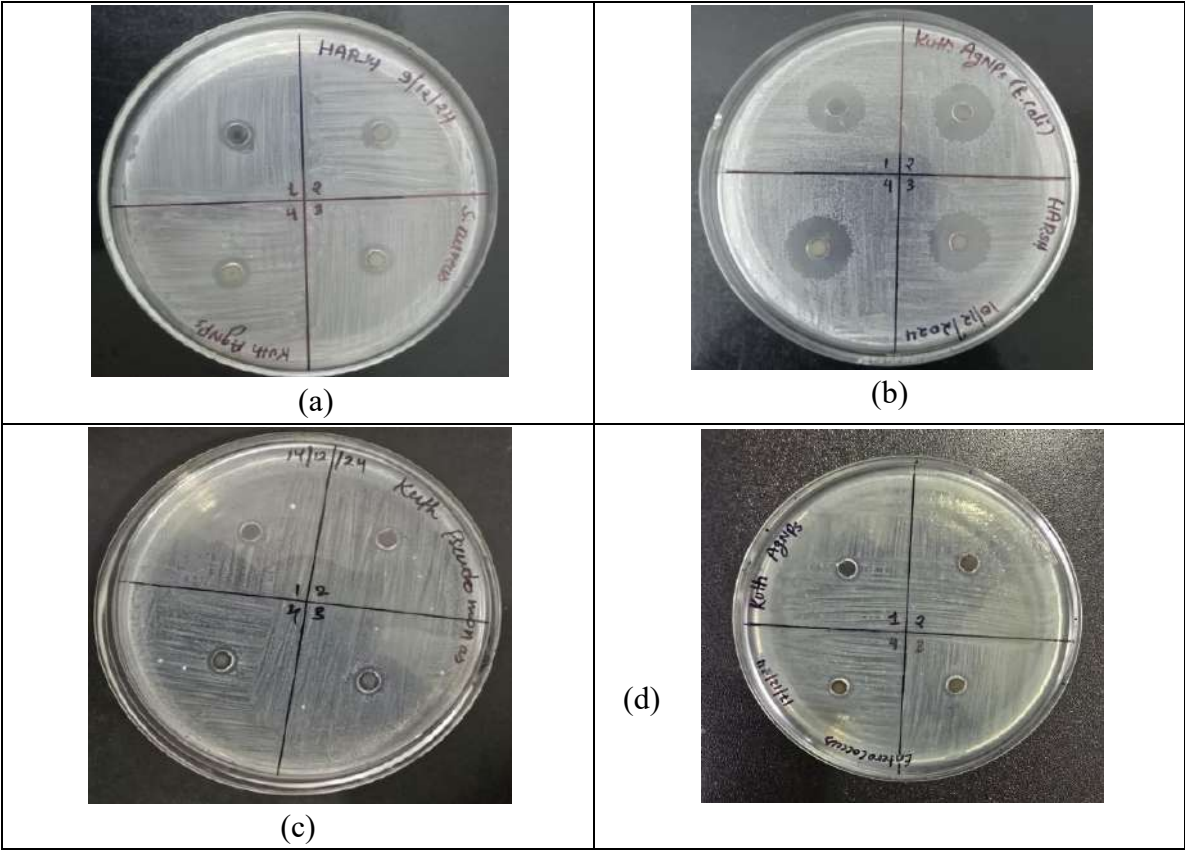


Figure 8: Growth suppression zone for silver nanoparticles of Glycyrrhiza glabra tested for its impact on (a)*Staphylococcus Aureus*, (b)*Escherichia coli*, (c)*Pseudomonas Aeruginosa* and (d)*Enterococcus*

Table 3 Antimicrobial action of *Saussurea Lappa* silver nanoparticles

| Synthesized plant nano silver particles (in uL) | <i>Staphylococcus</i> (In mm) | <i>pseudomonas</i> (In mm) | <i>Escherichia</i> (In mm) | <i>Enterococcus</i> (In mm) |
|---|-------------------------------|----------------------------|----------------------------|-----------------------------|
| 5 | 3.5 | 7.5 | 11.5 | 2.75 |
| 10 | 4.5 | 8 | 12.5 | 3.75 |
| 15 | 5 | 8.5 | 12.5 | 3.75 |
| 20 | 4 | 9.25 | 13.5 | 4.25 |

