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Research paper

Synthesis of silver nanoparticles using fresh bark of *Pongamia pinnata* and characterization of its antibacterial activity against gram positive and gram negative pathogens

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Abstract

The recent advancements in techniques for synthesis of zerovalent nanoparticles using green method show a clean, simple, less toxic and environmentally benign process. In this communication, silver nanoparticles were synthesized and characterized using the fresh bark extract of *Pongamia pinnata*. The bark extract was exposed to silver ions and the resultant biosynthesized silver nanoparticles characterized by UV–vis spectrophotometry shows the surface plasmon resonance band at 420 nm. X-ray diffraction spectrum shows crystalline structure while scanning electron microscope and transmission electron microscope analyses revealed the polydisperse distribution and particle size of 5–55 nm. The elemental analysis shows strong signal at 3 keV that corresponds to silver ions and confirms the presence of metallic silver. The antibacterial activity of silver nanoparticles was determined by agar well diffusion method against gram positive and gram negative bacteria. Maximum and minimum zones of inhibition were noted against *Klebsiella planticola* (15 mm) and *Staphylococcus aureus* (13 mm), respectively. This study reveals that silver nanoparticles possess good antibacterial activity at 100 µg/ml concentration.

Keywords: Antibacterial activity; Green synthesis; *Pongamia pinnata*; Silver nanoparticles; TEM

1. Introduction

Nanotechnology is a highly emerging field that has vast applications in many industries, medicine, cosmetics etc. Nanoscience is the study and creation of materials at nanoscale level with exclusive properties [1] for the vast range of applications in various fields. The properties of nonmaterial are related to the size of materials and differ significantly from the bulk materials [2,3]. Nanoparticles exhibit various morphological structures with shapes like rods, sphericals, tubes, hollow spheres, platelets etc. Different types of nanoparticles existed like metal, metal oxides, semiconductors, polymer, and core-shell particles. Among these types metal nanoparticles show high physical and chemical unique properties. Silver nanoparticles belonged to metallic nanoparticles.

Silver is a transition metal and used in an ancient medicinal system for curing various diseases [4]. Nanoscale silver has received considerable attention in various applications due to its unique properties [5]. Application of nanoparticles in various fields depends on the size and shape characteristics of nanoparticles [6]. Mainly, silver nanoparticle was used as a bactericide. It has more antibacterial effects due to the large surface to volume ratio. Silver nanoparticles have recently been used in many consumer products like soap, toothpaste, and socks due to its antibacterial properties. And also, it has been used in water purification systems, medical devices, cosmetics [7,8], bioremediation, heavy metal and pesticide removal in water and soil [9,10].

Nanoparticles can be synthesized by various methods like physical, chemical and biological methods. The physical and chemical methods are complicated, expensive and also generate hazardous by-products [11]. In contrast, the green method that uses plant extracts has recently been having considerable attention in nanoparticle synthesis with its clean, simple, less toxic and eco-friendly nature [12,13]. The plant *Pongamia pinnata* is classified in the family Fabaceae found throughout India. Different

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parts of this plant are used in medicinal systems for various treatments of ailments as antiseptic, blood purifier, and wound healing agent [14]. This plant has antioxidant, antiproliferative, anti-inflammatory and anticancer activities. The plant has various phytochemical constituents that include flavonoids, furane flavones, furanoflavonols, chromenoflavones, furanodiketones, flavonoid and glycosides [15–18]. In this investigation, synthesis of silver nanoparticles was achieved by greener method using fresh bark extract of *P. pinnata* and assessment of its characterization and antibacterial activity against pathogenic microorganisms.

2. Materials and methods

2.1. Synthesis of silver nanoparticles by using P. pinnata fresh bark extracts

The *P. pinnata* fresh barks were collected from a medicinal plant garden located in APCAS, Kalavai, Vellore Dist, India. The leaves were cut into small pieces and washed with the detergent TWEEN 20 followed by double distilled water for 2–3 times. It was slightly dried at room temperature. Individually, 10 g of *P. pinnata* bark was weighed and boiled with 100 ml double distilled water at 60–80 °C for 10 min. After that, the solution was filtered through nylon mesh cloth and stored at 4 °C for nanoparticle synthesis process.

