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RESEARCH PAPER

Preparation of silver nanoparticles by *Garcinia mangostana* stem extract and investigation of the antimicrobial properties

Perumal Karthiga

Sri Paramakalyani Centre for Environmental Sciences, Alwarkurichi, Tamil Nadu, India

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Abstract The synthesis of nanoparticles using green chemistry method is an intensifying research area due to the prospective applications in nanomedicines. The purpose of this present study was to develop a simple phyto-assisted method for the synthesis of silver nanoparticles (AgNPs) using *Garcinia mangostana* stem aqueous extract as reducing agent. The formation of AgNPs was confirmed by surface plasmon resonance as determined by UV–visible spectra at 430 nm. The morphology (SEM), diffraction pattern (XRD), elemental analysis (EDX) and visible absorption spectroscopy (UV–vis) confirmed the reduction of silver ions to AgNPs with the above characterization. The XRD data predicted the characteristic diffraction peaks of the elemental silver phases and the SEM image showed spherical nanoparticles in monodisperesed nature. The antibacterial assessment of AgNPs was analyzed by measuring the inhibitory zone.

Introduction

Metal nanoparticles are being extensively used nowadays in the fields of medicine, biology, material science, physics and chemistry. Metal nanoparticles have been shown to hold enormous application in the areas such as catalysis, biological labeling, optoelectronics (Prathana, Chandrasekaran, Raichur, & Mukerjee, 2011), removal of heavy metal contaminants (Gupta, Srivastava, Mohan, & Sharma, 1997; Gupta, Ali, Saleh, Nayak, & Agarwal, 2012; Saleh & Gupta, 2012), organic pollutants (Gupta et al., 2012; Rajendran et al., 2016) and dyes from water (Mittal, Mittal, Malviya, Dipika, & Gupta, 2009, 2010). There is a rising interest in AgNPs due to its unique antimicrobial properties (Choi et al., 2008; Gopinath et al., 2012). AgNPs are significant materials that have been studied widely with electrical, optical, biological properties and are thus applied in catalysis, biosensing, imaging, drug delivery, fabrication and especially in medicine (Jain, Huang, El-Sayed, & El-Sayed, 2008; Nair & Laurencin, 2007). Synthesis of AgNPs was broadly studied by employing chemical and physical methods, but the development of dependable technology to produce nanoparticles is a vital feature of nanotechnology (Natarajan, Selvaraj, & Ramachandra, 2010). Nanoparticles are synthesized by various methods like physical, chemical and biological. The physical and chemical

E-mail: karthibiotec@gmail.com

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methods are expensive and also produce hazardous by-products (Magudapatty, Gangopadhyayrans, Panigrahi, Nair, & Dhara, 2001). In contrast, green synthesis using plant extracts having considerable attention in nanoparticles synthesis with simple, non-hazardous and eco-friendly product (Nair & Pradeep, 2007).

The plant extract act as reducing and capping agents for nanoparticles development, are more favorable more than other biological processes (Valli & Vaseeharan, 2012), for the reason that they destroy the elaborated process of culturing and maintaining of the cell, and can also be scaled up for large-scale nanoparticle synthesis (Saxena, Tripathi, Zafar, & Singh, 2012). Different plant materials such as fruit, bark, stem, fruit peels, root and callus have been studied until now for the synthesis of silver, gold, platinum and titanium nanoparticles in various sizes and shapes (Gopinath et al., 2012).

Garcinia mangostana Linn, belongs to the family Clusiacea/Guttiferae. It is a tropical plant generally known as mangosteen originates in Southeast Asia. The plant is rich in xanthones and known to hold a wide range of naturally occurring polysaccharide (Bennett and Lee, 1989). Xanthone compounds have anti-inflammatory, antioxidant, anti-proliferative, anti-plasmodial and powerful antibacterial activity (Pedraza-Chaverri, Reyes-Fermin, Nolasco-Amaya, Orozco-Ibarra, & Medina, 2009). The major compound was recognized by using thin layer chromatographic technique (TLC). The green synthesis of AgNPs evaluated based on green chemistry perspectives, solvent medium, reducing agent, and assortment of nontoxic substances for the AgNPs (Raveendran, Fu, & Wallen, 2003). Mainly, AgNPs were used as a bactericide. Silver nanoparticles have been recently used in many consumer products like soap, toothpaste, and socks due to its antibacterial properties (Rajeshkumar, 2016).

