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ECO-FRIENDLY SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM PUTATIVE MEDICINAL PLANT *ANNONA RETICULATA*

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ABSTRACT

Synthesis of silver nanoparticles using green method is an emerging aspect of nanoscience; it is safe and eco-friendly. Particularly medicinal plants used for synthesis gain prominent success in the metallic nanoparticle. In this work, silver nanoparticles were synthesized by using fresh leaves extract of *Annona reticulata* plant. The synthesized nanoparticles were characterized by UV-Visible Spectrophotometer, Fourier Transform Infrared (FTIR) spectroscopy; Scanning Electron Microscope (SEM) and Transmission Electron Microscopy (TEM). Results confirmed the formation silver nanoparticles and are spherical in shape and the average 50 nm in size. The biosynthesized silver nanoparticles were studied for their antibacterial activity against gram-positive bacteria *Staphylococcus aureus, Micrococcus luteus,* and gram-negative *Escherichia coli, Pseudomonas putida* bacteria. It was observed that synthesized silver nanoparticles have a maximum zone of inhibition for gram-positive bacteria *Staphylococcus aureus* followed by *Micrococcus luteus* bacteria.

KEYWORDS: Biological synthesis, antibacterial studies, Transmission electron microscope, silver nanoparticles,

INTRODUCTION

Nanotechnology is one of the modern technologies which create waves in the present research era. It provides an alternative to the potentially hazardous chemical additives and leads to more eco-friendly synthesis methods of nanomaterials. Many attractive nanodevices are effectively used in the biomedical field for the improved cancer detection, diagnosis and treatment. Metal nanoparticles are very important nanomaterials due to their outstanding physical, chemical, electrical, magnetic, optical and biological properties. Silver has long been recognized as having restrictive action on microbes present in medical and industrial processes.[1,2] Silver nanoparticles (SNPs) exhibit enhanced properties based on their size and morphology. SNPs are also called as nanosilver; possess different properties compared to the bulk material due to its extremely smaller size and large surface area. Nanosilver exhibits a high extinction coefficient, high surface plasmon resonance and superior anti-microbial properties.[3,4] It is a popular additive in many health products due to its unique ability to fight with infectious diseases, slow down the growth of bacterium, mould and germs. All these properties make nanosilver, the new "wonder-drug" of the nanotechnology world. Silver nanoparticles have found huge applications in the field of high sensitivity bio-molecular detection and diagnostics, antimicrobials and therapeutics, catalysis and microelectronics. The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burn and open wounds.[5,6]

Many researchers reported various approaches for the synthesis of silver nanoparticles using plant extracts such as leaves, seeds, stem, and roots of a variety of plants.[7-9] These approaches have many advantages over chemical, physical, and microbial syntheses.[10-13] Number of approaches reported for the synthesis of silver nanoparticles are reduction in solutions[14], chemical and photochemical reactions in reverse micelles[15], thermal decomposition of silver compounds[16], radiation assisted[17], electrochemical[18], sonochemical[19], microwave-assisted processes[20] and recently via green chemistry route.[21-22] With the development of new chemical or physical methods, the concern for environmental contaminations is also intensified. This is because; the chemical procedures involved in the synthesis of nanomaterials generate a large number of hazardous by-products. Thus, there is a need for economic, commercially feasible as well as environmentally clean synthesis route to synthesize silver nanoparticles that includes a clean, non-toxic and eco-friendly method of synthesis. Biosynthesis of silver nanoparticles is important in the field of nanotechnology, which has economic and eco-friendly benefit for chemical and biophysical characterization. The use of environmentally benign materials like plant extract[21],

bacteria[23], fungi[24] and enzymes[25] for the synthesis of silver nanoparticles offer numerous benefits such as eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis process.

