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GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM MANGROVE PLANT *Rhizophora stylosa*

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author MLMKA designed the study and collected all the plant materials and characterized the work and managed the draft and carried out corrections and got approved from both authors. Author SI carried out the TEM studies in his lab and draft the manuscript. Author FM has carried out the SEM and XRD studies in her lab and managed the draft and carried out corrections

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ABSTRACT

The green synthesis of nano-sized particles with specific functions is of great significance in the present bionanotechnology. In this study, the biosynthesis of silver nanoparticles from the aqueous leaf extract of *Rhizophora stylosa*, mangrove plant from southern India, and its activity against some bacterial pathogens are reported for the first time. A green procedure for the synthesis of silver nanoparticles using the mangrove extract as reducing agent was used. Synthesized silver nanoparticles were investigated using UV–visible spectrophotometry, Scanning electron microscope, Transmission electron microscope, X-ray diffraction, Selected area electron diffraction and Fourier transform infrared spectroscopy. The biosynthesized silver nanoparticles were characterized by UV–visible spectrophotometry at a wavelength of 422 nm confirmed the synthesis of silver nanoparticles. The results from the X-ray diffraction analysis approved the formation of crystalline silver nanoparticles with crystallinity percentage of 85.87. As shown by Transmission electron microscope evaluation, silver nanoparticles had the same spherical morphology. The size of the biosynthesized silver nanoparticles was between 1 and 75 nm with average size of 38.62 nm. Scanning electron microscope images identified silver nanoparticles ranging in size from 18 to 42 nm. Fourier transform infrared spectroscopy analysis distinguished different functional groups such as aromatic loops, alcohol, phenol group, alkanes and alkyl halides in the biosynthesis process. Green biosynthesis of silver nanoparticles using aqueous extract of mangrove *R. stylosa* appears rapid, reliable, nontoxic, and eco-friendly.

Keywords: Silver nanoparticles; mangrove plant; *Rhizophora stylosa*; characterization.

1. INTRODUCTION

Biotechnology has emerged as an integration between biotechnology and nanotechnology to develop an environmentally friendly and biosynthetic technology for the synthesis of nanomaterials. Generally the nanoparticles synthesized from chemical methods, but this method of synthesis are medically non-applicable

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because of contamination from precursor chemicals [1]. Today, different types of nanoparticles of copper, zinc, titanium [2], manganese, magnesium, silver and gold are synthesized [1]. In the metal nanoparticles mentioned above, silver nanoparticles are biologically active and therefore have immense application in the field of medicine [3,4,5]. Silver nanoparticles are gaining more and more attention due to their enormous applications, which include biomarkers in optical receptors, catalysts in many chemical reactions, and also have different biological activities such as antibacterial, antifungal, antioxidant, antiviral and anti-inflammatory activities [6,7,8,9]. Green nanotechnology has received a lot of attention due to its cost-effective and green approach. Among the various sources available, plants are considered the preferred option due to their potential for biological reduction and stabilization [10]. Plants serve as sources for many biochemical compounds including alkaloids, flavonoids, tannins, phenols, and saponins that could act as effective reducing agents for metal bioreduction in nanoparticles that have a wide range of biological applications [11,12]. The synthesis of nanoparticles through plant extracts is relatively fast since it is not necessary to maintain specific culture and medium conditions, unlike microbial synthesis. In this context, *Rhizophora stylosa*, a mangrove plant was selected for the study. However, there is no scientific report on the use of leaves of selected plants for the synthesis of AgNPs, therefore, in the present study, we attempted to synthesize AgNPs from selected plant.

2. METHODOLOGY

2.1 Chemicals

Silver salt (silver nitrate- $AgNO_3$), analytical grade were purchased from Merck chemicals. Mumbai, India.

2.2 Preparation of Plant Extract

Fresh leaves of *Rhizophora stylosa* were collected from Pichavaram mangrove forest, Tamil Nadu, India. The leaves were washed with distilled water, shade-dried and powdered. Prior to use, the powdered leaves were soaked in millipore water in the rotary shaker at RT (25°C) for overnight. The plant extract was strained and centrifuged at 10,000 rpm for 20 min. The supernatant was filtered using Whatman No. 1 filter paper and used for subsequent experiments.