Based on this approach, the present study aims to synthesize silver nanoparticles by a green biological route, using an extract derived from mangosteen stem, and characterization of the synthesized nanoparticles utilizing UV–visible spectroscopy (UV–vis), scanning electron microscope (SEM), energy dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FT-IR) analysis. In addition, the silver nanoparticles thus produced are tested for its bactericidal activity.

Materials and methods

Chemicals

All the chemicals used for synthesis [silver nitrate ($AgNO_3$), potassium bromide (KBr)], were of analytical grade from Merck Limited, Mumbai, India. Nutrient Agar, Nutrient Broth, Agar Agar, Muller Hinton Agar (MHA) purchased from Himedia Laboratories, Mumbai, India. The aqueous solutions were prepared with triple distilled water.

Preparation of stem extract

Healthy and fresh stem of *Garcinia mangostana* (Fig. 1) was collected from Courtallam, Tamil Nadu, India. Fine stems were collected and carefully washed with deionized water several times to remove the dust particles and then air dried to remove the residual moisture and cut into small pieces and stored in air-tight container. 30 g of stem in a round bottomed flask with 100 ml deionized water and refluxed for half an hour, cooled at room temperature and filtered with Whatman No. 1 filter paper.

Figure 1 Fresh and healthy plant and stem of *Garcina mangostana*.

Qualitative analysis of secondary phytochemicals

Extracts of plant part stem was evaluated for preliminary screening of secondary phytochemicals such as alkaloids, Quinones, Coumarin, Flavanoid, Steroid, Alkaloids, Phenol and Sugar/Glycosides following the reported methods with minor modifications.

Identification of mangostin in the crude extract

Mangostin extract obtained through cold extraction method was dissolved in methanol. It was spotted on thinlayer chromatography (TLC) plate using capillary tubes. Standard mangostin was also spotted equivalent to crude extract on TLC plate. Then TLC pate was kept into the developing tank containing the mobile phase to develop. Then after the movement of the mobile phase near the end of the plate (stationary phase), it was removed and dried. Then the visualizing reagent, modified vanillin sulphuric acid reagent was spread over the plate which was then allowed to air dry and then kept over the hot plate for development of the spots. Toluene and Methanol was used as the solvent system in the ratio of 8:2

$$\text{Retention factor } (R_f) = \frac{\text{distance travelled by solute}}{\text{distance travelled by solvent}}$$

Preparation of silver nanoparticles

The nanoparticles were synthesized by known concentration of *G. mangostana* broth was interacted with 1 mM silver nitrate. For the reduction of silver ions, 15 ml of stem extract make up to 100 ml volume in 250 ml Erlenmeyer flask. The flask was incubated in a rotary shaker at 150 rpm speed for a desired time at room temperature for the development of silver nanoparticles. The reduced solution was centrifuged using REMI at 5000 rpm for 30 min to get clear

supernatant. The supernatant was discarded and the particle obtained was centrifuged with water repeatedly to get pure nanoparticles.

Characterization of Ag nanoparticles

The nanoparticle solution thus obtained was purified by repeated centrifugation at 20,000 rpm for 30 min followed by redispersion of the pellet in distilled water. UV–vis spectra were recorded as a function of reaction time on a Perkin Elmer UV-Vis spectrophotometer operated at resolution of 1 nm UV–visible spectral analysis was carried out using Perkin Elmer Lambda 25 spectrophotometer. Bioreduced silver nanoparticles were characterized to reveal their structure using X-ray diffraction technique. The XRD pattern was recorded using the instrument PANALYTICAL X'PERT powder X' Celerator diffractometer model. The obtained result was compared and confirmed with the built-in software corresponding to the Bragg's reflection. The surface morphology and size of the nanoparticles were studied by scanning electron microscope FEI QUANTA FEG 200. Sample constituents are obtained from the Energy Dispersive X-ray spectroscopy.

Bactericidal studies

The bacterial strains used were *Escherichia coli* and *Bacillus subtilis*. The strains were obtained from the Department of Microbiology, Sri Paramakalyani College, Alwarkurichi, Tamil Nadu. Stock cultures were maintained at 4 °C on slopes of nutrient agar. Cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller Hinton Broth (MHB) for bacteria. The cultures were incubated for 24 h at room temperature. Agar well diffusion method (Nwokem, Asgbaji, Kagbu, & Ekanem, 2010) for bactericidal susceptibility was carried out according to standard method to assess the presence of antibacterial activity of the synthesized silver nanoparticle. The concentration of the nanoparticle used in the experiment was 20, 40, 60, and 80 μL. Well of about 6 mm diameter were made aseptically using gel puncture instrument. The plates were swabbed with gram negative and positive strains like *E. coli*, *Klebsiella planticola* and *B. subtilis*. Then the plates were incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the diameter of the zone of inhibition around the well.