Annona reticulata is deciduous plant belongs to Annonaceae family, growing up to 30 meters in height, spread in the tropical regions of the world[26], bearing nutritious fruits.[27] This plant having a number of herbal medicinal use, many active compounds and chemicals have been found in fruits as well as leaves. A novel set of active chemicals called acetogenins shows significant cytotoxicity and potential anticancer agents.[28-29]

In the present study, the SNPs were synthesized by using the aqueous leaf extract of *Annona reticulata.* Accordingly, heat and stirrer based biosynthesis of SNPs using the fresh leaves of *Annona reticulata* extracts were used. The SNPs were characterized by spectroscopic and microscopic analysis and the potential antimicrobial studies of synthesized SNPs were analyzed by both gram positive and gram negative bacteria. The nobleness of this current study is that the synthesis of SNPs from a potent medicinal plant extract is an ideal cost-effective, eco-friendly route and thus, can be economical and effective alternative for the large-scale synthesis of silver nanoparticles applicable for various drug therapies.

MATERIALS AND METHODS

Annona reticulata leaves were collected from the botanical garden in the Osmania university science college, Hyderabad. Materials used for the synthesis of silver nanoparticles are silver nitrate ($AgNO_3$ AR grade, Merck Company-purchased from India), Yeast extract, Tryptophan and bacterial grade Agar-agar (purchased from HiMedia laboratories, Mumbai, India).The Bacterial test strains that were used are *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus and Pseudomonas putida* (from IMTECH, Chandigarh).

Preparation of leaf extract: 5 gm of *Annona reticulata* leaves were thoroughly cleaned for at least three times with distilled water to remove the dust particles and other contaminants. The leftover moisture was removed by shade drying. The shade dried leaves were then cleanly chopped and were taken into a clean 250 ml conical flask then 100 ml of Millipore water was added and kept at 60° C on sand bath for 20 min. It makes the formation of aqueous leaf extract easy and then the extract was filtered using Whatman No. 1 filter paper. The collected filtrate is the leaf extract and is used for the synthesis of silver nanoparticles.

Synthesis of silver nanoparticles: 1mM $AgNO_3$ solution was prepared by dissolving 0.169gms of $AgNO_3$ in 1000 ml of Millipore water. In order to avoid auto-oxidation of silver, the solution was stored in an amber colored bottle. Then, 1:9 ratios of *Annona reticulata* leaf extract and $AgNO_3$ solutions were mixed and incubated in the sand bath at 60 ^{0}C for 15 min. A color change from transparent pale yellow to orange reddish was observed due to the reduction of Ag^+ ions and formation of silver nanoparticles (SNPs). Consequently formed SNPs were centrifuged at 15000rpm for 20 min for washing thrice with millipore pore water and vacuum dried in the concentrator at 60°C.

Characterization of Silver Nanoparticles: Synthesized SNPs using *Annona reticulata* leaf extract were initially confirmed by using UV Spectrophotometer (Elico SL-159). A small aliquot of SNPs was taken in UV-Visible Spectrophotometer and absorption spectra against the aqueous leaf extract were measured between 300-700nm. The Thermo Nicolet Nexus-670 Spectrophotometer has used for the Fourier-Transform Infrared Spectroscopic (FTIR) analysis of reduced SNPs and also for the aqueous extract. The spectrum was recorded in the mid-IR region of 400-4000 cm^{-1} in KBr pellets with diffuse reflectance mode. The redispersed SNPs were kept in an oven at 60°C in order to obtain the powdered form with high purity. The size and morphology of the prepared SNPs were known by Scanning Electron Microscope (SEM) analysis using Ziess 700 Scanning Electron Microscope. Thin films of synthesized and stabilized silver nanoparticles were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid for SEM analysis.

To determine the size and shape of the synthesized Ag nanoparticles precisely Transmission Electron Microscopy (TEM) analysis was carried out by using Philips model CM 200 instrument at an accelerated voltage 200 KV.

