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**Original Research Article**

# Green Synthesis of Silver Nanoparticles Using the Plant Extract of *Leucas aspera* (Willd) Link and their Characterization

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## Abstract

***Objective:*** Silver nanoparticles (AgNPs) were synthesized through the high-efficient, cost-effective green and facile process, using the *Leucas aspera* (Willd.) Link extract as a bio-reduction and capping agent at room temperature. ***Methods:*** The greenly synthesized AgNPs were subjected to UV-Visible spectrophotometer, Fourier-transform infrared spectroscopy (FTIR), and X-ray diffraction spectroscopy (XRD) analyses. ***Results:*** The surface plasmon resonance found at 410 nm confirmed the formation of AgNPs. FTIR analysis was carried out to identify possible biomolecules responsible for the bio-reduction of silver ions. Furthermore, the crystallographic structure was confirmed by XRD and the average particle size of the synthesized silver nanoparticles was calculated which was found to be 36 nm. ***Conclusion:*** This study exhibits one step innovative green approach for the synthesis of silver nanoparticles from *L. aspera* plant extract. The method stands out primarily because it is eco-friendly and advantageous over conventional physical and chemical methods.

**Keywords**: Silver Nanoparticles, *Leucas aspera*, UV-Visible spectrophotometer, FTIR, XRD.



## INTRODUCTION

Nanoparticles have multifunctional properties and very exciting applications in various fields such as medicine, nutrition, and energy [1]. Nanoparticles have created remarkable advantages in the pharmacological industry to cure various bacterial and viral diseases [2].

Different types of nanoparticles such as Silver (Ag), Gold (Au), Platinum (Pt), and Palladium (Pd) have been synthesized in the recent past by chemical, physical and biological methods. The chemical methods are the most popular but the use of toxic chemicals during synthesis produces toxic by-products. The physical methods require a large amount of energy to maintain the high pressure and temperature required for the reaction. Thus, the chemical and physical methods have their limitations; these are considered expensive and unsuitable for a sustainable ecosystem. The synthesis of nanomaterials using biological entities is gaining momentum as biological methods are providing nontoxic and environmentally acceptable green chemistry procedures [3]. Synthesis of nanoparticles using plant extracts is the most accepted technique of eco-friendly production of nanoparticles and additionally has a distinct advantage that the plants are extensively distributed, easily available, much more secure to handle, and act as a source of numerous metabolites [4].

Among all-noble metal nanoparticles, silver nanoparticles (AgNPs) are an arch product from the field of nanotechnology which has gained inestimable interests because of their unique properties such as chemical stability, good conductivity, catalytic, antibacterial, anti-viral, antifungal, and anti-inflammatory activities. AgNPs are incorporated into composite fibres, cryogenic superconducting materials, cosmetic products, the food industry, and electronic components [5]. Given the above reasons, in the present study, *Leucas aspera* (Willd.) Link was collected from

Krishnapuram, Tirunelveli district, Tamil Nadu, and was used for silver nanoparticle synthesis and characterization. *L. aspera* commonly known as 'Thumbai' is found along roadsides and fallow fields from plains to 400 m. It is normally found in India, Bangladesh, Indo-China, and Malesia. The plant is used traditionally as an antipyretic and insecticide. Many veterinary medicines are prepared from the leaves. Leaves and flower buds are consumed as a vegetable. Its leaves are rich in calcium, magnesium, potassium, iron, and vitamins such as vitamin C, vitamin D, and vitamin E. A mixture of leaves and charcoal was applied to the wounds of cattle to kill worms. It is considered medicinal for liver ailments, snake bites, scorpion sting sinusitis, etc. It is used as a stimulant, anti-helminthic, laxative, and diaphoretic. Also being reported for use orally in the treatment of headache, asthma, bronchitis, etc [6]. Medicinally, it has been proven to possess various pharmacological activities like antifungal, antioxidant, antimicrobial, antinociceptive, and cytotoxic activity [7, 8].

