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Original Article

CHARACTERIZATION AND ANTIBACTERIAL STUDIES OF LEAF ASSISTED SILVER NANOPARTICLES FROM *CARICA* PAPAYA: A GREEN SYNTHETIC APPROACH

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ABSTRACT

Objective: The aim of this study is to synthesis silver nanoparticles using C*arica papaya* leaf extracts and testing its antibacterial activity.

Methods: Silver nanoparticles (Agnps) were prepared by treating *Carica papaya* leaf extracts with 1 mm silver nitrate ($AgNO_3$). The bioreduced silver nanoparticles were characterized by ultraviolet visible spectroscopy (UV-Vis), Fourier transform infrared spectroscopy (FTIR) and the size and shape of the silver nanoparticles were obtained from transmission electron microscopy (TEM). The synthesized silver nanoparticles were tested for their antibacterial activity by disc diffusion method.

Results: From the UV spectrum, it was observed that the peak at 430 nm showing the reduction of silver ions to stable silver nanoparticles. The size of the biosynthesized nanoparticles was in the range of 10-50 nm obtained from TEM image. FTIR measurements provided the bands at different wave numbers, indicating the stretching of alcohols, carboxylic acids, esters and ethers. The synthesized silver nanoparticles exhibited an excellent bactericidal activity against bacterial strains like *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus*.

Conclusion: Green synthesis of silver nanoparticles using papaya leaf extract exhibited effective antibacterial property.

Keywords: Green synthesis, Silver nanoparticles, Carica papaya, Characterization and antibacterial activity.

INTRODUCTION

Green nanotechnology refers the utilization of various plant resources for biosynthesis of noble metallic nanoparticles. Green synthesis field is advancement for chemical and physical method as it is cost effective, environmental friendly, easy scale up for large scale synthesis and in this method there is no need high pressure, energy, temperature and toxic chemicals [1]. Silver nanoparticles (Agnps) have become the focus of intensive research owing to their wide range of applications in areas such as catalysis, optics, antimicrobials, and bio material production. Silver has a long and intriguing history as an antibiotic in human health care. The silver ion is biologically active and readily interacts with proteins, amino acid residues, free anions and receptors on mammalian and eukaryotic cell membranes. Silver nanoparticles exhibit new or improved properties depending upon their size, morphology, and distribution [2]. However, there is still need for commercially viable as well as environmentally clean synthesis route to synthesize the silver nanoparticles. Various approaches using plant extract have been used for synthesis of noble metal nanoparticles.

Plant extracts, with their antioxidant or reducing properties are responsible for the reduction of metal compounds into their respective nanoparticles in cheaper ways for nanoparticles synthesis. These approaches have many advantages over chemical, physical, and microbial syntheses [3–13] because there is no need of the elaborated process of culturing and maintaining the cell, hazardous chemicals, high-energy requirements, and wasteful purifications. Several researchers developed and reported metallic nanoparticles using various plants and their extracts such as *Jatropha curcas* (Latex), *Clove, Brassica, Morganella, Cinnamon zeylanicum* (Bark extract), *Jatropha cruces* (seed) etc [13]. Biosynthesis using leaves extracts of *Murraya koenigii* [14] *Eucalyptus hybrida* [15], *Artocarpus heterophyllus* [16], *Camellia Sinensis* [17], *Mollugo nudicaulis* [18], *Panicum virgatum* [19] and fruit extracts of *Capsicum annum* L. [20], *Carica papaya*. L [21] *Citrullus colocynthis* [22] and *Lantana camara* [23] have been reported so far. But information on silver nanoparticle synthesis using papaya leaves at room temperature is scanty. Hence the present work focused on to study the synthesis of silver nanoparticles using papaya leaves and to evaluate its antibacterial activity against pathogenic bacterial strains.

MATERIALS AND METHODS

Preparation of leaf extract

The experimental work was carried out by preparing leaf extract with 10 g of fresh papaya leaves collected locally from Tirupati and thoroughly washed the leaves with running tap water and distilled water for 3–4 times. The leaves were then chopped into fine pieces and boiled in a 250 ml Erlenmeyer flask by stirring at 60 °C for 1h. After cooling, the extract was filtered using whatman No.1 filter paper and stored at 4 °C for further experimental work.

Synthesis and characterization of silver nanoparticles

Ten milliliters of leaf extract was added into 90 ml of an aqueous solution of 1 mm silver nitrate for bio reduction of Ag^+ ions in the solution and kept at room temperature for 24 hours and observed for change in colour from green to dark brown.

UV-Vis spectra analysis

The reduction of pure silver ions was monitored under UV–Vis spectrophotometer (Thermo Scientific-Genesys 10S). Sample of 3 ml was withdrawn and the absorbance was measured. UV–Vis spectral analysis was done between 300-700 nm.