Analysis of Antibacterial Activity

The antibacterial activity of the green synthesized silver nanoparticles from an aqueous extract of *Annona reticulata* leaf was tested by the disc diffusion method against an antibiotic, ampicillin (1µg/µl) as a standard drug.[30] The bacterial strains were Gram-positive bacteria *Staphylococcus aureus, Micrococcus luteus* and Gram-negative bacteria, *Escherichia coli and Pseudomonas putida* were used for the measurement of antibacterial activity with disc diffusion method. The Agar plates were prepared and 100ul of overnight grown bacterial culture was spreaded over the plate and aseptically inserted autoclaved paper discs, then known amount of ArAgNPs and ampicillin was spotted carefully in laminar hood. Later the plates were incubated at 37^{0}C overnight to know the zone of inhibition.

RESULTS AND DISCUSSION

The present study reports that aqueous leave extract of *Annona reticulata (Ar)* acted as reducing and capping agent for the synthesis of silver nanoparticles (SNPs). When the aqueous leaf extract of *Annona reticulata* was mixed with the aqueous solution of silver ion complex (1:9 ratio), resulted in the colour change of extract from transparent yellow to orange reddish coloured solution

due to the reduction of Ag^+ ions. *Annona reticulata* leaf extract and *Annona reticulata* silver nanoparticles (ArAgNPs) were shown in Figure 1 & 2.

Fig. 1: Annona reticulata leaves.

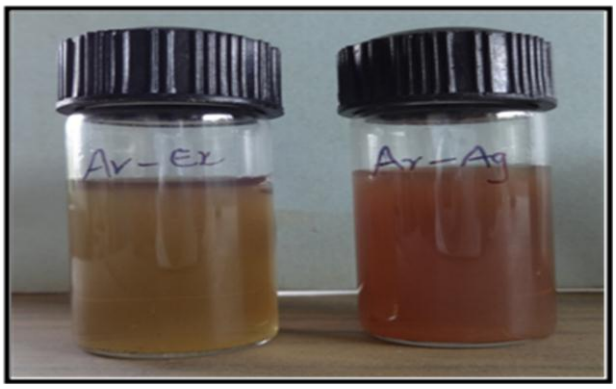

Fig. 2. Annona reticulata leaf extract and Annona reticulata leaf extract and silver nanoparticles.

UV-VIS spectra analysis: Formation of silver nanoparticles were analyzed by performing UV-Vis spectra between 300-700nm, which shown the maximum chromatogram at 420- 440 for aqueous leaf extracts of *Annona reticulata* silver nanoparticles (ArAgNPs), indicating that presence of silver nanoparticles (SNPs) in the sample. *Annona reticulata* leaf extract was taken as blank against SNPs solution for measuring UV-Vis spectra (Fig. 3). Characteristics surface plasmon absorbance band was observed at 440 nm indicates conformation of SNPs in UV visible spectrum analysis.