Results and discussion

Qualitative analyses of phytochemicals were carried out for stem extract of *G. mangostana*. The biosynthesized Ag nanoparticles from the extract were characterized by using spectroscopic and microscopic techniques such as UV–vis, XRD and SEM-EDX, The AgNPs using stem assisted extract of *G. mangostana* shows good antimicrobial activity against clinically important pathogens.

Identification of compounds

The extract crude from the different extraction produces were subjected to TLC. For analyzing the presence of mangostin, it was confirmed by comparison with the standard mangostin on precoated silica gel 60F254 plates shown in Fig. 2. Toluene and methanol in the ratio of 8:2 serves as the mobile phase. The developed plate was viewed by wing visualizing reagent modified vanillin sulphuric acid reagent. The crude from the different extraction procedures were found to have mangostin, which was conformed with reference to the standard. The R_f value of mangostin is calculated using the formula and predicted as 0.5.

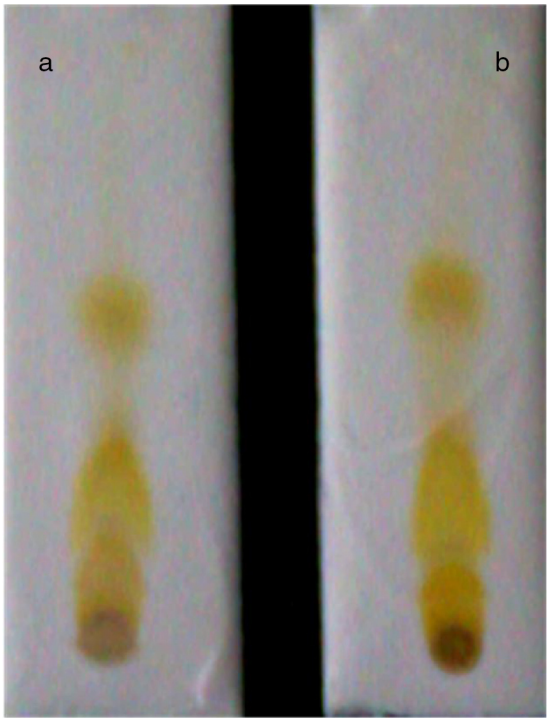

Figure 2 Mangostin the major compound isolated from stem of *Garcina mangostana* (a – standard and b – isolated compound).

Table 1 Qualitative analysis of secondary phytochemicals.

S. No	Test	Presence/Absence
1	Quinones	Presence
2	Coumarin	Absence
3	Flavanoids	Presence
4	Steroids	Presence
5	Alkaloids	Absence
6	Phenols	Presence

The secondary metabolites present in the extract were predicted in Table 1.

Visual observation and UV–visible spectroscopy

Numerous approaches have been engaged to obtain a better synthesis of silver nanoparticles such as chemical and biological methods. Lately, synthesis of silver nanoparticles using plant extracts getting more popular (Li et al., 2007; Song & Kim, 2009). Chandran, Chaudhary, Pasricha, Ahmad, and Sastry (2006) synthesized silver nanoparticles by

Figure 3 Visual identification of the development of silver nanoparticles from pale yellow to brown color.

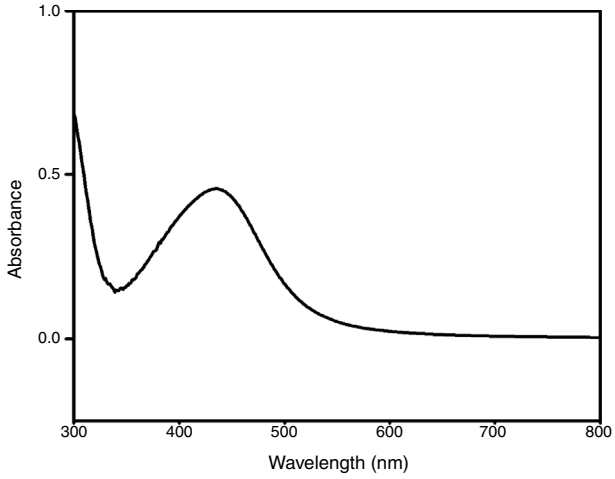

Figure 4 UV–visible absorption spectra of synthesized silver nanoparticles showing the surface Plasmon resonance peak at 430 nm.

