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BIOSYNTHESIS OF CHARACTERIZATION OF SILVER NANOPARTICLES USING SEEDS OF P*ONGAMIA PINNATA* (L) PIERRE

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ABSTRACT

The goal of research work is to synthesis and characterization of silver nanoparticles using seeds of Pongamia pinnata. 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 seed extract of Pongamia pinnata. The resultant biosynthesized silver nanoparticles characterized by UV-vis spectrophotometry shows the surface plasmon resonance band at 427 nm. FTIR-Fourier Transform Infrared spectrometer analysis was carried out to determine the capping agents in the seed extract. X-ray diffraction shows crystalline structure. TEM image divulges that silver nano particles are quite polydispersity, the size ranging from 10 nm to 20 nm with an average of 15 nm. The elemental analysis shows strong singal at 3keV 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 *Staphylococcus aureus (20 nm), Escherichia coli (18 nm),* respectively. This study reveals that silver nanoparticles possess good antibacterial activity 100µg/ml concentration.

KEYWORDS: Bio synthesis, *Pongamia pinnata*, silver nanoparticles, TEM, antibacterial.

INTRODUCTION

Nanoscience poses a basic scientific challenge as it requires a control over the connections between atoms. All physiochemical method of nanoparticles synthesis is having inherent limitations up to a certain extent which impose an important hurdle in the maturation of this science. The improvement in the experimental procedure of controlling shape and size of nanoparticles is one of the challenges for materials scientists. Equally, the development of eco-friendly cost effective method is also in demand. Consequently, viable production of metal nanoparticles is mostly studied.[1]

Silver is a transition metal and used in an ancient medicinal system for curing various diseases.[2] Nanoscale silver has received considerable attention in various applications due to its unique properties.[3] Application of nanoparticles in various fields depends on the size and shape characteristics of nanoparticles.[4] Mainly, silver nanoparticles were 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 systems, medical devices, cosmetics[5,6], bioremediation, heavy metal and pesticide removal in water and soil.[7,8]

Nanoparticles physical and chemical methods are complicated, expensive and also generate hazardous by-products.[9] 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.[10,11] The plant P*ongamia pinnata* is classified in the family Fabaceae found throughout India. The plant has various flavones, furanoflavonols, chromenoflavones, furanodiketones, flavonoid and glycosides.[13-14] In this investigation, synthesis of silver nanoparticles was achieved by using seed extract of *Pongamia pinnata* and assessment of its characterization and antibacterial activity against pathogenic microorganisms.

MATERIALS AND METHOD

Collection of P*ongamia pinnata* Linn plant seeds

Healthy dried seeds of P*ongamia pinnata* were collected from QUAID-E-MILLETH COLLEGE FOR WOMEN, CHENNAI, INDIA. The fresh seeds were dried under shade. The dried seeds were powdered by using blender. The powder was used for extraction. The extract was used for further works.

Preparation of 1Mm silver nitrate aqueous solution

Accurately weighed 0.017g of silver nitrate was dissolved in 100ml distilled water and stored in amber colour bottle until further use.

Synthesis of silver nanoparticles by using P. *pinnata* seed extracts

Prior to the experiment, seed extract was prepared by taking 10g of seed powder weighed, mixed with 100ml of distilled water in a 250ml Erlenmeyer flask and mixture was boiled for 10 minutes before decanting. The solution was filtered through Whatmann No.1 filtre paper and stored at 4°c for nanoparticles synthesis process.

For silver nanoparticles synthesis, about 10 ml of P.*pinnata* seed extract was added separately to 100ml aqueous solution of $AgNO_3$ (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* seed extract with silver metal ions. A control was maintained without an addition of silver nitrate, which shows no colour changes, it confirm the occurrence of silver nanoparticles in the reaction mixture where the colour changes take place.

UV-Visible spectrophotometer

The bioreduction of silver nitate iorns in aqueous solution UV–Vis analysis was done. The reaction mixture using UV–Visible Spectrophotometer (Shimadzu UV-1650 pc) from 200 to 700nm at a resolution of 1nm.

Transmission Electron Microscopy

The size of silver nanoparticles was measured by TEM (Tecnai G 2 F 20), which operated at an acceleration voltage of 200 kV.

X-ray powder diffractometer

The compounds were checked by X-ray diffraction (XRD) spectrum. The patern was measured by drop coated films of AgNO3 on glass plate. which carried out at 40 kV and 40 mA.