FTIR analysis

The chemical composition of silver nanoparticles was analyzed using FTIR spectrometer (Thermo Nicolet Nexus 670 spectrometer) after bio reduction with silver nitrate. For FTIR measurements, dry powders of silver nanoparticles were obtained by centrifuging the reduced leaf extracts particles at 10, 000 rpm for 15 min, following which the pellet was dispersed in sterile distilled water to get rid of any uncoordinated molecules. The process of centrifugation and dispersion in sterile distilled water was repeated three to four times to ensure better separation of free entities from the metal nanoparticles. The purified pellets were then dried and the powder that obtained was subjected to FTIR spectroscopy measurement. The samples were scanned using infrared in the range of 4000-400 cm^{-1}.

Transmission electron microscope

Transmission electron microscope was performed for characterizing size and shape of silver nanoparticles. The sample was sonicated (VS 80, Vibronics) for 15 min. A drop of this solution was loaded on carbon-coated copper grids, and the solvent was allowed to evaporate under Infrared light for 30 min. TEM measurements were performed on the Philips Tecnai F12 instrument operated at an accelerating voltage at 200 kV.

Antimicrobial assay

Antibacterial activity of silver nanoparticles was assayed using standard well diffusion method against human pathogenic bacteria (*Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, and Staphylococcus aureus*). Nutrient agar was prepared for cultivation of bacteria. Fifty microliters of fresh overnight grown cultures of the bacteria were spread on nutrient agar containing petri plates and with a sterile borer 1 mm holes were punched in the medium. Fifty microliters of the solution containing nanoparticles was inoculated in this hole and the plates were incubated at 37 °C for 24 h. Further zone of inhibition was measured.

RESULTS AND DISCUSSION

Biosynthesis of silver nanoparticles by using *C. papaya* leaves

The silver nanoparticles were synthesized from papaya leaves by adding 1 mm $AgNO_3$ to the leaf extract filtrate. The original filtrate, which is greenish in color gradually, changes to the dark brown color within minutes of the addition of silver nitrate to the filtrate (fig. 1). The color got stabilized during 24 h of the reaction as the formation of the silver nanoparticles by the reduction of the Ag^+ions takes place extracellular. It is known that silver nanoparticles exhibit yellowish brown color in water; this color arises due to excitation of surface Plasmon vibrations in the metal particles [24].

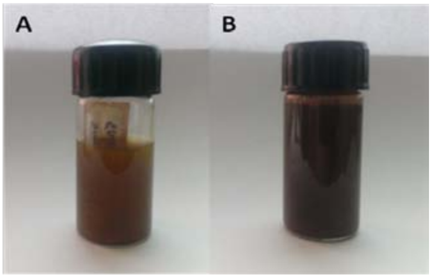

Fig. 1: Papaya leaf extract (A) before addition of $AgNO_3$ and (B) after addition of $AgNO_3$ and incubated for 72 h

UV-Vis Spectrophotometer analysis

The silver nanoparticles formation and the stability of the reduced silver nanoparticles in the filtrate were monitored by using UV-Vis Spectrophotometer (fig. 2). An UV-Vis Spectrophotometer is one of the important techniques to ascertain the formation of metal nanoparticles. The results were plotted and are shown in fig. 2. The UV–Vis spectrum Showed an SPR peak of silver nanoparticles at 430 nm. It is well known that the size and shape of the silver nanoparticles reflects the absorbance peak [25, 26]. The absorption spectrum obtained showed a strong surface plasmon resonance band maximum at 434 nm (fig. 2) a characteristic peak of silver nanoparticles [27].

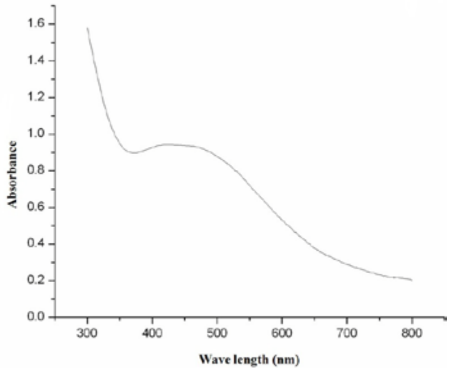

Fig. 2: UV-Visible spectral analysis of synthesized silver nanoparticles

Characterization of silver nanoparticles by FTIR and TEM analysis

The FT-IR spectrum of synthesized silver nanoparticles was shown in fig. 3. The spectrum showing a band at 3441 cm^{-1} corresponds to-OH and-H bonded alcoholic and phenolic groups. The band at 1700 cm^{-1}, 1114 cm^{-1} and 1050 cm^{-1} signifies the-C=O stretch in acids, esters, ethers etc. The carbonyl group from amino acid residues and peptides of proteins has the stronger ability to bind to metal [28].