using the Aloe vera extract at 24 h of incubation. Similarly, in the present study silver nanoparticles were synthesized using stem extract of *G. mangostana*. Interestingly, silver nanoparticles were synthesized rapidly within 30 min of incubation period. The aqueous silver nitrate solution was turned to brown color within 30 min, with the addition of stem extract shown in (Fig. 3). Intensity of brown color increased in direct proportion to the incubation period. The reaction was completed within 24 h. The improvement of silver nanoparticles was constantly monitored by visual inspection by measuring with UV–visible spectral analysis. It may be due to the excitation of surface plasmon resonance (SPR) effect and reduction of $AgNO_3$ (Mulvaney, 1996). The control $AgNO_3$ solution (without stem extract) showed no change of color. The characteristic absorption peak at 420 nm in UV–vis spectrum confirmed the formation of silver nanoparticles and broadening of peak indicated that the particles are polydispersed (Fig. 4).

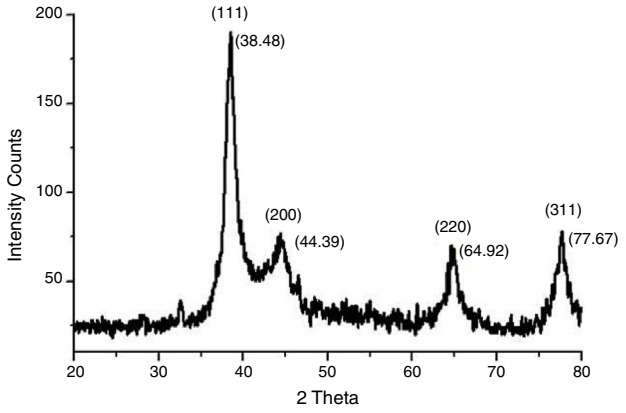

Figure 5 X-ray diffraction of stem mediated silver nanoparticles.

XRD analysis

The crystalline nature of silver nanoparticles was confirmed by the analysis of XRD pattern as shown in (Fig. 5). The distinctive diffraction peaks at 2 theta values of 38.15, 44.30, 64.53 and 76.96 degrees can be indexed to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) reflection planes of face centered cubic structure of silver. In addition to the Bragg peaks representative of silver nanocrystals, additional peaks were also observed. These peaks are due to the organic compounds which are present the extract and responsible for silver ions reduction and stabilization of resultant nanoparticles.

Morphological analysis with energy dispersive X-ray

SEM analysis is used to visualize the size and shape of the nanoparticles. SEM micrographs of silver nanoparticles are given in (Fig. 6) with different magnifications. The absorption of Ag NPs shows the broad peak which represents that the particles were in monodispersed in nature. In this, the secondary metabolites present in the plant were also plays a vital role in the morphological changes. Among the secondary metabolites capsaicin, this was the major compound present in the leaf extract of *G. mangostana* involved in the synthesis part. The particles get aggregated with one another and the image was taken after 24–48 h, this similar result was discussed by Veerasamy et al. (2011). However the particles aggregate may due to cross linking. The particle size obtained from SEM images is well correlated with the particle size determined from XRD using according to the Scherrer formula and the average of the synthesized nanoparticles was in the range of 15–20 nm.

Energy dispersive X-ray technique indexed to verify the presence of element involved in the development of nanoparticles which was shown in Fig. 7. Also it showed some small peaks along with the specific element as C and O. The peak observed around 3 keV was clearly predicted in the figure and the binding energy of AgL which proves the confirmation of pure silver due to the surface plasmon resonance (Kalimuthu, Babu, Venketraman, Bilal, & Gurunathan, 2008).