Table 4 Antimicrobial action of *Glycyrrhiza glabra* silver nanoparticles

| Synthesized plant nano silver particles (in uL) | <i>Staphylococcus</i> (In mm) | <i>pseudomonas</i> (In mm) | <i>Escherichia</i> (In mm) | <i>Enterococcus</i> (In mm) |
|---|-------------------------------|----------------------------|----------------------------|-----------------------------|
| | | | | |

| | | | | |
|----|-----|------|-----|------|
| 5 | 6.5 | 4.5 | 4.5 | 5.25 |
| 10 | 7.5 | 8 | 6 | 6.5 |
| 15 | 7 | 6.5 | 7.5 | 6.5 |
| 20 | 7.5 | 8.25 | 8.5 | 7.5 |

CONCLUSION

The productive amalgamation of nano silver particles was accomplished through bio-reduction of silver nitrate arrangements utilizing extracts from *Saussurea lappa* and *Glycyrrhiza glabra*. The properties of these nanoparticles were examined through UV-visible spectroscopy for characterization. Their stability, biocompatibility, and antimicrobial properties also make them suitable for industrial and environmental remediation. In summary, our findings indicate that herbal extracts facilitate the production of Nano silver particles. These nanoparticles demonstrate effective antibacterial properties against both monoderm bacteria and diderm bacteria.

The inhibitory potential of silver nanoparticles from *Saussurea lappa* was more articulated against Gram-negative microbes, while their action on Gram-positive bacteria was moderate, as their ability to induce bacterial death was comparatively lower. *Glycyrrhiza glabra*-derived silver nanoparticles showed significant suppression of Gram-positive and Gram-negative bacterial development. According to this study, nano silver particles of *Saussurea lappa* and *Glycyrrhiza glabra* can be utilized as successful antibacterial agents against different microorganism which can threaten human life.

REFERENCES

1. Alothman M, Abd-El-Aziz ARM. Effect of green synthesis silver nanoparticles from five fruits peel on protein capped and anti-fungal properties. International journal of advance research in biological science. 2019; 6(2):156–165. Available from: <https://ijarbs.com/pdfcopy/2019/feb2019/ijarbs18.pdf>
2. Wijnhoven SWP, Peijnenburg WJGM, Herberts CA, Hagens WI, Oomen AG, Heugens EHW, Roszek B, Bisschops J, Gosens I, Van De Meent D, Dekker S, Jong WHD, Zijverden MV, Sips AJ and Geertsma RE. Nano-silver— A review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicology. 2009; 3(2):109–138. Available from: <https://doi.org/10.1080/17435390902725914>
3. Alt V, Bechert T, Steinrucke P, Wagener M, Seidel P, Dingeldein E, Domann E, Schnettler R. An in vitro assessment of the antibacterial properties and cytotoxicity of nanoparticulate silver bone cement. Biomaterials. 2004; 25(18):4383–4391. Available from: <https://doi.org/10.1016/j.biomaterials.2003.10.078>
4. Kumar J and Pundir M. Phytochemistry and pharmacology of *Saussurea* genus (*Saussurea lappa*, *Saussurea costus*, *Saussurea obvallata*, *Saussurea involucrata*). Materialstoday: Proceedings. 2022; 56(3):1173-1181. Available from: <https://doi.org/10.1016/j.matpr.2021.11.145>

