Original Article

Leaf extract of *Leucas aspera* serves as a potential reducing source: A study on the synthesis and characterization of silver nanoparticles

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Abstract

Background: Plant mediated biosynthesis of metallic nanoparticles is gaining importance due to its simplicity, accuracy, cost-effectiveness and eco-friendliness.

Objectives: The present study aimed to synthesize silver nanoparticles (AgNPs) using ethanolic leaf extract of *Leucas aspera* (LA).

Materials and Methods: The enrichment of flavonoids such as rutin, gallic acid, luteolin and unknown compound in the LA extract was confirmed through High-performance liquid chromatography (HPLC) quantitation using different standard flavonoids. The extract of *Leucas aspera* reduced silver nanoparticles (LA-AgNPs) was confirmed from its color formation and UV absorption. The shape and size of LA-AgNPs were characterized by Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) analyses. The contributions of functional groups for the reduction and stability of LA-AgNPs were identified by Fourier Transform Infra-Red Spectroscopy (FT-IR) analysis.

Results: The synthesized LA-AgNPs were showed a dark brown color, maximum UV absorption at 420 nm, and spherical morphology with sizes of 8-30 nm under SEM and TEM. The FTIR spectrum of LA-AgNPs was showed the peaks for several functional groups revealed that LA phytochemicals mediated the effective reduction and stabilization of LA-AgNPs.

Conclusions: This study suggests that the flavonoids enriched LA extract may be considered as an efficient reducing source for the synthesis of potent AgNPs with reduced size for the biological applications.

Key words : Leucas aspera, Flavonoids, Silver nanoparticles, Physical properties

Introduction

Synthesis and characterization of metal nanoparticles received considerable attention in recent years because of their wide range of applications in various fields including medicine.[1] To date, metallic nanoparticles are prepared from noble metals such as Ag, Pt, Au and Pd.[2] Among the noble metals, Ag is the metal of choice for the preparation of nanoparticles because it is a health additive in traditional and Indian Ayurvedic medicine and also exhibits strong toxicity against a wide range of microorganisms.[3,4] Metallic nanoparticles are synthesized through three different methods such as chemical, physical and biological methods. Among these, synthesize of nanoparticles through the biological method has been gaining more advantages than the other approaches. The biological route offers rapid synthesis, gives stable nanoparticles, without the usage of external stabilizers and also environmentally safe.[5,6] Whereas, the use of chemicals, reagents and high temperatures for synthesizing nanoparticles in physical or chemical methods are not eco-friendly and also highly toxic to human health hence become invalid for biological applications.[7,8] Thus, the present study is aimed to synthesize silver nanoparticles through the green method by using *Leucas aspera* leaves extract.

Leucas aspera (LA) is a medicinal plant widely distributed in India. It is commonly known as "Thumbai" in Tamil.[9] It is widely used in the field of medi

cine and agriculture. It is a traditional medicine used to treat snake and scorpion bites. The leaves of LA are traditionally used to treat nasal congestion, cough, cold, headache & fever.[10] The juices of its flower are used to kill the intestinal worms. Studies have shown that leaves extract of LA display various pharmacological activities such as antimicrobial, antioxidant and anticeptive, etc.[11] This demonstrates that multiple pharmacological actions of CE leaves may be due to the enrichment of bioactive compounds. Therefore, the present study was hypothesized that the preparation of ethanolic extract of LA would serve as the best-reducing source for the synthesis of silver nanoparticles and this would also enhance or promote its therapeutic efficacy through minimizing herbal therapy limitations.[12] The present study is prepared ethanolic leaves extract of LA and quantitated its flavonoids through high-performance liquid chromatography (HPLC) analysis. Then, the LA extract reduced silver nanoparticles were characterized through the microscopic and spectral analyses.

Materials and Methods

Collection of Leucas aspera

The plant *Leucas aspera* (LA) was collected from Chennai, Tamil Nadu. The leaves of LA were removed and rinsed with tap water and distilled water and shade dried for one week.

Preparation of LA leaves extract

The dried leaves of LA were powdered by using a ball mill. Then, 10 g of LA leaves powder was transferred into a 250 mL Erlenmeyer flask and mixed with 100 ml of ethanol and kept in a magnetic stirrer for 24 h. The resulting ethanolic extract of LA was filtered by using a muslin cloth and stored in a brown bottle at 4°C and used for further studies.