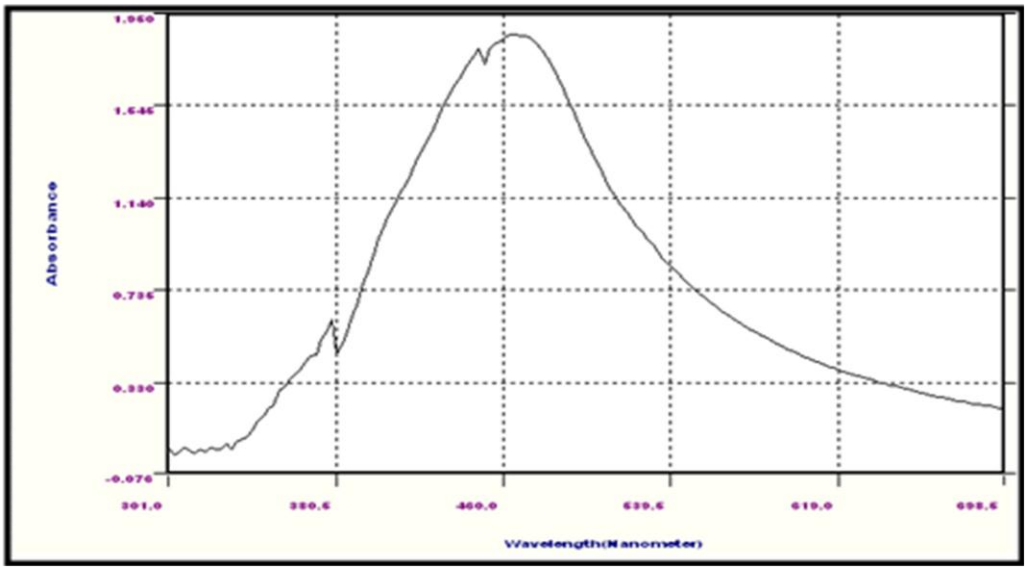

Fig. 3. UV-Vis spectra of *Annona reticulata* silver nanoparticles (ArAgNPs).

FTIR analysis: The FTIR analysis was carried out to identify the possible biomolecules involved in the reduction of Ag^+ ions and capping of the bioreduced nanoparticles synthesized by the aqueous leaf extract of *Annona reticulata.* The representative spectra of ArAgNPs obtained where the peaks are located at 3383cm-1 (-OH groups of carboxylic acids and also –NH group of amine, 2131cm-1 C-N, C-C, C-H stretch), 1786 cm-1 (-C=O of ketones and esters), 1643 cm-1 (C=O of amides and carboxylic acids stretch) and 1045 cm-1 C=O of esters. These functional groups are involved in the formation of Ar AgNPs and may copped to NPs and even that functional group corresponding molecule also bind to SNPs. This will help in biomedical research to establish the medicinal values of nanoparticles synthesized by eco-friendly method.

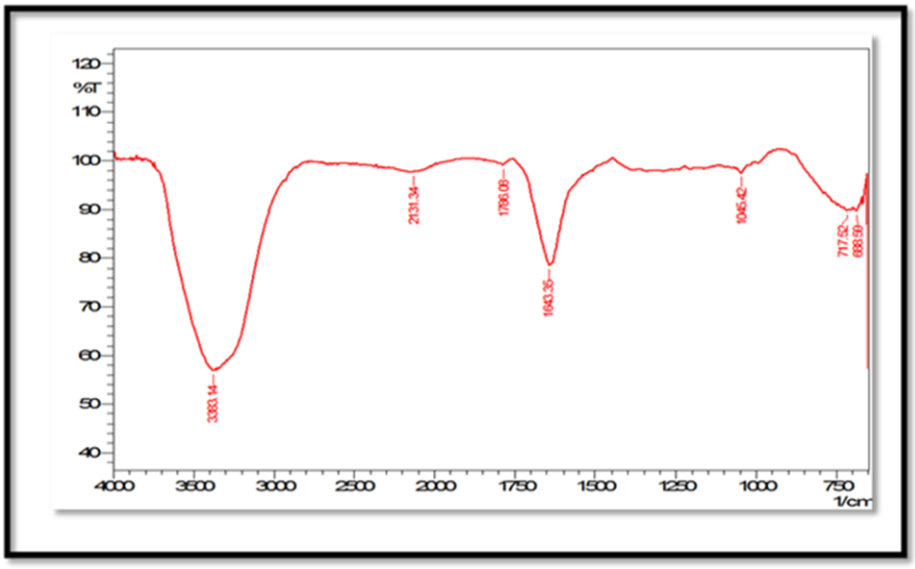

Fig. 4: FTIR spectra of *Annona reticulata* silver nanoparticles (ArAgNPs).

Scanning Electron Microscope (SEM): SEM images were recorded from the drop-coated films of the Ag-NPs synthesized using aqueous leaf extract of *Annona reticulata* was shown in figure 5. It is known that the shape of the metal nanoparticles considerably changes their optical and electronic properties. The SEM image showed relatively polydispersed shape nanoparticle formed with most of them are circular and uniform distribution at a voltage of 10KV and under 50.00 KX magnifications.

