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Research Article

Green Synthesis of Silver Nanoparticles from Aqueous Leaf Extract of *Medinilla beddomei* C B Clarke and its Antimicrobial Activity

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ABSTRACT

Silver nanoparticles were synthesized from aqueous leaf extract of *Medinilla beddomei* C B Clarke and evaluated its antimicrobial activity against different bacterial and fungal strains. Here silver nanoparticles were synthesized through green route using leaves of *M. beddomei*, confirmed by color change and UV-visible spectroscopy. The silver nanoparticles were characterized by transmission electron microscopy (TEM), fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) studies. The antimicrobial activity of the silver nanoparticles was evaluated by agar well diffusion method against four bacterial strains and two fungal strains. The formation of silver nanoparticles after treatment was confirmed by the colour change of the aqueous leaf extract into dark brown. TEM and XRD studies revealed that the synthesized silver nanoparticles are almost spherical, with an average size of 18.88 nm. The silver nanoparticles synthesized from *M. beddomei* showed high antimicrobial activity for 50 and 100 μg/mL concentrations. The highest antimicrobial activity was found against *Aspergillus niger*. The zone of inhibition of fungal strain shown by *A. niger* (36.00 ± 1.50 mm) at 100 μg/mL was higher than that of ciprofloxacin (28.00 ± 1.57 mm) at 200 mg/mL, the positive control. The silver nanoparticles synthesized from the aqueous leaf extract of *M. beddomei* possess high antimicrobial efficacy against pathogenic bacterial and fungal strains. It can be exploited well in the pharmaceutical industry and for nanomedicine also.

Introduction

Indian flora is a rich source of valuable medicinal plants. For centuries plants and its products are widely used for various medicinal purposes. However, a lot more plants need to be exploited for their unique constituents and applications in various fields of science.[1] Recently, nanotechnology has emerged as the most vibrant area of research in material science and modern medicine with significant potential. Particles with a size less than 100 nm are referred as nanoparticles which possess some unique characteristics due to its size, distribution and morphology.[2] Metal nanoparticles like silver, gold, platinum and palladium are widely studied due to their wide range of applications in electronics, catalysts, surface coating agents, antimicrobials and information storage.[3,4] Silver nanoparticles gained more attention because of its unique properties in catalysis, chemical sensing, electronics and pharmaceuticals.[5] It has high antimicrobial activity against pathogenic microorganisms and also has good anti-inflammatory properties.[6] Currently, silver nanoparticles are widely used in shampoos, soaps, detergents, cosmetics, toothpaste and pharmaceutical products.[7]

Various methods are used for the synthesis of silver nanoparticles like chemical, thermal, electrochemical and radiation methods.[8] All these approaches are expensive and require toxic chemicals which are hazardous to the environment.[9] To avoid these problems, the synthesis of silver nanoparticles from microorganisms and plant extracts has emerged as an alternative method. The synthesis of silver nanoparticles from plants is more

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suitable than the synthesis of silver nanoparticles from microorganisms. Plants contain primary and secondary metabolites which act as nanoparticles' reducing and capping agents without generating any hazardous substances to the environment.[10] Synthesis of silver nanoparticles from plant extract is simple and less expensive process. In the current scenario, green synthesis of silver nanoparticles became the most acceptable approach for meeting the increasing need and demand of silver nanoparticles.

Medinilla beddomei is an epiphytic climbing shrub that belongs to the family melastomataceae which is endemic to the South Western Ghats.[11] *M. beddomei* is an ethanobotanically important plant. Reports of phytochemical studies revealed the presence of various phytochemical compounds in the leaves of *M. beddomei* namely alkaloids, phenols, flavonoids, tannin, terpenoids and glycosides.[12] According to an extensive literature survey done, it was found that little research and analytical studies were conducted on *M. beddomei.*

This inspired us to study the antimicrobial action of green synthesized silver nanoparticles of this plant extract. In the present study, an evaluation of the antimicrobial efficacy of silver nanoparticles against selected human pathogenic bacterial and fungal strains were conducted.

Materials and Methods

Collection and Identification of the Plant Material

M. beddomei is a climbing epiphytic shrub, branches are terete and pendulous with opposite leaves. Leaves are very succulent with 5 mm long petiole. Inflorescences are axillary cymes with 1–3 white or pink colored flowers.