High-performance liquid chromatography (HPLC) analysis of LA extract

The reversed-phase HPLC analysis was performed for the quantitation of flavonoids in LA extract. The liquid chromatography was operated under [LCGC—Agilent, injection volume 20 L; Nova-Pak C—18 column (4.6 mm 9 24 cm); mobile phase (methanol, water and phosphoric acid; 100:100:1); UV detection at 270 nm and flow rate 1.5 mL/min] the optimized conditions. The duplicates of the sample were analyzed by using the flavonoids standard. The obtained HPLC chromatogram of LA extract was compared with standard flavonoids.

Synthesis of LA extract reduced silver nanoparticles (LA-AgNPs)

The prepared ethanolic extract of LA was used for the reduction of Ag^{+} ions to Ag^{0}. Briefly, 10 mL of LA extract was mixed with 90 mL of 1 mM silver nitrate solution and stirred at 37°C for 24 h. The colour changes and patterns of UV absorption were followed for the confirmation of bio reduced LA-Ag-NPs.

Preliminary analysis of LA-AgNPs

The colour developed after the process of reductions was followed by visibility and photographed. After the period of reduction, the pattern of UV absorption of LA-AgNPs was recorded by using (Shimadzu 1601 model, Japan).

Scanning electron microscopy (TEM) analysis of LA-AgNPs

A thin film of LA-AgNPs was prepared on a carbon-coated copper grid by placing a very small drop of sample and dried under a mercury lamp for a few minutes. Then, a thin film on the grid was analyzed by using SEM (VEGA3TESCAN).

Transmission electron microscopy (TEM) analysis of LA-AgNPs

The sample LA-AgNPs was coated on carbon-coated copper grids and analyzed by using TEM (JEOL model 3010). The instrument was operated at 200 kV and a beam current of 104.1 µA.

Fourier transform-infrared (FT-IR) spectroscopy analysis of LA-AgNPs

FTIR measurements were taken for both LA extract and LA-AgNPs. Briefly, the solution of LA-AgNPs was centrifuged at 10,000 rpm for 30min and the resulting pellet was further washed with de-ionized water and dried. Then, the sample was mixed with KBr pellets powdered and used FT-IR measurements. The FT-IR scanning was performed at 4000–450 cm^{-1}.

Results

Phytochemicals of LA extract

Figure 1. shows the HPLC chromatogram of LA flavonoids. The HPLC quantitation of ethanolic extract of LA revealed the presence of flavonoids such as rutin (0.00093g/mL), gallic acid (0.0080g/mL), luteolin (0.0212g/mL) and unknown compound (0.108g/mL).

Colour and UV absorption of LA-Ag-NPs

Figure 2 depicts (a) Colour and (b) UV absorption spectrum of LA-AgNPS. The ethanolic extract of LA reduced AgNPs showed the greenish light brown color after the period 24 h. The maximum rage of UV absorption for the reduced sample was found at 420 nm.

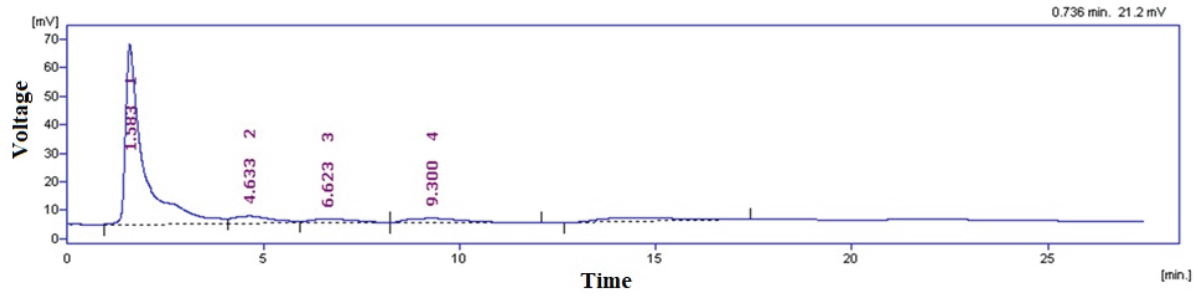

Figure 1: The HPLC chromatogram of LA flavonoids. Peaks 1-4 indicates the retention times of LA flavonoids such as rutin, gallic acid, luteolin, and unknown compound, respectively.