For silver nanoparticle synthesis, about 10 ml of *P. pinnata* fresh bark extract was added separately to 90 ml aqueous solution of AgNO3 (1 mM) and kept at room temperature. The colour changes from pale yellow to brown colour, which indicates that the silver nanoparticles could be formed due to the reaction of *P. pinnata* fresh bark extract with silver metal ions. A control was maintained without an addition of silver nitrate, which shows no colour changes. It confirms the occurrence of silver nanoparticles in the reaction mixture where the colour changes take place.

2.2. Characterization of silver nanoparticles

The silver nanoparticles were synthesized at different time intervals and the absorption spectra of the reaction solution were evaluated by UV–vis spectrophotometry. The reaction mixture was centrifuged and washed with distilled water 4–5 times. The pellet was collected and dried in a hot air oven. The dried particles were scanned on a Philips scanning electron microscope (SEM) and later with an XDL 3000 powder X-ray diffractometer operated at a voltage of 40 kV and a current of 30 mA. The size and morphology of the air-dried silver nanoparticles were characterized by SEM. The TEM analyses for nanoparticles were performed by Philips CM200 with an operating voltage of 20–200 kV and the line resolution was 2.4 Å. For TEM analysis, the samples were prepared on carbon-coated copper grids and examined the size and morphology of the silver nanoparticles. The presence of elemental silver in the solution mixture was analyzed by EDAX analysis.

2.3. Antimicrobial activity of silver nanoparticles

The antibacterial activity of synthesized silver nanoparticles was determined by agar well diffusion method against pathogenic bacteria: *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Klebsiella planticola*. One millilitre of fresh overnight culture (1 × 108 CFU) of each strain was swabbed uniformly onto the individual plates containing sterile Luria–Bertani agar and 3 wells were made with the diameter of 6 mm using cork borer. Then 25 µl (100 µg/ml) of purified silver nanoparticles, *P. pinnata* fresh bark extract (100 µg/ml) and silver nitrate solution (100 µg/ml) were poured into each well using sterile micropipettes. Inoculated Petri dishes were incubated for 24 h at 370 °C. After incubation, different levels of zonation formed around the well and were measured. This experiment was repeated three times.

3. Results and discussion

3.1. Visual identification

The identification of colour change is a preliminary tool that confirms the ability of plant extract in nanoparticle synthesis. Formation of brown colour in the reaction mixture could confirm the presence of silver nanoparticles (Fig. 1c and d). Initially, the reaction mixture showed no colour change and turned into brown colour after 10 min of incubation. In *P. pinnata*, the brown colour formation after 10 min indicates that the process of silver nanoparticle production has started and the intensity of brown colour was increased after 24 h (Fig. 1d). After 24 h, the settling of synthesized silver nanoparticles at the bottom of the conical flask reveals the reduction of silver metal into silver nanoparticles was completed. Similar colour observations were noted in the plant extracts of *Coleus aromaticus*, *Calliandra haematocephala*, and marigold flower [19–21].

3.2. UV–vis spectroscopic analysis

The silver nanoparticle formation was confirmed by the positioning of SPR in the UV–vis spectroscopic analysis. Fig. 2 shows the UV–vis spectra of the reaction mixture of silver nitrate solution with *P. pinnata* fresh bark extracts that were exposed with different time intervals such as 10 min, 30 min, 1 h, 2 h. Herein, the peaks observed at 420 nm indicate the presence of silver nanoparticles which is synthesized by *P. pinnata* extracts, the peak was raised due to the effect of surface plasmon resonance of electrons in the reaction mixture. The silver nanoparticle synthesis with reaction time 10 min–2 h shows no considerable changes in the shift of SPR band and indicates that no changes were found in the size of nanoparticles [22]. The narrow peaks formed indicate the synthesis of small-sized nanoparticles. Formation of nanoparticles was gradually increased with reaction periods up to 2 h, which was determined based on the absorbance intensity of silver nanoparticles in the UV spectra. After 2 h, the intensity was decreased due to the completion of nanoparticle formation.