Family: Melastomataceae

Genus: *Medinilla*

Species: *beddomei*

Common Name: Elavallikkodi/Thali

Plant material was collected from Mathikettan Shola National Park, Idukki. The specimen was taxonomically identified[13] and the voucher specimen No: 18374 was deposited at the herbarium of KFRI, Thrissur for further studies.

Preparation of Leaf Extract and Silver Nitrate Solution

Fresh leaves of *M. beddomei* were collected and washed well with distilled water to remove dust particles. The fresh leaves were cut into fine pieces, 10 g of the fine chopped leaves were boiled for 10 min in distilled water. The extract was filtered with Whatman filter paper. The filtrate collected was stored at 4°C for further studies.[14] The silver nitrate (A. R. grade) was obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. 1 mM silver nitrate ($AgNO_3$) solution was prepared by dissolving 0.169 g of $AgNO_3$ in 1L distilled water and stored in dark bottles for future uses.[15]

Green synthesis of silver nanoparticles

About 10 mL of the plant extract was slowly added to 90 mL of 1 mM $AgNO_3$ solution in Erlenmeyer flask under agitation. Then the mixture was transferred to the water bath at 60°C for few hours. All the procedures were conducted in darkness at room temperature. After the complete reduction of Ag^+ to Ag^0 the mixture was wrapped with aluminium foil and stored in the refrigerator for further use.[16]

Characterization of Silver Nanoparticles

UV-visible Spectroscopy

The change of color of the solution from pale yellow to dark brown confirmed the presence of silver nanoparticles. The absorption spectra of silver nanoparticles were measured at a wavelength of 200–800 nm using a UV-vis spectrophotometer (Thermo Scientific evolution 201/220, USA). Equal amounts of colloidal mixture and distilled water were taken separately in quarts cuvettes. Distilled water was used as blank. The absorption spectrum of the mixture was read between 200–800 nm.[17]

Fourier Transform Infrared Spectroscopy

The silver nanoparticles were separated and collected from the reaction mixture by centrifugation at 1000 rpm for 30 minutes. The pellet was washed with distilled water to remove impurities. Then the pellet was dried and stored in a vial. FTIR (Thermo Nicolet Is50, USA) analysis at a spectral region between 4000–400 cm^{-1} was used to determine the functional groups capped in the silver nanoparticles.[18]

Transmission Electron Microscope

The pellet of silver nanoparticles was dissolved in distilled water. A thin film of silver nanoparticles was prepared by dropping a small amount of sample on a carbon-coated copper grid. The sample was allowed to dry. This film was scanned using high-resolution TEM (Jeol/Jem 2100, Japan) to visualize the shape and morphology of the silver nanoparticles.[19]

X-ray Diffraction Analysis

The crystalline nature of silver nanoparticles was measured with XRD peaks and average size of the silver nanoparticles was measured using Deby Scherrer equation.

$$D = k\lambda/\beta cos\theta$$

Where D = Thickness of the silver nanoparticles, K = Scherrer constant, 0.94, λ = Wavelength of the X ray, 1.5406 × 10-10, β = Full width of half maxima of reflection of Bragg's angle, it is calculated by fitting a Gaussian curve, θ = Bragg diffraction angle.[20] For XRD analysis thin film of silver nanoparticles was made on a glass plate and scanned under XRD (Bruker D8, Germany).

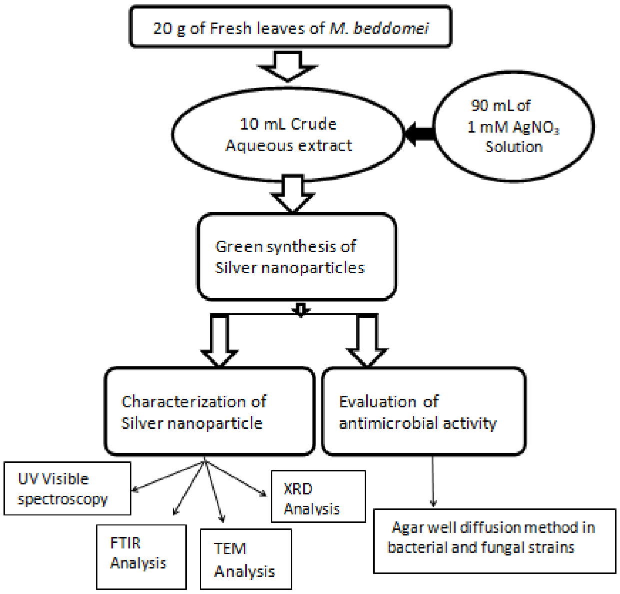


Fig. 1: Schematic representation of the analytical procedures done.

