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ORIGINAL ARTICLE

Biosynthesis of Silver nanoparticle using aqueous extract of *Saraca asoca* leaves, its characterization and antimicrobial activity

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Abstract

The use of less hazardous chemicals or natural material in place of toxic chemical for the formation of metal nanoparticle is known as green synthesis. The present paper deals with greener approach for the synthesis of silver (Ag) nanoparticles. The *Saraca asoca* plant leaves extract solution was used for the silver nanoparticles. Confirmation of Ag nanoparticles has been done using various characterization techniques viz. structure by X-Ray Diffraction (XRD), morphological analysis by Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM) and elemental analysis by Energy Dispersive X-ray Spectroscopy (EDX). The particle size of silver nanoparticle is found to be 24.85 nm. The particle exhibits good antibacterial properties against *Staphylococci aures, Streptococci pyogens, Salmonella typhi.*

Keywords: *AFM; Ag-Nanoparticle; EDX; Green Synthesis; Saraca Asoca; SEM; XRD.*

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INTRODUCTION

From the ancient Babylonians and Greek period, silver is extensively used as an antimicrobial agent [1]. Nowadays research is going on synthesis and application of Ag nano particles all over the world due to its wide applications. Silver nanoparticles can be synthesized by various methods viz. sol-gel, ion sputtering, chemical reduction, reverse micelle, sono-chemical reactions, electrochemical reduction, microwave assisted synthesis etc. [2-4]. The nano materials can be synthesized using micro-organism, plant extract, diatoms, etc. [5]. Researchers are exploring green synthesis of Ag nanoparticles to decrease the use of toxic and hazardous reactants and formation of by-products formed during the reaction. Green approach is simple and eco-friendly to adopt the twelve fundamental principle of green chemistry, biological and biometric approach is used to reduce environmentally toxic chemical waste [6]. The biosynthesis of nano-silver crystals has been successfully reported using different plant extract such as leaf extracts of *Rosa rugosa* [7], Coriandrum sativam leaf extract [8], *Eucalyptus Chapmaniana* leaves extract [9], Papaya fruit extract [10], *Parthenium* leaf extracts [11], *Citrullus colocynthis* stem extract [12], Aspergillus niger [13], *Iresine herbstii* leaf extract [14]. To the best of our knowledge till date nobody has used *Saraca asoca* leaves extract for the synthesis of silver nanoparticles.

The plant extract mediated synthesis of silver nanoparticles show enhanced antibacterial properties. When nanoparticles enter into the cell, it interferes with the bacterial growth signalling pathway by modulating tyrosine phosphorylation of putative peptides substrate which is critical for cell viability and division [15]. These properties make them useful in topical ointment and creams to prevent infection of burns and open wounds [16]. The antibacterial property of silver nanopar-

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ticles depends on particle size. Antibacterial activity decreases with increase in the particle size [17]. The silver salt and silver ions are also used as antimicrobial agent, due to its growth inhibitory activity. As silver ion is reduced to silver nanoparticles, its antimicrobial property increases [18].

EXPERIMENTAL

A stock solution of 0.001M $AgNO_3$ (S.D fine chem. Ltd.), was prepared in double distilled water and stored in the amber color air tight bottle. The plant leaves of *Saraca asoca* was collected from the premises of Maulana Azad College, Aurangabad. The plant is authenticated (Acc. No. 0639) by Dr. Narayan Pandure and index in herbarium Dr. BAMU Aurangabad. It was dried in shadow and grinded to fine powder. The powder was extracted with water. The solvent was evaporated. The mass is dried and 500 ppm extract solution was prepared in distilled water.

Biosynthesis of silver nanoparticles

Synthesis of silver nanoparticle was performed as per reported method in literature [19]. Extract solution prepared in 500 ppm concentration in 50 mL quantity was transferred to 250 mL beaker and then 3.78 g of sodium bicarbonate added slowly with stirring. 10 mL of this solution was pipetted out and diluted to 50 mL in standard flask. In another beaker, 100 mL of 0.001M $AgNO_3$ was taken and heated up to boiling and to the hot solution 10 mL of extract solution was added drop by drop. During addition, solution was mixed vigorously [20]. The solution was heated till its color changed to pale yellow. The obtained nanoparticles were separated and washed with de-ionized water. The obtained silver nanoparticles were further characterized by SEM, EDX from Savitribai Phule University, Pune and AFM from Department of Physics, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad.