The proteins could be most possibly from a coat covering the metal nanoparticles to prevent agglomeration of the nanoparticles and stabilizing them in the medium. This evidence suggests that the biological molecules could possibly perform the function for the formation and stabilization of the silver nanoparticles in aqueous medium [29].

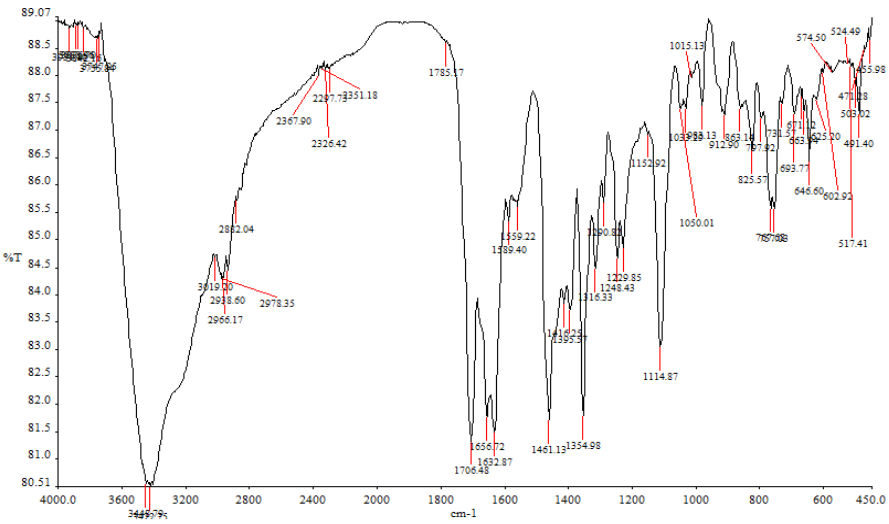

Fig. 3: FTIR analysis of silver nanoparticles

TEM image of silver nanoparticles derived from papaya leaf extract was shown in fig. 4. The morphology of our silver nanoparticles was very different from other studies that reported which is showing spherical, oval and pentagonal. The particle size of silver nano particles are found to be in the range of 10–50 nm.

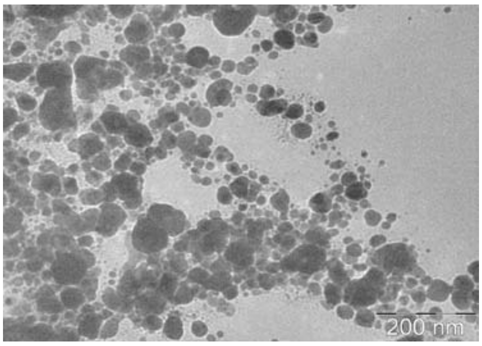

Fig. 4: TEM image of formed silver nanoparticles

The synthesized silver nanoparticles derived from papaya leaf extract exhibited an excellent antibacterial activity against both gram positive and gram negative bacteria. The results suggest that silver nanoparticles undergo an interaction with bacterial cell and displayed the strong bactericidal action against *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus*. Similarly, Jaidev and Narasimha 2010 [30] and Murali sastry *et al.*, 2005 [29] demonstrated that reactive antibacterial activity of silver nanoparticles and metal oxides.

Table 1: Antibacterial activity of silver nanoparticles

S. No.	Organism	Zone of inhibition (cm)
1	*Escherichia. Coli*	1.0±0.2
2	*Staphylococcus aureus*	1.1±0.1
3	*Pseudomonas aeruginosa*	0.9±0.3
4	*Bacillus subtilis*	1.4±0.4
5	streptomycin	1.2±0.1

*All values represented in the table are average of conducted experiment

It has been known for a long time that silver ions and silver compounds are highly toxic to most bacterial strains. Recently it was shown that the highly concentrated and non hazardous nanosized silver nanoparticles can easily be prepared in a cost effective manner and tested as a new type of bactericidal agents. In this study, formation of clear zone (restricted bacterial growth) around the cavity is an indication of antibacterial property of silver nanoparticles (table 1).

CONCLUSION

Green synthesis of nanoparticles using C. papaya leaf extract is a natural, simple, eco-friendly and cost-effective process. The synthesized silver nanoparticles were in the range of 10-50 nm as characterized with TEM. The synthesized silver nanoparticles exhibited an excellent anti bacterial activity against gram positive and gram negative pathogenic bacterial strains like *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, and Staphylococcus aureus.* This type of green synthetic approach in the production of nanoparticles is getting importance in industrial scale and biomedical fields as they are stable for a long time with a very little volume of plant or leaf extracts.

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CONFLICT OF INTERESTS

Declared None

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