5. Oliveira MSD, Nollet LML. Bioactive Compounds Identification and Characterization of their Food and Pharmacological Potential. CRC Press. 2025. Available from: <https://doi.org/10.1201/9781003462804>
6. Noreen S, Mubarik F, Farooq F, Khan M, Khan AU, Pane YS. Medicinal Uses of Licorice (*Glycyrrhiza glabra* L.): A Comprehensive Review. Macedonian Journal of Medical Sciences. 2021; 9(F):668-675. Available from: <https://doi.org/10.3889/oamjms.2021.7526>
7. Fayaz AM, Balaji K, Girilal M, Yadav R, Kalaichelvan PT, Venketesan R. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: A study against gram-positive and gram-negative bacteria. Nanomed. Nanotechnol. Biol. Med. 2010; 6(1): 103–109. Available from: <https://doi.org/10.1016/j.nano.2009.04.006>
8. Pal S, Tak YK, Song JM. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. Appl. Environ. Microbiol. 2007; 73:1712–1720. Available from: <https://doi.org/10.1128/AEM.02218-06>
9. Al-Deen AMT, ALhaidari SAA, Al-Kaf AG, Al-Hadi FA, Mahbashi AA. Phytochemical screening and thin layer chromatographic of *Prunus dulcis* (almond) medicinal plant leaves used in folk medicine for treatment of wounds and burns in hufash district Al mahweet governorate–Yemen. Universal Journal of Pharmaceutical science. 2019; 4(2):19-23. Available from: <https://doi.org/10.22270/ujpr.v4i2.252>
10. Shahverdi AR, Fakhimi A, Shahverdi HR, Minaian S. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*. Nanomedicine. 2007; 3(2):168–171. Available from: <https://doi.org/10.1016/j.nano.2007.02.001>
11. Shankar SS, Ahmad A, Sastry M. Geranium leaf assisted biosynthesis of silver nanoparticles. Biotechnol. Prog. 2003; 19(6):1627–1631. Available from: <https://doi.org/10.1021/bp034070w>
12. Kumar V, Yadav SK. Plant-mediated synthesis of silver and gold nanoparticles and their applications. J. Chem. Technol. Biotechnol. 2009; 84(2):151–157. Available from: <https://doi.org/10.1002/jctb.2023>
13. Mueller JH, and Hinton J. A protein-free medium for primary isolation of gonococcus and meningococcus. Proc. Soc. Exp. Biol. Med. 1941; 48(1): 330-333. Available from: <https://doi.org/10.3181/00379727-48-13311>
14. Singhal G, Bhavesh R, Kasariya K, Sharma AR, Singh RP. Biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screening its antimicrobial activity. J Nanoparticle Res. 2011; 13:2981–2988. Available from: <https://doi.org/10.1007/s11051-010-0193-y>
15. Namratha N, Monica PV. Synthesis of silver nanoparticles using *Azadirachta indica* (Neem) extract and usage in water purification. Asian J Pharm Tech. 2013; 3:170–174. Available from: <https://ajptonline.com/AbstractView.aspx?PID=2013-3-4-9>
16. Gogoi S, Gopinath P, Paul A, Ramesh A, Ghosh S, Chattopadhyay A. Green fluorescent protein-expressing *Escherichia coli* as a model system for investigating the antimicrobial activities of silver nanoparticles. Langmuir. 2006; 22:9322–9328. Available from: <https://doi.org/10.1021/la060661v>

17. Kim SH, Lee HS, Ryu DS, Choi SJ, Lee DS. Antibacterial activity of silver-nanoparticles against *Staphylococcus aureus* and *Escherichia coli*. Korean. J. Microbiol. Biotechnol. 2011; 39:77–85. Available from: [https://pdf.medrang.co.kr/MBL/2011/039/11-10-12002\(77-85\).pdf](https://pdf.medrang.co.kr/MBL/2011/039/11-10-12002(77-85).pdf)
18. Pahal V, Kumar P, Kumar P, Kumar V. Antibacterial activity and hormetic response of silver nanoparticles synthesized using leaflet extract of wheat (*Triticum aestivum*) and rice (*Oryza sativa*) crop plants. Journal of Applied Biology & Biotechnology. 2022; 10(2):154-167. Available from: 10.7324/JABB.2022.100219
19. Li WR, Xie XB, Shi QS, Duan SS, Ouyang YS, Chen YB. Antibacterial effect of silver nanoparticles on *Staphylococcus aureus*. Biometals. 2011; 24:135–141. Available from: <https://doi.org/10.1007/s10534-010-9381-6>