2.3 Synthesis of Silver Nanoparticles

The optimal parameters for the green synthesis of Ag nanoparticles were studied. Different concentrations of the aqueous leaf extract (*R. stylosa*) were interacted with varying concentration of metal salt solution in different mixing ratio and incubated at RT in a rotary shaker at 130 rpm.

2.4 Characterization of Silver Nanoparticles

The biosynthesized AgNPs were characterized by optical techniques.

2.4.1 Uv–visible spectroscopy

The preliminary characterization of the synthesized AgNPs was carried out by using double beam spectrophotometer (UV-Vis, Systronics-2201). The spectra were recorded from 300-700 nm at a resolution of 0.1 nm. To study the evolution of NPs the optical absorbance spectra was measured at regular intervals until constant optical density was reached.

2.4.2 Fourier transform infrared (Ftir) spectroscopy

The functional groups present in the aqueous extract before and after biosynthesis of AgNPs was evaluated by the FTIR analysis. The lyophilized powder (Micro modulo 230, freeze dryer, Thermo Electron Corporation, USA) of aqueous extract and the metal nanoparticles produced after synthesis was mixed with KBr and then transparent tablets were made using the pelleting machine. The infrared spectra were obtained using the Fourier transform infrared spectrometer (Nicolet 6700 FT-IR Spectrometer, Thermo Scientific Instruments Groups, Madison, Wisconsin, USA). The spectra were recorded within a scanning range of 4,000-400 cm^{-1}.

2.4.3 X-Ray diffraction (Xrd)

The crystalline nature of the formed nanoparticles was determined using XRD technique. The lyophilized powders were subjected to X-ray diffraction analysis (D8 Advance, Bruker, Germany). The target was Cu Kα (λ = 1.54 A°).

2.4.4 Selected area electron diffraction (Saed) analysis

X-ray diffraction pattern of dry nanoparticles powder was obtained using Siemens D 5005. X-ray diffractometer with Cu Kα radiation to confirm the crystalline nature of nanoparticles.

2.4.5 SEM analysis

The NPs were characterized for their morphology by S-3400N scanning electron microscopy (SEM), Hitachi, Japan. SEM analysis was used for initial morphology evaluation of NPs. Analysis provided the information about size and shape of NPs.

2.4.6 TEM analysis

Morphology and size of the silver nanoparticles were investigated by TEM images using Phillips, TECHNAI FE 12 instrument. Thin film of the sample was prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid and drying under lamp.

3. RESULTS

3.1 Optical Characterizations

3.1.1 Synthesis of silver nanoparticle

The interaction of silver nitrate and aqueous leaf extract led to change in the colour of the solution from green to brown as shown in (Fig. 2). The brown coloration is a spectroscopic signature for the formation of AgNPs. The effect of various process parameters in the synthesis of AgNPs was studied using UV-Vis spectrophotometer.

3.1.2 UV–Vis spectroscopy

SNPs have characteristic brown color due to the excitation of surface plasmon resonance (SPR). This phenomenon is due to collective oscillation of conductance electrons of metallic NPs at different wavelengths. The absorbance around 422 nm is characteristic of SNPs and is being used to confirm the synthesis of SNPs. The reaction mixture upon incubation of 1mM $AgNO_3$ with mangrove extract showed absorbance around 422 nm (Fig. 3). Also, the color of reaction mixture was changed from green to brownish. The appearance of peak around 422 nm and change in the color of reaction mixture to brownish has indicated the synthesis of SNPs by mangrove extract.