## MATERIALS AND METHODS

### Preparation of plant extracts

The fresh healthy plants of *Leucas aspera* (Willd.) Link (Family: Lamiaceae) was collected from the Krishnapuram, Tirunelveli district, Tamil Nadu, and was authenticated by using The Flora of the Tamil Nadu Carnatic [9]. Using The Plant List [10] and the newly launched Plants of the World Online [11] correct accepted botanical name for the species identified was revised. The voucher specimen (Voucher No. VV-BOT-VOCC-2) was also maintained in the Department of Botany, V.O. Chidambaram College, Tuticorin, Tamil Nadu, India. The plants were surface cleaned with running tap water to remove debris and other organic contents, followed by double distilled water and air-dried at room temperature.

A 50g of fresh chopped whole plant material was added in a conical flask containing 100 ml of double-distilled water and boiled for 20 min. After cooling, the conical flask was kept under stirring on an orbital shaker for 2 h for complete extraction. The aqueous extract was filtered through Whatman (No.1) filter paper and the filtrate was gathered in an Erlenmeyer flask and was used for the reduction procedure of $Ag^+$ to $Ag^0$ [12].

### Green synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, 5 ml of plant extract was mixed with 95 ml of 1 mM aqueous silver nitrate solution (1 mM silver nitrate was prepared by dissolving 0.169 g $AgNO_3$ in 1000 ml deionized water and stored in an amber coloured bottle to prevent the self-oxidation of silver nitrate solution) and was incubated in a dark chamber to minimize photo-activation of silver nitrate at room temperature [13]. The reaction progression for silver nanoparticles synthesis was visually examined based on the colour alteration of the reaction mixture. After that 50 ml of colloidal silver nanoparticle suspension was stored in the refrigerator (4ºC) for further studies like UV-Visible spectrophotometer spectral analysis. The remaining suspension was poured into a Petridis and kept at 80ºC ± 2 for 12 h in the hot-air oven for drying. The dried sample was scraped, stored in a screw-capped bottle, and was used for FTIR analysis.

### Characterization of silver nanoparticles

#### Visual inspection

The confirmation of silver nanoparticles formation was made visually by the colour change from greenish-yellow to reddish-brown [13].

#### UV-Visible spectrophotometer spectral analysis

To observe the optical property of biosynthesized silver nanoparticles, 1 ml of the colloidal silver nanoparticle suspension was taken in a test tube and was diluted with 2 ml of de-ionized water. Then the sample was scanned in a UV-Visible Spectrophotometer between wavelengths of 350 to 750 nm [14].

#### Fourier-transform infrared (FTIR) spectroscopic analysis

FTIR analysis of the dried silver nanoparticles was carried out by the potassium bromide (KBr) pellet method. 1 mg of silver nanoparticle was mixed with 100 mg of dry potassium bromide (1:100 ratio) and then the mixture was compressed into a pellet using a hydraulic press (5000-10000 PSI). The compressed pellet was put into the sample holder and the FTIR (Systronics 166) spectra were recorded in the range of 400-4000 $cm^{-1}$. To alleviate the moisture content in the sample, a blank disc was put in the reference beam [15].

#### X-ray diffraction (XRD) analysis

The colloidal silver nanoparticle suspension stored withinside the refrigerator was centrifuged at 15000 rpm for 10 min. The supernatant was discarded and the pellet was taken. The pellet was re-dissolved in 10 ml of de-ionized water. While preparing samples for X-Ray Diffraction (XRD) analysis, a thin film of the sample (100 µl) was applied on a glass slide and allowed to dry for 30 min. The XRD pattern was recorded using X'Pert PROP Analytical-PW 3040/60 X-ray Diffractometer with an operating voltage of 30 kV at a 20 mA current strength. The sample was subjected to Cu-Kα radiation with nickel monochromator in the 2θ range of 20–80º [16]. The size of the silver nanoparticle was calculated by Debye–Scherrer equation [17] as follows:

$$S = \frac{k\lambda}{\beta_{0.5}\cos\theta}$$

Where, S is the crystallite size of the silver nanoparticle, k is the Scherrer constant that varies from

0.9 to 1, λ is the wavelength of the X-ray source (1.54056 Å) used in XRD, $\beta_{0.5}$ is the Full Width at Half Maximum (FWHM) of the diffraction peak in radian, and θ is the Bragg angle in radian.

