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Original Article

Synthesis of Pomegranate Peel Extract Mediated Silver Nanoparticles and its Antibacterial Activity

M. Shanmugavadivu[1], Selvam Kuppusamy[*2] and R. Ranjithkumar[1]

[1]Department of Biotechnology, Dr. N.G.P Arts and Science College, Coimbatore, Tamilnadu- 641048, India
[2]Department of Botany, Periyar University, Salem, Tamilnadu- 636011, India

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Address for Correspondence
Department of Botany, Periyar University, Salem, Tamilnadu - 636011, India.

Tel. +91-9894453888.
E-mail: vadivurajesh1981@gmail.com

ABSTRACT

In this report a simple and eco-friendly biosynthesis of silver nanoparticles using Pomegranate peel extract as the reducing agent from 1 mM $AgNO_3$ had been investigated. The formation of silver nanoparticles was characterized by UV-Vis spectrum, Fourier Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscopic (SEM) analysis. The UV-Vis spectra results show a strong resonance centered on the surface of silver nanoparticles (AgNPs) at 371 nm. The Fourier Transformation Infrared Spectroscopy spectral study demonstrates pomegranate peel extract acted as the reducing agent. The scanning electron microscopic (SEM) analysis shows nanoparticles with the average particles size ranges about 5-50 nm. Further the antibacterial activity of AgNPs was evaluated against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* pathogens. This route is rapid, simple, without any hazardous chemicals as reducing or stabilizing agents and economical to synthesize AgNPs.

Keywords: Pomegranate peel, AgNPs, Green synthesis, FTIR.

INTRODUCTION

Nanotechnology is emerging as a rapidly growing field with its application in Science and Technology for the purpose of manufacturing new materials at the nanoscale level[1]. The word “nano” is used to indicate one billionth a meter or 10^{-9}. The term Nanotechnology was coined by Professor Noria Taniguchi of Tokyo Science University in the year 1974 to describe precision manufacturing of materials at the nanometer level. “Nano” is a Greek word synonymous to dwarf meaning extremely small. Nanoparticles are beginning viewed as fundamental building blocks of

nanotechnology[2]. The use of nanoparticles is gaining attention in the present century as they posses defined chemical, optical and mechanical properties[3.] Nanoparticles exhibit completely new or improved properties compared with larger particles of the bulk materials and these novel properties are derived due to the variation in specific characteristics such as size, distribution and morphology of the particles. Nanoparticles present a higher surface area to volume ration with decrease in the size of the particles.

Specific surface area is relevant to catalytic activity and other related properties such as antimicrobial activity of AgNPs[4-6]. As the specific surface area of nanoparticles in increased, the biological effectiveness can also increase on the account of a rise in surface energy. Nanoparticles of noble metals, such as silver, gold and platinum are widely applied in products that directly come in contact with the human body, such as shampoos, soaps, detergent, shoes, cosmetic products, and toothpaste, besides medical and pharmaceutical application. The metallic nanoparticles are most promising as they showed good antibacterial properties due to their large surface area to volume ration, which is coming up as the current interest in the researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains[7]. Among several metals, silver is well known as a disinfecting agent; in its nanoparticles forms induce their ability in applications from medicine to culinary items.

A number of approaches are available for the synthesis of silver nanoparticles for example facile method[8], thermal decomposition of silver compounds[9], electrochemical[10], sono-chemical[11], microwave assisted process[12] and recently via green chemistry route[13]. Unfortunately, many of the nanoparticles synthesis or production methods involve use of hazardous chemicals, low material conversions, high energy requirements, difficult and wasteful purifications. Therefore, there is a growing need to develop environmentally friendly processes for nanoparticles synthesis without using toxic chemicals. Biosynthetic methods employing either microorganisms or plant extracts have emerged as a simple and viable alternative to chemical synthetic procedures and physical methods. The use of plant materials for the synthesis of nanoparticles could be more advantageous, because it does not require elaborate processes such as intracellular synthesis and multiple purification steps or the maintenance of microbial cell cultures. In the present study we have explored the synthesis of silver nanoparticles using fruit peel extract of *Punica granatum* (Pomegranate). The synthesized nanoparticles were confirmed by colour changes and characterized by UV-Visible spectroscopy. Fourier transform infrared (FTIR) spectral measurements were carried out to identify the potential biomolecules. The size of the nanoparticles was observed by SEM (Scanning Electron Microscope). Furthers its efficacy to inhibit different pathogenic bacterial growth were evaluated.