3.3. X-ray diffraction analysis

The XRD spectra are used to confirm the crystalline nature of the silver nanoparticles synthesized by using *P. pinnata* fresh bark extract and the pattern was exhibited in Fig. 3. The spectra of XRD clearly indicated that the synthesized silver

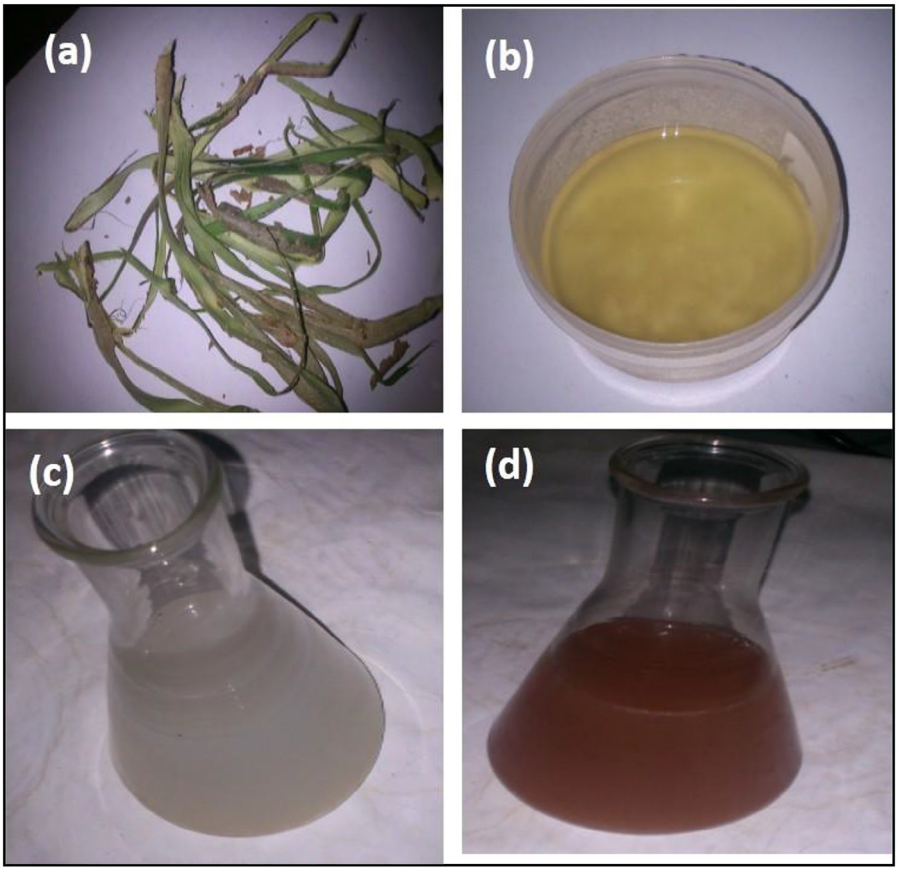

Fig. 1. Visual observation showing (a) bark, (b) bark extract and (c) colour changes after adding bark extract to $AgNO_3$ solution after reaction time of 0 h (d) 24 h.