## RESULTS AND DISCUSSION

### Visual observation and UV-Visible spectrophotometer study

In the aqueous solution of 1 mM silver nitrate when mixed with extract of *L. aspera*, a visible colour change from greenish-yellow to reddish-brown was noted within 10 min at room temperature. Reduction of silver ions exhibited reddish-brown colour in aqueous solution due to surface plasma vibration in silver nanoparticles. When the plant extract was added to the aqueous solution of the silver ion complex, the colour started to change from greenish-yellow to reddish-brown due to the reduction of silver ions [18]. According to Sasikala *et al.* [19], secondary metabolites present withinside the plant system may be accountable for the reduction of silver ions and the synthesis of nanoparticles. The biogenic route is the energy (or) electron released during Glycolysis (photosynthesis) for conversion of NAD to NADH led to the transformation of Ag $(NO_3)_2$ to form nanoparticles and another mechanism is releasing of an electron when the formation of ascorbate radicals from ascorbate reduces the silver ions.

UV-visible spectroscopy is a very useful and reliable technique for the primary characterization of synthesized nanoparticles [20]. UV-visible spectrum analysis for the biosynthesized silver nanoparticles using the extract of *L. aspera* shows the highest absorbance peak at 410 nm (Fig.1).

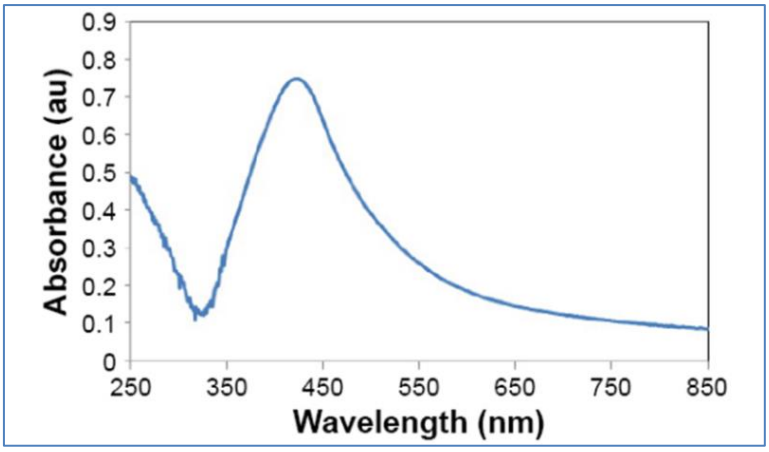


**Fig-1: UV-Vis Spectrum of *Leucas aspera* Silver Nanoparticles**

The occurrence of the highest absorbance peak at 410 nm is due to the phenomenon of surface plasmon resonance, which occurs due to the excitation of the surface plasmons present on the outer surface of the silver nanoparticles which gets excited due to the applied electromagnetic field. Compared with the earlier studies, our result is different in the absorbance spectrum. For instance, when using *Orthosiphon thymiflorus* (Lamiaceae) leaf extract to synthesis silver nanoparticles, the highest peak of absorbance appeared at 421 nm [21]. In some other studies, *Salvia spinosa* (Lamiaceae) extract was used to synthesize silver nanoparticles and the highest peak of absorbance was regarded at 450 nm [22].