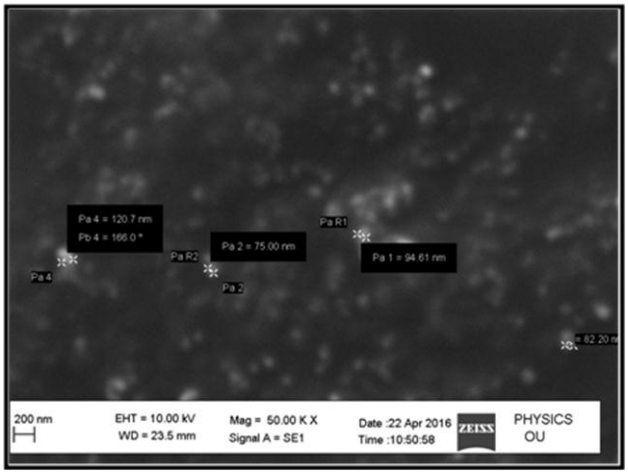

Fig. 5. SEM images of *Annona reticulata* silver nanoparticles (ArAgNPs).

Transmission Electron Microscope (TEM): TEM analysis of *Annona reticulata* silver nanoparticles (ArAgNPs) revealed that the synthesized silver nanoparticles are of size 5-50 nm in size, which is evident from the histogram given below (Fig 6c). Nanocrystalline formation of Ag particles along with particles broadening can be observed from the concentric electron diffraction pattern. The TEM image along with electron diffraction pattern was presented in Fig 6a and 6b.

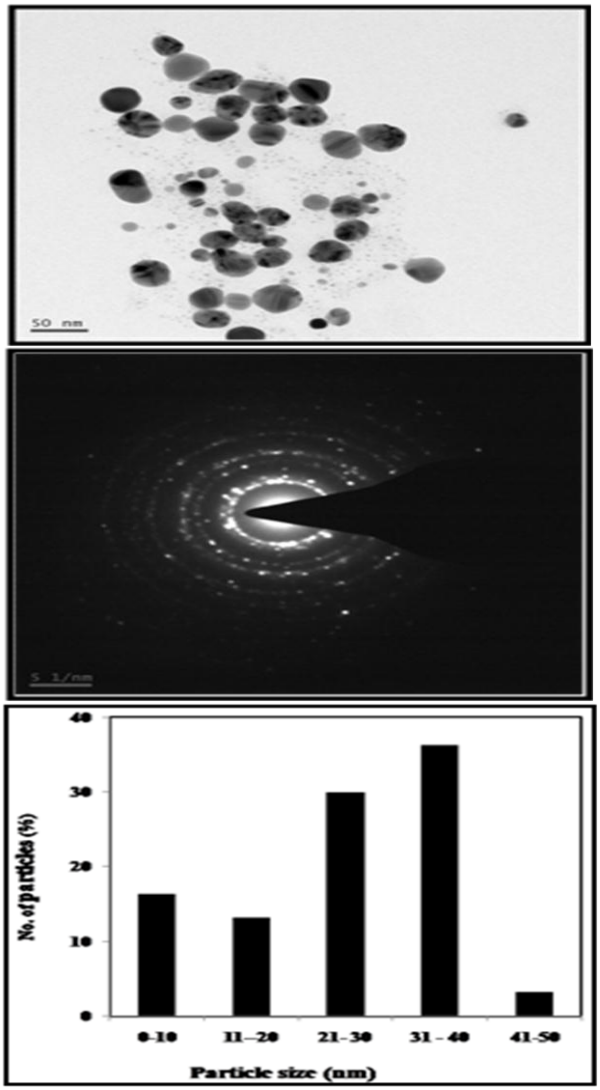

Fig. 6. a) TEM images of *Annona reticulata* silver nanoparticles (ArAgNPs), b) Electron diffraction pattern, c) Particle size histogram.

