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Short communication

Phytosynthesis of silver nanoparticles using *Mangifera indica* flower extract as bioreductant and their broad-spectrum antibacterial activity

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ABSTRACT

The present study focused on the evaluation of antibacterial property of silver nanoparticles (AgNPs) synthesized using mango flower extract. The morphology of the synthesized AgNPs was observed under transmission electron microscopy and the particles have shown spherical shape in the range of 10–20 nm. X-ray powder diffraction analysis confirmed the crystalline nature of the AgNPs. The atomic percentage of the Ag element in the nanoparticles was about 7.58% which is greater than the other elements present in the sample. The AgNPs showed extensive lethal effect on both Gram-positive (*Staphylococcus* sp.) and Gram-negative (*Klebsiella* sp., *Pantoea agglomerans*, and *Rahnella* sp.) bacteria. The extensive lethal effect of AgNPs against clinically important pathogens demonstrated that the mango flower mediated AgNPs could be applied as potential antibacterial agent to control the bacterial population in the respective industries.

1. Introduction

Bacterial contaminations remain as a one of the most severe issues in medical devices, water treatment and food industries [1]. Both Gram-positive and Gram-negative bacteria are common contaminants and cause diseases in human beings [2,3]. Several antibacterial agents are available in the market to control bacterial contaminations in various products. However, these available antibacterial agents have numerous drawbacks such as, reduced solubility, high cost, toxicity and side effects [4]. Thus, there is much interest to investigate safe and effective antibacterial compounds [5,6]. Currently, in several products, such as in disinfectants, metal nanoparticles are used as the active substance. Silver nanoparticles have proved their efficiency against pathogenic bacteria [7]. Silver nanoparticles (AgNPs) synthesis has gained much attention because they potentially offer a solution to overcome bacterial contaminations and/or infections in an effective and safe approach.

Several physico-chemical routes, including electro-chemical [8], photochemical [9], and radiation methods [10] are commonly used to produce AgNPs. These methods, however, have disadvantages due to environmental contamination or toxic residues in the nanoparticles [11]. Therefore, new environmentally friendly techniques, which use biological organisms to mediate the nanoparticle synthesis, are still searched.

Efficient biological substrates used in the production of AgNPs are various, for instance, plant extracts [12,13], microorganisms [14], algae [15], panchakavya [16], oilcake [17], vegetable waste [18], seaweed [19], enzymes [20], and metabolites of arthropods [21]. It has been generally suggested that plant based materials are promising substrates for the AgNPs synthesis because the process is simple to scale up [22].

Mango (*Mangifera indica* L.) is an important tropical and sub-tropical crop belonging to the *Anacardiaceae* family [23,24]. The leaves of this native South-Asian species have various properties, including antidiabetic, antimicrobial and anti-inflammatory activities [25]. AgNPs have been successfully synthesized using mango peel [26] and leaves [27]. Mango flowers have not been reported for their efficiency of AgNPs synthesis and its broad-spectrum antibacterial activity. Hence, the study aimed (i) to produce AgNPs using a mango flower extract, (ii) to characterize the biosynthesized AgNPs using various spectroscopic techniques, and (iii) to measure their antibacterial property.

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2. Methods

2.1. Plant material

Analytical grade chemicals (Sigma-Aldrich Chemicals (USA) were used in this study. Fresh and healthy flowers of *M. indica* were collected from Mallasamudram, Tamil Nadu, India. The flowers were first rinsed with tap water and then with autoclaved water to remove all dust particles. The fresh material (25 g) was mixed with autoclaved water (250 ml), grinded using a juicer and filtered through Whatman filter paper (No. 1) and stored at 4 °C for the AgNPs synthesis.

2.2. AgNPs biosynthesis

AgNPs synthesis was carried out as described in Govarthanan et al. [15]. Briefly, the plant extract was mixed with1 mM $AgNO_3$ until the color changed indicated a successful synthesis. After centrifugation, the pellet was dispersed and dried. An aliquot of 4 ml of the prepared extract was added to 96 ml of $AgNO_3$ (1 mM) and mixed with vigorous magnetic stirring for 30 min until the color change from pink to brown indicated the synthesis. The mixture was centrifuged at 3000 rpm for 15 min, the pellet was dispersed in double-distilled water and dried in lyophilizer.

2.3. Spectroscopic analysis

The formation AgNPs were initially screened by UV–vis spectrophotometer (Elico-SL 164) at 400–800 nm. All other characterization was done according to Govarthanan et al. [15]. The morphology of the synthesized AgNPs was examined using biological transmission electron microscopy (Bio-TEM; H-7650, Japan, HITACHI). The elemental composition of the synthesized AgNPs was confirmed by scanning electron microscopy-energy dispersive spectra (SEM–EDS; JEOL-64000, Japan). The Fourier transform infrared spectrum (FTIR) of the AgNPs was obtained on a Perkin-Elmer FTIR spectrophotometer (USA) in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets. The X-ray powder diffraction (XRD) was carried out using Rigaku X-ray diffractometer (Rigaku, Japan). The scanning was performed in the region of $2\theta = 30–80°$ at 0.041°/min with a time constant of 2 s.