MATERIALS AND METHODS

Pomegranate fruits were collected from the local market. The plant was authenticated as *Punica granatum* by Dr. S. Murugesh, Professor and Head, Department of Botany, Periyar University, Salem. All glass wares and the pomegranate fruits were washed properly with deionized water. The glass wares were dried in hot air oven and from the washed pomegranate fruit the peel was removed and washed again with deionized water and air dried. 20g of pomegranate fruit peel was weighted and

added in 100 ml of distilled water in 250 ml Erlenmeyer flask and boiled for 10 minutes. With the help of Whatmann filter paper (NO.3), the boiled materials were filtered to get aqueous fruit peel extract which was used as such for metal nanoparticles synthesis.

1mM aqueous solution of silver nitrate was prepared for 100 ml. To this 5 ml of filtrate was added and kept for 24 hours incubation with intermittent shaking. After 24 hours the brown colour development indicated the formation of silver nanoparticles. The bioreduction of Ag+ ion in aqueous solution was monitored with the help of UV-visible spectroscopic analysis. UV-Visible spectroscopic analysis of silver nanoparticles was carried out as a function of time needed for bioreduction at room temperature on UV-2600 series Shimadzu spectrophotometer at a resolution of 1nm. The residual solution containing the nanoparticles was centrifuged at 8,000 rpm for 10 minutes and the resulting suspension was redispersed in 100 ml of sterile distilled water. The purified suspension was then analyzed by IR-Tracer-100 Shimadzu for FTIR. The purified silver nanoparticles after centrifugation were dried to powder form and analyzed by Fb-quanta 200 Scanning Electron Microscopy (SEM) for the structure, composition and average size identification.

The silver nanoparticles synthesized using Pomegranate Peel extract was tested for antimicrobial activity by disc diffusion method against *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922). Approximately 20ml of molten and cooled media (Mullar-Hinton agar) was poured into sterilized Petri dishes. The plates were left overnight at room temperature to check for any contamination to appear. The bacterial test organisms were grown in nutrient broth. 0.1ml from 10^{-8} dilution of different pathogenic bacteria suspension was spread on Muller-Hinton agar plates. Filter discs (5mm in diameter) were impregnated with synthesized silver particles and placed on the plates. Streptomycin served as the standard for measuring the antibacterial activity. The plates were then incubated at 37°C for 24 hrs and the zone of inhibition was measured in nm.

RESULTS AND DISCUSSION

Various methods have been employed for the synthesis of silver nanoparticles such as chemical and biological methods. Currently, syntheses of silver nanoparticles using plant materials are getting more popular[14,15]. In this study, when we are adding the pomegranate fruit peel extract to the aqueous solution of the silver nitrate the colour of the reaction medium changed rapidly from colorless to brown. Similar results were shown by early workers. The brown colour indicated the formation of silver nanoparticles with the reduction of silver ion, where as the control $AgNO_3$ solution did not show any colour change (Figure 1).

Figure 2, showed the UV-Vis spectrum of silver nanoparticles synthesized with the help of pomegranate fruit peel extracts as a reducing agent. While no absorbance peak was observed in control, a characteristics surface plasmon absorption bands were observed at 371 nm after 24hrs incubation[16].

The FT-IR measurements were carried out to identify the possible biomolecules responsible for the reduction of Ag+ ions to AgNPs by pomegranate fruit peel extract. The spectrum showed in the figure 3 indicated the major peak at 3371 $cm^{-1}$and other peaks were obtained at 1635 cm^{-1} 1373 cm^{-1}, 2924 cm^{-1} respectively. The bands at 3371 cm^{-1} and 2924 cm^{-1} were assigned to the stretching of primary and secondary amines respectively, while their corresponding vibrations seen at 1635 cm^{-1}. The band observed at 1373 cm^{-1} can be assigned to the

C-N stretching vibrations of aromatic groups[17].

Scanning Electron Microscopy (SEM) technique was employed to visualize the size and shape of silver nanoparticles synthesized using pomegranate fruit peel extract. The dried silver nanoparticles were mounted on a copper coated grid. The formations of silver nanoparticles as well as their morphological dimensions in the SEM study demonstrated that the average size was 5-50 nm (Figure.4) with inter particles distance[18].

In the study the AgNPs synthesized using pomegranate fruit peel extract as a reducing agent has exhibited a fairly significant antibacterial activity against *S. aureus* and *E. coli*. Streptomycin was used as the positive control. Maximum zone of inhibition 26mm was observed against *S. aureus* by 2mg/ml concentration of silver nanoparticles (Table.1 and Figure. 5). Shrivastava *et al.* found that the major mechanism through which silver nanoparticles manifest antibacterial properties was either by anchoring or penetrating the bacterial cell wall and modulating cellular signaling by dephosphorylating peptide substrate on tyrosine residues[19].

