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Green synthesis approach on the anti-bacterial credentials and characterization of silver nanoparticle-coated leafy extracts of *Mimusops elengi*

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ABSTRACT

In view of their wide applicability in electronics, catalysis, chemistry, energy, and medicine, there is an increased commercial demand for nanoparticles (NPs) in biotechnology. Metallic NPs are traditionally synthesized by wet chemical techniques that reportedly use toxic and inflammable agents. Herein, we describe an eco-friendly technique for green synthesis of silver (Ag) NPs from silver nitrate ($AgNO_3$) solution using leafy extracts of *Mimusops elengi (M. elengi*) as reducing as well as capping agent. The NP preparations were characterized using UV–VIS absorption spectroscopy, X-ray diffraction (XRD), Fourier Transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) analyses. XRD and SEM investigations showed a 50nm average particle size as well as structural configurations. The synthesized NPs also exhibited antibacterial potentials against ATCC strains of *Staphylococcus aureus* and *Escherichia coli*. Our study highlights the importance of the development of value-added products from *M. elengi* for potential applications in biomedicine and nanotechnology-based industries.

KEYWORDS

Antibacterial;
M. elengi;
Nanoparticles;
Nanotechnology;
Silver.

INTRODUCTION

Noble metal nanoparticles (NPs) are widely used in electromagnetics, optoelectronics, and information storage[1]. The intrinsic properties of the NPs are determined by their size, shape, composition, crystallinity and structure (solid or hollow). Silver (Ag) NPs, a member of noble metal NPs, serves as an excellent substrate for surface-enhanced Raman scattering (SERS)[2] to probe single molecules, and used as catalysts for accelerating the rate of reactions[3]. A wide array of approaches is adopted to synthesize Ag NPs namely, chemical reduction of Ag+[4] electrochemical[5], radiation[6], photochemical[7], the Langmuir–Blodgett[8,9] and biological methods[10]. Amongst these, biological syntheses are effective in fabricating benign nanostructure materials. Notably, biological means lessens the release of hazardous substances, which provides a facile and convenient entry to producing multiple inorganic NPs. Accumulating evidence suggests the use purified natural materials to generate noble metal NPs[11], such as vitamin B2 to synthesize gold (Au) and platinum NPs with interesting morphologies[11], and vitamin C (ascorbic acid) to generate Au NPs for use liquid crystal systems[12].

Of late, there has been renewed interest in green synthesis research; especially the use of bio-organisms (e.g. plants) that secrete functional molecules for a reaction is compatible with green chemistry principles. Bio-organisms are as eco-friendly as are the reducing agents employed currently, and serve as capping agents in reactions. The use of live alfalfa (*Medicago sativa*) to synthesize Au and Ag NPs[13] and broth of lemongrass (*Cymbopogon citratus*) and geranium (*Pelargonium graveolens*) leaves to synthesize Au and Ag NPs[14] have been widely reported heretofore. Importantly, the lesser levels of toxicity attributes relative to other reducing agents make bio-organisms the ideal multifunctional agents for the production of nanomaterials.

Mimusops elengi Linn., (*M. elengi)* commonly known as '*bakul*' belongs to the family *Sapotaceae* and is a small to large evergreen ornamental tree noted for its aromatic flowers distributed widely in India, Bangladesh and Pakistan[15]. Almost all parts of *M. elengi* are being used in the indigenous system of medicine for treating various ailments. Therapeutic applications, such as cardiotonic, alexipharmic, stomachic, antihelmintic and astringent, anti-diarrhoeal, antirhinorrhoeal and laxative roles have been ascribed to parts of *M. elengi*[15, 16] and several triterpenoids, steroids, steroidal glycosides, flavonoids, and alkaloids have been reported from this species[16, 17]. Phytochemical review shows the presence of taraxerol, taraxerone, ursolic acid[18], betulinic acid, v-spinosterol, w-sitosterol, lupeol and alkaloid isoretronecyl tiglate[19]. A mixture of triterpenoid saponins of the bark of *M. elengi* could serve as potential reductants reacting with Ag+ and as scaffolds to direct the formation of Ag NPs in solution. To the best of our knowledge, the use of *M. elengi* plant extracts at room temperature for green synthesis of noble metal NPs, such as Ag NPs, has not been reported. Hence, we studied the synthesis of NPs from extracts of *M. elengi* to understand the basic mechanisms underlying the biological synthesis with a view to explore novel green ways to prefabricate nanomaterials.

