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Synthesis of silver nanoparticles using *Rotheca serrata* leaves and evaluation of their antimicrobial potential

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Abstract

A biosynthesis of Silver nanoparticles (AgNPs) was obtained from an extract of *Rotheca serrata* leaves. The synthesized AgNPs were characterized by various techniques such as UV–Vis spectroscopy, Infra-Red spectroscopy (IR), X-ray diffraction (XRD) and transmission electron microscope (TEM) analysis. Surface plasmon resonance in UV–Vis analysis exhibited a characteristic peak at 390 nm. TEM analysis described the spherical shape of the synthesized nanoparticles. The average size of biosynthesized AgNPs was found to be ~26 nm which was also confirmed by XRD calculations. The synthesized AgNPs were then evaluated for antibacterial and antifungal activity. The antibacterial activity was found remarkable against *B. cerus* and *E. coli*. Antifungal activity against *A. niger* and *C. albicans* confirms the superiority of the material. It has been demonstrated that biosynthesized AgNPs are affordable, safe for the environment, stable, repeatable, and very effective against fungi.

Keywords: *Rotheca serrata*; Silver Nanoparticles; Anti-bacterial; Silver nitrate; Biosynthesis

1. Introduction

The antibacterial hostility has emerged as one of the main threats to human health and illness as the death rate gradually increased due to ineffective cure of common bacterial diseases [1]. Resistance genes have recently been found to be emerging as a result of inappropriate and overuse of antibiotics [2]. Thus, the development of new military tactics is desperately needed to combat germs that are resistant to many drugs and to lessen the side effects of chemical medication consumption in order to combat microbial illnesses [3-4].

The medications made from plants or plant materials are less hazardous, less expensive, and have fewer adverse effects. Nowadays, plant extract-based nanoparticles are being considered as a potential antibiotic replacement [5]. Specifically, silver nanoparticles, or SNPs, have drawn a lot of interest from several scientific disciplines [6-7]. Because of their excellent optical qualities, stability, electric conductivity, and antimicrobial activity, they are being used in a variety of products, including as sensors, filters, nano-electronic devices, and antimicrobial agents [8-9] as they are effective against bacterial agents and not hazardous for the human cell.

Several techniques are being used to synthesize SNPs. Even though synthetic SNPs are widely used, the chemical method for synthesis comes with hazardous materials that are expensive and environmentally dangerous. The biosynthesis method, which employs microorganisms and plant extracts to overcome the drawbacks of physical and chemical

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methods, has emerged as an environmentally friendly synthetic method [10-13]. Because plant extracts are easy to utilize, fast, affordable, non-toxic, and practicable and they provide a convenient and advantageous starting point for the biosynthesis of SNPs. There are several publications that describe the production of SNPs utilizing plant extract [14-15].

The synthesis of silver nanoparticles by reacting aqueous root extracts of *Rubus ellipticus* with 1 mM silver nitrate solution was reported by Khanal *et al.* [16]. The antifungal activity of silver nanoparticles synthesized from bamboo leaf extract was assayed against *Bipolaris maydis*, *Exserohilum turcicum*, and *Curvularia lunata* [17]. The Fenugreek leaf extract was found to contain certain bioactive molecules that led to the reduction of silver ions to their nano form. These nanoparticles showed potential antibacterial activity against *S. aureus*, *E. coli*, *P. aeruginosa,* and *V. cholera* [18]. The biosynthesized nanoparticles have been shown to be a good antimicrobial agent. The stability of nanoparticles over a longer period of time must be assured. Although the antibacterial activity of silver nanoparticles synthesized from plant extract has been reported, no study has investigated the synthesis of SNPs from leaves of *Rotheca serrata* and evaluated their biological activity such as antibacterial and antifungal.

