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Green synthesis of silver nanoparticles from leaf extract of *Mimusops elengi*, Linn. for enhanced antibacterial activity against multi drug resistant clinical isolates

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ABSTRACT

Green synthesis of metallic silver nanoparticles has attracted nowadays and alternative to physical and chemical approaches. In the present study, silver nanoparticles (AgNPs) were synthesized from leaf extract of *Mimusops elengi*, L. at room temperature. Formation of stable AgNPs at 1 mM concentrations of silver nitrate ($AgNO_3$) typically gave spherical shape particles with diameter range from 55 to 83 nm. The kinetic properties of particle formation were proportional to the effect of concentration of $AgNO_3$ solution. In order to identify the compounds responsible for the bioreduction of Ag^+ ion and the stabilization of AgNPs produced, the functional group present in *Mimusops elengi*, L. leaf extract was investigated using FTIR. The formation of nanoparticle was confirmed using the surface plasmon resonance band shown in UV–vis spectrophotometer. The topography and morphology of the particles were determined using scanning electron microscopy. The crystalline nature of nanoparticles was confirmed from the XRD pattern. Furthermore these green synthesized AgNPs were found to show higher antimicrobial efficacy against multi drug resistant clinical isolates.

1. Introduction

Natural sources have the potential to reduce metal ion into metal nanoparticles [1,2]. AgNPs were used in various applications such as catalytic, electrical conducting [3–7] and antimicrobial activity [8–10]. The size, shape and surface morphology of nanoparticles plays a vital role in controlling the physical and chemical properties. The synthesis of metal nanoparticles by chemical reduction method was often performed in the presence of stabilizing agent to prevent the unwanted agglomeration of colloids. Furthermore, the chemically synthesized metal nanoparticles are expensive, hazardous to environment and require high energy consumption. Biological approaches using plant extracts for metal nanoparticles synthesis have been suggested as valuable alternative tool towards chemical methods [11–13]. Since metal nanoparticles are widely applied in biomedical field, sequentially there is an increasing need to produce metal bio-nanoparticles through eco-friendly process which are highly stable for the large scale production (with absence of toxic chemicals). There are so many reports related to the usage of natural sources like plants and micro organisms, for synthesizing silver nanoparticles by green chemistry approach [14]. The synthesis of bioactive silver nanoparticles using several plant extracts, particularly neem leaf broth (*Azadirachta indica*), *Pelargonium graveolens*, Geranium leaves *Medicago sativa* (Alfalfa), *Aloe vera, Emblica officinalis* (Amla, Indian Gooseberry), *Acalypha indica* leaf, *Sorbus aucuparia* leaf, *Cinnamomum camphora* has been already documented in various approaches [15–21].

In this present study, a novel approach for the biosynthesis of silver nanoparticles using leaf extract of *Mimusops elengi*, L. at room temperature has been reported, which belongs to the family of Sapotaceae. It has been used in the traditional medicine for long, the aqueous extract of flowers, fruits and bark are mainly used for dental ailments (pyorrhea, dental caries), and also being used against heart diseases like menorrhagia and leucorrhoea. A ripened fruit of *Mimusops elengi*, L. was crushed and mixed with water, which has been given to promote delivery during childbirth. Powder form of dried flowers was used as a brain tonic and helpful to reduce pain in head and neck [22]. Hence, the phyto-synthesis of AgNPs from leaf extracts of *Mimusops elengi*, L. were found to be stable for

Abbreviations: AgNPs, silver nanoparticles; $AgNO_3$, silver nitrate; FTIR, Fourier transform infrared spectroscopy; MDRB, multi drug resistant bacteria; MHA, muller hinton agar; XRD, X-ray diffraction; SEM, scanning electron microscope; SPR, surface plasmon resonance; ZOI, zone of inhibition; L., linnaeus.

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long time, eco-friendly and reproducible. The reduction of silver ion in aqueous solution exhibits colloidal silver with particle diameter in nanometer. Furthermore these phyto-synthesized AgNPs were found to show higher antimicrobial activity against different multi drug resistant bacterium isolated from clinical specimen.

2. Experimental details

2.1. Biosynthesis of AgNPs

Mimusops elengi, L. leaf was collected from Thiagarajar College campus, Madurai where the work has being carried out. The silver nitrate ($AgNO_3$) was used as the starting materials obtained from Sigma–Aldrich chemicals, USA. Fresh leaf of *Mimusops elengi*, L. was washed repeatedly with milli-Q deionized water (Millipore Water Unit, Bangalore, India). 5 g of leaf was finely cut and boiled with 100 ml of milli-Q deionized water for 5 min to get the extract. The crude extract thus developed, was undergone a filtration method by using Whatman No.1 filter paper. To optimize this reaction, the following method was adopted: 100 ml aqueous filtrate of *Mimusops elengi*, L. extract has being taken into 250 ml Erlenmeyer flask. Then the extract was mixed with $AgNO_3$, to make the final volume concentration of 1 mM aqueous solution. This reaction mixture was kept uninterrupted until its dark brown colour change was arisen.

2.2. Characterization of AgNPs

Preliminary characterization of the AgNPs was carried out using UV–vis spectroscopy. The measurement was carried out by using a Jasco dual-beam spectrophotometer (model V-530, Japan) having operational range of wavelength between 300 nm and 800 nm. The synthesized AgNPs were characterized by UV–vis spectroscopy periodically at different time intervals up to a month in order to observe rapid reduction of silver nanoparticles by the action of aqueous leaf extracts of *Mimusops elengi*, L. using constant 1 mM $AgNO_3$ solution. The colour change was observed in every minute and the colour of the reaction mixture was set up to change from light green to dark brown within 15 min.