Fig. 2: Aqueous leaf extract of *M. beddomei* before and after the addition of silver nitrate solution.

Antimicrobial Activity

Antibacterial activity of the silver nanoparticles from the aqueous extract of *M. beddomei* was tested through agar well diffusion method[21] against two gram-positive bacteria, *Staphylococcus aureus* (NCIM 2127), *Bacillus subtilis* (NCIM 2063) and two gram-negative bacteria *Escherichia coli* (NCIM 2065), *Salmonella typhi* (NCIM 2501). The antifungal activity was tested using two fungal strains *Candida albicans* (NCIM 3102), *Aspergillus niger* (1004), respectively (Fig. 1). All the test microorganisms were procured from National Collection of Industrial Microorganisms, NCL, Pune. Sterilized agar plates were prepared using Muller Hinton Agar. Molted agar was poured uniformly in petri plates and allowed to solidify. Inoculum of each microbial strain was swabbed on the plates with cotton swab. Wells of 6 mm diameter were made on the medium using gel cutter. Different concentrations of silver nanoparticles dissolved in DMSO were poured onto the wells. Streptomycin (1-mg/mL) was used as standard for bacterial culture. Fluconazole (2 mg/mL) was used as the

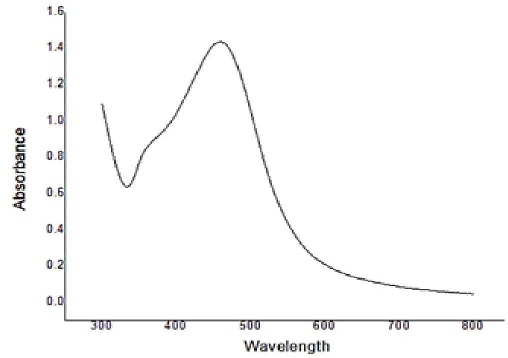


Fig. 3: UV-visible spectrum of silver nanoparticles synthesized from aqueous leaf extract of *M. beddomei.*

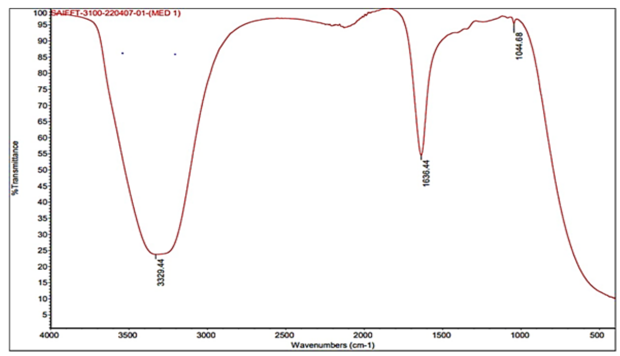


Fig. 4: FTIR graph of silver nanoparticles from aqueous leaf extract of *M. beddomei.*

positive control for *C. albicans*. Clotrimazole (200 mg/mL) was the standard for *A. niger* culture. DMSO was used as negative control. The plates were incubated for 48 hours at 35°C and the zone of inhibition was measured.

Statistical Analysis

Antimicrobial assays were carried out in triplicates and the results were represented as mean ± standard deviation. The statistical analysis was done using Origin software (version 7.0383, origin Lab Corporation, Northampto, MA 01060, USA).

Results

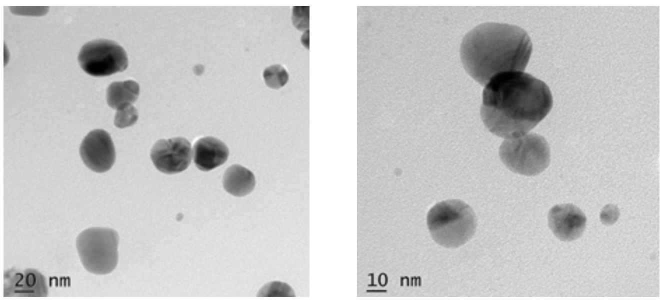
UV-visible Spectroscopic Analysis

Synthesis of silver nanoparticles in aqueous leaf extract of *M. beddomei* was assessed through UV-vis spectroscopy. The observation of colour change in solution from pale yellow to dark brown indicated the presence of silver nanoparticles (Fig. 2). A broad absorption peak was observed at 460 nm wavelength, the characteristics feature of uniform spheres of silver nanoparticles. It is due to the surface plasmon resonance of particles.[22] All the Ag^+ ions were reduced into Ag^0 ions in the solution. The SPR peak obtained between 410–460 nm indicated[23] the presence of silver nanoparticles (Fig. 3).