CONCLUSION

In this experiment, pomegranate peel extract was used as reducing and capping agent. This method of AgNPs synthesis has many advantages like, low cost, economic viability, eco friendly etc. Analytical techniques such as UV-visible spectroscopy, FT-IR and SEM are applied to characterize the synthesized nanoparticles. The results showed the synthesis of AgNPs with average size of 5-50 nm. The silver nanoparticles synthesized using pomegranate fruit peel extract showed maximum antibacterial activity against *Staphylococcus aureus.*

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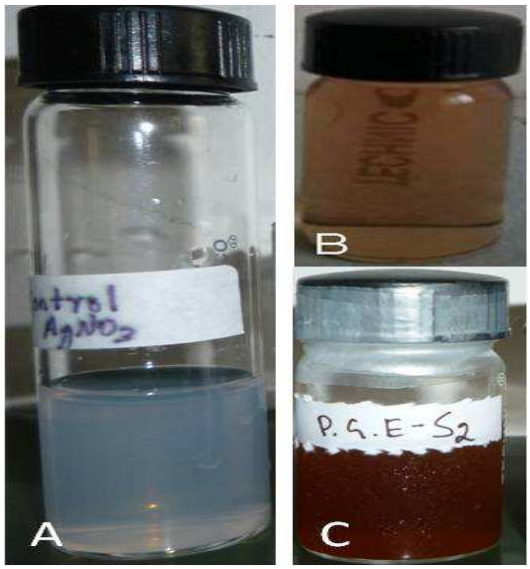

Figure 1. Colour changing during synthesis of AgNPs. A) 1mM $AgNO_3$ B) Pomegranate Peel extract C) AgNPs after 24h incubation in room temperature (Reaction Mixture)

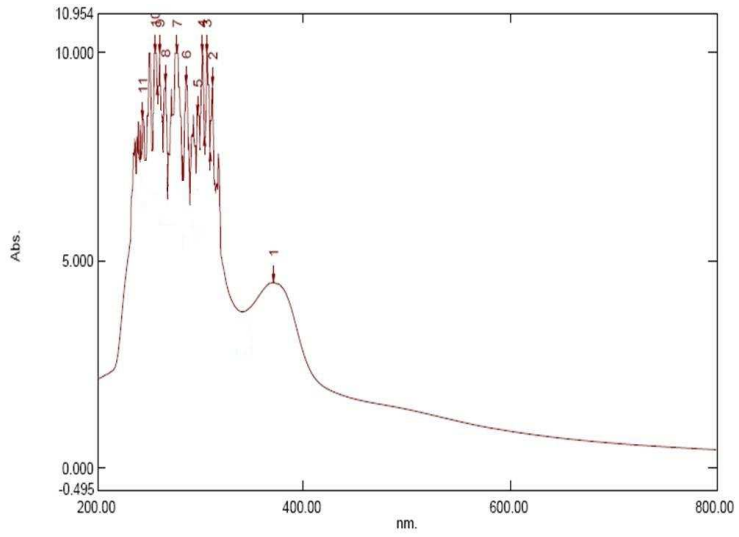

Figure 2. UV/Vis absorption spectra of reducing of silver ions to silver nanoparticles

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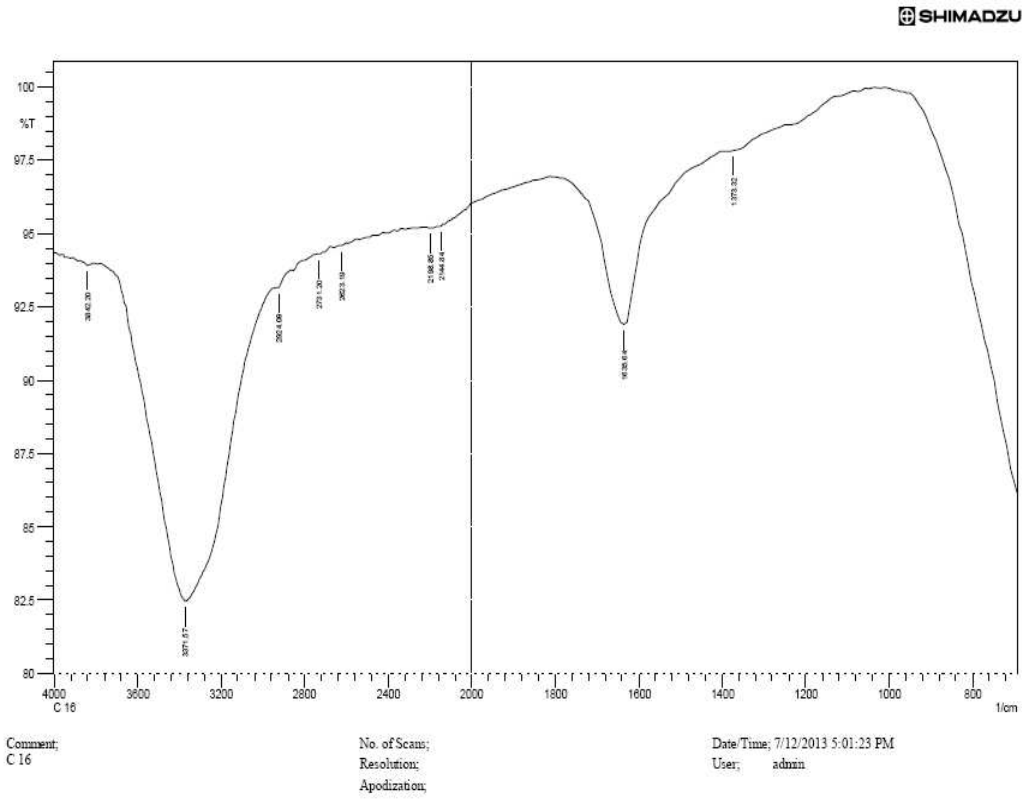