FTIR Analysis

FTIR analysis of the dried Ag NPs was carried out through potassium bromide (KBr) pellet method and the spectrum was recorded using Fourier Transform Infrared Spectrometer.

RESULTS AND DISCUSSION

Development of easy, reliable and eco-friendly methods helps in endorsing extra interest in the synthesis and application of nanoparticles which are good for mankind.[17] In this context the utilization of biological systems for nanoparticles synthesis is notable alternative for the advancement of multifaceted approach. Biological systems have shown the ability to interact with metal ions and reduce them to metallic nanoparticles.[18-19]

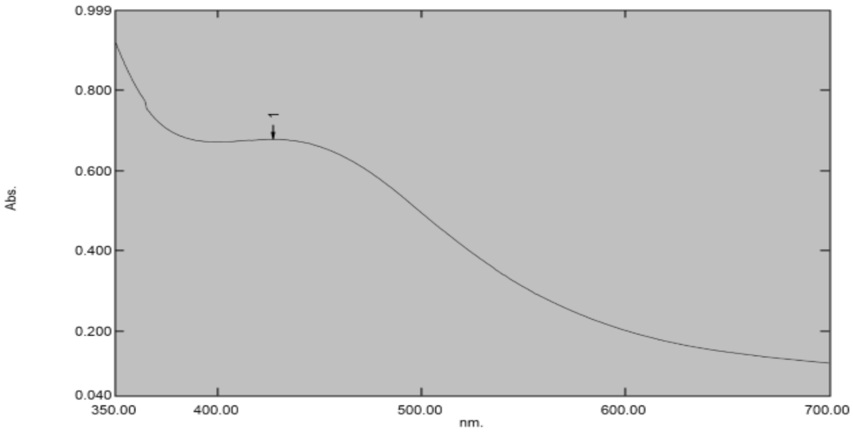
Visual identification

The identification of colour change is a preliminary tool that confirms the ability of plant extract in nanoparticles synthesis. Formation of brown colour in the reaction mixture could confirm the presence of silver nanoparticles (Fig 1). Initially, the reaction mixture showed no colour change and turned into brown colour after incubation in 10 min and the intensity of brown colour was increased after 24h (Fig 1). The settling of synthesized silver nanoparticles at the bottom of the conical flask reveals the reduction of silver metal into silver nanoparticles was completed after 24 hrs.[20-22]

Fig 1: Visual observation (A) P.pinnata seeds, (B) Seed extract, (C) $AgNO_3$, (D) $AgNO_3$+Seed extract.

UV-vis spectroscopic analysis

The silver nanoparticles 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 siver nitrate solution with Pongamia pinnata seed extracts. The peak observed at 427.40 nm indicates the presence of silver nanoparticles which is synthesized by P*ongamia ponnata* extract, the peak was raised during to the effect of surface plasmon resonance of electrons in the reaction mixture. The silver nanoparticles synthesis with reaction time 10 min-2 h shows no considerable changes in the shift SPR band and indicates that no changes were found in the size of nanoparticles.[23] The narrow peaks formed indicate the synthesis of small-sized nanoparticles.



No.	P/V	Wavelength	Abs.	Description
1	●	427.40	0.677	

Fig 2: UV-vis spectra of silver nanoparticlessynthesized by P.*pinnata* seed extract show the SPR band at 427.40nm could confirm the formation of silver nanoparticles in the reaction mixture.

X-ray diffraction analysis

The XRD spectra are used to confirm the crystalline nature of the silver nanoparticles synthesized by using P*ongamia pinnata* seed extract and the pattern was exhibited in (Fig 3). The spectra of XRD clearly indicated that the synthesized silver nanoparticles using the above-mentioned extracts were crystalline in nature. The Bragg reflections of silver nanoparticles were observed at 2 theta values of 22.13, 32.14 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.[20, 24]