2.4. Antibacterial activity

The activity of the AgNPs against human pathogenic bacteria was tested as described previously by Govarthanan et al. [16]. The bacterial strains used in the present study were *Pantoea agglomerans* (KR296735), *Rahnella* sp. (KX656894), *Staphylococcus* sp. (KC688883), *Klebsiella* sp. (KC899845). The pathogens were cultured in different AgNPs concentrations (0 (Control), 2.5, 5, 7.5 and 10 mM) in nutrient broths. At regular intervals (0–48 h), the bacterial growth was observed using an UV–vis spectrophotometer (600 nm). Using the adsorption units (AU),

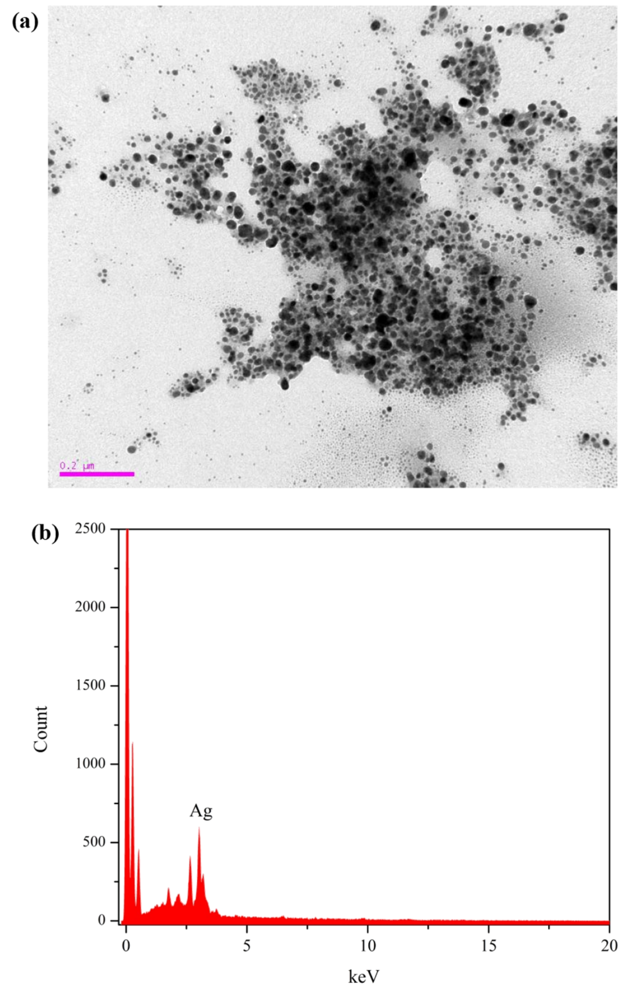

Fig. 2. (a) TEM image of bigenic AgNPs. (b) EDS of AgNPs obtained from flower extract of *Mangifera indica.*

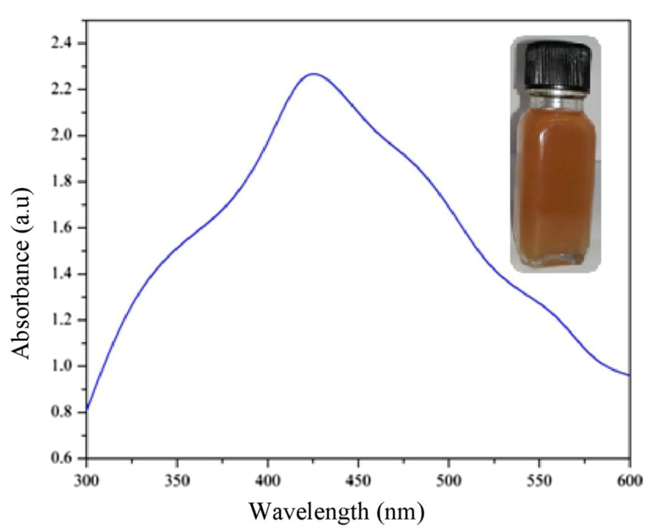

Fig. 1. UV–Vis spectroscopy analysis of AgNPs.

