

Research Article

Biosynthesis, Characterization, and Antidermatophytic Activity of Silver Nanoparticles Using Raamphal Plant (*Annona reticulata*) Aqueous Leaves Extract

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The present work investigated the biosynthesis of silver nanoparticles using *Annona reticulata* leaf aqueous extract. The biosynthesised silver nanoparticles were confirmed by visual observation and UV-Vis spectroscopy. Appearance of dark brown colour indicated the synthesis of silver in the reaction mixture. The silver nanoparticles were found to be spherical, rod, and triangular in shape with variable size ranging from 23.84 to 50.54 nm, as evident by X-ray diffraction studies, TEM. The X-ray diffraction studies, energy dispersive X-ray analysis, and TEM analysis indicate that the particles are crystalline in nature. The nanoparticles appeared to be associated with some chemical compounds which possess hydroxyl and carbonyl groups, confirmed by FTIR. This is the first and novel report of silver nanoparticles synthesised from *Annona reticulata* leaves extract and their antidermatophytic activity.

1. Introduction

The field of nanotechnology is one of the most active areas of research in modern materials science and technology. It provides the ability to create materials, devices, and systems with fundamentally new functions and properties [1]. Recently, research in synthesis of nanoparticles using microbes and plant extracts gained more importance due to its eco-friendliness; flexible and main point is the evasion of toxic chemicals [2]. When compared to microbes, plant mediated synthesis is actively being practiced by the researchers for its positive advantages like avoidance of maintaining the microbial culture, being time-consuming, and being cost effective [3]. Previously, various plants have been successfully used for the synthesis of biogenic metal nanoparticles [4]. Nanoparticles are synthesized using plant materials such as, *Mucuna pruriens* [5], *Cassia occidentalis* [6], banana peel [7], *Azadirachta indica* [8], *Aloe vera* [9], *Emblica officinalis* [10], *Capsicum annuum* [11], *Cinnamomum camphora* [12],

Gliricidia sepium Jacq. [13], *Carica papaya* [14], *Opuntia ficus-indica* [15], *Murraya koenigii* [16], *Ocimum sanctum* [17], and *Saururus chinensis* [18]. The various phytochemicals present within the plant result in effective reduction of silver salts to nanoparticles but their chemical framework is also effective at wrapping around the nanoparticles to provide excellent robustness against agglomeration [19]; the synthesised silver nanoparticles were used effectively against multidrug resistant bacteria [20]; it can be used in many antimicrobial preparations [21]; Durán et al. [22] successfully developed silver nanoparticle impregnated wound dressings and textile fabrics which can be used for burnt patients. Silver nanoparticles are also used for the preparation of surgical masks [23]. *Annona reticulata* is a semievergreen plant belonging to the family Annonaceae. *A. reticulata* commonly known as raamphal plant or bullock's heart is widely distributed all over India. The leaves have been using in the treatment of insecticides, helminthic, styptic epilepsy, toothache, tumor, fever, dysentery and are also used externally as suppurant. The bark is used in

treating antidiarrheal and vermifuge. The root, bark, leaves, and stem of this plant possess isoquinoline alkaloids [24], whereas still there was no reports on biosynthesis of AgNPs (silver nanoparticles) using leaves of raamphal plant.

In the present study, *A. reticulata* leaf aqueous extract was used for the synthesis of silver nanoparticles and their antidermatophytic activity was evaluated.

2. Materials and Methods

2.1. Collection of Material. Fresh leaves of *Annona reticulata* were collected from botanical garden of Gulbarga University campus. Silver nitrate (AgNO_3) is procured from High Media Laboratories. Solutions were prepared with triply distilled water.

2.2. Preparation of the Extract. 25 g of *A. reticulata* fresh leaves was weighed, thoroughly washed in distilled water, cut into fine pieces, and smashed into 100 mL sterile distilled water and plant extract was boiled for 5 to 6 min and filtered through Whatman No. 1 filter paper (pore size $0.45 \mu\text{m}$) and was further filtered through $0.22 \mu\text{m}$ sized filters. The extract was stored at 4°C for further experiments.

2.3. Synthesis of Silver Nanoparticles from *Annona reticulata* Leaf Extract. The aqueous solution of 1 mM silver nitrate (AgNO_3) was prepared and used for the synthesis of silver nanoparticles. 1 mL of *A. reticulata* leaf extract was added into 100 mL of 1 mM silver nitrate aqueous solution for reduction into Ag^+ ions and kept for incubation for 20 min at room temperature.