Table 1: Antimicrobial activity of silver nanoparticles synthesized from aqueous leaf extract of *M. beddomei.*

Zone of Inhibition in mm				
Test organisms	*Positive control*	*25 µg/mL*	*50 µg/mL*	*100 µg/mL*
Staphylococcus aureus (NCIM 2127)	23 ± 0.50^{S}	–	12.25 ± 1.15	13.40 ± 0.50
Bacillus subtilis (NCIM 2063)	31 ± 0.82^{S}	10.31 ± 0.32	12.21 ± 1.23	13.42 ± 1.52
Escherichia coli (NCIM 2065)	22 ± 0.29^{S}	–	10.45 ± 0.58	11.50 ± 0.52
Salmonella typhimurium (NCIM 2501)	25 ± 0.50^{S}	11.32 ± 0.78	12.92 ± 1.38	13.45 ± 1.25
*Aspergillus niger (*NCIM 1004)	28 ± 1.57^{C}	–	10.35 ± 1.15	36.00 ± 1.50
*Candida albicans (*NCIM 3102*)*	33 ± 1.29^{f}	11.50 ± 1.88	12.31 ± 0.52	22.80 ± 2.32

Values are presented as mean ± SD, s: inhibition to Streptomycin (1 mg/mL), c: inhibition to Clotrimazole (200 mg/mL), f: inhibition to Fluconazole (2 mg/mL).



A B

Fig. 5: TEM images (A and B) of silver nanoparticles.

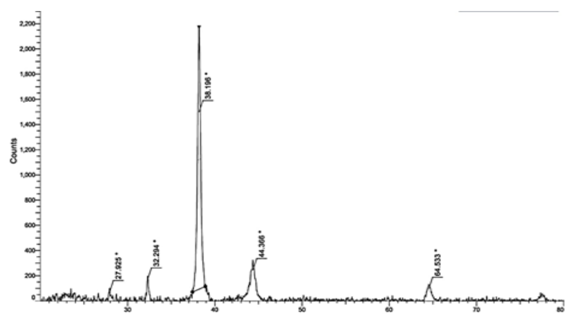


Fig. 6: XRD pattern of silver nanoparticles obtained from *M. beddomei* leaf extract.

FTIR, TEM and XED studies

FTIR spectrum was used to identify the functional groups or biomolecules that are bound and stabilize the nanoparticles. FTIR spectrum of synthesized nanoparticles from aqueous extract of *M. beddomei* is shown in Fig. 4. It presents three main bands at 3329, 1636 and 1044 cm^{-1}. Band at 3329 cm^{-1} indicates the presence of –OH group.[24] Peak at 1636 cm^{-1} corresponds to C=O stretching vibrations[25] and the band 1044 cm^{-1} is assigned to C-H bending vibrations.[26] TEM images visualized the shape and size of the particles clearly. TEM images confirmed the presence of well dispersed spherical and plate like silver nanoparticles with a size between 10–30 nm. Spherical silver nanoparticles were the dominant ones (Fig. 5). XRD patterns showed a number of peaks of Bragg reflection with 2θ values at 32.29°, 38.14°, 44.36° and 64.53° (Fig. 6) which corresponded to (101), (111), (200) and (220) planes of face centred cubic (fcc) lattice of silver (JCPDS File No: 03-065–2871). It is indicated that the synthesized nanoparticles were crystalline in nature with an average size of 18.88 nm.