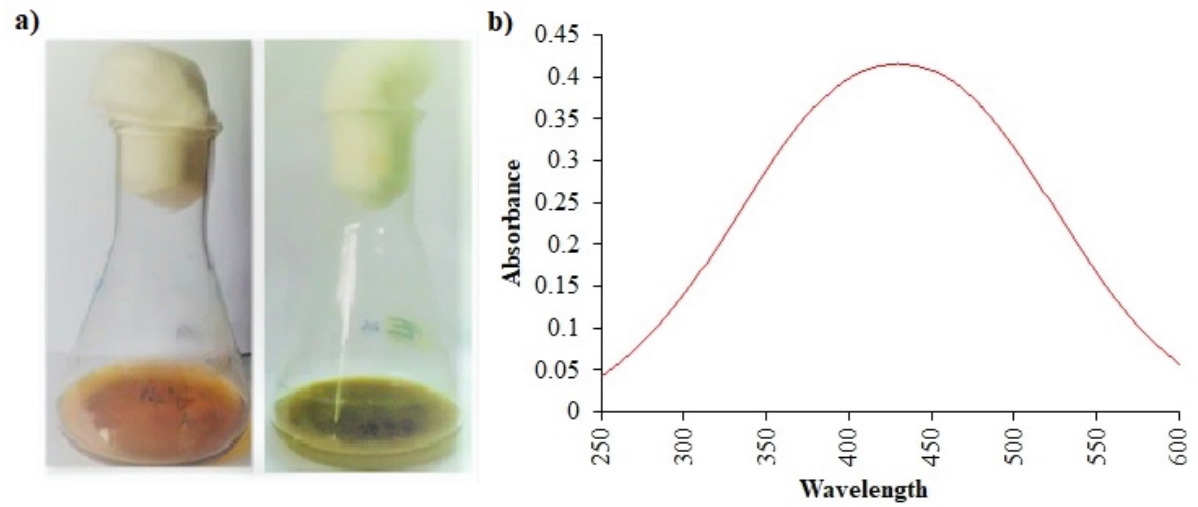

Figure 2: a) The color and b) UV absorption spectrum of LA-AgNPS. LA extract appears in orange color and LA-AgNPs appear in greenish light brown. LA-AgNPs shows λmax at 420nm.