2.4. Characterization. The synthesized AgNPs were characterized using UV-vis Elico double spectrophotometer operated at with 1 nm resolution with optical length of 10 mm. UV-vis analysis of the reaction mixture was observed for a period of 300 s. For the study of crystallinity, films of colloidal AgNPs formed on Si(III) substrates by drop coating were used for X-ray-diffraction (XRD) study. The data was obtained using Ricago X-Ray Diffractometer (Japan), operated at 30 kV and 20 mA current with Cu Ka ($\lambda = 1.54 \text{ \AA}$). The transmission electron microscopy (TEM) images were obtained using Technai-20 Philips instrument operated at 190 keV. Biosynthesized silver nanoparticles solution drops on carbon coated copper grids were kept for 5 min; the extra solution was removed using blotting paper. The film of TEM grid is exposed to IR light for drying. The powder sample of AgNPs was prepared by centrifuging the synthesized AgNPs solution at 10,000 rpm for 20 min. The solid residue was washed with deionized water to remove any unattached biological moieties to the surface of the nanoparticles, which are not responsible for biofunctionalization and capping. The resultant residue is then dried completely and the powder obtained was used for FTIR analysis carried out on a Nicolet iS5 FTIR with diamond ATR.



FIGURE 1: *Annona reticulata* leaves used for the synthesis of AgNPs.

2.5. Antidermatophytic Activity of AgNPs Synthesised from *A. reticulata* Leaves Aqueous Extract

2.5.1. Test Microorganisms. Three fungi *Trichophyton rubrum*, *Trichophyton tonsurans*, and *Microsporum gypseum* and three bacterial strains *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* were used in the present study; all the tested strains were obtained from M.R.M.C. Medical College, Gulbarga, Karnataka, India. These testcultures were grown in SDB, nutrient broth (Himedia, M002), at 37°C and maintained on nutrient and potato agar slants at 4°C .

2.5.2. Agar-Well Diffusion Method [25]. The assay was conducted by agar-well diffusion method. About 15 to 20 mL of potato dextrose agar medium was poured in the sterilized petri dishes and allowed to solidify. The fungal strains were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 McFarland standards (108 CFU/mL). 1 mL of fungal strains was spread over the medium using a sterilized glass spreader. Using flamed sterile borer, wells of 4 mm diameter were punctured in the culture medium. Required concentrations (80, 40, 20, and $10 \mu\text{L/well}$) were added to the wells. The plates thus prepared were left for diffusion of extracts into media for one hour in the refrigerator and then incubated at 37°C . After incubation for 48 h, the plates were observed for zones of inhibition. The diameter zone of inhibition was measured and expressed in millimetres. 1 mM AgNO_3 solution and aqueous plant extract were used as negative control. Ketoconazole, streptomycin were used as positive control against fungi and bacteria ($1000 \mu\text{g/mL}$ conc. $40 \mu\text{L/well}$). The experiments were conducted in triplicate. The same method was followed for testing antibacterial activity using nutrient agar medium incubated at 37°C for 18 h.

3. Results and Discussion

The biosynthesis of AgNPs using *A. reticulata* (Figure 1) fresh leaf aqueous extract (light yellowish) was carried out

TABLE 1: Antidermatophytic activity of AgNPs synthesised from aqueous leaf extract of *Annona reticulata*.

Dermatophytic fungi and bacterial strains	Zone of inhibition in mm at different conc. of Ag NPs					Leaf aqueous extract	1 mM AgNO ₃ solution	Standard K & S (1000 µg/mL conc.)
	80 µL/well	60 µL/well	40 µL/well	20 µL/well				
<i>T. rubrum</i>	13.00	10.00	07.00	05.00	—	—	22.00	
<i>T. tonsurans</i>	11.00	09.00	06.00	05.00	—	—	23.00	
<i>M. gypseum</i>	14.00	12.00	07.00	05.00	—	—	20.00	
<i>S. aureus</i>	20.00	17.00	13.00	09.00	—	—	32.00	
<i>B. subtilis</i>	18.00	14.00	11.00	07.00	—	—	35.00	
<i>E. coli</i>	22.00	20.00	16.00	11.00	—	—	30.00	

T. rubrum: *Trichophyton rubrum*, *T. tonsurans*: *Trichophyton tonsurans*, *M. gypseum*: *Microsporium gypseum*, *S. aureus*: *Staphylococcus aureus*, *B. subtilis*: *Bacillus subtilis*, *E. coli*: *Escherichia coli*. Standards K: ketoconazole against fungi, S: streptomycin against bacteria.