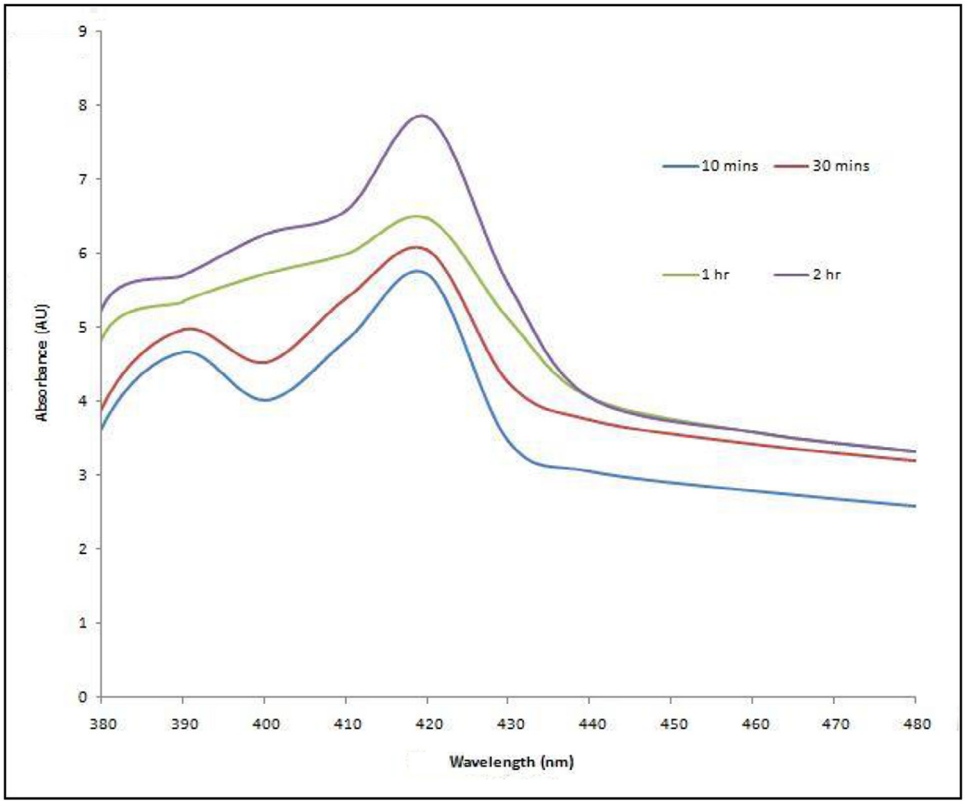

Fig. 2. UV–vis spectra of silver nanoparticles synthesized by *P. pinnata* bark extract show the SPR band at 420 nm could confirm the formation of silver nanoparticles in the reaction mixture.

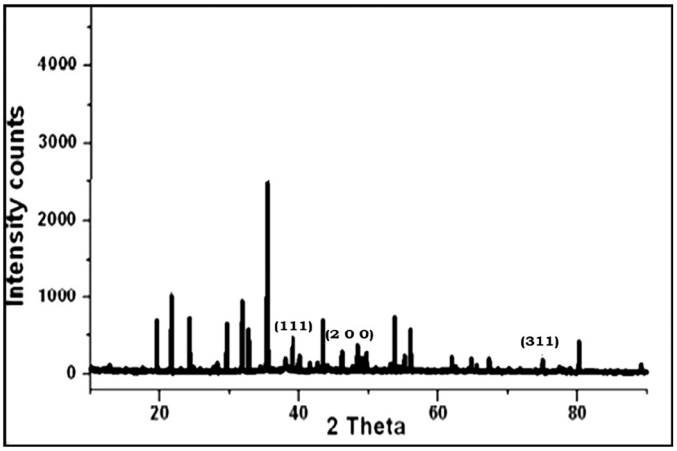

Fig. 3. XRD spectrum of the crystalline nature of silver nanoparticles.

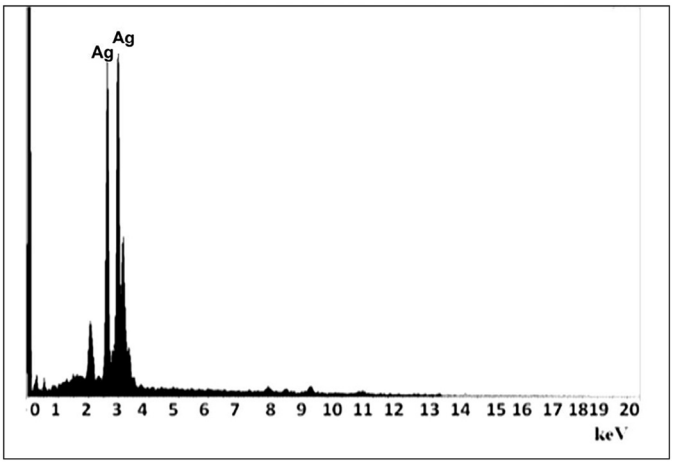

Fig. 5. EDAX spectrum of synthesized silver nanoparticles using *P. pinnata* bark extract shows typical absorption peak at 3 keV for silver.

nanoparticles using the above-mentioned extracts were crystalline in nature. The Bragg reflections of silver nanoparticles were observed at 2 theta values of 38.09°, 46.1°, and 77.3° corresponding to the lattice planes (1 1 1), (2 0 0) and (3 1 1) which were indexed for silver. Some of the unassigned peaks were identified due to the presence of phytochemicals from extracts that may be capping on the surface of nanoparticles [19,23].