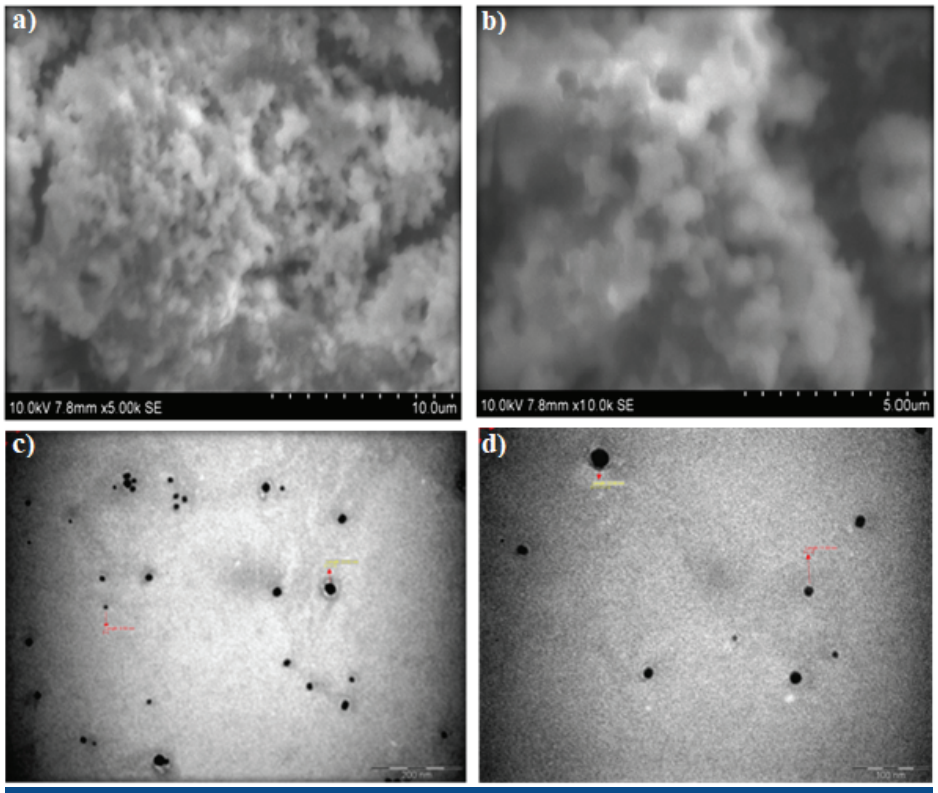

Figure 3: (a & b) the SEM and (c & d) TEM images of LA-AgNPs. LA-AgNPs show spherical shape with sizes ranging from 8-30nm.