2. Material and methods

2.1. Synthesis and characterization of AgNPs

The AgNPs were synthesized form leaves of *Rotheca serrata.* The aqueous extract of *Rotheca serrata* acts as a reducing and stabilizing agent. Fresh leaves of *Rotheca serrata* were collected from Radhangri region in October. To get rid of any dust, the gathered plant was repeatedly rinsed in running water and then in distilled water. After being shade-dried to eliminate any remaining moisture, the plant was crushed into a fine powder. The plant extract was prepared by boiling 10 g of leaves powder in 200 mL distilled water for 25 min. The filtrate was then filtered through Whatmann filter paper No. 1 and bio-residue was rejected. The filtrate was used as an extract for the reduction of silver ions in the $AgNO_3$ solution. Synthesis of AgNPs was achieved by treating 10 mL of leaf extract with 80 mL $AgNO_3$ (1 mM) and allowing it to react at room temperature for 24 h. The solution was then centrifuged at 3500 rpm for 15 minutes. The supernatant solution containing AgNPs was collected in a beaker and dried in an oven at 75 °C to afford bio-synthesized AgNPs. The bio-synthesized AgNPs were initially monitored spectrophotometrically by taking a UV–visible spectrum in the range of 400 to 700 nm using Perkin-Elmer lambda 950 spectrophotometer. FT-IR analysis was performed on JASCO Japan, FT/IR-4700. The crystalline nature and structure were confirmed by XRD analysis by using Bruker Ltd Germany: D2 Phaser. The surface morphology of AgNPs was assessed by SEM and TEM analysis on TESCAN Brno Czech Republic EU: MIRA3 LMH and Hitachi: H7650 Zero 'C' 120KV instrument, respectively.

2.2. Antimicrobial activity

Stock solutions of the respective nanoparticle were prepared at a concentration of 1 mg/mL in DMSO and HPLC grade sterile distilled water (Sigma-Aldrich and SRL Chemicals India). Next, 100 µg/mL concentrations of respective nanoparticle samples were examined for their antimicrobial activity against respective pathogens. Each sample was loaded into wells in plates spread with the respective test organisms to assess its antimicrobial activity. The antibacterial activity of DMSO-suspended nanoparticles was performed against Gram-negative (*E. coli* NCIM 2832 *and P. aeroginosa* NCIM 5032, *S. typhi* NCIM 2501) and Gram-positive *(B. cerus* NCIM 2703) bacterial strains by modified agar well diffusion method. The suspension of respective test pathogens was prepared in sterile saline and used for further study. For the antimicrobial activity test pathogens were inoculated on the surface of sterile Muller and Hinton agar and spread on plates by using a sterile spreader. After that agar well was prepared aseptically with the help of a sterilized crock borer having a 0.7 cm diameter. Then 100 µL of the nanoparticle solution of all the synthesized nanoparticles was loaded into the wells prepared on the agar plates. Then plates were placed at 4 °C for 20 min for sample diffusion in a culture medium and transferred to an incubator at 37 °C for 24 hrs. Furthermore, the obtained results were compared with the well containing 1000 µg/mL Streptomycin as the positive control and DMSO and Water as a negative control.

2.3. Antifungal activity

The antimicrobial activity of the nanoparticles was determined by using the agar well diffusion method against fungal pathogens *Candida albicans* (NCIM 3103), and *A. niger* (NCIM 814). For further study, the fungal pathogen suspension was prepared in sterile saline then the pathogen was spread on the surface of sterile MGYP agar (Malt extract, Glucose, Yeast extract, Peptone) using a sterile spreader for the antimicrobial activity test. After that, an agar well was created aseptically using a 0.7 cm diameter sterilized cork borer. Then 100 µL of DMSO-suspended nanoparticles was added aseptically into the respective well. Then plates were placed at 4 °C for 10 min for sample diffusion in a culture medium and transferred to an incubator at 27 °C for 48 h. Furthermore, the diameter of the inhibition zone was measured in mm

and the results were recorded and compared against the standard antifungal compound Ketoconazole which was a concentration of 100 µg/mL.

