RESEARCH PAPER

# Extraction, Standardization And Synthesis Of Silver Nanoparticles From Root Extract Of *Nardostachys Jatamansi*

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**Received:** 20th Feb, 2026; **Revised:** 4th Mar, 2026; **Accepted:** 25th Mar, 2026; **Available Online:** 10th Apr, 2026

**Abstract:**

The burgeoning interest in nanotechnology has catalyzed extensive investigations into the fabrication and multifunctional applications of nanomaterials, particularly silver nanoparticles (AgNPs), owing to their distinctive physicochemical properties and broad-spectrum bioactivity. This study delineates the extraction, phytochemical standardization, and green synthesis of AgNPs employing the root extract of *Nardostachys jatamansi*, a medicinal herb renowned for its diverse secondary metabolites. Initially, the root extract was meticulously prepared and standardized to ensure batch-to-batch consistency in the concentration of the bioactive constituents. The biosynthesis of AgNPs was facilitated via a sustainable, phytochemical-mediated reduction process in which the extract functioned as both a reducing and capping agent. The physicochemical attributes of the synthesized nanoparticles were comprehensively characterized using ultraviolet-visible (UV-Vis) spectroscopy, X-ray diffraction (XRD), and transmission electron microscopy (TEM) to elucidate their optical properties, crystalline nature, morphology, and particle size distribution. Furthermore, the antifungal potential of the synthesized AgNPs was evaluated against Candida albicans to assess their therapeutic efficacy in vitro. These findings underscore a facile and eco-compatible approach for AgNP synthesis, with promising implications for biomedical applications, environmental remediation, and catalytic systems.

**Keywords:** Silver nanoparticles, *Nardostachys jatamansi*, green synthesis, antifungal activity, characterization.

**How To Cite This Article:** Bhagwat A, Shelar Mu, Kadam Jn, Nagare S, Gondhale-Karpe P, Dhumal Js, Jadhav Ny, Warude Bj. Extraction, Standardization And Synthesis Of Silver Nanoparticles From Root Extract Of Nardostachys Jatamansi. Int J Drug Deliv Technol. 2026;16(4):438-445. Doi: 10.25258/ijddt.16.4.43

## Introduction:

Pharmaceutical and biomedical research is benefiting greatly from nanotechnology, which is able to create new types of engineered products that use engineered nanomaterials (i.e., nanoparticles) at the nanoscale. Manufactured nanoparticles have unique physical, chemical, and biological characteristics compared to their respective bulk materials. These typically have greater surface area reactivity, increased biosafety, and increased functional capabilities than their larger counterparts. Therefore, nanoparticles as engineered materials may become an invaluable resource for drug delivery systems, antimicrobial treatments, diagnostic tools, and therapeutic material development. One type of engineered nanomaterial that has been extensively used in both the pharmaceutical and biomedical fields is silver nanoparticles (AgNPs) because of their proven success as an antimicrobial, antifungal, catalysts and other biomedical applications. [1-3]

The broad spectrum of biological activities exhibited by silver nanoparticles has led to increased interest in using them as antifungal agents, for medical coatings, and for wound care systems as well as other nano-enabled therapeutic platforms. However, with Physical and Chemical Methods of synthesis commonly requiring high energy inputs, toxic reducing agents and synthetic stabilisers, conventional processes often pose an environmental threat, create biological toxicity risks, and incur high costs. Therefore, there has been a strong research push towards Green Synthesis Methods using biological sources to provide the reducing and stabilizing systems necessary to create these products.[4-6]

Use of plants to create nanoparticles has become popular because they are easy to find and inexpensive[7-9]. Phytochemicals in plants can act as both reducing and capping agents when making nanoparticles using plants[7-8] . The use of phytochemicals to make nanoparticles will also improve how well they work with living organisms and it will keep you away from using toxic materials and dangerous chemicals [9-10].

Research into green nanoparticles has experienced tremendous growth over a relatively short period of time; however, one major barrier to the continued growth of this research area continues to be the variability of plants and lack of standardization of Phytochemicals and analytical validation of plant extracts used for the synthesis of nanoparticles. Due to these issues, the majority of research studies that describe how nanoparticles have been synthesized lack an establishment of extract consistency or quantification of the active marker compounds used in nanoparticle formation, which results in limited scientific validity and translational potential[10].