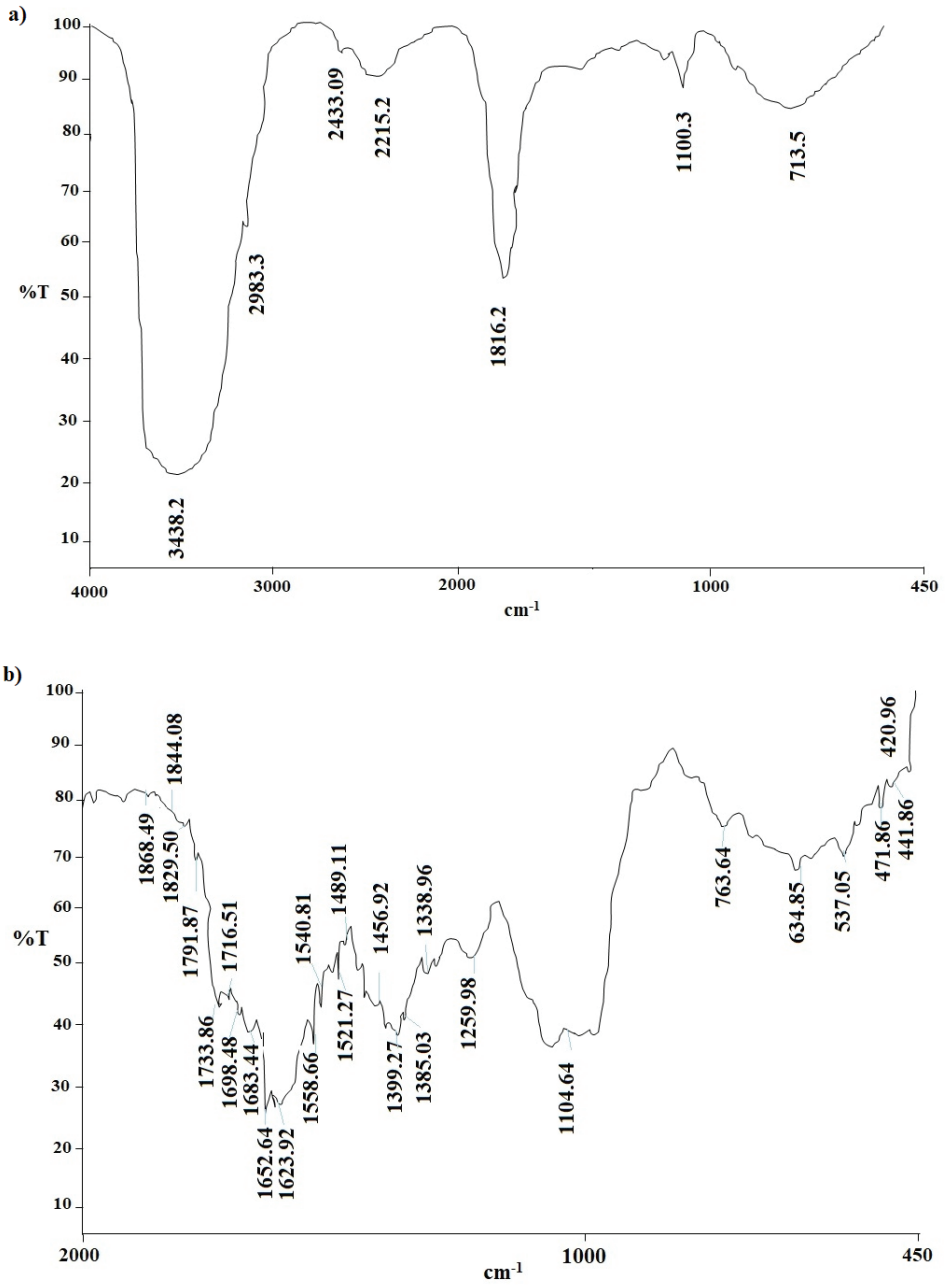

Figure 4: (a)FT-IR spectrum of the plain LA extract (b) FT-IR spectrum of LA-AgNPs

SEM and TEM micrographs of LA-Ag-NPs

Figure 3 represents (a & b) the SEM and c & d) TEM images of LA-AgNPs. The SEM and TEM micrographs of LA-AgNPs showed spherical shaped particles with sizes ranging from 8-30 nm. Furthermore, the appearance of surface-attached moieties demonstrating that LA extracts derived moieties.

FT-IR spectrum of LA-AgNPs

Figure 4 (a) displays the FT-IR spectrum of LA extract and (b) LA-AgNPs. The FT-IR spectrum of the ethanolic extract of LA showed seven peaks (i.e) stretching vibrations. However, in the FT-IR spectrum of LA-AgNPs, most of the observed LA peaks get vanished and appeared more numbers of additional peaks confirming that the formation of LA phytofabricated silver nanoparticles.

Discussion

This study was synthesized silver nanoparticles using the ethanolic leaves extract of LA and further characterized through advanced microscopic and spectral analyses. It has been documented that the leaves of LA contain multiple bioactive compounds such as tannins, phenols, flavonoids, saponin, quinone, protein, carbohydrates, cyanin and terpenoids.[13] This

suggests that LA leaves are the main source of multiple phytochemicals. The present study has identified the enrichment of phytochemicals such as rutin, gallic acid, and luteolin in the ethanolic leaves extract of LA.

Plants are a good reservoir of bioactive phytochemicals. Thus, several studies have been proven their bio reducing properties through the synthesis of various metallic nanoparticles. Generally, the development of metallic nanoparticles through the green method has been preliminarily confirmed through color transformation and pattern of UV absorption.[14, 15] In the present study, the ethanolic leaves extract of LA reduced silver nanoparticles had produced the characteristic brown color and UV absorption at 420nm, confirming that the reducing ability of LA phytochemicals is flavoured the conversion of Ag^{+} to Ag^{0}.[16,17]

The physical properties of metallic nanoparticles such as shapes and size are determined through SEM and TEM analyses. The SEM and TEM analyses of LA-AgNPs revealed that spherical shaped particles with sizes of 8-30 nm. Furthermore, the appearance of a layer around the silver nanoparticles demonstrated the surface integrated bio moieties of LA. It has been reported that biomolecules can interact with the surface of metallic nanoparticles through their free amino or sulfhydryl groups which eventually providing the stability to nanoparticles.[18]

FT-IR analysis provides the contributions of different functionalities in the process of reduction and structural stabilization of metallic nanoparticles. The FT-IR spectrum of plain LA extract showed the peaks for different functionalities. However, most of these peaks get disappeared and various new peaks were appeared in the FT-IR spectrum of LA-AgNPs, demonstrating that the contributions of functional groups of bioactive flavonoids (rutin, gallic acid, luteolin) for the reduction and stabilization/capping of LA-AgNPs. It has been reported that the functional groups of biomolecules such as C-O, C-OH, NH, and COO display a strong affinity to metal for their surface interaction and stability of nanoparticles.[19] Furthermore, it has shown that the peak at 1634 cm–1 is due to –C = C– aromatic stretching and suggested that AgNPs might be capped by secondary plant metabolites like flavonoids.[20] In the present study, the appearance of new peaks at the above-mentioned range confirmed that the surface integrating bioactive flavonoids of LA.

Conclusion

The obtained silver nanoparticles with the capping of multiple functionalities inferred that the flavonoids enriched leaf extract of LA could serve as the best alternative reducing agent for the rapid synthesis of silver nanoparticles with the desired size for the use of biological applications.

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