Figure 3. FR-IR of dried silver nanoparticles

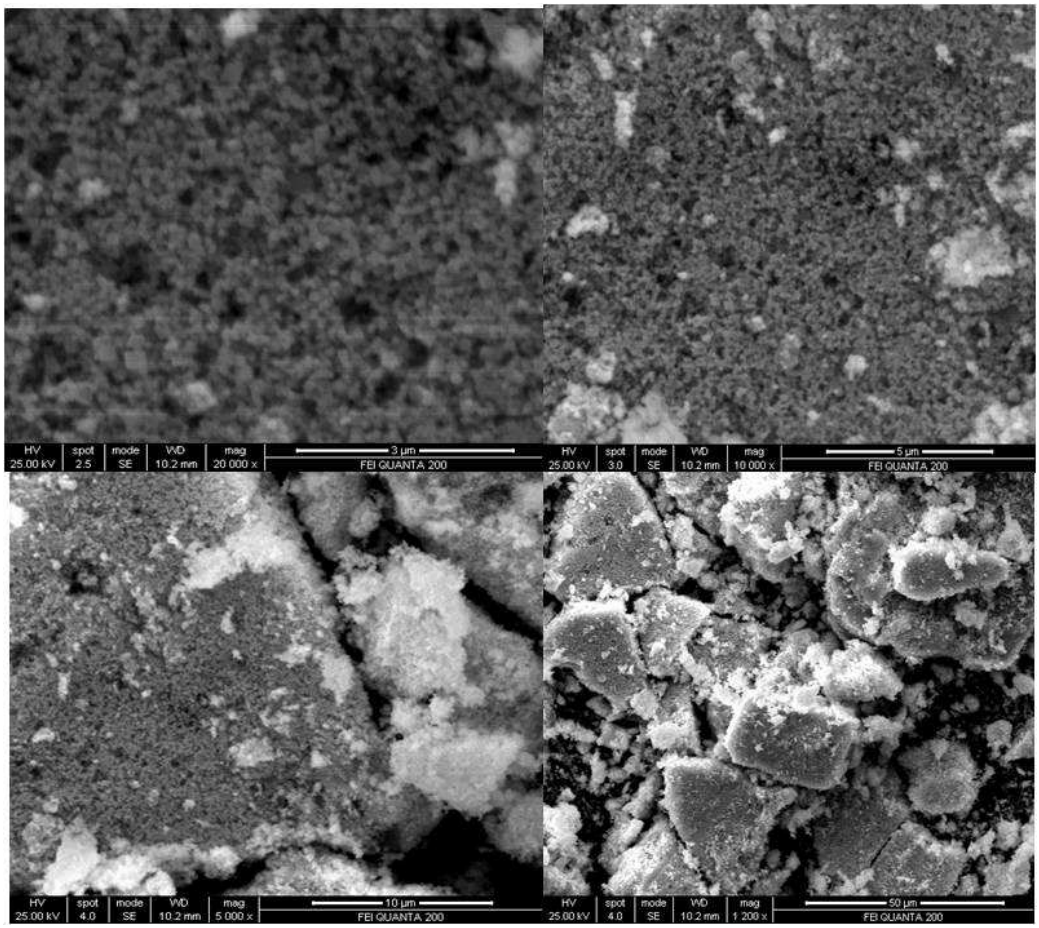

Figure 4. SEM images of AgNPs at different magnification levels

Figure 5. Antibacterial activity of silver nanoparticles against *Staphylococcus aureus*

Table 1. Antibacterial activity of synthesized silver nanoparticles

Name of the Organism	1mM $AgNO_3$ 50µL	(2mg/mL) SNPs 50µL	(3mg/mL) SNPs 50µL	(Streptomycin) (2mg/ml) 50µL
	Zone of inhibition (mm)			
Staphylococcus aureus (ATCC 25923)	20	26	06	25
Pseudomonas aeruginosa (ATCC 27853)	06	06	06	20
Escherichia coli (ATCC 25922)	09	08	11	28