The plant known as *Nardostachys jatamansi* is a medicinal herb that contains phenolics, coumarins, and other bioactive compounds with known antibacterial and antifungal properties[10-11]. However, research on the synthesis of silver nanoparticles from botanical sources has been plentiful, yet there has been little research that includes phytochemical standardization, analytical validation based on markers, and controlled green nanoparticle synthesis specifically from *Nardostachys jatamansi*[10-12]. Therefore, this lack of integrated methodologies creates a current gap for researchers in the field of green nanotechnology.

Thus, this study is aimed at producing a standardized extract from *Nardostachys jatamansi* roots and validating its phytochemical composition using reverse phase high-performance liquid chromatography (RP-HPLC), and utilizing the standardized extract to provide a green method of synthesizing silver nanoparticles. The silver nanoparticles produced are fully characterized through spectroscopic and morphological techniques, and their antifungal activity is investigated. Therefore, this project will create a reproducible and analytically supported framework for nanoparticle synthesis through the green approach using *Nardostachys jatamansi*[10-12].

## Materials and Methods

### Preparation and Standardization of *Nardostachys jatamansi* Extract

#### Plant Material Collection and Authentication

Roots of *Nardostachys jatamansi* were collected from Manikarnika Aushadhalaya, Pimpri-Chinchwad, Pune, in November 2023. The roots were thoroughly cleaned, dried, and crushed into a coarse powder[13]. The plant material was authenticated by the Agharkar Research Institute, Pune, Maharashtra, India.

#### Physicochemical Analysis of Plant Material

The coarse powder of *N. jatamansi* roots was subjected to preliminary identification tests as per the Ayurvedic Pharmacopoeia of India. Parameters evaluated included color, odor, taste, moisture content, alcohol-soluble extractives, and water-soluble extractives[14].

#### Preparation of Ethanolic Extract

Ethanolic extract of *N. jatamansi* (EEM) was prepared by cold maceration. Briefly, 200 g of dried root powder was macerated in 2.5 L of 90% ethanol for 15 days at room temperature. The mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure to yield the EEM[15].

#### Physicochemical Characterization of EEM

The EEM was characterized for organoleptic properties (color, odor, taste), melting point, solubility profile, preliminary phytochemical tests, UV-visible absorption maxima ( $\lambda_{max}$ ), and Fourier-transform infrared (FTIR) spectroscopy[16-17].

#### HPLC Method Development and Validation for Gallic Acid Quantification

Quantification of gallic acid was performed using a Jasco HPLC system equipped with an isocratic pump, ChromatoStage software, and UV-visible detector. Separation was achieved on a Licrospher C18 RP column (250 × 4.6 mm, 5µ ID)) with a Phenomenex guard column (4.6 × 10 mm, 5µ ID). The mobile phase consisted of methanol (solvent A) and water (solvent B) (60:40 v/v), delivered at 1 mL/min. The injection volume was 20 µ L, and detection was at 272 nm. Chromatograms were evaluated for peak shape and

retention time ($R_t$); the mobile phase was optimized for optimal peak symmetry and minimal $R_t$.

The method was validated per ICH guidelines for linearity, precision, accuracy, specificity, and limits of detection/quantification.

**Standardization of Extracts**

The EEM, Gallic acid standard, and silver nanoparticles loaded with *N. jatamansi* extract were standardized for Gallic acid content using the validated RP-HPLC method

**Green Synthesis of *N. jatamansi*-Mediated Silver Nanoparticles (NJ-AgNPs)**

**Synthesis and Optimization**

Initial screening used 1 mM silver nitrate (AgNO3) solution. Volumes of 1, 2, 3, 4, or 5 mL EEM were added to 1 mM $AgNO_3$, stirred magnetically for 6–7 hrs, and incubated in the dark at room temperature to prevent photo-activation. Formation of AgNPs was indicated by colour change from colourless to brown and confirmed by UV-visible spectroscopy.

