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Original Article

BIOFABRICATION OF SILVER NANOPARTICLES USING AQUEOUS LEAF EXTRACT OF *MELIA DUBIA*, CHARACTERIZATION AND ANTIFUNGAL ACTIVITY

VASUDEVA REDDY NETALA[1], VENKATA SUBBAIAH KOTAKADI[2], SUKHENDU BIKASH GHOSH[3],PUSHPALATHA BOBBU[1], VENKATESWARLU NAGAM[4], SHARMA K. K.[5], VIJAYA TARTTE[4]*

[1]Department of Biotechnology, Sri Venkateswara University, Tirupati, A. P. India, [2]DST-PURSE Centre, Sri Venkateswara University, Tirupati, A. P. India, [3]NABTD, DAE-BRNS, BARC, Mumbai, M. H. India, [4]Department of Botany, Sri Venkateswara University, Tirupati, A. P. India, [5]Department of Microbiology, SVIMS University, Tirupati, A. P. India.
Email: tvijayasvu@yahoo.com

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ABSTRACT

Objective: To investigate the bio-fabrication of silver nanoparticles (AgNPs) using aqueous leaf extract of *Melia dubia* (ALM) and test the antifungal activity of AgNPs against pathogenic fungi *Aspergillus niger* and *Candida tropicalis.*

Methods: 10 ml of aqueous leaf extract of *Melia dubia* was added to 90 ml of 1 mM silver nitrate and incubated for 8h at room temperature. After incubation, the color change was observed from light yellow to dark brown. The synthesized AgNPs were characterized using UV-Vis spectroscopy, Fourier Transform Infra red spectroscopy (FTIR), Energy Dispersive X-ray Spectroscopy (EDX), Scanning Electron microscopy (SEM), X-ray diffraction analysis (XRD) and Atomic Force Microscopy (AFM). Antifungal activity against *Aspergillus niger* and *Candida tropicalis* was carried out by employing the disc diffusion method.

Results: UV-Vis spectra confirmed the synthesis of AgNPs by showing characteristic peak between 380-450 nm*. FTIR spectra showed the functional groups possibly involved in the synthesis of AgNPs. EDX confirms the presence of elemental silver at 3 Kev. SEM and AFM showed the synthesized AgNPs were spherical in shape with size ranging between 20-40 nm*. XRD analysis revealed the crystalline nature of AgNPs with face centred cubic (FCC) lattice. AgNPs was found to be very effective against the tested pathogenic fungi *A. niger* and *C. tropicalis* and formed the inhibition zones 13.0 and 14.5 mm respectively.

Conclusion: It is concluded that the bio-fabrication of AgNPs using aqueous leaf extract of *Melia dubia* was robust and rapid. The AgNPs were stable and proved to be excellent antifungal agents.

Keywords: *Melia dubia*, Silver nanoparticles, FTIR, SEM, Antifungal activity.

INTRODUCTION

In the recent years, research on silver nanoparticles (AgNPs) has attracted many scientists a considerable attention due to their remarkable optical, electrical, magnetic and catalytic properties [1]. Due to these properties, AgNPs find several applications in different fields. AgNPs was employed in electrical batteries [2], polarizing filters, catalysts, sensors [3], drug delivery [4], DNA sequencing [5], anticytotoxic agents [6] and antimicrobial agents [7,8]. Many recent studies reported that AgNPs are proved to be excellent antifungal agents [9]. Many physical and chemical methods which include sonochemical assisted [10], thermal assisted [11], and polyol [12] processes were reported for the synthesis of AgNPs. But all of these methods involved the application of toxic, hazardous chemicals leading to environmental and health risks. By considering all these risks, researchers moved to biosynthetic approach. Biological approaches are simple, eco-friendly and rapid. Biological approaches includes plant extract mediated synthesis, microbial assisted and enzyme assisted synthesis. Microbial and enzyme mediated approaches are involved elaborate culturing processes, aseptic environment, while plant extract mediated synthesis does not involve all of these processes. AgNPs has been extensively synthesized using various plant extracts including *Acalypha indica* [13], *Eucalyptus citriodora*, *Ficus bengalensis* [14], *Cissus quadrangularis* [15], *Cassia alata* [16], *Catharanthus roseus* [17] and *Andrographis paniculata* [18]. In the present work, we have reported the bio-fabrication of AgNPs from the aqueous leaf extract of *Melia dubia* (ALM), an important medicinal plant belongs to the family Meliaceae. The characterization of biofabricated AgNPs was done to determine their formation, size, shape and crystalline nature by employing different spectroscopic methods which include UV-Vis spectroscopy, Fourier Transform Infra Red spectroscopy (FTIR), Scanning Electron microscopy (SEM), Energy dispersive X-ray spectroscopy (EDX), Atomic force microscopy (AFM) and X-ray diffraction (XRD) analysis. Antifungal activity of the biofabricated AgNPs was carried out to evaluate their biomedical importance. AgNPs were very effective against *Aspergillus niger* and *Candida tropicalis*

MATERIALS AND METHODS

Collection of plant material

Leaves of *Melia dubia* were collected and authenticated by Taxonomist, Department of Botany,

Sri Venkateswara University, Tirupati, A. P. India. The voucher specimen was deposited in the herbarium (VJ-0912).