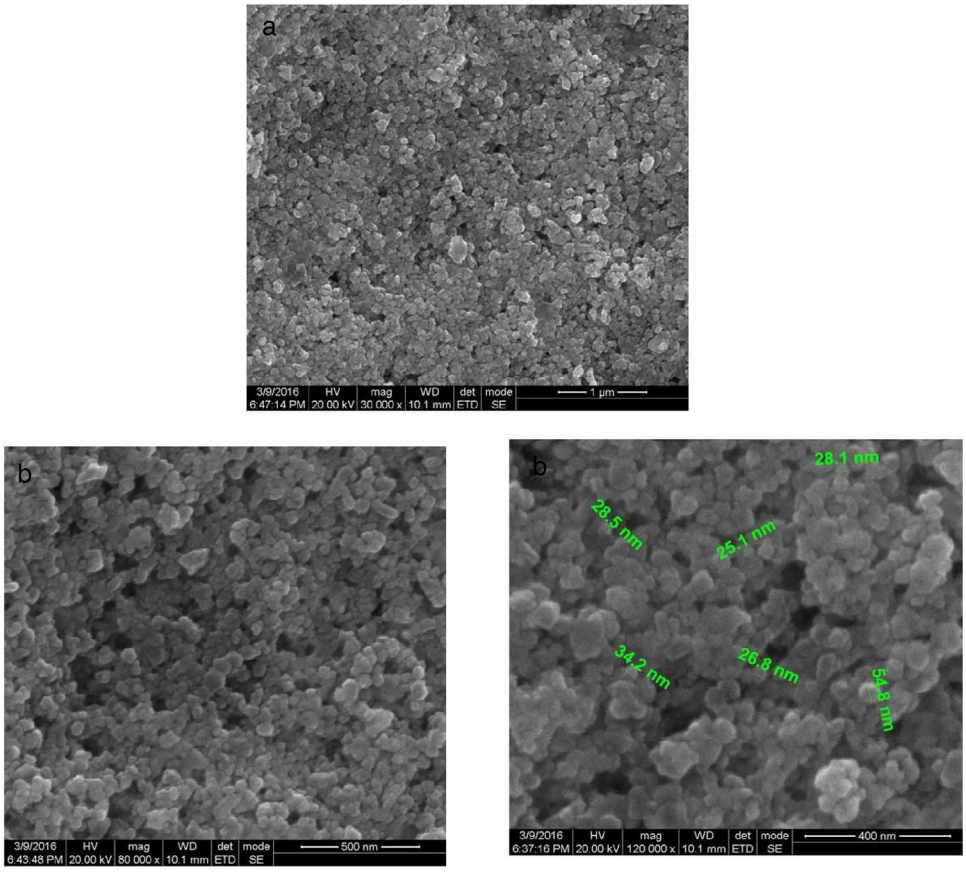

Figure 6 SEM micrograph of silver nanoparticles with scale bar corresponds to 400 and 500 nm.

Bactericidal studies

Biological synthesis of metal s a traditional method and the use of plant extracts has a new awareness for the control of disease, besides being safe and no phyto-toxic effects (Gardea-Torresdey et al., 2003). The silver nanoparticles using medicinal plants were found to highly toxic against different pathogenic bacteria of selected species. Well diffusion assay was performed for evaluating the antibacterial efficacy of AgNPs against *E. coli*, *K. planticola* and *B. subtilis*. Results for the well diffusion assay are shown in (Fig. 8) which represents zone of inhibitions around the well, inoculated with different suspensions of Ag NPs. When compared to *B. subtilis* and *K. planticola E. coli* was more effective because the gram negative bacteria poses weaker cell wall due to less peptidoglycan content as compared to gram positive bacteria (Chaloupka, Malam, & Seifalian, 2010).

Metallic silver, silver nanoparticles and sparingly soluble silver salts released the silver ions when they came in contact with water. These ions were biochemically active agents (Brett, 2006). It must be stressed that those silver ions will react with sparingly soluble salts, which precipitate or remain in colloidal dispersion and will also undergo complexation with proteins and other bio-molecules if they were released in the media. Interactions of free silver ions of silver nitrate with vital enzymes of bacteria provides antibacterial activity due to the phytochemicals particularly mangosteen the major compound which plays a vital role.

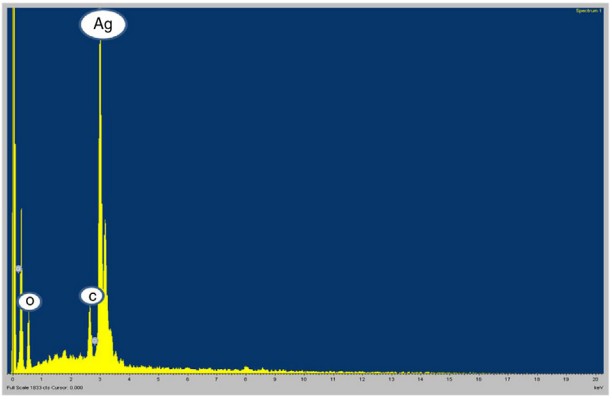

Figure 7 EDX profile of the synthesized silver nanoparticles.