This AgNPs dried at 50 °C for 6 h and mixed with KBr and convert into a pellet form by using bench press. Thereafter the formed pellet was used to test for functional groups by IR spectroscopy. In addition, to identify which functional group is present in the aqueous leaf extract causing the reduction? For that, the dry leaf powder of *Mimusops elengi*, L. *was* pelletized and FTIR (Jasco 460 plus, Japan) spectra were recorded between 4000 and 400 cm^{-1}. The spectrum of AgNPs in the aqueous extract and the spectrum for pure dry leaf powder were compiled.

The size and morphological characterization of the synthesized and lyophilized AgNPs were studied using scanning electron microscope (SEM). A thin film was prepared by drop coating biologically synthesized purified silver nanoparticles on to carbon coated copper SEM grids. The thin film on the SEM grid was allowed to stand for 5 min to dry prior to measurement and then the extra sample solution was removed using a blotting paper. The SEM images were recorded (JEOL-JEM 6390, Japan) at 40,000× magnifications operating with 20.00 kV. Furthermore, the X-ray thin film diffraction measurement for bio-reduced AgNPs were also carried out in a Goniometer = PW3050/60 (Theta/Theta) using Cu kα radiation which facilitate at 40 KV and 25 °C. Subsequently the X-ray patterns were obtained in the 2θ range of 20–80 °C.

2.3. Determination of antibacterial activity of AgNPs

The antibacterial efficacy was assayed by the standard Kirby–Bauer disc diffusion method and antibacterial activity of synthesized silver nanoparticles were observed against multi drug resistant clinical isolates such as *Klebsiella pneumoniae*, *Micrococcus luteus* and *Staphylococcus aureus* were obtained from SRM Medical College and Research centre. The bacterial suspension was swabbed on the Muller Hinton Agar (MHA) plates using sterile cotton swab. The sterile Whatman No 1 paper disc at 6 mm dimension was impregnated with AgNPs in different concentrations (5 μg, 10 μg and 15 μg). The disc with aqueous leaf extract, located on the plates was maintained as control. These discs were gently pressed in Muller Hinton Agar plates and incubated in inverted position for 24 h at 37° C. However, the susceptibility of the test organisms were determined by measuring the diameter of the zone of inhibition using Hi-Media zone scale and tabulated.

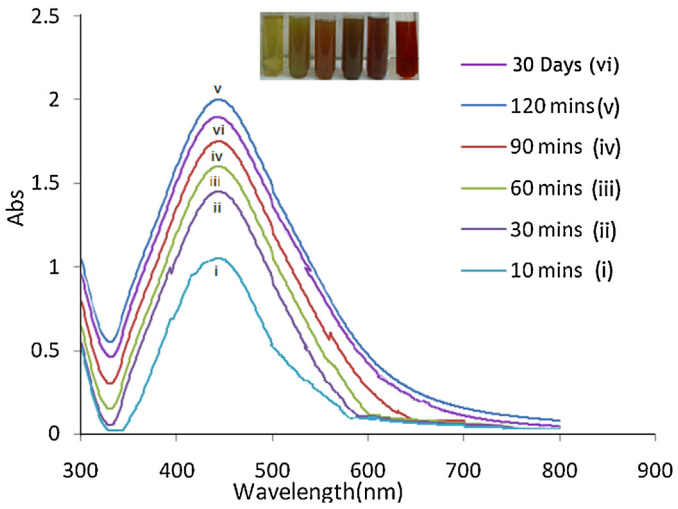

Fig. 1. UV spectra of AgNPs (A) UV–vis absorption spectra of AgNPs synthesized from *Mimusops elengi*, L. leaf extract at different time intervals. Curves i, ii, iii, iv and v correspond to the readings taken on aliquots removed for analysis at 10, 30, 90, 120, and 30 days respectively.

3. Results and discussion

The plant leaf *Mimusops elengi*, L. appears to be a potential source for hydrocarbons. It has been reported that the leaves contain various organic compounds such as alkaloids, flavonoids, tannins, terpenoids, steroids, glycosides and benzenoids [23,24]. The water soluble compounds present in the aqueous extract were found to be responsible for efficient stabilization of nanoparticles and reduction of metal ions.

UV–vis spectroscopy is a significant technique to authenticate the formation and stability of AgNPs in aqueous solution. It is renowned that AgNPs exhibit dark brown colours, depending on the intensity and the size of nanoparticles; the colours arise due to the excitation of surface plasmon resonance (SPR) of the AgNPs [25]. The UV–vis spectra were recorded for aqueous leaf extract of *Mimusops elengi*, L. at different time intervals. The absorption peaks were indexed as i, ii, iii, iv and v correspond to the readings taken on aliquots removed for analysis at 10 min, 30 min, 90 min, 120 min and 30 days respectively (Fig. 1). It is observed from the spectra that the AgNPs SPR peak occurs at 434 nm with high absorbance, which is very specific for silver nanoparticles [8].

Vilchis-Nestor et al. revealed that the reduction of silver ion and formation of stable nanoparticles within 4 h of reaction with *Camellia sinensis* extract [1]. Chandran et al. synthesized silver nanoparticles within 24 h of reaction using Aloe vera [17]. In this study the stable AgNPs formed within 2 h. UV–vis spectra were recorded in every 30 min interval and its absorption peak steadily increased. However, the intensity of colour does not intensify after