Antimicrobial activity: Antibacterial analysis of *Annona reticulata* silver nanoparticles (ArAgNPs) reveals that gram-positive bacteria *Staphylococcus aureus* and *Micrococcus luteus* showed more inhibition than gram negative bacteria *E. coli* and P. *putida.* Zone of inhibition was calculated by disc diffusion method shown in fig 7. The bacterial plates were examined for the presence of growth inhibition, which is indicated by a clear zone surrounding each disc. From the figure and table, it is clear that ArAgNPs showed maximum zone of inhibition with gram-positive bacteria *Micrococcus luteus* and *Staphylococcus aureus* bacteria than gram negative bacteria *Escherichia coli* and *Pseudomonas putida*. The cell wall properties of gram positive bacteria in outer membrane is absent and no lipids linked peptidoglycan, so that more inhibition occur compare to gram negative bacteria.

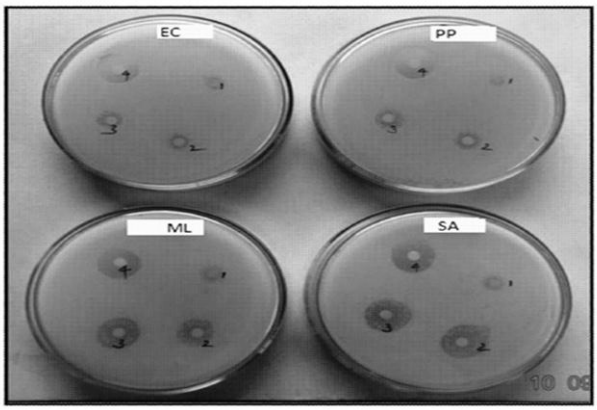

Fig. 7 Antimicrobial activity of *Annona reticulata* silver nanoparticles (ArAgNPs), EC= *Escherichia coli*; ML= *Micrococcus luteus*; PP= *Pseudomonas putida*; SA= *Staphylococcus aureus*. (1= *Annona reticulata* leaf extract, 2= 10µg of ArAgNPs; 3= 20µg of ArAgNPs, 4= 10µg of Ampicillin).

Table: Antibacterial Activity of aqueous SNPs against the reference drug Ampicillin.

S. No	Name of the Organism	Diameter of Zone of Inhibition (mm)			
		Extract (10µl)	ArAgNPs (10µg)	ArAgNPs (20µg)	Ampicillin (10µg)
1.	*E. coli*	2.5	6.0	12.8	16.8
2.	*P. putida*	5.4	6.6	11.2	15.2
3.	*S. aureus*	5.2	9.1	13.9	14.9
4.	*M. luteus*	4.5	8.8	13.1	16.1

DISCUSSION

Plant materials are the best source for preparation of NPs in green synthesis apporaches. In this study, the authors exposed the equivalent potential of the *Annona reticulata* to expand the scope of non-toxic biological systems for the biogenic synthesis of SNPs. Here, eco-friendly, SNPs were successfully synthesized from potent medicinal plant *Annona reticulata* fresh leaf extracts. The synthesized particles were characterized by spectroscopic and microscopic analysis and its potential activity was measured. The SNPs were shown superior antibacterial activity against gram-positive bacteria *Staphylococcus aureus* and *Micrococcus luteus* and less against *Escherichia coli*, as well as *Pseudomonas putida* bacteria. Maximum zone of inhibition was observed for *Staphylococcus aureus* bacteria. The cell wall properties of Gram positive bacteria and gram negative bacteria is different, in gram positive bacteria outer membrane is absent and no lipids linked peptidoglycan, more inhibition occurs compare to gram negative bacteria.

CONCLUSION

In this study our findings representing that green biosynthesis of SNPs using medicinal plant sources is a distinctive approach towards the development of nanomedicine. The medicinal property of plant brings up greater value to the SNPs. It gives the immense idea further medicinal values of plant material bind to the SNPs reveals the greater extent towards nanomedicine.

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