Moreover the activity is happen due to the destruction of cell wall as well as DNA damage (Jones & Hoek, 2010). It seemed, the formation of reactive oxygen species in the presence of silver depends on the involved cell type (Greulich et al., 2011; Luther, Koehler, Diendorf, Epple, & Dringen, 2011). Thus, there were different possibilities for silver to distract biological system.

Conclusion

The Stem of *G. mangostana* was successfully utilized for the rapid and simple procedure for the synthesis of silver nano-

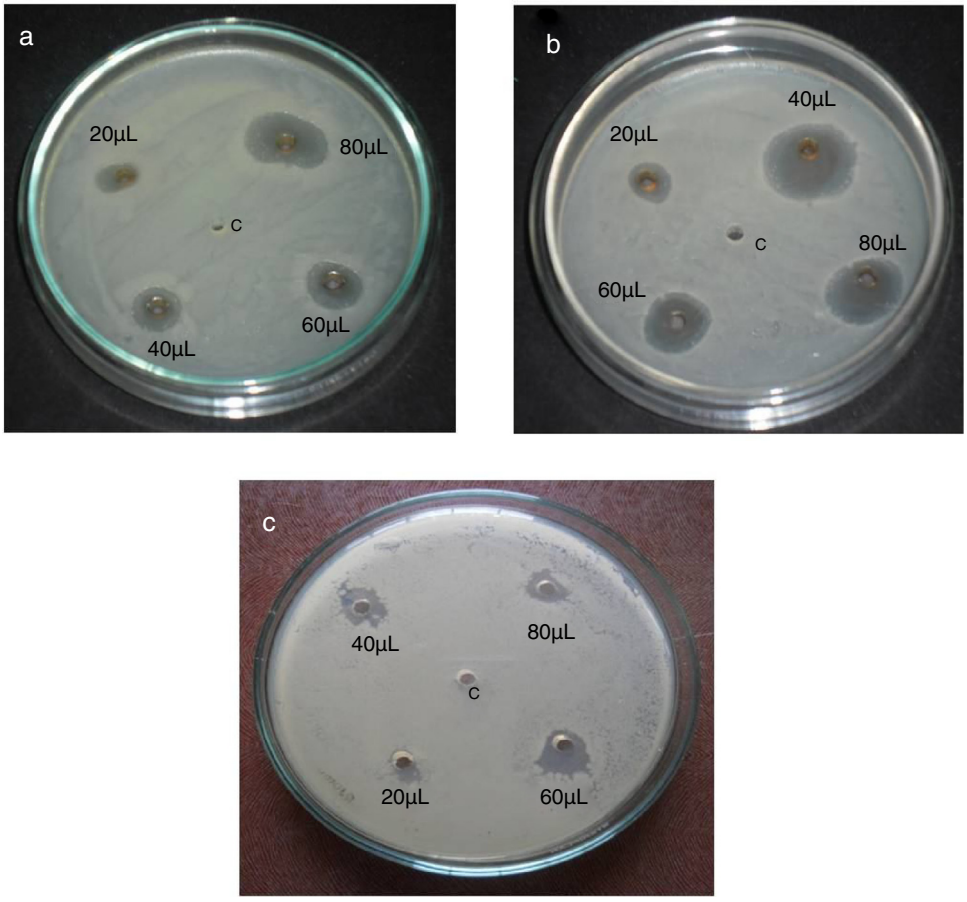

Figure 8 Bactericidal activity of silver nanoparticles against clinical pathogens. (a) *K. planticola*, (b) *E. coli* and (c) *B. subtilis*.

particles. This phyto-assisted nanoparticle was in uniform, crystalline, spherical and monodispersed with the average particle size 30 nm. The phytochemical, mangosteen in this extract can be responsible for the reduction of silver ions and stabilization of nanoparticles. Excellent bactericidal activity can be applied to a wide range of materials for various branched nanostructures, which may serve as potential building blocks in nanomedicine, especially it plays a vital role in wound healing properties. These have several advantages such as cost effective, compatibility for medical and delivering drugs as for commercial production scale.

Conflicts of interest

The author declares no conflicts of interest.

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