Preparation of aqueous leaf extracts

Fresh leaves were washed with tap water and then with distilled water (DW) for 2-3 times. 10 g of leaves were weighed, cut into fine fragments, boiled in 100 ml of DW for 15 m at 60 º C, cooled to room temperature and then filtered. The filtrate named. Aqueous leaf extract (ALM) was used for the synthesis of AgNPs.

Bio-fabrication of AgNPs

Silver nitrate ($AgNO_3$) was purchased from Molychem, India. 10 ml of ALM was added to 90 ml of 1 mM $AgNO_3$ and incubated in a dark place at room temperature for about 8h. After incubation, the synthesis of AgNPs was initially observed visually and further detected by UV-Vis spectral analysis.

Characterization of AgNPs

UV-Vis analysis of AgNPs

1 ml of AgNPs solution was diluted with 10 ml of DW. An aliquot of this diluted sample was used for UV-Vis spectroscopic (Analytical

technologies Ltd, India) analysis. UV-Vis spectrum was recorded between 300-700 nm* with a resolution of 1 nm*.

FTIR analysis of AgNPs

100 ml of AgNPs solution was taken in a rotator evaporator, evaporated, concentrated and dried to get fine powder of AgNPs. This fine powder was used for FTIR analysis. FTIR analysis was carried out using FTIR spectrometer (ALPHA interferometer; Make: Bruker, Germany) between 500-4000 cm^{-1} range at 2 cm^{-1}resolution.

SEM and EDX analysis of AgNPs

To confirm the morphology and size of the bio-fabricated AgNPs, SEM analysis was performed. An aliquot of the suspension of AgNPs was taken on a clean glass slide and evaporated to dryness. SEM analysis was done at 15 kV with magnification X 2.5 k, followed by EDX spectroscopy to confirm elemental silver (SEM Hitachi- S520).

AFM analysis of AgNPs

The AgNPs were tested for surface morphology by using AFM (Solver NEXT). Surface topographical image was obtained in tapping mode at a high resonance frequency by oscillating the cantilever using piezoelectric materials. Characterization was done by observing the patterns of surface topography and data analysis was done using Nova PX software.

XRD characterization of AgNPs

The AgNPs powder was used for XRD characterization to know the crystalline nature of synthesized AgNPs. X-ray diffractometer (Rigaku, Tokyo) was operated at a voltage of 40 kV and a current of 50 mA with Cu Kα as a radiation source with step size of 2θ.

Antifungal activity of AgNPs

Antifungal activity of the AgNPs was evaluated for their biomedical importance. Antifungal activity of the AgNPs was checked against fungal species *Aspergillus niger* and *Candida tropicalis.* Disc diffusion method was employed for antifungal activity [19]. 15 ml of autoclaved PDA medium was taken into each plate and allowed to solidify. After solidification, 200 µl of fungal inoculum were swabbed with cotton. Each PDA plate comprises of 3 discs. One disc impregnated with ALM. Second with antibiotic Voriconazole, third one impregnated with 25 µl of AgNPs solution. PDA plates were incubated for 26^0C for 24 h and tested for inhibition zone.

RESULTS AND DISCUSSION

In the present investigation, bio-fabrication of AgNPs from the ALM was carried out. The bio-fabricated AgNPs were well characterized and then evaluated for their biomedical importance as antifungal agents. The bio-fabrication of AgNPs initially detected by the color change of the solution from light yellow to dark brown. This color change was due to Surface Plasmon Resonance (SPR) vibrations within the AgNPs [17,18].

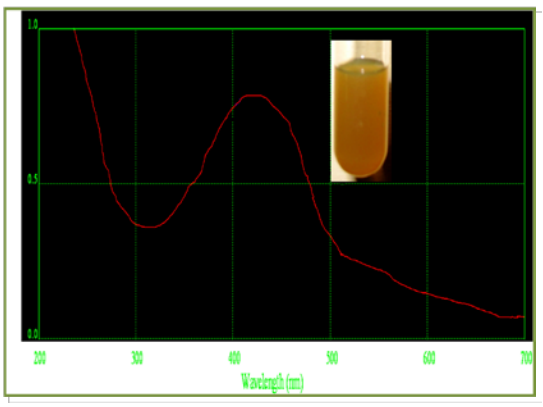

Fig. 1: UV-Vis spectra of AgNPs showing specific SPR peak. (Inset shows the dark brown colored solution of AgNPs).