Antibacterial activity

As per the composition, Mueller Hinton Agar was prepared by using sterile distilled water and it was sterilized at 121°C at 15 lb pressure for 15 min in an autoclave. The medium was cooled to room temperature and poured in sterile petri plates and were allowed to solidify. Bacterial inoculums were swabbed over the medium using sterile cotton swab. Sterile disc was placed on medium, on which 20 µl of complex suspension was added. Zone of inhibition were observed and measured after incubation at 30°C for 18-20 hrs.

RESULTS AND DISCUSSION

Fourier Transform Infrared Spectroscopy (FTIR)

A pellet of KBr with silver nanoparticle was prepared and IR bands are shown in Fig. 1. The absorption bands at 1625, 1554, 1411 cm^{-1} observed for Ag Nanoparticles along with additional bands. The additional IR bands observed due to the presence of residual plant extract materials.

The plant residue contains various phytochemicals, particularly secondary metabolites, hence there are several compounds with different func-

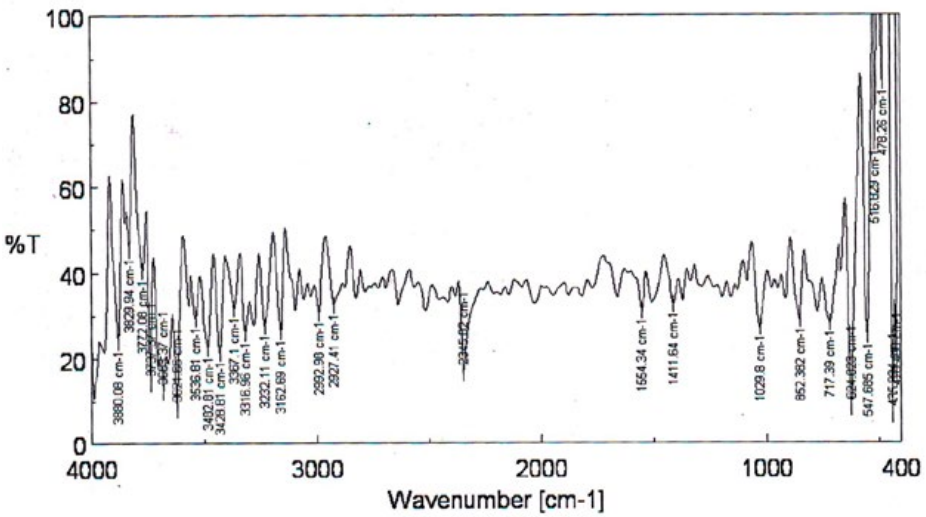

Fig. 1. FTIR spectra of synthesized silver nanoparticles.

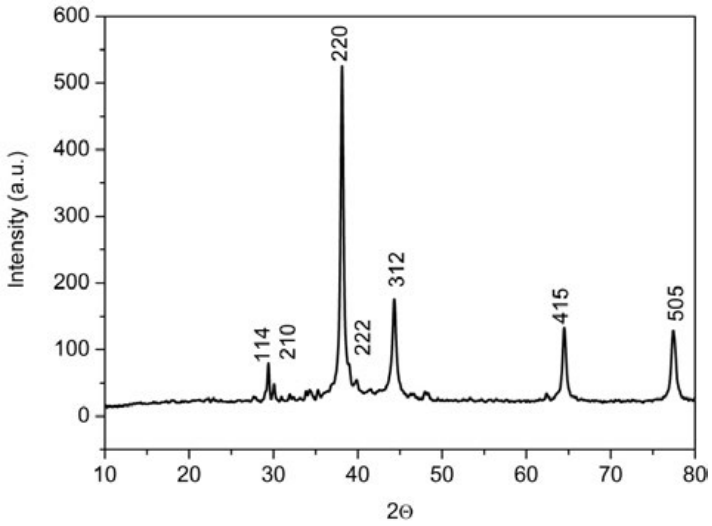

Fig. 2. XRD Pattern of Ag nanoparticle.