3.4. Scanning electron microscope and energy dispersive X-ray analysis

Scanning electron microscope is one of the powerful tools to identify the morphology of the synthesized nanoparticles. The silver nanoparticles synthesized by the *P. pinnata* fresh bark extract are predominantly spherical in shape (Fig. 4) (scale bar 500 nm). The aggregation of nanoparticles is acquired resulted in the formation of large sized nanoparticles. This report is well matched with the report of UV–vis spectra. SEM image showed well dispersed small and large sized spherical and irregularly shaped silver nanoparticles.

Further, elemental analysis was carried out to confirm the formation of metallic silver nanoparticles in the reaction mixture. Fig. 5 shows the EDAX analysis of *P. pinnata* fresh bark extract mediated synthesis of silver nanoparticles. The EDAX analysis showed an intense signal at 3 keV, which indicates the presence of elemental silver [24].

3.5. Transmission electron microscope

The TEM image of *P. pinnata* fresh bark extract synthesized silver nanoparticles is shown in Fig. 6 and the image signifies that the synthesized silver nanoparticles are polydisperse. The synthesized silver nanoparticles attain different shapes. The size of the synthesized nanoparticles that ranges from 5 to 15 nm (small) and 22–55 nm (large) could be observed. Entirely, the synthesized silver nanoparticles are spherical in shape and some undefined shapes are also observed with slight aggregation. The formation of small, large sized and undefined shaped nanoparticles is dependent on the presence of phytochemicals

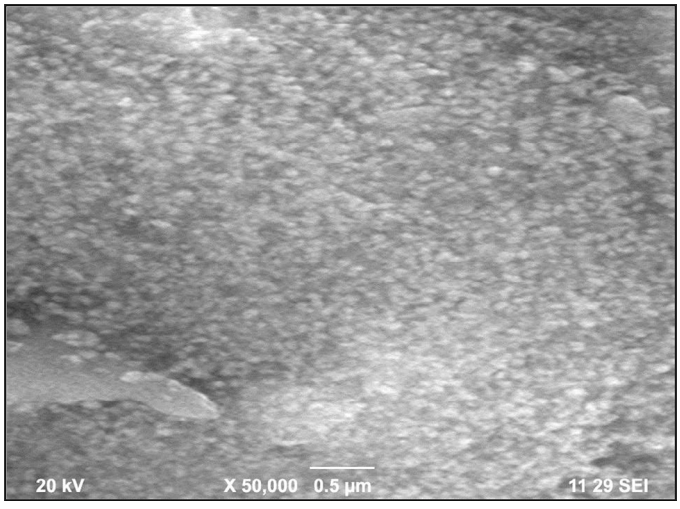

Fig. 4. SEM image of silver nanoparticles synthesized by *P. pinnata* fresh bark extract.

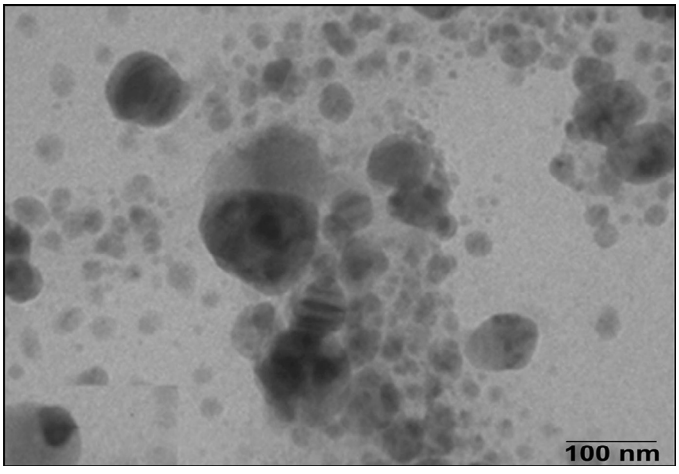

Fig. 6. Transmission electron microscopic image of silver nanoparticles synthesized by *P. pinnata* fresh bark extracts.