### FTIR analysis

Fourier-transform Infrared Spectroscopy (FTIR) was used to analyse the functional groups present in the silver nanoparticles synthesized using plant extract. The FTIR spectroscopy investigation of *L. aspera* extract with 1 mM silver nitrate solution displays peaks at 3448.08 $cm^{-1}$, 2397.04 $cm^{-1}$, 2364.46 $cm^{-1}$, 2341.56 $cm^{-1}$, 1763.29 $cm^{-1}$, 1622.70 $cm^{-1}$, 1384.42 $cm^{-1}$, 1101.72 $cm^{-1}$, 826.09 $cm^{-1}$, 668.42 $cm^{-1}$, and 600.68 $cm^{-1}$ absorption peaks are known to be associated with the stretching vibration for amines/N-H (1°-amines) 2 bands, phosphorus/P-H phosphene, carboxylic acids and derivatives/C=O (2-bands), amines/$NH_2$ scissoring (1°-amines), alcohols and phenols/O-H bending (in-plane), phosphorus/P=O phosphine oxide, arenes/C-H bending & ring puckering, amines/$NH_2$ & N-H wagging and alkynes/C-H deformation (Fig. 2 and Table 1). The FTIR spectrum has more than 5 absorbance bands and henceforth, the sample may be a complex molecule.

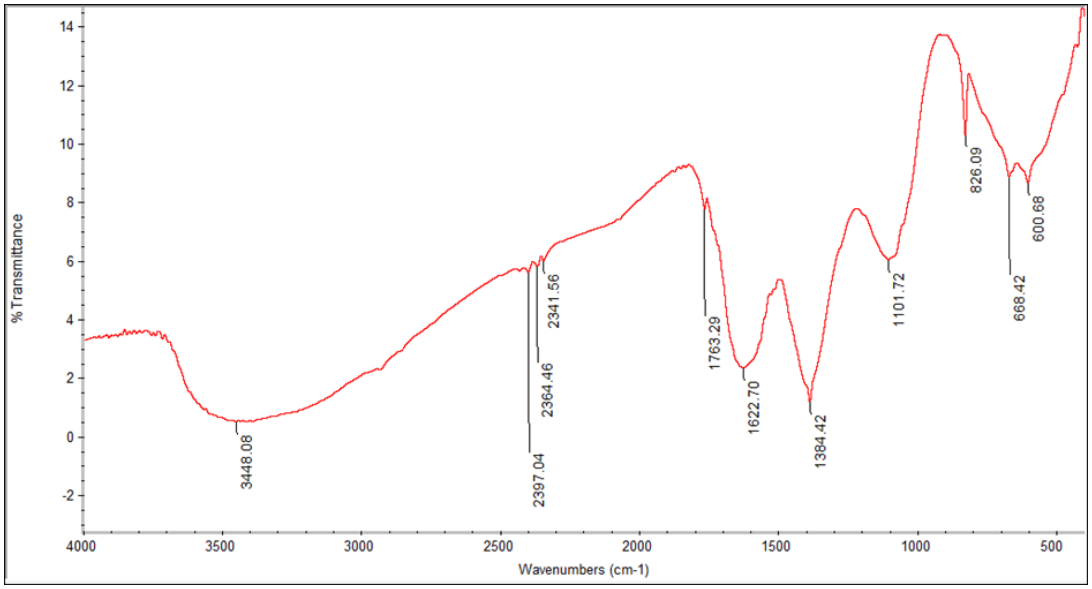


**Fig-2: FTIR Spectrum of *Leucas aspera***

**Table-1: FTIR Spectral Qualities Interpretation of the Comparative Shift in Functional Peaks of Critical Value (*Leucas aspera*)**

| Frequency Range ($cm^{-1}$) | Functional Group/assignment |
|---|---|
| 3448.08 | Amines/N-H (1°-amines) 2 bands |
| 2397.04 | Phosphorus/P-H Phosphene |
| 2364.46 | Phosphorus/P-H Phosphene |
| 2341.56 | Phosphorus/P-H Phosphene |
| 1763.29 | Carboxylic Acids & Derivatives/C=O (2-bands) |
| 1622.70 | Amines/$NH_2$ Scissoring (1°-amines) |
| 1384.42 | Alcohols & Phenols/O-H bending (in-plane) |
| 1101.72 | Phosphorus/P=O Phosphine oxide |
| 826.09 | Arenes/C-H bending & ring puckering |
| 668.42 | Amines/NH2 & N-H Wagging |
| 600.68 | Alkynes/C-H deformation |