Optimization trials refined conditions (Table 1). The optimized protocol (Trial 4) involved dissolving 50 mg EEM in 20 mL methanol, adding 2–4 drops of 1 mM $AgNO_3$ in 80 mL distilled water, stirring for 5 h, and incubating for 1–2 hrs[18].

**Table 1. Optimization trials for NJ-AgNPs synthesis.**

| Trial | Extract (mg) | Solvent/Volume | $AgNO_3$ | Stirring (hrs) | Incubation (hrs) | Result |
|---|---|---|---|---|---|---|
| 1 | 10 | 20 mL methanol | 10 mL in 80 mL water | 4–5 | 7–8 | Partial reduction |
| 2 | 20 | 20 mL methanol | 10 mL in 80 mL water | 4 | 7–8 | Moderate yield |
| 3 | 50 | 20 mL methanol | 10 mL in 80 mL water | 6 | 12 | Good stability |
| 4 (Optimized) | 50 | 20 mL methanol | 2–4 drops in 80 mL water | 5 | 1–2 | Optimal (brown colour, stable SPR) |

**Characterization of NJ-AgNPs**

Hydrodynamic size and zeta potential were determined by dynamic light scattering (DLS) using a Horiba SZ-100 analyzer (Z-type, Ver. 2.20) in distilled water (pH 7.0, 25°C; 3 replicate runs). Entrapment efficiency (EE%) and drug content were quantified by extracting 5 mg NJ-AgNPs in methanol, followed by RP-HPLC analysis, calculated as EE% = [(total drug - free drug)/total drug] × 100. Surface morphology was examined by scanning electron microscopy (SEM) on a FEI Nova NanoSEM 450 (5–15 kV, gold-sputtered samples). Crystallinity was assessed by powder X-ray diffraction (XRD) using a Bruker D8 Advance diffractometer (Cu Kα radiation, λ = 1.5406 Å; 2θ 10–80°, 0.02° step size, 0.6 s/step)[19-20].

**Result and Discussion**

**RESULTS AND DISCUSSION**

**Physicochemical Assessment of *Nardostachys jatamansi* Root Powder**

The powdered form of the root of *Nardostachys jatamansi* had typical organoleptic features, such as brown color, characteristic aroma, and bitter taste, which indicated that this sample belongs to the said plant according to the criteria established by the Indian Pharmacopoeia.

It is important that no excessive amount of water could be detected in the samples, which means that the process of degradation due to microbial activity could not occur. In addition, alcohol-soluble extractive was higher than water-soluble extractive, indicating that the plant contains moderately polar or non-polar compounds (phenolic substances and sesquiterpenes).

**Preparation and Yield of Ethanolic Extract (EEM)**

A dark brown ethanolic extract of *Nardostachys jatamansi* (EEM) was obtained by cold maceration method from the powdered root material.The percentage yield 90% was obtained, suggests that this solvent is highly efficient for extraction of bioactive compounds, especially phenolic compounds like Gallic acid.

**EEM Physico-Chemical Properties**

The EEM exhibited good solubility in methanol and ethanol, with moderate solubility in water. Phytochemical analysis indicated the existence of phenolics, flavonoids, alkaloids, and terpenoids, which have antioxidant and reducing capabilities.

**HPLC Method Development and Validation**

A RP-HPLC technique was used to quantify the gallic acid. The separation process was done through the use of a mobile phase comprising of methanol and water in a 60:40 ratio, which offered excellent peak shape with a retention time of 2.8 minutes with detection at 272 nm wavelength. This choice of parameters helped achieve good peak shape, sharpness, and sensitivity. It was successfully utilized in determining the amount of Gallic acid in the extracted ethanolic solution and in synthesizing the nanoparticles.