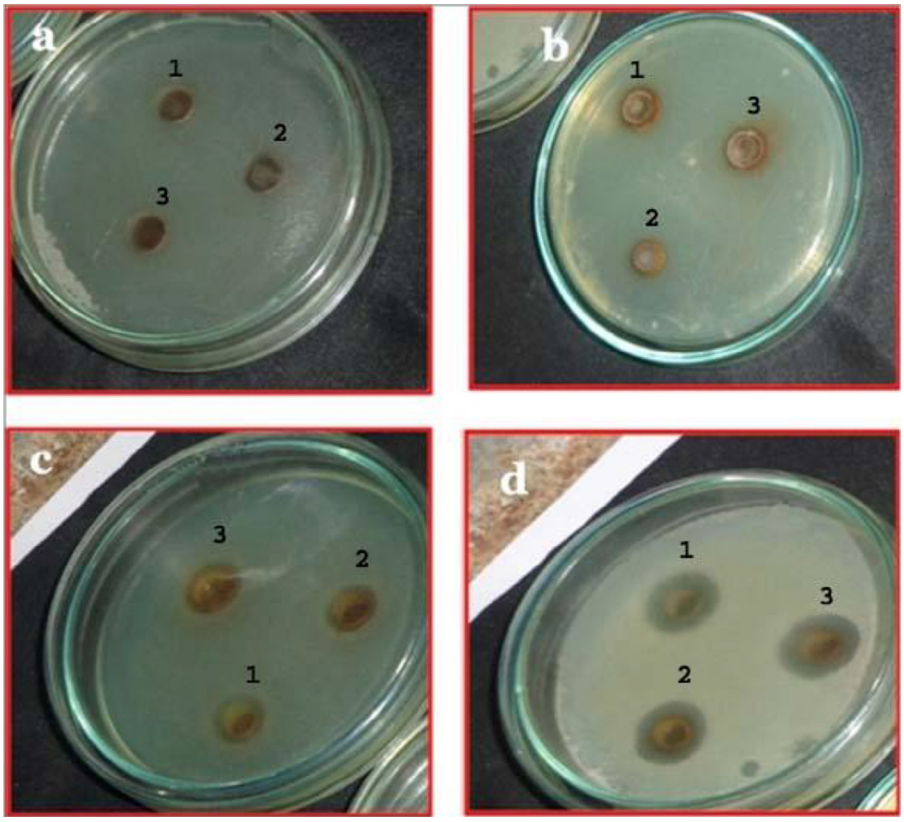

Fig. 7. Antimicrobial activity of silver nanoparticles against pathogenic microorganisms (a) *Bacillus subtilis* (b) *Staphylococcus aureus* (c) *Klebsiella pneumoniae* (d) *K. planticola* (1 – plant extract; 2 – silver nitrate; 3 – silver nanoparticles).

phenolic amides, piperine, polysaccharides and other reducing sugars that might have [25,26] played an important role in the synthesis of silver nanoparticles.

3.6. *Antimicrobial activity of silver nanoparticles synthesized using* P. pinnata

The green synthesized silver nanoparticles' antibacterial activity was assessed against pathogenic microorganisms. Silver nanoparticles show a significant inhibition activity against both gram positive and gram negative microorganisms. Predominantly, the maximum zone of inhibition was noted against *K. planticola*, a gram negative bacterium (Fig. 7d). In contrast, minimum zone inhibition was measured against *S. aureus*, a gram positive bacterium (Fig. 7b). Indeed, the antibacterial activity was higher than the plant extract and silver nitrate solution. The difference in inhibition activity of silver nanoparticles against gram positive and gram negative bacteria is due to the composition of the cell wall. The mechanisms of antibacterial activity of silver nanoparticles are by binding on the membrane of microorganisms through electrostatic interactions, cell wall disruption and affecting the intracellular processes such as DNA, RNA and protein synthesis [27–30].

4. Conclusion

The green synthesis and characterization of silver nanoparticles using bark extract of *P. pinnata* were performed and confirmed by spectroscopic and microscopic techniques. This synthesis method is uncomplicated, environmentally benign and low cost due to the availability of the source of reducing agent bark of *P. pinnata*. The very good results of antibacterial activity reveals the biomedical application of silver nanoparticles for diseases related to both gram positive and gram negative bacterial strains.

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