The absorbance bands found with *L. aspera* extract at 3448.08 cm-1 (Amine functional group because of N-H stretch in proteins) endorse the presence of proteins at Ag-core particles' surface and withinside the nanoparticles shell. As plant molecules get absorbed onto the AgNPs surface, the amine groups intend to form more potent bonds with Ag atoms to break the maximum of the H-bonds between the N-H groups. These results confirm the presence of proteins acting as the reducing and stabilizing agents [23].

**XRD (X-ray diffraction) analysis**

The specific nature of the synthesised silver nanoparticles may be deduced from the XRD spectrum of the sample. The XRD pattern of the *L. aspera* plant-derived AgNPs (Figure 3) showed five intense peaks in the whole spectrum of 2θº values ranging from 20º to 80º. XRD spectra of the pure crystalline silver structure have been published by the Joint Committee on Powder Diffraction Standards (File. No. 04-0783). A comparison of our XRD spectrum with the Standard conformed that the silver particles formed in our experiments were in form of nanocrystals, as evidenced by the peaks at 2θº = 27.3823º, 29.6207º, 32.8229º, 44.7744º, and 55.2904º, corresponding to (1 1 1) (1 1 1) (2 0 0) (2 2 0) and (2 2 2) lattice planes respectively, to the face-centered cubic (fcc) structure of metallic silver.

The mean particle diameter of AgNPs was calculated from the XRD pattern, consistent with the line width of the maximum intensity reflection peak. The Full Width at Half Maximum (FWHM) values measured for (1 1 1) (1 1 1) (2 0 0) (2 2 0) and (2 2 2) lattice planes of reflection had been used with the Debye-Scherrer Equation to calculate the size of the nanoparticles. The XRD pattern clearly showed that the plant extract mediated synthesized silver nanoparticles were crystalline and the average size of nanoparticles was calculated as 36 nm (Fig. 3 and Tables 2 & 3). Logeswari *et al.* [24] reported that the size of the silver nanoparticles is 26 nm, 26nm, 59nm, 20nm, and 24nm corresponding to *Ocimum tenuiflorum*, *Syzygium cumini*, *Citrus sinensis*, *Solanum tricobatum*, and *Centella asiatica* respectively.