**HPLC Method Validation [21-23]**

The analytical method was validated as per ICH guidelines. The calibration curve of gallic acid was very linear at the concentration range of 80-180 ppm with correlation coefficient of 0.9965 signifying a high level of linearity of the method (Figure 2, Table 2). Analyses indicated that %RSD of both intra-day (1.15-1.72%) and inter-day (0.95 – 1.74%) precision could be reproducible as the values were less than 2% (Table .3) Recovery was revealed to be 98.96 - 100.17% which is summarized in Table 4. The test of robustness

to small changes in the chromatographic conditions revealed a little deviation (< 2) indicating that the method was stable (Table 5). LOD and LOQ were estimated to be 19.99 µg/mL and 60.60 µg/mL respectively which is good sensitivity.

**Standardization of *Nardostachy jatamansi* Extract.**

Gallic acid estimation in EEM and NJ-AgNPs through the validated RP-HPLC method proved effective. Gallic acid was present in both extracts; hence, it was evident that the phytocompound was stable during the preparation of NJ-AgNPs.

There was an insignificant difference in the quantity of gallic acid between extract and NJ-AgNPs; thus, there was minimal participation of phenolics in the reduction of silver ions.

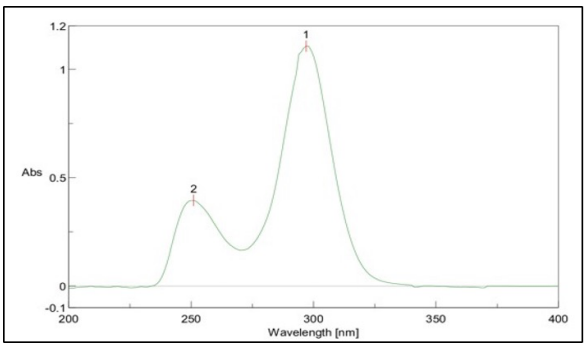


**Figure 1: UV spectrum of gallic acid showing λmax at 272 nm**

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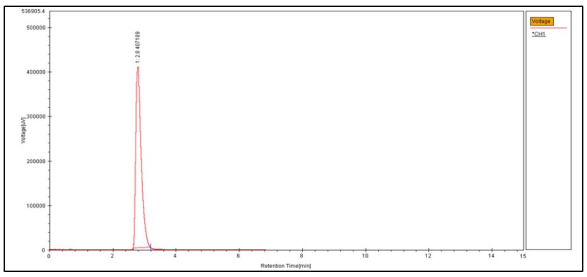

**Figure 2: Chromatogram of Gallic acid standard.**

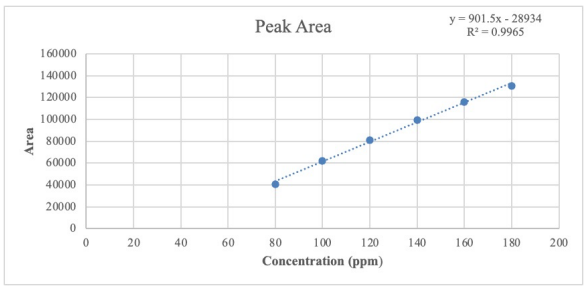


**Figure 3: Calibration curve of gallic acid.**

**Table 2: Linearity regression data for gallic acid.**

| Parameters | Result |
|---|---|
| **Calibration Range (ppm)** | 80-180 |
| **Detection Wavelength (nm)** | 272 |
| **Solvent (Methanol: Water)** | 60:40 |
| **Regression Equation (y*)** | Y=901.5x-28934 |
| **Slope (b)** | 901.5 |
| **Intercept (a)** | 28934 |
| **Correlation Coefficient ($R^2$)** | 0.9965 |

**Table 3: Intraday and Inter day Precision**

| Concentration | Intraday | | Interday | |
|---|---|---|---|---|
| | Mean ± SD (n=3) | % RSD for peak area | Mean ± SD (n=3) | % RSD for peak area |
| 100 | 52613 ± 909.96 | 1.72 | 51502 ± 896.21 | 1.74 |
| 120 | 57912 ± 839.81 | 1.45 | 56854 ± 541.61 | 0.95 |
| 140 | 67657 ± 784.60 | 1.15 | 60320 ± 879.03 | 1.45 |

**Table 4: Percent recovery of Gallic acid**

| Level (%) | Amount Added (µg/mL) | Amount Found (µg/mL) | % Recovery |
|---|---|---|---|
| 80 | 35 | 34.98 | **99.94** |
| 100 | 44 | 43.50 | **98.86** |
| 120 | 52 | 52.09 | **100.17** |