3. Results and discussion

3.1. Synthesis of Silver Nanoparticles

The biosynthesis of AgNPs was achieved by leaves of *Rotheca serrate.* Initially, the leaves collected from the source, were washed thoroughly with running water and then distilled water to remove adsorbed dust particles on the surface. The leaves were selected randomly to ensure proper growth. The washed leaves were kept for shade drying to remove any remaining moisture. The dried leaves were then crushed into fine powder. The fine powder was used for the preparation of plant extract. The plant extract was prepared by boiling 10 g of leaves powder in 200 mL distilled water for 25 min. After completion of the boiling, leaf extract was filtered through Whatman filter paper No.1. The bio-residue was rejected and the filtrate was collected and used for synthesis of AgNPs. The filtrate was used as extract for the reduction of silver ions in the $AgNO_3$ solution. The 10 mL of leaves extract treated with 80 mL $AgNO_3$ (1 mM) solution and allowed to react at room temperature for 24 h. The solution was then centrifuged at 3500 rpm for 15 minutes. The supernatant solution containing Silver nanoparticles was collected in a beaker and dried in an oven at 75 °C to afford bio-synthesized AgNPs.

3.2. Characterization of biosynthesized AgNPs

3.2.1. UV- visible spectroscopy

UV-visible Spectroscopy is special technique widely used to confirm the formation, size, and shape distribution of silver nanoparticles in colloidal solution. The evidence for the successful formation of stable AgNPs achieved from UV-visible absorbance spectral studies [fig. 1]. The initial mixture of silver nitrate solution and plant extract exhibited milkfish color. The formation of AgNPs was revealed by a change in color from milkfish to brownish black after five hours stirring. The biosynthesized AgNPs showed maximum surface plasma absorbance at 390 nm confirming successful synthesis of AgNPs.

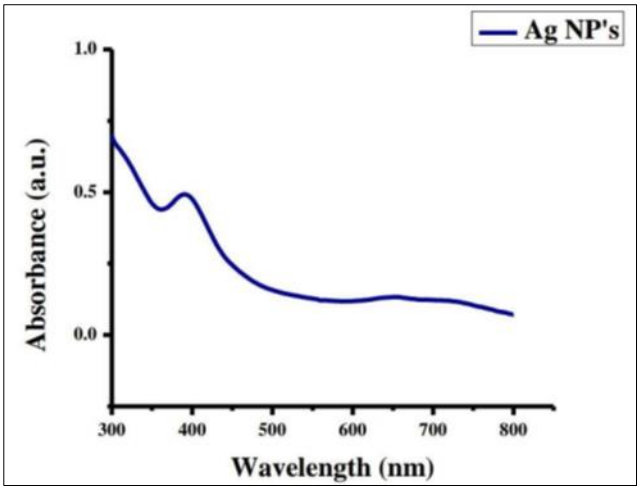


Figure 1 UV-Visible spectrum of biosynthesized AgNPs

3.2.2. Fourier Transform Infrared (FT-IR) spectroscopy

In order to emphasize the functional groups and explore a potential process for the synthesis of these silver nanoparticles, FT-IR spectroscopy analysis was conducted. The FT-IR results from significant bands with respect to corresponding stretching and bending vibrations. [Fig. 2] The peak that appeared at 3357 cm^{-1} corresponds to the O-H stretching frequency of hydroxyl groups (-OH) of water. The C-H stretching vibration bands of methyl or methylene groups appeared at 2917 and 2846 cm^{-1} represent. The peak at 1746, 1460, 1154, and 1014 cm^{-1} could be due to stretching vibration of the carbonyl group (-C=O), C-C aromatic groups, and ester bonds. The peak at 682.67 cm^{-1} assigned to CH out of plane bending vibrations are substituted ethylene systems –CH=CH.