Fig. 1. Figure showing the mangrove plant *Rhizopora stylosa*

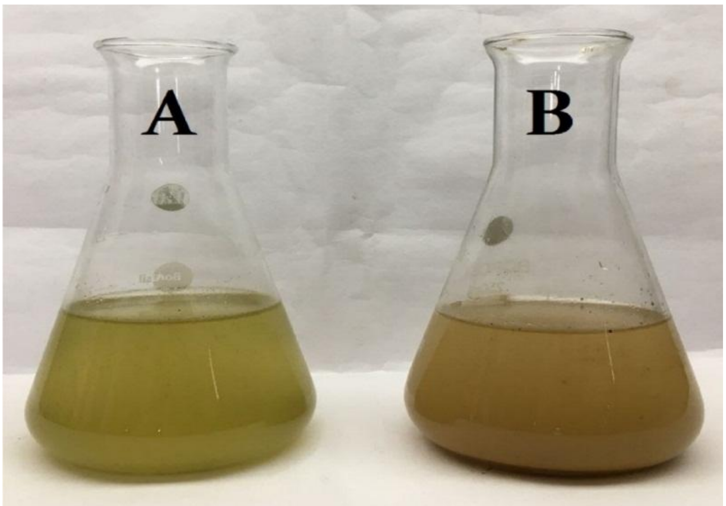

Fig. 2. A) Aqueous extract of *Rhizopora stylosa* B) Silver Nanoparticles synthesized *Rhizopora stylosa*

3.1.3 Fourier transform infrared spectroscopy (ftir)

The peaks observed in the spectra of aqueous extract were 3463, 2927, 1710, 1641, 1280, 1450 and 1041 cm^{-1} respectively (Fig. 4). The wavenumber at 3463 cm^{-1} corresponds to the presence of hydroxyl groups. The C–H stretching at 2,927 cm^{-1} was observed. The wave numbers at 1280 cm^{-1} and 1450 cm^{-1} is attributed to symmetric and asymmetric stretching of C– H bond. The band at 1,710 cm^{-1} is from the C–O stretch of phenols. 1041 cm^{-1} (ether linkages) was also observed. The FTIR analysis confirmed the presence of functional group on the surface of AgNPs.

3.1.4 X-Ray diffraction (Xrd) technique

The diffraction patterns of AgNPs at (111), (200), (220) and (311) planes of face centered cubic is illustrated in (Fig. 5). The planes corresponded to 38°, 44°, 64° and 77° diffraction angles. In both the diffraction spectra the (111) plane is dominant. The peaks obtained were in accordance with the JCPDS No. 96-901-3037 and 96-901-3036 respectively.

3.1.5 Selected Area Electron Diffraction (SAED)

Selected area electron diffraction (SAED) pattern of the silver nanoparticles was studied for confirmation of their crystal nature. The ring-like diffraction pattern indicates that the particles are crystalline (Fig. 6). The diffraction rings could be indexed on the basis of the FCC structure of silver. Four rings arise due to reflections from lattice planes of FCC silver nanoparticles, respectively. This is evident by sharp Braggs reflection observed in the XRD spectra.

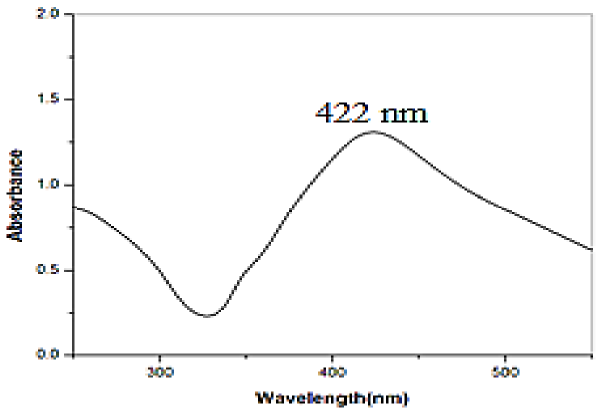

Fig. 3. UV-vis spectra of newly synthesized Silver Nanoparticles of *Rhizopora stylosa*