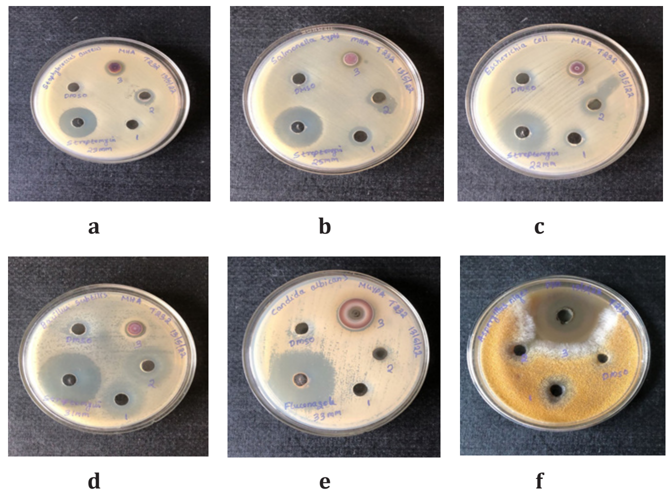


Fig. 7: Antimicrobial activity silver nanoparticles from aqueous leaf extract of *M. beddomei* Antimicrobial effects on (a) *S. aureus* (b) *S. typhi* (c) *E. coli* (d) *B. subtilis* (e) *C. albicans* (f) *A. niger* at different concentrations of silver nanoparticles 1; 25 µg/mL, 2; 50 µg/mL and 3; 100 µg/mL.

Antimicrobial Effect of Silver Nanoparticles

Antimicrobial activity of silver nanoparticles against pathogenic bacterial and fungal strains is represented in Table 1. Different concentrations of silver nanoparticles such as 25, 50 and 100 µg/mL were taken for the assay. Streptomycin, fluconazole and clotrimazole were used as positive control. The table shows that maximum zone of inhibition of silver nanoparticles is 36.00 ± 1.50 mm for a concentration of 100 µg/mL against *A. niger*. The nanoparticles showed greater inhibition for *C. albicans* (22.80 ± 2.32). The silver nanoparticles did not show any inhibition at a concentration of 10 µg/mL against *S. aureus, B. subtilis* and *C. albicans*. A lesser zone of inhibition of synthesized nanoparticles was found against *E. coli* (10.31 ± 0.32) at 10 µg/mL of concentration (Fig. 7).

From this data, the zone of inhibition increases with the increasing concentration of silver nanoparticles.

Discussion

The synthesis of silver nanoparticles from aqueous extract of *M. beddomei* is confirmed by the visual effect of colour change of the solution from pale yellow to dark brown. Several studies revealed that silver nanoparticles have pale yellow to dark brown colour in the solution.[27] The absorption spectrum of nanoparticles obtained in UV-vis spectroscopy analysis showed a broad peak at 460 nm wavelength. Similar absorption spectra have been observed in earlier studies of silver nanoparticles.[28] According to Gilaki[29] pH, concentration of silver nitrate solution, plant source, time, temperature and methods adopted are the key factors of making different sized silver nanoparticles. FTIR analysis of this study showed that hydroxyl (-OH), Carboxyl (-C=O) and alkyl (-CH) groups are capped and stabilized the silver nanoparticles. Studies reported that –OH, -C=O, -C-O, -C-H, -C=C, -C-N and –NH groups are directly or indirectly responsible for the reduction of silver ions into its nanoscale.[30,31] Vidipriya Arya stated that proteins, carbohydrates, phenols and flavonoids are the important biomolecules that helped in the capping and stabilizing silver nanoparticles.[32] TEM images proved the spherical and well-dispersed nature of silver nanoparticles.

XRD analysis indicated the crystalline nature of silver nanoparticles with an average size of 18.88 nm. The average size was calculated with Deby Scherrer equation. According to Zahir shah *et al*.[33] the size of silver nanoparticles from aqueous extract of *Planatgo lanceolata* is 20 nm. Tailor *et al*., reported that the aqueous leaf extract of *Ocimum canum* produced silver nanoparticles with a size of 15.72 nm by green synthesis.[34] The smaller sized silver nanoparticles have greater biological activity. The study of the antimicrobial activity of silver nanoparticles from *M. beddomei* has showed high antibacterial and antifungal activities. Smaller sized silver nanoparticles kill the bacteria and fungi efficiently. Singh *et al.*[26] reported that silver nanoparticles from *Premna integrifolia* have good antibacterial effect against gram-positive and gram-negative bacteria. Silver nanoparticles have also shown antiviral, antifungal and anti-inflammatory properties.[35] From this study, green synthesis of silver nanoparticles is a simple, rapid and efficient method for obtaining silver nanoparticles. Silver nanoparticles are efficient antimicrobial agents. Nowadays many microorganisms have evolved with multidrug resistance and cause severe threats to animals.[36] The present study suggests green synthesized nanoparticles as an alternative tool to efficiently eradicate the multidrug resistant organisms.

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