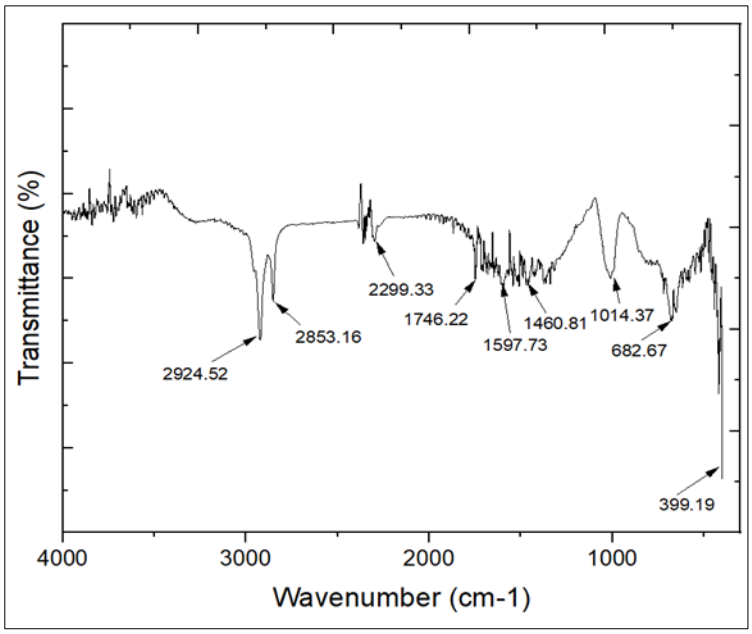


Figure 2 FTIR Spectrum of biosynthesized AgNPs

3.2.3. X-Ray Diffraction (XRD) analysis

The X-ray diffraction pattern of the silver nanoparticles synthesized by electrolysis method is shown in Fig 3. The XRD pattern exhibit number of strong Bragg reflections correspond to (111), (101), (200), (211) and (202) for FCC silver (Table 1). The intensity of peaks reflected the high degree of crystallinity of AgNPs. The size of the Ag nanoparticles estimated from the Debye–Scherrer formula (Instrumental broadening) is 25.82 nm.

$$\text{Debye–Scherrer formula,} \quad D = \frac{k\lambda}{\beta cos\theta}$$

Table 1 Debye-Scherrer parameters for determining size

D	2θ	I fix	Hkl
2.36	38.101	101	111
2.04	44.370	54	200
1.45	64.179	28	220
1.23	77.549	54	311
1.18	81.506	6	222
1.02	98.085	2	400

Six peaks at 2θ values of 38.101, 44.370, 64.179, 77.549, 81.506 and 98.085 corresponding to (111), (200), (220), (311), (222), and (400) planes of silver were observed respectively. The data was compared and confirmed with joint committee on powder Diffraction Standards (JCPDS) silver File No. 00-001-1167.