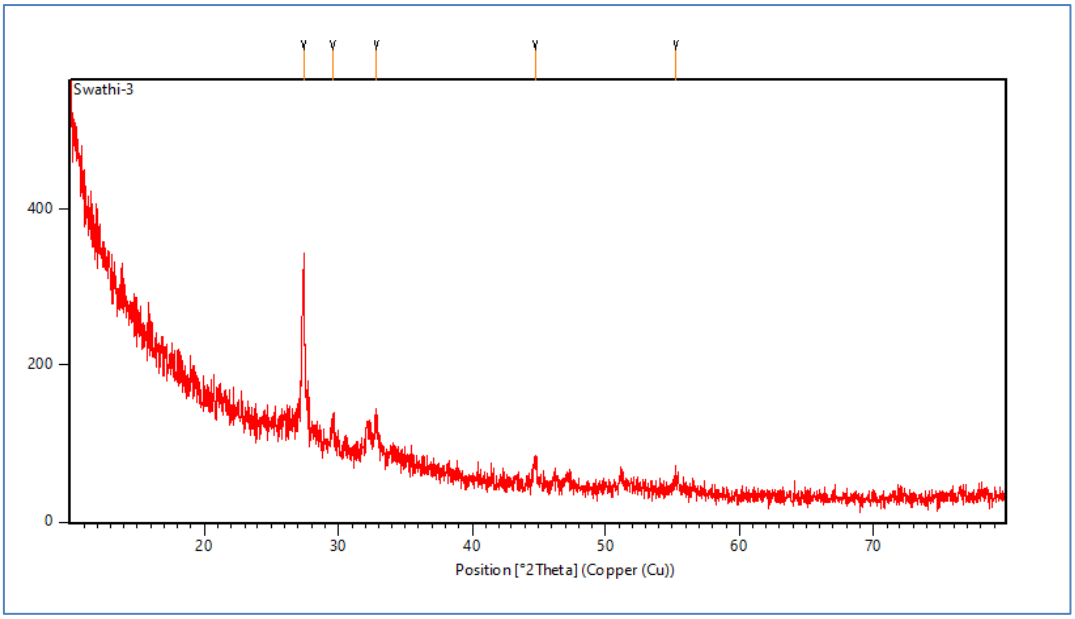


**Fig.-3: XRD Analysis of Biosynthesised Silver Nanoparticles Using *Leucas aspera***

**Table-2: Peak Indexing from XRD Spectra (*Leucas aspera*)**

| 2θ | θ | Sin θ | $Sin^2\theta$ | $\frac{3 \text{ X } Sin^2\theta}{Sin^2\theta_{min}}$ | $h^2+k^2+l^2$ | h k l |
|---|---|---|---|---|---|---|
| 27.3823 | 13.6911 | 0.2367 | 0.0560 | $\frac{3\ x\ 0.0560}{0.0560} = 3$ | $1^2+1^2+1^2$ | 1 1 1 |
| 29.6207 | 14.8103 | 0.2556 | 0.0653 | $\frac{3\ x\ 0.0653}{0.0560} = 3$ | $1^2+1^2+1^2$ | 1 1 1 |
| 32.8229 | 16.4114 | 0.2825 | 0.0798 | $\frac{3\ x\ 0.0798}{0.0560} = 4$ | $2^2+0^2+0^2$ | 2 0 0 |
| 44.7744 | 22.3872 | 0.3809 | 0.1451 | $\frac{3\ x\ 0.1451}{0.0560} = 8$ | $2^2+2^2+0^2$ | 2 2 0 |
| 55.2904 | 27.6452 | 0.4640 | 0.2152 | $\frac{3\ x\ 0.2152}{0.0560} = 12$ | $2^2+2^2+2^2$ | 2 2 2 |

**Table-3: Particle Size Derived from XRD Spectrum (*Leucas aspera*)**

| S. No | h k l | 2θ | Θ | FWHM (º) | β (radian) | Size (nm) |
|---|---|---|---|---|---|---|
| 1 | 1 1 1 | 27.3823 | 13.6911 | 0.1338 | 0.0023 | 63 |
| 2 | 1 1 1 | 29.6207 | 14.8103 | 0.4015 | 0.0070 | 20 |
| 3 | 2 0 0 | 32.8229 | 16.4114 | 0.2007 | 0.0034 | 42 |
| 4 | 2 2 0 | 44.7744 | 22.3872 | 0.2007 | 0.0034 | 45 |
| 5 | 2 2 2 | 55.2904 | 27.6422 | 0.8029 | 0.0140 | 11 |
| | | | | | Mean | 36 |

## CONCLUSION

To conclude, this study exhibits one step innovative green approach for the synthesis of silver nanoparticles from *L. aspera* plant extract. The method stands out primarily because it is eco-friendly and advantageous over conventional physical and chemical methods. These particles are predicted to have significant applications in numerous industries. The formation of silver nanoparticles became shown with the aid of using the colour change. The particle size and crystalline nature were determined by XRD. The plant material used is of wide occurrence as a weed and as far as known used for the first time in green synthesis of nanoparticles. Still, extra-scientific trials are had to help its healing uses.

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