UV-Vis spectra confirmation of AgNPs

To further strengthen the evidence that the biofabrication of AgNPs was carried out using ALM, UV–Vis spectral analysis was carried in the range of 300– 800 nm to see the appearance of specific SPR peak of AgNPs. The spectrum showed SPR peak between 380-450 nm and confirmed the synthesis of AgNPs from ALM (Fig 1)

FTIR Analysis

FTIR analysis of the AgNPs synthesized showed intensive peaks at 1025 cm^{-1}, 1265 cm^{-1}, 1610 cm^{-1} and 2273 cm^{-1}. The peak at 1025 cm^{-1} corresponding to C-N stretching vibrations of aliphatic amines. Peak at 1265 cm^{-1} corresponding to O-H group of the phenols. Peak at 1610 cm^{-1} could be assigned to amide I band. The peak at 2273 cm^{-1} corresponding to methylene stretch of the proteins. FTIR results indicated that phenols play an important role in the biosynthesis of AgNPs, while proteins involved both in synthesis and stabilization of AgNPs.

SEM and EDX characterization of AgNPs

The SEM characterization determined that AgNPs were 20-40 nm in size with the roughly spherical shape. SEM micrograph (Fig 2) showed that AgNPs were partially aggregated. EDX showed the characteristic peak of elemental silver by sharp signals 3 Kev and thus confirmed the presence of elemental silver. The absorption peak in the range of 3 to 4 KeV is characteristic for the elemental silver [8].

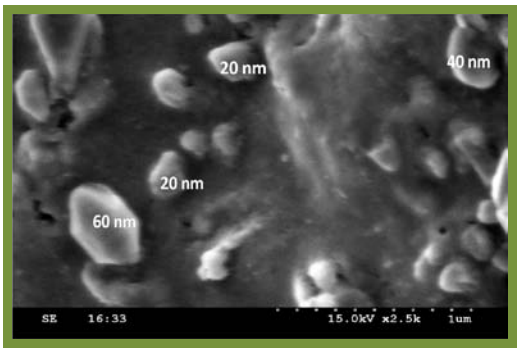

Fig. 2: Representative SEM micrograph of synthesized AgNPs from ALM.

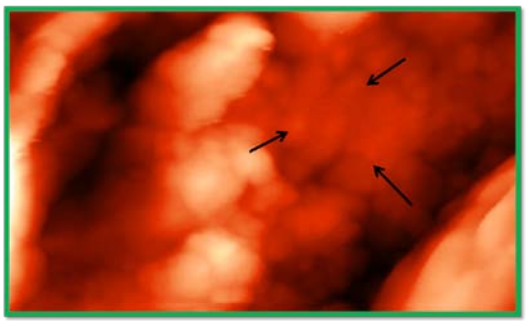

Fig. 3: AFM micrograph showing the topography of the AgNPs synthesized from ALM. (Arrows indicates roughly spherical shaped AgNPs with 20-40 nm* in size)

AFM analysis of AgNPs

The surface topology micrograph of AgNPs was shown in Fig 3. The nicrograph shows AgNPs were uneven distributed, aggregated with roughly spherical shape and the size ranged between 20-40 nm*.

XRD characterization of AgNPs

XRD characterization of AgNPs revealed the crystalline nature. The XRD pattern showed the two different diffraction peaks at 38.3^0 and

46.4^0 which were indexed to (111) and (200) planes respectively of the lattice of face centred cubic (FCC) symmetry and thus confirmed the crystallization of AgNPs [18,19].

Antifungal activity of the AgNPs

Antifungal activity of the AgNPs was tested against fungal pathogens *Aspergillus niger* and *Candida tropicalis.*

The results were shown in the Table 1. It is noted that ALM also showed antifungal activity against *Aspergillus niger* and *Candida tropicalis.* and formed of inhibiton zones of 6 mm and 8 mm diameter respectively. AgNPs synthesized from ALM formed the inhibiton zones against *Aspergillus niger* and *Candida tropicalis* of 13 mm and 14.5 mm respectively and thus proved their biomedical importance.

Table 1: Antifungal activity of AgNPs synthesized, compared with ALM and Voriconazole, a standard fungicide (Mean values ± SD of three replicates).

S. No.	Tested Pathogen	Diameter of inhibition zone (mm)		
		ALM	AgNPs	Voriconazole
1.	*Aspergillus niger*	6.0±0.1	13.0±0.3	16.5±0.2
2.	*Candida tropicalis*	8.0±0.4	14.5±0.2	16.2±0.3

CONCLUSION

In the present study, we have investigated the bio-fabrication of Silver nanoparticles using aqueous leaf extract of *Melia dubia*. Color change of the reaction solution and UV-Vis spectra confirmed the formation of silver nanoparticles. The possible mechanism was reported using FTIR spectra. Plant secondary metabolites which include phenols, flavonoids and proteins were possibly involved the reduction of Silver ions (Ag^+)to silver nanoparticles (Ag^0). SEM and AFM studies showed that the size ranging from 20-40 nm with the spherical shape. XRD confirmed their crystalline nature. The bio-fabricated AgNPs were proved as excellent antifungal agents. This method is very simple, eco-friendly and very rapid approach.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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