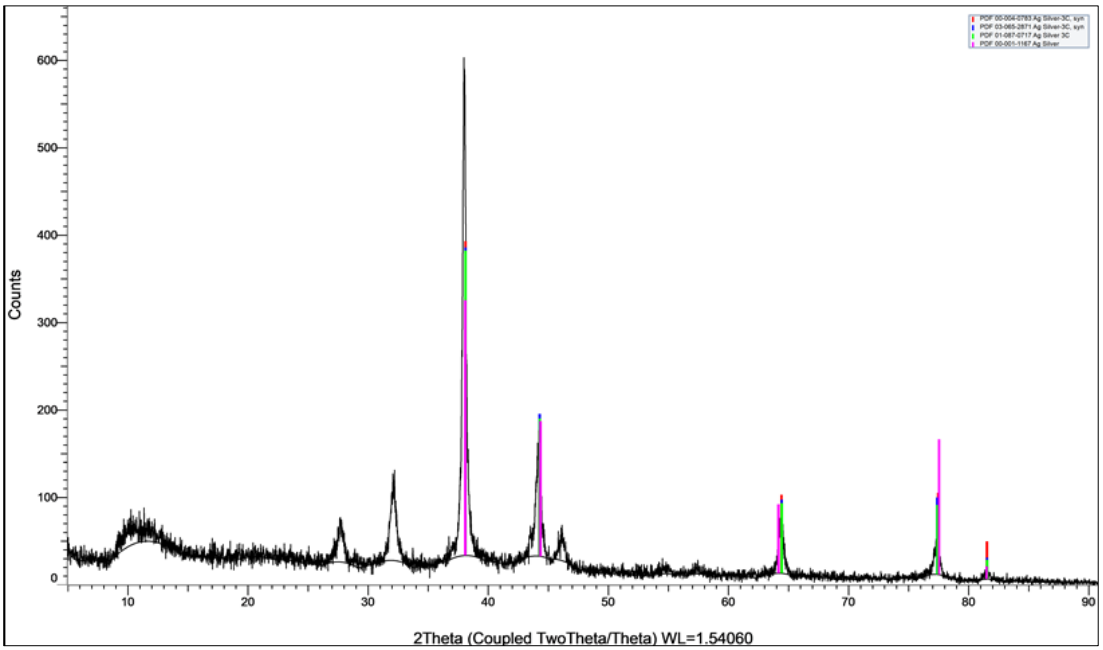


Figure 3 XRD spectrum of AgNPs

3.2.4. Transmission Electron Microscopy (TEM) analysis

Morphology and size of AgNPs was examined by transmission electron microscopy. Fig. 4 indicate the TEM micrograph for AgNPs (a and b) and particle size distribution curve (c). TEM micrograph revealed that the shaper of AgNPs are polydispersed and spherical [19]. The size distribution histogram for AgNPs confirmed average particle size of ~26 nm.

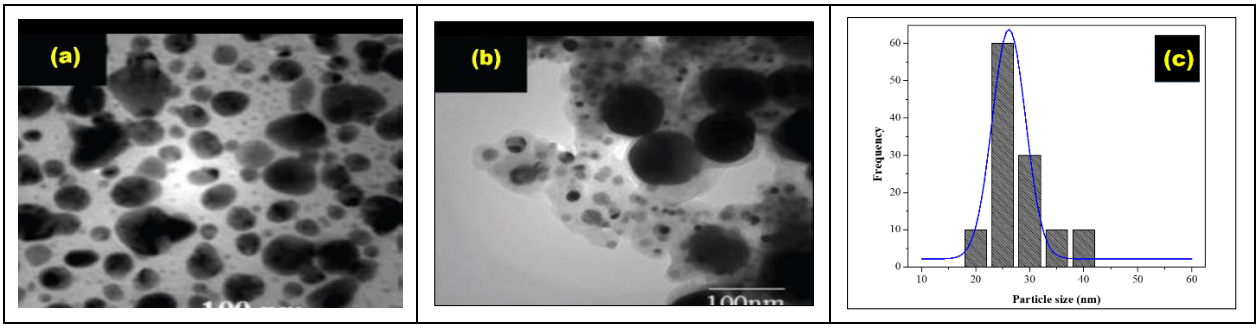


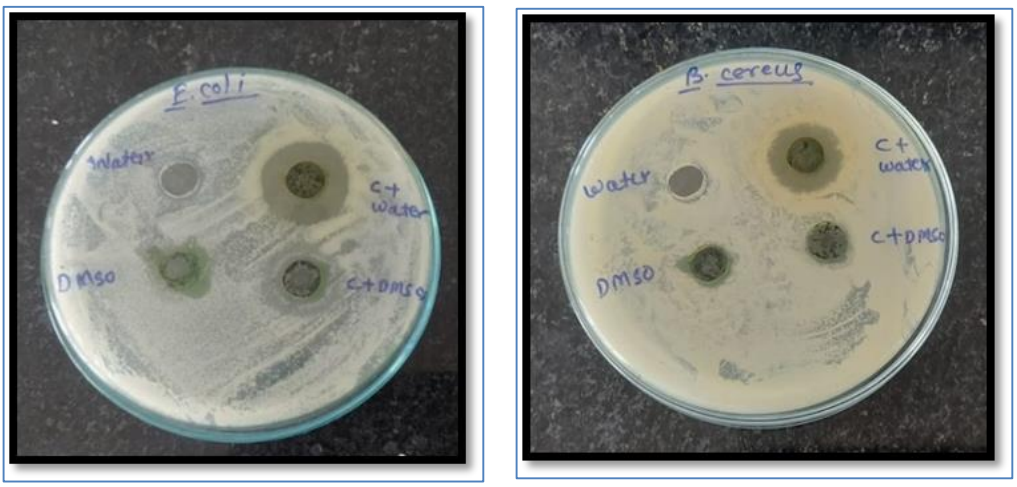
Figure 4 TEM analysis of biosynthesized AgNPs, micropraph (a) and (b) and Size distribution curve (c)