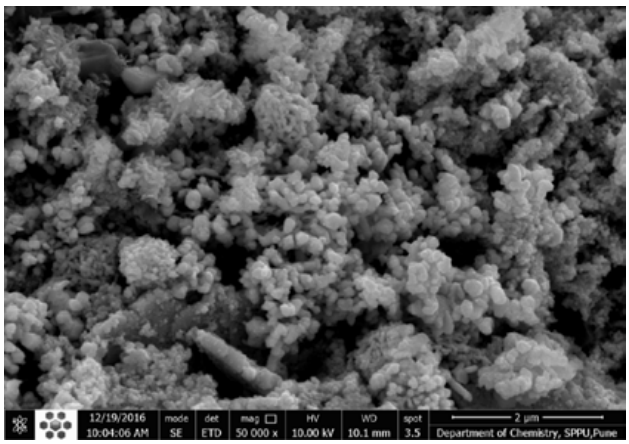

Fig. 3. SEM image of silver nanoparticle.

tional groups present in it. Some of these compounds act as capping agent and they stabilized nanoparticles. Therefore the IR spectrum shows that various peaks representing different functional group are present in constituents of leave extract.

X-ray diffraction

The XRD pattern of nanoparticles obtains using *Saraca asoca* is shown in Fig. 2. The peak at (220) indicates fcc crystal structure of silver nanoparticles. Similar peak also obtained when silver nanoparticles were synthesized using a marine alga, *Sargassum wightli* [20] and leaf extract of *Ocimum Sanctum* (tulsi) [21]. There are few unassigned peaks also observed at (114), (210), (222), (312) and (415) due to the presence of phyto-chemicals which are acting as capping agent. Particle size was determined by using Scherer formula

$$t = 0.94 \lambda / \beta \cos \theta$$

The particle size is found to be 24.84 nm.

Scanning Electron Microscope (SEM) and Atomic Force Microscope (AFM)

SEM images are used to study the morphology of silver nanoparticles. It has been observed that the size differences, size distribution, and capacity for aggregation depends on experimental conditions, stabilities etc. [22]. The shapes of silver nanoparticles, synthesized using plant extract, are like spheres, rods, prisms, plates, needles, leaf or

Table 1. Roughness parameters of silver nanoparticle.

Sr. No.	Parameters	Values (nm)
1.	Average roughness (Ra)	32.779
2.	Root mean square roughness (Rq)	132.275
3.	Max height of profile (Rz)	27.399
4.	Max profile peak height (Rpv)	41.344

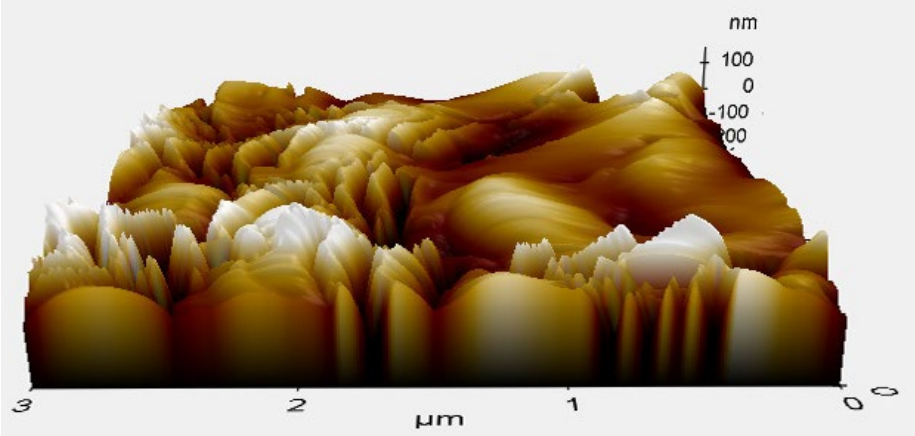

Fig. 4. AFM images of silver nanoparticle.