EXPERIMENTAL

Plant extracts

Fresh leaves of *M. elengi* obtained from Adambakkam, Chennai were used to prepare the aqueous extract. Dry leaves weighing 25g were thoroughly washed in distilled water (d.H_2O), chopped into pieces and boiled in 100mL sterile d.H_2O and filtered through Whatman no.1 filter paper (pore size 25µm). Subsequently, the filtrate was filtered through a 0.6µm filter and the elute stored for experimental procedures.

Synthesis of silver nanoparticles

An aqueous solution (5mM) of $AgNO_3$ was prepared and used for the synthesis of Ag NPs. Briefly, 10mL of the plant extract was added to 50mL of aqueous solution of 5 mM $AgNO_3$ for reduction into Ag+ ions and left to stand at room temperature for 5 hours.

UV-VIS spectra analysis

To monitor the reduction of pure Ag+ ions, the UV-VIS spectrum of the reaction medium was screened (UV-2450, Shimadzu, Japan) using a small aliquot of sample diluted in d.H_2O.

X Ray diffraction (XRD)

The Ag NPs solution obtained was purified by repeated centrifugation at 5000 rpm for 20 min followed by redispersion of the pellet of Ag NPs in 10mL of deionized water. The freeze-dried Ag NP was analysed to determine the formation of Ag NPs by an X'pert pro X-ray diffractometer (PANalytical BV, Almelo, The Netherlands).

Scanning electron microscopy (SEM)

Thin films of sample were prepared on a carbon-coated copper grid the film on SEM (Hitachi S-4500, Tokyo, Japan), dried under a mercury-vapor lamp for 5 minutes and analysed.

FTIR analysis

To remove any free biomass residue or compound that are not capping the ligands of the NPs, 100mL of the residual solution after the reaction was centrifuged at 5000rpm for 10min and the resulting suspension was redispersed in 10mL sterile d.H_2O. The centrifugation and redispersion steps were repeated three times. Thereafter, the purified suspension was freeze-dried and analyzed by FTIR using a Nicolet Avatar 660 (Nicolet, USA).

Antibacterial assays

The antibacterial potential of the NPs prepared was tested against certain common bacterial pathogens viz., *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213),by the Kirby-Bauer disc diffusion method. Sterile paper discs of 5mm diameter (containing 20mg/L Ag NPs) along with standard antibiotic discs (HiMedia, Mumbai, India) ampicillin (10 mcg), cefotaxime (30 mcg), ciprofloxacin (5 mcg), erythromycin (15 mcg), tetracycline (30 mcg) and ofloxacin (5mcg) were used in each plate as described elsewhere[20].

RESULTS & DISCUSSION

Once the leafy extract of *M. elengi* was mixed in the aqueous solution of Ag+ complex, it assumed color change from watery to yellowish brown largely owing to reduction of Ag+, thereby indicating the formation of Ag NPs. It is generally recognized that UV–VIS spectroscopy could be used to examine size- and shape-controlled NPs in aqueous suspensions. Absorption spectra of Ag NPs formed in the reaction media had

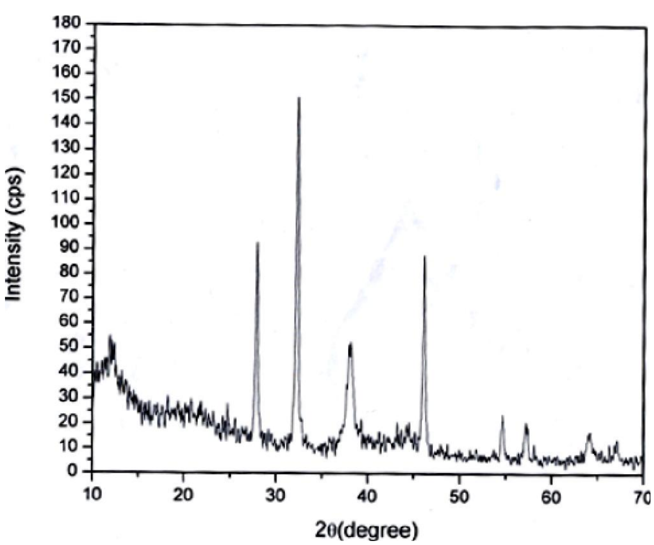

Figure 1 : XRD pattern of silver nanoparticles synthesized by treating 10% Mimusods leaf extract with 5mM AgNO3 solution.