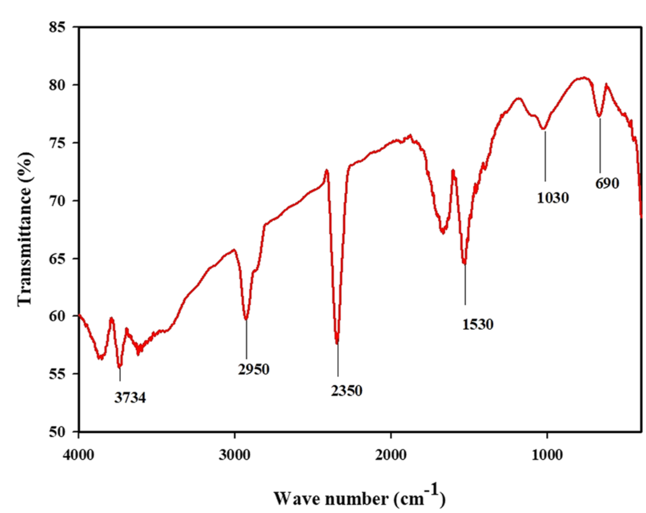

Fig. 3. FT-IR spectra of *purified* AgNPs.

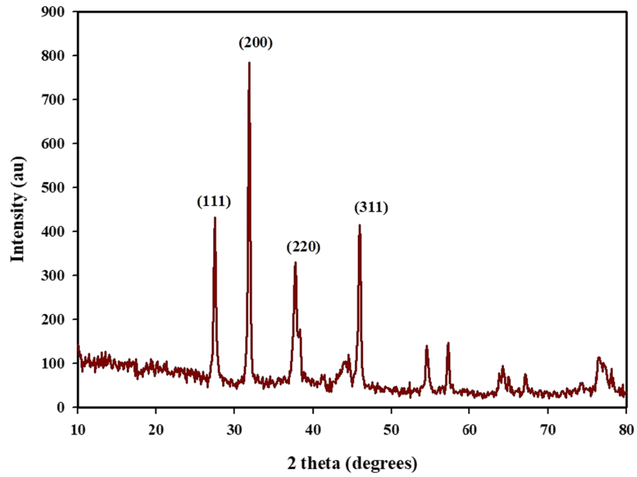

Fig. 4. X-ray Diffraction of AgNPs obtained from *Mangifera indica* flower extract.

the inhibition of the growth was calculated as a test sample AU percentage of the control sample AU according to Elegbede et al. [28].

3. Results and discussion

The green AgNPs synthesis with plants as bioreductants has attracted over other biological sources, because plants are more suitable for a large-scale synthesis. In this study, a mango flower extract was used. The color change and the UV–vis analysis confirmed the success in the synthesis (Fig. 1). As reported previously, an absorption peak around 450 nm is indicating to the surface plasmon resonance (SPR) of AgNPs [29,30].

The TEM image shows the spherical shape of the AgNPs and the size range of 10–20 nm (Fig. 2a). Fig. 2(b) shows the SEM-EDS pattern of AgNPs. The atomic percentage of Ag was 7.58% (Table 1). This is greater than the other elements present in the sample. The sharp peak at ~3 keV confirmed that elemental silver was present in the pure form. This was and consistent with previous reports using other biological materials [31].

The secondary metabolites were revealed by an FT-IR spectrum that showed a small peak at 3734 cm^{-1} assigned to —OH stretching vibrations (Fig. 3). The peak at 2950 cm^{-1} indicated the presence of alkane —CH stretching. The band at 2350 cm^{-1} could be assigned to the aliphatic C—H stretching vibration. The peaks at 1530, 1030 and 690 cm^{-1} correspond to the C—O and N═O stretches of the ester, and to nitro groups, respectively. The FT-IR analysis indicated phyto-chemicals, such as alkaloids, flavonoids, amino acids and proteins, which seem to be responsible for the AgNPs synthesis and also affect to the stability and capping of the AgNPs. Ajitha et al. [32] reported that polyphenols and other secondary metabolites of the extract used mediate the reduction of ions to the atomic nanoparticle form. The biosynthesized AgNPs were in a face-centered cubic crystal form, which was indicated by the XRD spectrum peaks (Fig. 4) as described previously by Guan et al. [12].

The AgNPs had antibacterial activity against Gram-negative bacteria *Klebsiella* sp., *P. agglomerans*, and *Rahnella* sp. at 10 mM of AgNPs (Fig. 5). The calculated inhibition percentages were 65.9%, 62.1%, and 51.4%, respectively (Fig. 5). Statistical analysis showed significant differences ($P < 0.05$) were observed in the different AgNPs concentration groups. Lateef et al. [33] observed that *Petiveria alliacea* L. leaf extract mediated AgNPs inhibited 100% of the growth of *Klebsiella*