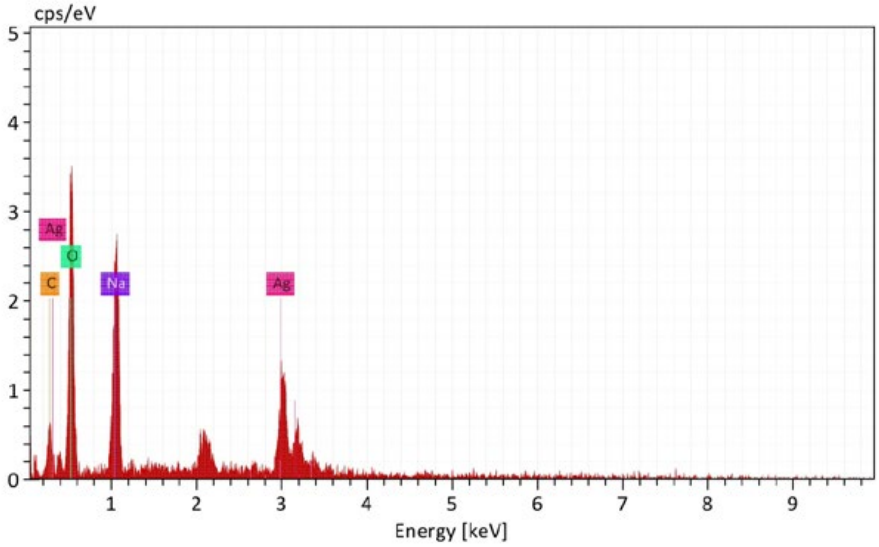

Fig. 5. EDX spectra of silver nanoparticle.

dendrites. It depends on concentration of plant extract, part of the plant used, rate of addition or mixing of plant extract and metal salts, time taken for reaction, pH, temperature etc. [23].

In the present imaging of the surfaces of silver nanoparticles grains are clearly highlighted in Fig. 3 and Fig. 4. It is also observed that silver nanoparticles are granular in shape. From AFM data the average roughness of surface was evaluated and it is found to be 32.779 (nm), which confirm the formation of nano-silver. The other parameters are given in Table 1.

Energy dispersive X-ray analysis EDX

The energy dispersive X-ray analysis (EDX) of silver nanoparticles synthesized using leaves of *Saraca asoca* reveals strong signal in the silver region. Silver nanoparticles shows typical optical absorption peak approximately at 3 KeV due to surface Plasmon resonance [24]. The EDX result of

Table 2. Anti-bacterial activity of silver nanoparticles.

Type of bacteria	Bacteria	Zone of inhibition for silver nano particles	Zone of inhibition for azithromycin
GM +ve	Staphylococci aures	10 mm	25mm
	Streptococci pyogens	10 mm	
	Diplococci sp.	10 mm	
GM –ve	Escherichia coli	---	
	Pseudomonas fluroscens	---	
	Salmonella typhi	10 mm	25mm

silver nanoparticle shows the percentage of silver, carbon, sodium and oxygen is 31.69, 3.11, 27.98, 37.22 respectively Fig. 5. The sodium present in silver nanoparticles may be due to the sodium carbonate added during the synthesis.

Antibacterial activity

Silver nanoparticle synthesized using aqueous extract of *Saraca asoca* leaves possess antibacterial activity against *Staphylococci aures, streptococci pyogens, Diplococci sp.* and *Salmonella typhi*. Antibacterial activity was investigated using disc method using azithromycin as standard. With the help of sterile wire loop, the test was inoculated into a test tube containing Mueller Hinton broth. The O.D of the inoculums was adjusted in between 0.08-0.1.Surprisingly it does not show any activity against *Escherichia coli* and *Pseudomonas fluroscens*.

CONCLUSION

Saraca Asoka leave extracts has been used successfully for the synthesis of silver nano particles. Absorption band of FTIR at 1625, 1554, 1411 supports the formation of Ag Nano particles. Nano particles are granular (SEM) and it has average rough surface 32.779 (nm) obtained from AFM data and particle size 24.84 nm evaluated by XRD. The results confirm the formation of silver nano particles using *Saraca asoka*. Nano particles thus obtained shows anti-bacterial activities against gram positive bacteria.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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