**Table 5: Robustness (n=6), concentration - 100 µg/ml**

| Sr. No. | Parameter | % RSD |
|---|---|---|
| | Flow rate (± 0.1 ml) | 1.74 |
| | Mobile phase composition (± 5 ml) | 1.03 |
| | Wavelength (± 2 nm) | 1.48 |

**Green synthesis and optimization of silver nanoparticles.**

The reduction of $Ag^+$ to $Ag^0$ in the synthesis of silver nanoparticles (AgNPs) with *N. jatamansi* extract was followed by changes in colourless to brown. In four trials of optimization, Trial 4 gave the best results due to fast formation, intense brown color, and good stability. The decrease in silver nitrate concentration and optimized extract ratio led to proper nucleation and no aggregation.

Phytochemicals in *Nardostachys jatamansi* extract were responsible for reducing and stabilizing silver nanoparticles without any use of additional chemicals.

**Silver Nanoparticles Characterization**

**UV–Visible Spectroscopy**

The produced nanoparticles had a typical absorption peak at 245 - 250 nm which indicated the presence of AgNPs as a result of surface plasmon resonance formation. The steady peak value was an indication of constant nanoparticle formation, whereas the growing absorbance as a function of concentration was an indication of increased nanoparticle production and stabilization as illustrated in Figure 4.

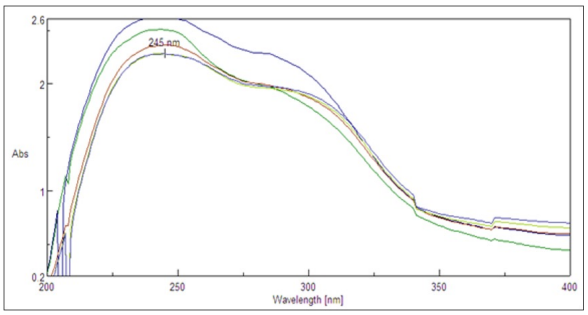


**Figure 4: UV spectra for nanoparticles solution taken five times at time interval of 1 hr**

**Particle Size Analysis**

According to the graph, there is a clear, single peak in the range of about 150–300 nm, with the highest intensity occurring at approximately 200 nm, which agrees well with the average particle size reported in this case (202.3 nm). This implies that the particles have been successfully distributed in a rather narrow range of sizes.

The unimodal distribution pattern suggests that the synthesized nanoparticles exhibit good uniformity and homogeneity. The fact that there are no multiple peaks suggests that there is little to no presence of secondary populations of particles, including very small nuclei, or large aggregates. Nevertheless, the minimal broadness on the base of the peak indicates the moderately high degree of polydispersity, which is typical of biologically synthesized nanoparticles. In general, the graph supports the fact that the synthesized silver nanoparticles have a major homogeneous size distribution with an average diameter of approximately 202 nm. The controllable size distribution also allows the reproducibility of the synthesis process and indicates efficient stabilization of nanoparticles by the plant extract.

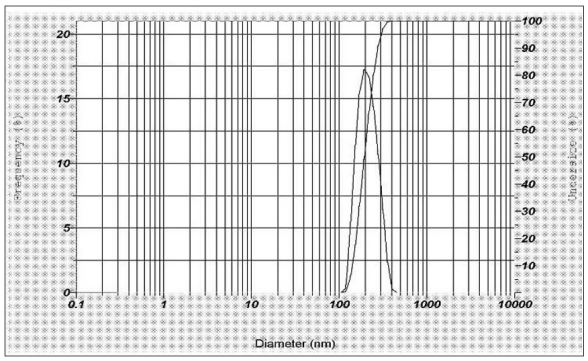


**Figure 5: Particle Size Distribution of Silver Nanoparticles**

**Zeta Potential**

The zeta potential of the nanoparticle suspension is -25.0 mV which implies moderate stability of the suspension. The repulsive effect of the negative charge between particles indicates that aggregation is minimized and dispensability is enhanced.