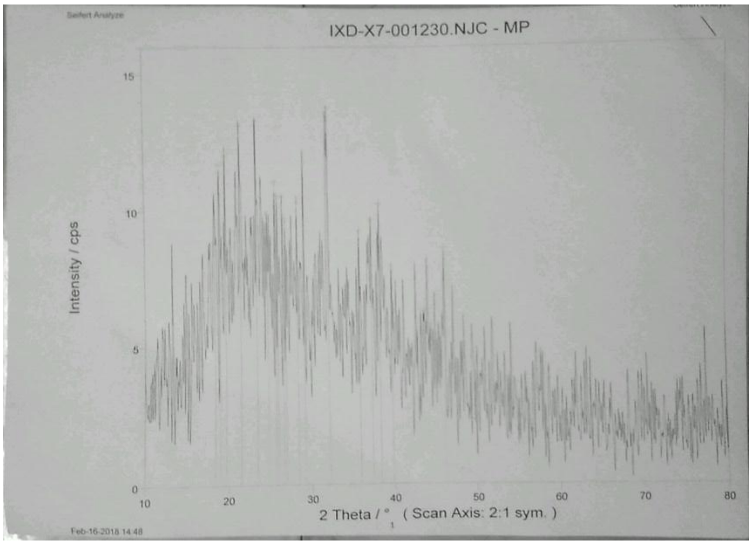


Fig 3: XRD Spectrum of the crystalline nature of silver nanoparticles.

Fourier Transform Infrared (FTIR) spectrometer analysis

Fourier Transform Infrared spectrometer analysis to determine the nature of the capping agents in the seed extract. The band at 1341.6 cm (Fig: 4).

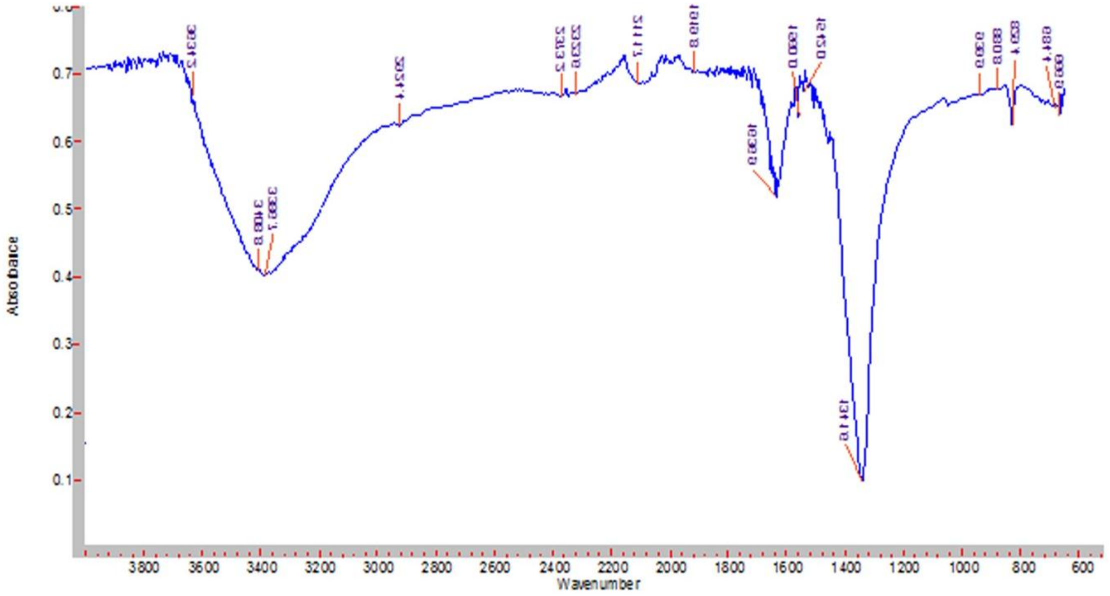


Fig 5: FTIR spectra of Silver Nanoparticles.

Transmission electron microscope (TEM)

The TEM image of Pongamia pinnata seed extract synthesized silver nanoparticles is shoem in fig.6 and the image signifies that the synthesized silver nanoparticles are polydisperse. The synthesized nanoparticles that ranges from 10 to 20nm with an average of 15nm. The formation of small, large sized and undefined shaped nanoparticles are dependent on the presence of phytochemicals phenolic amides, piperine, polysaccharides and other reducing sugars that is[26, 27] an important role in the synthesis of silver nanoparticles.