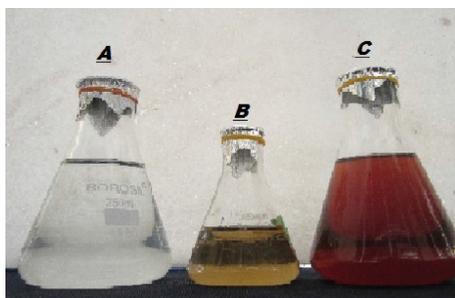


FIGURE 2: Synthesis of silver nanoparticles using *Annona reticulata* aqueous leaves extract treating with AgNO₃ solution at room temperature. (A) Silver nitrate (AgNO₃) solution; (B) *A. reticulata* aqueous leaf extract; (C) after formation AgNPs change the colour of AgNO₃ solution.

and reported in the present work. 2.5 mL of *A. reticulata* leaf aqueous extract was added to 250 mL of 1 mM AgNO₃ solution. The colour of the reaction mixture after 20 minutes at room temperature changes from transparent to dark brown colour; this observation is strong sign for the formation of AgNPs (Figure 2). The formation and stability of the reduced AgNPs in the colloidal solution was examined by using UV-vis spectral analysis. The UV-vis spectrum recorded from reaction mixture was plotted (Figure 3). The synthesized AgNPs were evaluated through Elico double beam spectrophotometer at a wavelength range of 400–500 nm; a characteristic peak at 420 nm showed that the typical optical spectra for silver nanoparticles was 350 nm–550 nm in visible light region [26], confirming the formation of silver nanoparticles. The similar type of the silver nanoparticles peaks were reported in Geranium leaf [27]. The present wave length reports of nm were supported by the previous reports at similar nm [28, 29].

The XRD pattern of AgNPs suggests that the particles are crystalline in nature. The intense diffraction peaks due to AgNPs are clearly observed at (111), (220) and (311), (380). All the peaks match well with the standard JCPDS file 04-0783 of silver shown in Figure 4.

TEM procedure was employed to visualize the size and shape of AgNPs formed. A typical TEM image of biologically

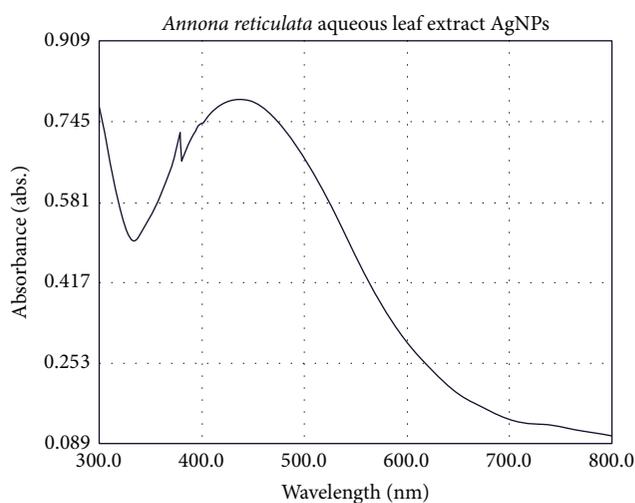


FIGURE 3: UV-vis spectrum of biofunctionalized AgNPs showing surface plasmon peak at 440 nm.

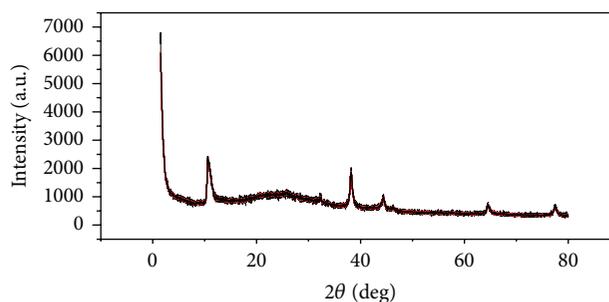


FIGURE 4: XRD patterns of biofunctionalized AgNPs.

synthesized AgNPs suggests that the particles are uneven in shape. Some are spherical, rod, and triangular shaped particles with a varying size of 23.84–50.54 nm shown in Figures 5(a) and 5(b). The FTIR measurement was carried out to identify the possible biomolecules in *A. reticulata* leaf extract responsible for capping leading to efficient stabilization of the AgNPs (Figure 6). The FTIR spectrum of silver nanoparticles manifests prominent absorption band located