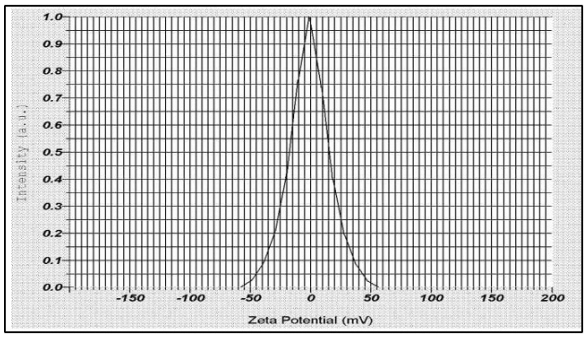


**Figure 6: Zeta Potential Analysis of Biosynthesized Silver Nanoparticles**

**Analyses of Nanoparticles by HPLC.**

The HPLC analysis showed the presence of gallic acid in the nanoparticle formulation which revealed that phytoconstituents had been incorporated successfully in AgNPs (Figure 7). The retention time was kept constant at 2.8 min, which indicates specificity and stability of the method.

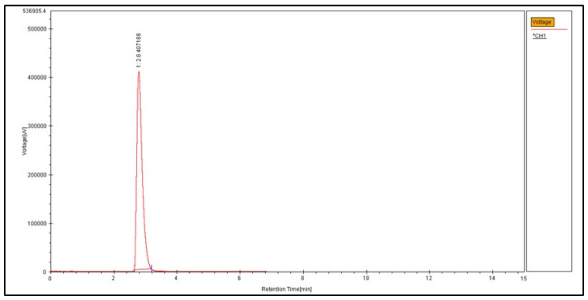

**Figure 7: Chromatogram of silver nanoparticles**

**SEM Analysis**

The SEM images (Figure 8), the silver nanoparticles are irregular and nearly spherical in nature and also seem to have clustered. The particle sizes highlighted within the SEM image vary from approximately ~25 nm to 50 nm, showing that the nanoparticles have been formed in the nanoscale range.

Aggregation could possibly result from strong interaction between the particles and the drying process used while preparing samples for observation under the SEM.

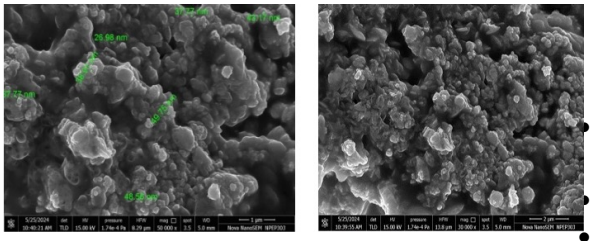

**Figure 8: SEM images of synthesized silver nanoparticles**

**XRD Analysis**

The XRD pattern (Figure 9) shows multiple distinct diffraction peaks at different 2θ values, indicating the crystalline nature of the synthesized silver nanoparticles. The prominent intense peak around ~38° (2θ) corresponds to the (111) plane of face-cantered cubic (fcc) silver, confirming the formation of metallic Ag nanoparticles.

Additional peaks observed near ~44°, ~64°, and ~77° can be indexed to the (200), (220), and (311) planes, respectively, further supporting the crystalline face structure.

The presence of sharp peaks indicates good Crystallinity, while minor additional peaks may arise from bio-organic capping agents or impurities from the plant extract.