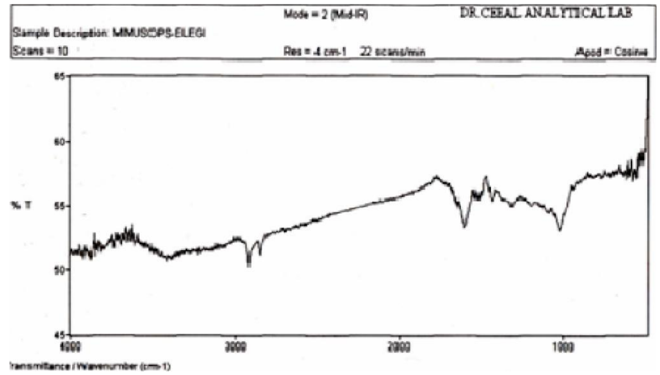

Figure 2 : FTIR Spectra of nano particles synthesized by 10% Mimusops elengi leaf extract.

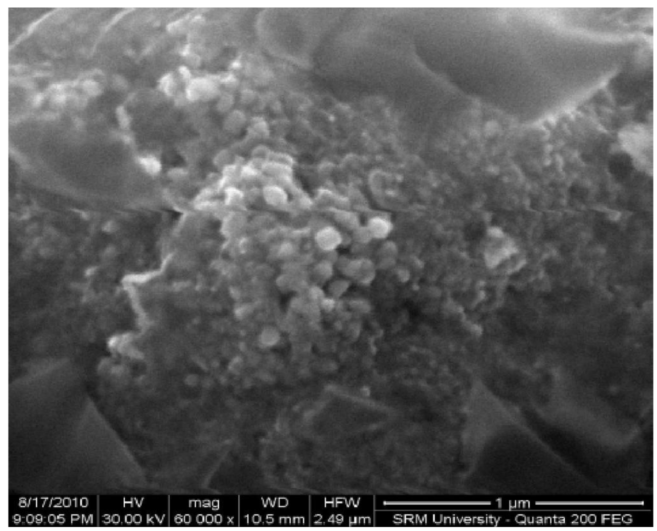

Figure 3 : Scanning electron microscopy imaging of nanoparticles.

absorbance peak at 450nm, and broadening of the peak indicated that the particles were polydispersed. The Ag nanostructure biosynthesised by employing leafy extracts of *M. elengi* was further confirmed by characteristic peaks observed in XRD imaging (Figure 1), FTIR analysis (Figure 2) and structural configurations under SEM analysis (Figure 3). The XRD pattern showed three intense peaks in the whole spectrum of 2θ value ranging between 20 and 60. The average size of the particles ranged from 10 to 50nm with structures assuming cubic and hexagonal shapes. The typical XRD pattern (Figure 1) revealed that the sample contained mixed (cubic and hexagonal) structures of Ag NPs. The SEM imaging analysis showing the occurrence of high density Ag NPs (synthesized by *M. elengi*) further confirmed the development of Ag nanostructures.

Beguilingly, the Ag NPs synthesised also exhibited antibacterial activity against *E. coli* and *S. aureus* against ampicillin, cefotaxime, ciprofloxacin, erythromycin, tetracycline and ofloxacin. The antibacterial effects of Ag NPs were observed to be more pronounced in synergistic combination with antibiotics rather than the Ag NPs alone (TABLE 1 and 2).

TABLE 1 : Antibacterial activity of *mimusops elengi* leaf extract-coated nanoparticles on *Escherichia coli* ATCC 25922

Antibiotic	Diameter of zone of inhibition (mm)			
	Control	Antibiotic	Ag NP	Ag NP + Antibiotic
Ampicillin	0	16	18	19
Cefotaxime	0	24	25	28
Ciprofloxacin	0	17	24	37
Ofloxacin	0	27	30	32

TABLE 2 : Antibacterial activity of *mimusops elengi* leaf extract-coated nanoparticles on *Staphylococcus aureus* ATCC 29213

Antibiotic	Diameter of zone of inhibition (mm)			
	Control	Antibiotic	Ag NP	Ag NP + Antibiotic
Erythromycin	0	14	13	15
Ciprofloxacin	0	23	28	32
Ofloxacin	0	27	26	30
Tetracycline	0	27	24	28

To conclude, we herein report an eco-friendly green synthesis method to synthesize Ag NPs (using 5mM) $AgNO_3$ and the leafy extract of *M. elengi*. The bio-reduction of aqueous Ag+ by the leafy extract of *M. elengi* has also been demonstrated. Furthermore, the reduction of metal ions by the leafy extract leading to formation of Ag NPs of fairly well-defined dimensions has been shown. Notably, metallic silver in the form of Ag NPs has been shown to possess potential antimicrobial properties. This could be important as several pathogenic bacteria have developed resistance against various antibiotics over the last few years. Our study highlights the importance of the development of value-added products from *M. elengi* for potential applications in biomedicine and nanotechnology-based industries.

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