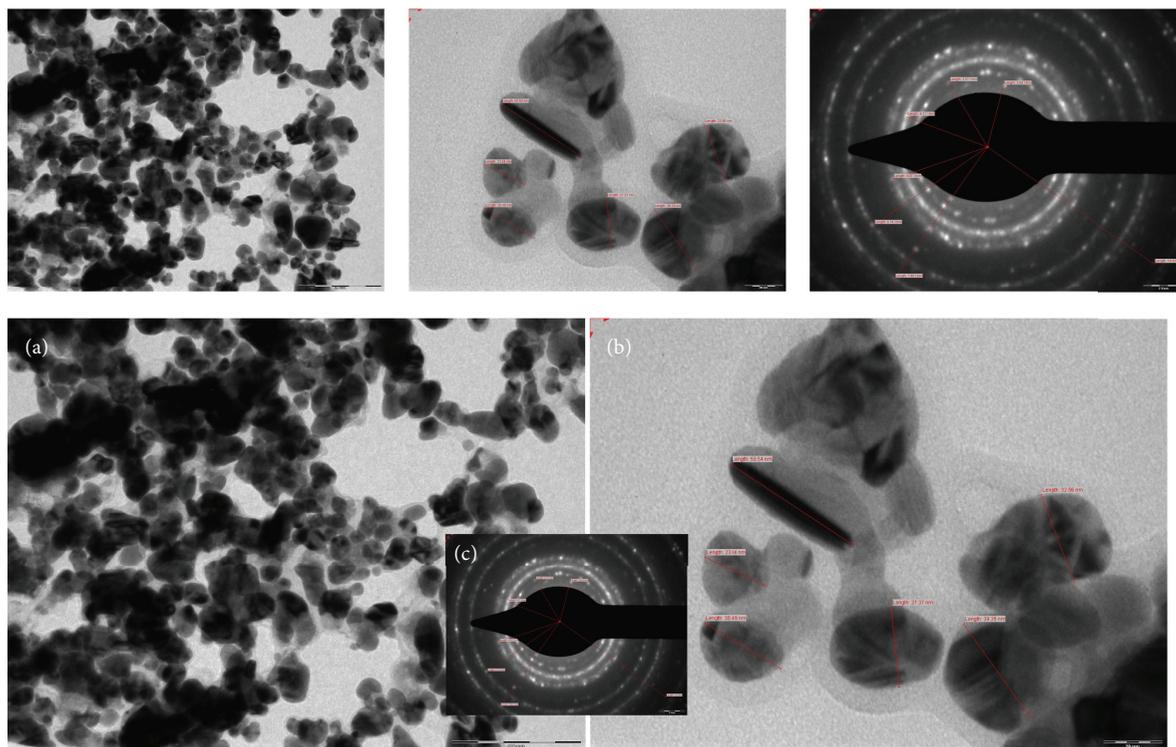


FIGURE 5: (a) and (b) TEM image of biofunctionalized AgNPs, (c) SAED pattern.

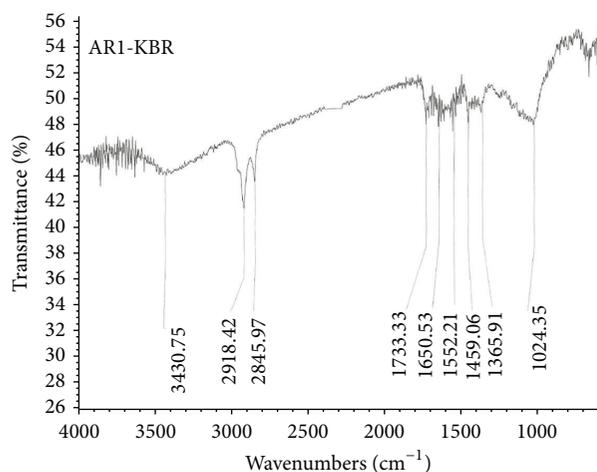


FIGURE 6: FTIR spectrum of biofunctionalized AgNPs.

at 1650, 53 and 1459, 06. The strong band at 1650 cm^{-1} may result from the N–H stretching vibration and can be assigned as absorption bands of C=H, –O–H, –S–H, –N=C=N, –C=O, and –S=O stretching vibration. These are derived from water soluble compounds such as flavonoids, alkaloids, and polyphenols present in leaves. Biological components are known to interact with metal salts via these functional groups and mediate their reduction to nanoparticles [30].

The AgNPs of *A. reticulata* leaves at $80\text{ }\mu\text{L/well}$ showed maximum antifungal activity against *M. gypseum* 14.00 mm followed by *T. rubrum* 13.00 mm and the least 11.00 mm

zone of inhibition showed by *T. tonsurans*. Similarly, against bacteria the maximum activity of 22.00 mm was recorded against *E. coli* followed by *S. aureus* 20.00 mm and *B. subtilis* 18.00 mm. The antidermatophytic activity was directly proportional to the concentration of AgNPs. Two negative controls, that is, plant aqueous extract and AgNO_3 solutions, did not show activity against tested strains. Streptomycin sulphate and ketoconazole showed the inhibition zones 23.00 mm and 35.00 mm, respectively (Table 1).

4. Conclusion

Biosynthesis of silver nanoparticles from *A. reticulata* leaf was shown to be stable and produce particles of crystallographic rod and irregular shapes. The synthesis procedure is ecofriendly in nature. The synthesized particles showed antidermatophytic activity, suggesting that they are useful as antimycotic agent.

Conflict of Interests

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

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