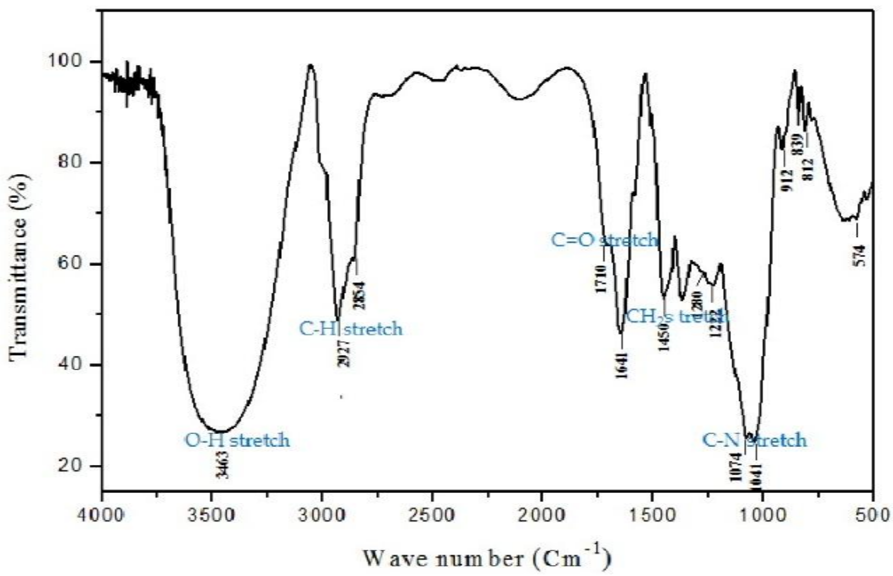

Fig. 4. FTIR spectra of mangrove *Rhizopora stylosa* synthesized silver nanoparticles

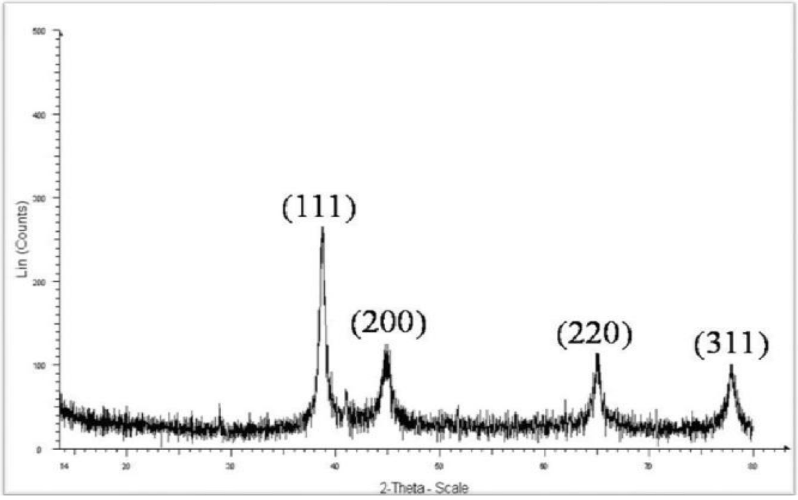

Fig. 5. XRD pattern of Silver Nanoparticles synthesized using the Mangrove *Rhizopora stylosa*

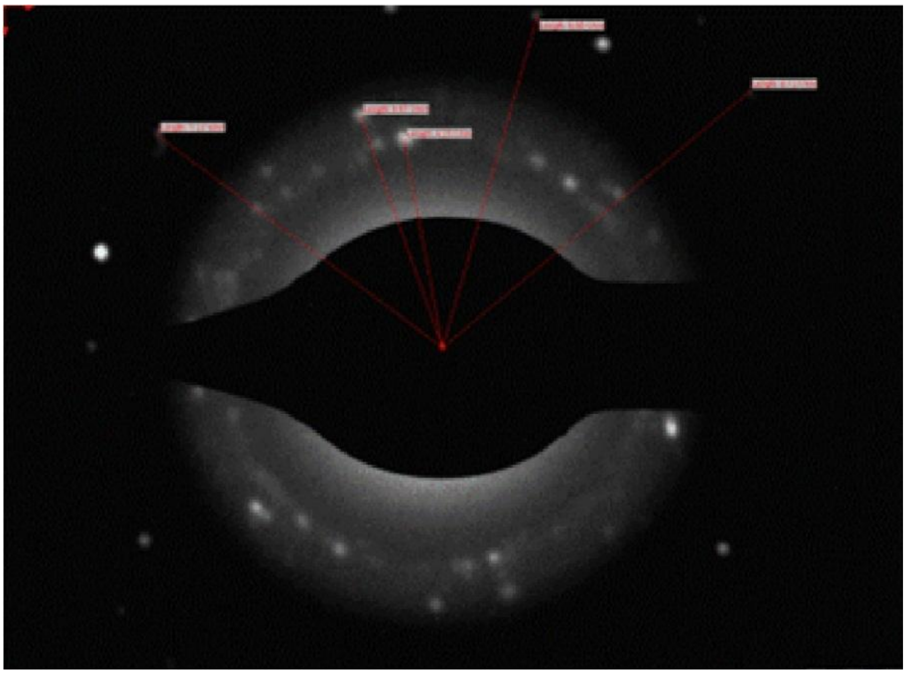

Fig. 6. SAED pattern of silver nanoparticles of *Rhizopora stylosa*

3.1.6 Scanning electron microscopic analysis

Surface and morphology of the synthesized AgNPs from *R. stylosa* was confirmed by the SEM studies. SEM studies shows the size of AgNPs of *R. stylosa* in the range of 18-42 nm with spherical shape (Fig. 7).