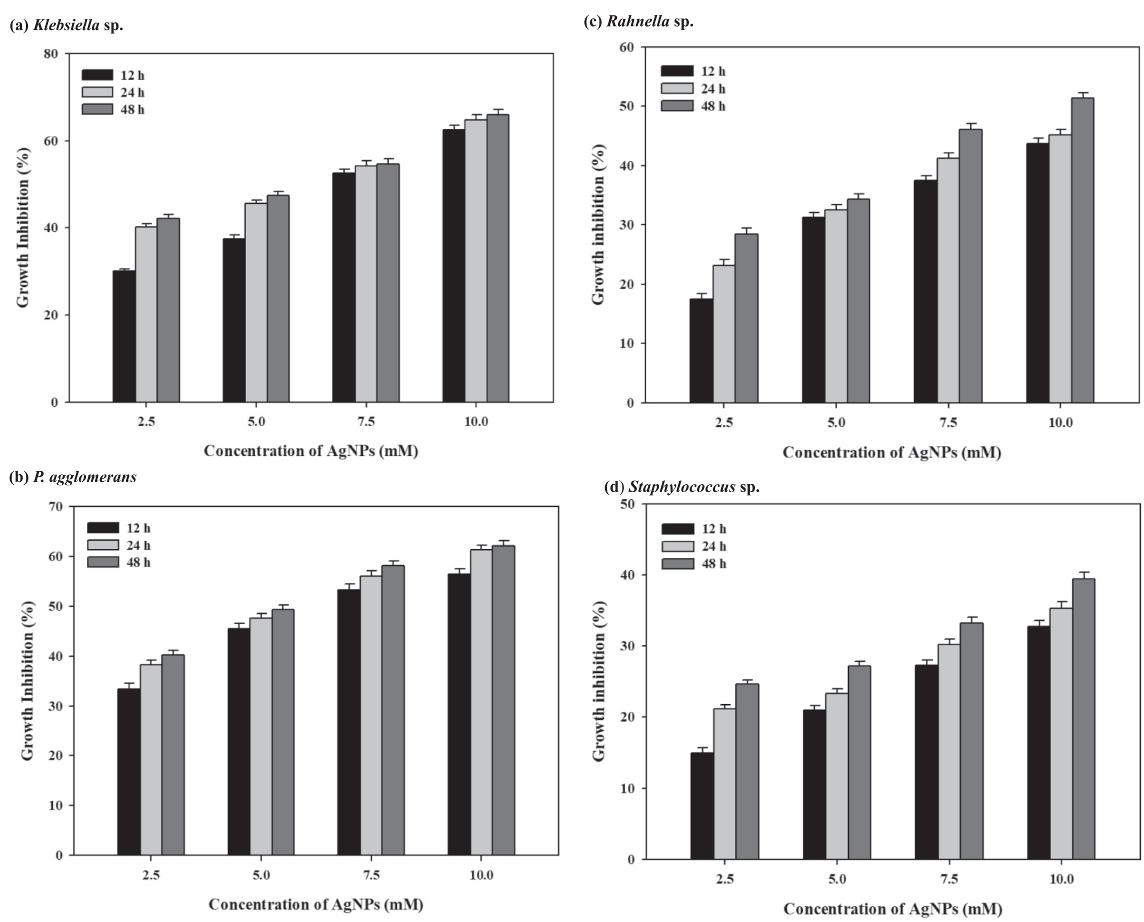

Fig. 5. Antibacterial activity of synthesized AgNPs, (a) *Klebsiella* sp., (b) *P. agglomerans*, (c) *Rahnella* sp., and (d) *Staphylococcus* sp.

pneumonia and *Staphylococcus aureus*. However, their used a relatively high concentration compared to us, 150 mM of AgNPs. In our studies, 10 mM AgNPs inhibited Gram-positive *Staphylococcus* sp. 39.4%. The inhibitory capabilities of AgNPs have been associated with the attack against the cell wall that is followed by the leaking of the cytoplasm and ultimately the cell death [28]. Several other mechanisms have been reported. The formation of reactive oxygen species (ROS), sulph-hydryl group bonding between Ag^+ ions, denaturation of bacterial proteins and cell wall damage also lead to lethal conditions [31,34].

Despite the huge amount of research about green nanomaterials and their medical applications published as reviewed recently by Saratale et al. [35], novel techniques and applications are still needed. Especially in less developed countries, economic and sustainable ways to treat pathogenic bacterial infections are under intensive study. It is important that local resources such as local plants could be used and find novel solutions to the increasing demand for antibacterial agents.

4. Conclusion

We investigated an ecological, economic, simple and rapid technology to the synthesizing of antibacterial nanoparticles using silver and mango flowers. The AgNPs we synthesized were of spherical shape with diameter ranging between 10 and 20 nm. The lethal effect of the AgNPs on clinically important bacterial strains was observed; *P. agglomerans*, *Rahnella* sp., *Staphylococcus* sp. and *Klebsiella* sp. were inhibited by the AgNPs. This study suggests the use of mango flower extract in the AgNPs synthesis and its potential in antibacterial applications in the field of clinical microbiology.

Declaration of Competing Interest

The authors declared that there are no conflicts of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.102970.

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