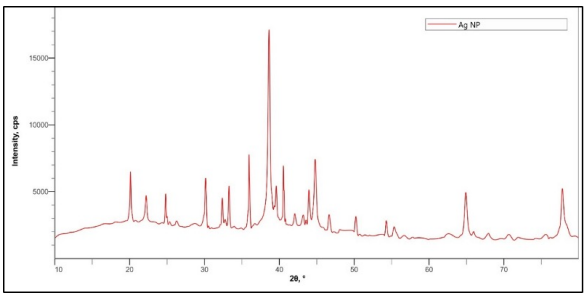


**Figure 9: X-ray Diffraction (XRD) Pattern of Silver Nanoparticles**

**Antifungal Activity** [24-25]

The well diffusion method was used to assess the antifungal properties of *Nardostachys jatamansi* root extract and synthesized silver nanoparticles (NJ-AgNPs) against Candida albicans. The results (Table 28 and Figure 14) clearly demonstrate a concentration-dependent increase in antifungal activity for both the extract and nanoparticles. The NJ extract had a bigger zone of inhibition (16 mm) at a low concentration (4 mg/mL) than NJ-AgNPs (10 mm) indicating that the crude extract displays superior activity at the beginning. Nevertheless, as the concentration of the silver nanoparticles increased (5 and 6 mg/mL), the antifungal activity of the silver nanoparticles was much greater, the zone of inhibition was 24 mm and 26 mm with the extract being 20 mm and 24 mm, respectively. This enhanced activity of silver nanoparticles at higher concentrations can be explained by:

- Bigger surface area of the nanoparticles, which increases contact with the fungal cells.
- Improved entry into microbial cell membranes.
- Silver ion ($Ag^{+}$) release, which disruption of cellular functions, proteins and DNA.
- Capping and reducing action of phytoconstituents as a synergistic effect.

The findings suggest that although the plant extract has got the intrinsic antifungal activity, nanoformulation remarkably amplifies its effectiveness particularly when used at a higher level. The action of the nanoparticles as antifungal agents is likely to be through membrane damage, oxidative stress and blockage of essential cellular processes in *Candida albicans*.

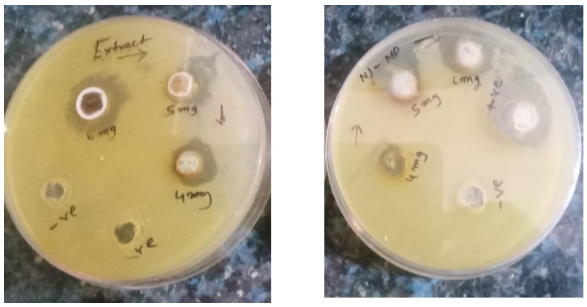


**Figure 10: Antifungal Activity**

**Table No. 6: Zones of inhibitions of silver nanoparticles obtained from antifungal activity.**

| Sr. No. | Bacterial Species | Concentration (mg/ml) | Diameter of inhibition zone (mean of triplicates) (mm) | |
|---|---|---|---|---|
| | | | *NJ* Extract | *NJ* - AgNPs |
| 1 | Candida Albicans | 4 | 16 mm | 10 mm |
| | | 5 | 20 mm | 24 mm |

| | | | | |
|---|---|---|---|---|
| | | 6 | 24 mm | 26 mm |

**Conclusion**

This study successfully established a robust standardization protocol for *Nardostachys jatamansi* root extract (EEM) through comprehensive physicochemical characterization and validated RP-HPLC quantification of gallic acid, ensuring batch-to-batch consistency and therapeutic reliability. The green synthesis of *N. jatamansi*-mediated silver nanoparticles (NJ-AgNPs) was optimized using 50 mg EEM in 20 mL methanol with 2–4 drops of 1 mM $AgNO_3$ in 80 mL water, yielding stable nanoparticles after 5 h stirring and 1–2 hrs dark incubation, followed by centrifugation and lyophilization.

Multimodal characterization confirmed NJ-AgNPs' superior attributes: hydrodynamic diameters of 26–48 nm (DLS), negative zeta potential indicating colloidal stability, polymorphic morphology (SEM), and face-centered cubic crystallinity (XRD). Notably, NJ-AgNPs exhibited markedly enhanced antifungal efficacy compared to native EEM, attributable to synergistic interactions between phytoconstituents and $Ag^0$, facilitating improved fungal cell penetration and reactive oxygen species generation.

These findings demonstrate the transformative potential of phytofabricated nanoparticles, amplifying the bioactivity of *N. jatamansi* while embracing eco-friendly, scalable synthesis. This convergence of Ayurvedic heritage and nanotechnology paves the way for next-generation therapeutics. Future investigations should elucidate in vivo pharmacokinetics, synergistic multi-drug formulations, and pilot-scale production to expedite clinical translation.

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