3.1.7 Transmission electron microscopic analysis

Transmission electron microscope image of silver nanoparticles derived from *R. stylosa* was shown in (Fig. 8). The morphology of the nanoparticles was spherical in nature. The obtained nanoparticles was in the range of sizes 1 and 75 nm with average size of 38.62 nm and few particles are agglomerated.

4. DISCUSSION

The study demonstrates the synthesis of silver nanoparticles using aqueous leaf extract of mangrove plants *Rhizophora stylosa*. The bioreduction of Ag^+ to $Ag^{(0)}$ is influenced by the concentration of aqueous extract, metal salt and their mixing ratios. The biogenic nanoparticles were functionalized with bioorganic components present in the mangrove plants. The appearance of dark brown color and yellowish brown color was also reported before as clear signs of the reduction of silver ions and the synthesis of silver nanoparticles using the mangrove *Avicennia marina* [13,14,15].

In the present study, according to the absorbance rate obtained by UV–visible spectrophotometry at 422 nm, the biosynthesis of AgNPs by aqueous extract of *R. stylosa* was confirmed. The biosynthesized AgNPs peak around 422 nm has also been reported by other studies [16,13].

The FTIR spectra of *R. stylosa*, AgNP is showed in (Fig. 4). The observed peaks of –OH, C-C and C=O corresponding to 3463, 2927 are 1710 cm−1 are characteristic of polyphenols. Mangrove plants contain high content of polyphenols, terpeniods and flavonoids. The mangrove plants are rich in polyphenols which act as metal chelators and reducing agents. The disappearance of the 1230 cm^{-1} band after the bioreduction is due to the role of polyphenols in the reduction of Ag+. Similarly, [17], have described that the bioreduction of metal ion to metal NPs was due to the oxidation of polyols present in the aqueous solution of black tea leaf extract. The decrease in the absorption intensity when compared with the aqueous extract indicates that the NPs are coated with the polyphenolic components. The minor shifts in the wavenumber after the synthesis of NPs could probably due to the restraint capping molecular motion, resulted from the coating of bioorganic components on the NPs [18]. These biomolecules have previously been described to own the potential to reduce the silver ions and form the AgNPs [19,20,15].

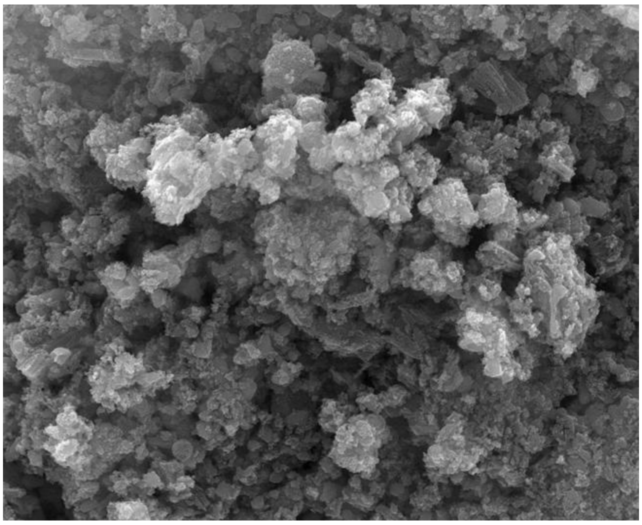

Fig. 7. SEM image of silver nanoparticles synthesized from aqueous leaf extract of *Rhizopora stylosa*