3.3. Antibacterial Activities of AgNPs

The antibacterial activity of DMSO-suspended AgNPs was performed against Gram-negative (*E. coli* NCIM 2832, *P. aeroginosa* NCIM 5032 and *S. typhi* NCIM 2501) and Gram-positive *(B. cerus* NCIM 2703) bacterial strains by modified agar well diffusion method [20-22] (Fig. 5). The suspension of respective test pathogens was prepared in sterile saline and used for further study. For the antibacterial activity test pathogens were inoculated on the surface of sterile Muller and Hinton agar and spread on plates by using a sterile spreader. After that agar well was prepared aseptically with the help of a sterilized cork borer having a 0.7 cm diameter. Then 100 µL of the nanoparticle solution of all the synthesized silver nanoparticles was loaded into the wells prepared on the agar plates. Then plates were placed at 4 °C for 20 min for sample diffusion in a culture medium and transferred to an incubator at 37 °C for 24 hrs. Furthermore, the obtained results were compared with the well containing 1000 µg /mL Streptomycin as the positive control and DMSO and water as a negative control. The diameter of the inhibition zone was measured in mm and the results were recorded (Table 2).

Table 2 Antibacterial activity of respective nanoparticles against respective bacteria

Sr. No.	Test organisms	Zone of inhibition of respective compounds in mm				
		Test		Streptomycin	water	DMSO
		C+ water	C+DMSO			
1.	*B.cerus*	23	00	20	00	00
2.	*S.typhi*	00	00	21	00	00
3.	*P.aeroginosa*	00	00	18	00	00
4.	*E. coli*	25	00	20	00	00
Here, C + Water means Compound dissolved in water. C+DMSO means Compound dissolved in DMSO.						



Escherichia coli *Bacillus cereus*

Figure 5 Antibacterial activity of AgNPs against *E. coli* and *B. cereus*

This study revealed that synthesized AgNPs exhibited good antibacterial against Gram-positive and negative pathogens except *P. aueroginosa* and *S. typhi* pathogen.

3.4. Antifungal activity of AgNPs

The antimicrobial activity of biosynthesized AgNPs was evaluated by using the agar well diffusion method [23-24] against fungal pathogens *Candida albicans* (NCIM 3103) and *A. niger* (NCIM 814). Initially, fungal pathogen suspension was prepared in sterile saline. The prepared pathogen was spread on the surface of sterile MGYP agar (Malt extract, Glucose, Yeast extract, Peptone) using a sterile spreader for the antimicrobial activity test. Agar wells were created aseptically using a 0.7 cm diameter sterilized cork borer. The prepare wells were filled by 100 µl of DMSO-suspended silver nanoparticles. The plates were then placed at 4 °C for 10 min for sample diffusion in a culture medium and transferred to an incubator at 27 °C for 48 h. Furthermore, the diameter of the inhibition zone was measured in mm and the results were recorded and compared against the standard antifungal compound Ketoconazole which was a concentration of 100µg/mL (Table 3).

Table 3 Antifungal activity of the synthesized AgNPs against respective test pathogens in mm

Test organisms	Zone of inhibition of respective compounds in mm		
	Sample	**Ketoconazole**	**Control**
A. niger	12	14	00
C. albicans	14	17	00

The study revealed that the biosynthesized AgNPs exhibited remarkable antifungal activity against test pathogen *A. niger* and *C. albicans* with respect to the standard antifungal agent, ketoconazole.

4. Conclusion

In the present protocol, AgNPs were biosynthesized from leaf extract of *Rotheca serrata* which was efficiently able to reduce silver nitrate to their nanoparticles. The remarkable characteristic properties were examined by various spectroscopic techniques. The AgNPs showed a crystalline nature with size of about ~26 nm with a spherical shape. The biosynthesized AgNPs were found highly active against antibacterial and antifungal organisms. Overall, the method was found highly effective, benign, inexpensive, and environmentally friendly.

Compliance with ethical standards

Acknowledgment

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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