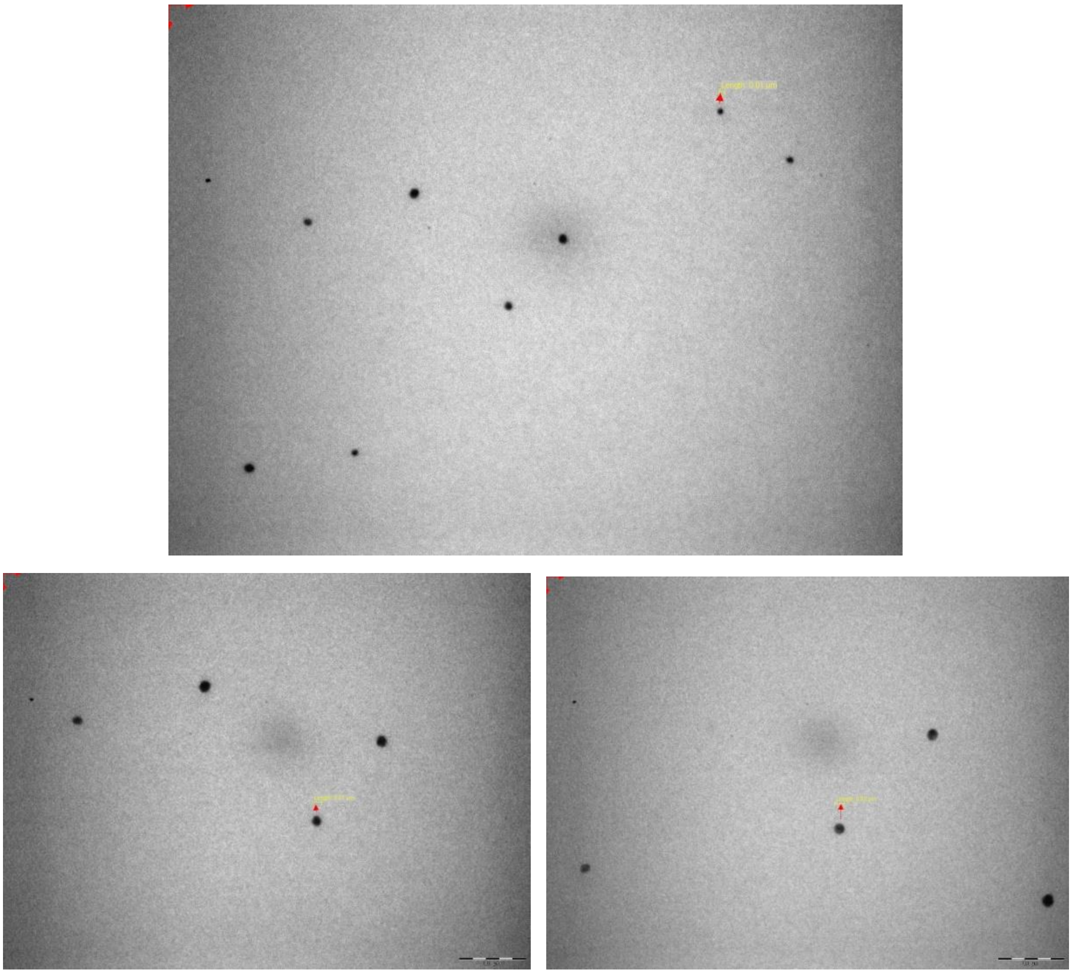


Fig 6: Transmission electron microscopic image of silver nanoparticales sun-the sized by P.*pinnata* seed extracts.

Antimicrobial activity of silver naoparticles synthesized using Pongamia *pinnata.*

The synthesised silver nanoparticles antibacterial activity was assessed against pathogenic microorganisms. Silver nanoparticles shows a significant inhibition activity against both gram positive and gram negative microorganisms. The maximum zone of inhibition was noted against *Staphylococcus aureus, a gram positive bacterium.* In contrast the minimum zone inhibition was measured against *Escherichia coli* gram negative bacterium. Indeed, the antibacterial activity was higher than the plant extract and silver nitrate solution. The mechanisms of antibacterial activities of silver nanoparticles are by binding on the membrane of microorganisms through electrostatic interaction cell wall disruption and affecting intercellular processes such as DNA, RNA and protein synthesis.[28-30]

No	**Micro organism**	**Zone of inhibition(mm)**							
		Aqueous plant Extract				**Nanoparticles**			
		C1	**C2**	**C3**	**C4**	**C1**	**C2**	**C3**	**C4**
1	*Stephylococcus aureus*	-	-	-	-	20	18	11	5
2	*Escherichia coli*	-	-	-	-	18	17	15	10

CONCLUSION

The synthesis and *characterization* of silver nanoparticles using seed extract of Pongamia 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 seed of *Pongamia pinnata*. The very good results of antimicrobial activity reveals the biochemical application of silver nanoparticles for diseases related to both gram positive and gram negative bacterial strains.

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