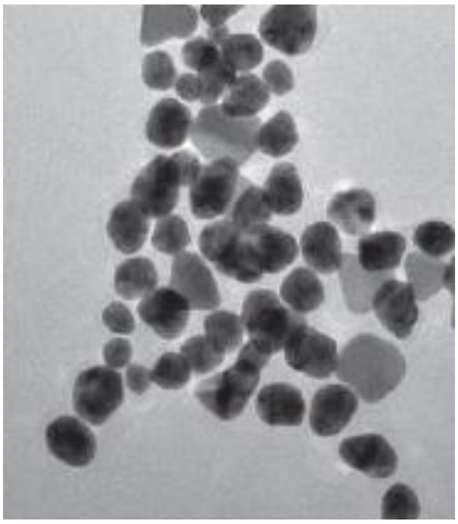

Fig. 8. TEM image of silver nanoparticles synthesized from aqueous leaf extract of *Rhizopora stylosa*

The characteristic XRD peaks of biogenic Ag nanoparticles corresponding to (111), (200), (220) and (311) planes (Fig. 5) confirms the crystalline nature of NPs. The diffraction planes contain energetically unique sites based on their atomic density which reacts with biological systems on interaction. Ag nanoparticles contains high atomic density on (111) plane [3]. The unassigned Bragg peaks around the vicinity of diffraction planes of NPs arises due to the crystallization of bioorganic components present in the aqueous extract on the surface of silver nanoparticles. The obtained diffraction planes corroborated with the results reported by [21,22,23] in the synthesis of Ag nanoparticle using neem leaf, mushroom extract and palm oil mill effluent. A same finding was reported using the mangrove plant *A. marina* [13].

The selected area electron diffraction pattern with bright circular rings corresponding to (111), (200), (220) and (311) planes exhibited the crystallinity of nanoparticles. Scherrer ring like diffraction pattern indicates that the particles are purely crystalline in nature. The electron diffraction rings could be indexed on the basis of the face centered cubic (fcc) silver structure. Four rings arise due to reflections from (111), (200), (220) and (311) planes of fcc silver nanoparticles. Newly synthesized silver nanoparticle's crystalline nature is in fairly good agreement.

The surface morphology, size and shape of the silver nanoparticles were analyzed by Scanning Electron Microscope. Fig. 5 shows the SEM image of silver nanoparticles synthesized from leaf extract. The SEM images show individual silver nanoparticles which are predominantly spherical in shape as well as number of aggregates with no defined morphology. The presences of biomolecules in the leaf extract has resulted in the synthesis of spherical silver nanoparticles and the aggregation may be due to the presence of secondary metabolites in the leaf extracts. The SEM image shows the size of the silver nanoparticles ranging from 18 to 42 nm. [24] reported an average particle size of about 27 nm for silver nanoparticles synthesized using the extract of leaves of the mangrove plant *E. agallocha*. An average particle size of 17.30 nm for AgNPs prepared from the leaf extract of the mangrove *Avicennia marina* has been reported (15). In synthesizing AgNPs using three mangroves of *Avicennia alba, Sonneratia caseolaris* and *Sonneratia apetela,* nanoparticles possessing a uniform spherical shape and in the size range of 20–40 nm have also been reported [25].

Transmission electron microscopy (TEM) has been used to identify the size, shape and morphology of nanoparticles. It reveals that the silver nanoparticles are well dispersed and predominantly spherical in shape, while some of the NPs were found to be having structures of irregular shape as shown in Fig. 8. The nanoparticles are homogeneous and spherical which conforms to the shape of SPR band in the UV–visible spectrum. The particle size agrees with that calculated from DLS histogram with average diameter of around 34 nm.

5. CONCLUSION

The potential outcome of the study from mangrove plant *Rhizophora stylosa* is to effectively use the Ag nanoparticles in medical, environmental and industrial applications. Benefit of synthesis of silver nanoparticles using plant extracts is that it is an economical, energy efficient, cost effective; provide healthier work places and communities, protecting human health and environment leading to lesser waste and safer products. UV–visible spectrophotometry confirmed the formation of silver nanoparticles from *R. stylosa* aqueous extract. The biosynthesized silver nanoparticles were crystalline and spherical based on XRD and TEM and according to SEM/TEM, in a size range between 1 and 75 nm. FTIR analysis revealed that different functional groups present in the mangrove extract caused the reduction of silver ions and helped in the formation of nanoparticles in the biosynthesis procedure. It is concluded that the biosynthesis of AgNPs using the extract of *R. stylosa* leaves is simple, environment friendly, clean and could be easily scaled up for large-scale syntheses. Hence in this regard; use of plant extract for synthesis can form an immense impact in coming decades.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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