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# BIOSYNTHESIS OF SILVER NANOPARTICLES BY MELIA DUBIA LEAF AQUEOUS EXTRACT AND ITS ANTIBACTERIAL ACTIVITY

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## ABSTRACT

Nanotechnology is an emerging field in the area of interdisciplinary research, especially in biotechnology. In this study the silver nanoparticles were synthesized by using a plant leaf extract of Melia dubia. The synthesized silver nanoparticles were confirmed by the change of colour after addition of leaf extract into the silver nitrate solution. The biosynthesized AgNPs were characterized by using UV-Vis analysis, Fourier Transform Infrared analysis (FTIR), X-ray diffraction analysis (XRD) and Scanning Electron Microscopy (SEM analysis. Antimicrobial activity of the silver bio-nanoparticles was performed by well diffusion method against Vibrio cholerae, Escherichia coli, staphylococcus aureus and Klebsiella pneumonia. The highest antimicrobial activity of silver nanoparticles synthesized by Melia dubia extract was found against Vibrio cholerae (22 mm) and E. coli (22 mm) respectively. The Ag NPs synthesized in this process has the efficient antimicrobial activity against pathogenic bacteria.

**KEYWORDS:** Green synthesis, Silver nanoparticles, FT-IR, SEM, XRD, Antimicrobial property.

## INTRODUCTION

Nanotechnology plays a very important role in modern research(Vasudev et al., and Asim umer et al., 2012. It is the most capable technology that can be applied in almost all fields such as pharmaceutical, electronics, health care, food and feed, biomedical science, drug and gene delivery, chemical industry, energy science, cosmetics, environmental health, mechanics and space industries. It has also been utilized for the treatments of infection( Furno et al.,

2004), cancer(Brigger et al., 2012), allergy(Roy et al., 1999), diabetes( Basarkar and Singh 2009) and inflammation(Wilson et al 2010).

Synthesis of nano particles can be performed using a number of routinely used chemical and physical methods. However, altogether these methods are energy and capital intensive, and they employ toxic chemicals and non polar solvents in the synthesis procedure and later on synthetic additives or capping agents, thus precluding their applications in clinical and biomedical fields. Therefore, the need for the development of a clean, reliable, biocompatible, benign, and eco-friendly process to synthesize nano particles leads to turning researchers toward "green" chemistry and bioprocesses(Jain et al., 2011).

Various plants were used for the synthesis of nanoparticles using green synthesis method. Nanoparticles were synthesized from all the parts of the plant separately like seed, stem, flower, leaf and skin of the fruits. The nanoparticles synthesized from plant extract were found to be covered by the medicinal properties of plant extract which could be used in drug, targeted drug delivery and cosmetic applications(Mallikarjunaa et al., 2011 and Abboud et al., 2013).

In this present investigation, the aqueous leaves extract of Melia dubia which mainly consists of fatty acids, alkaloids and flavonoids was used to synthesize AgNPs at various experimental conditions and thereby improving the importance of plant source and involving green chemistry for the synthesis of other nano particles as future research.

## MATERIALS AND METHODS

All the reagents used in this experiment were obtained from Sigma Aldrich chemicals India. Double distilled water was utilized for this process. Filtration was established by using Whatman no.1 filter papers. Glasswares used for the complete reactions were washed well, rinsed with double distilled water and dried in hot air oven.

### Preparation of Melia dubia leaf extract

The fresh Melia dubia leaves were collected from Thogamalai, India. The leaves were thoroughly washed several times using normal water and then followed by distilled water to remove impurities. The cleaned leaves were subsequently dried under sunshade to remove moisture completely, powdered by using mechanical grinder and then stored. The 5g of powdered plant leaves were taken into a beaker along with 100 ml of distilled water and

allowed to boil at 60°C for 30 min under reflux condition then it was cooled down to room temperature. The prepared solution was initially filtered through normal filter paper thereby powdered leafy materials will be filtered out. The filtrate was again filtered through Whatman No.1 filter paper to get clear solution. The filtrate were stored at 4°C for futher works.

**Synthesis of AgNPs**

Aqueous solution (3 mM) of silver nitrate ($AgNO_3$) was prepared in 250 mL Erlenmeyer flask and 25 ml solution leaf extract was added for reduction into $Ag^+$ ions. The composite mixture was then kept on magnetic stirrer for complete bioreduction for 5 hours. In the mean time, the colour change of the mixture from faint light to yellowish brown to reddish brown to colloidal brown was monitored periodically (time and colour change were recorded along with periodic sampling and scanning by UV-visible spectrophotometry) for maximum 5 hrs. Then, the colloidal mixture was sealed and stored properly for future use. The formation of Ag NPs was furthermore confirmed by spectrophotometric analysis(Priya Banarjee et al., 2014)

**Characterization Techniques**

The formation of the silver nanoparticles were confirmed by measuring the wave length of reaction mixture in the UV-Vis spectrum of the Perkin Elmer spectrophotometer at a resolution of 1 nm (from 200 to 800 nm) in 2 ml quartz cuvette with 1 cm path length. The Morphological characterization of the samples was done using JEOL Jsm-6480 LV for SEM analysis. The samples were dispersed on a slide and then coated with platinum in an auto fine coater. After that the material was subjected to analysis. The characterization of functional groups on the surface of AgNPs by plant extracts were investigated by FTIR analysis (Shimadzu) and the spectra was scanned in the range of 4000–400 $cm^{-1}$ range at a resolution of 4 $cm^{-1}$. XRD measurements of the reduced AgNPs perform were recorded on X-ray diffractometer (x'pertpananalytical ) instrument operating at a voltage of 40 kV and current of 30 mA with Cu K (α) radiation to determine the crystalline phase and material identification. The samples were taken in lids and put under instrument for analysis.

**Anti Microbial Activity**

The antimicrobial activity of pathogens was established using disc diffusion method.[12] The bactericidal effect of silver nanoparticles has been attributed to their high surface to volume ratio and small size which allows them to interact very closely with microbial membranes. The antimicrobial study of AgNPS was carried out using different pathogenic bacteria such

as Escherichia coli, Vibrio Cholerae, Staphylococcus aureus and Klebsiella pneumonia. To cultivate the bacteria, nutrient agar was used. After solidification of medium, the discs were placed on the solidified medium. The prepared silver nanoparticles were added into the discs with different concentrations varying from 25 µl to 100 µl. Petri dishes were incubated for 24 h at 37°C.Antibacterial capacity of the silver nano particles was measured by standard Zone of inhibition assay.

## RESULTS AND DISCUSSION

### UV-Visible Spectral Analysis

Figure- 1 shows the UV absorption peaks of Meliadubia .UV-Vis spectra were taken at different time period for 3mm $AgNO_3$ solution (30mins to 5hrs). The spectra showed the peak approximately at 438.00nm, clearly indicating the formation of spherical AgNPs in the aqueous extract of Meliadubia. The occurrence of the peak at 438 nm is due to the phenomenon of surface Plasmon resonance, which occurs due to the excitation of the surface plasmons present on the outer surface of the silver nanoparticles which gets excited due to the applied electromagnetic field(Krishnamoorthy et al., 2012).

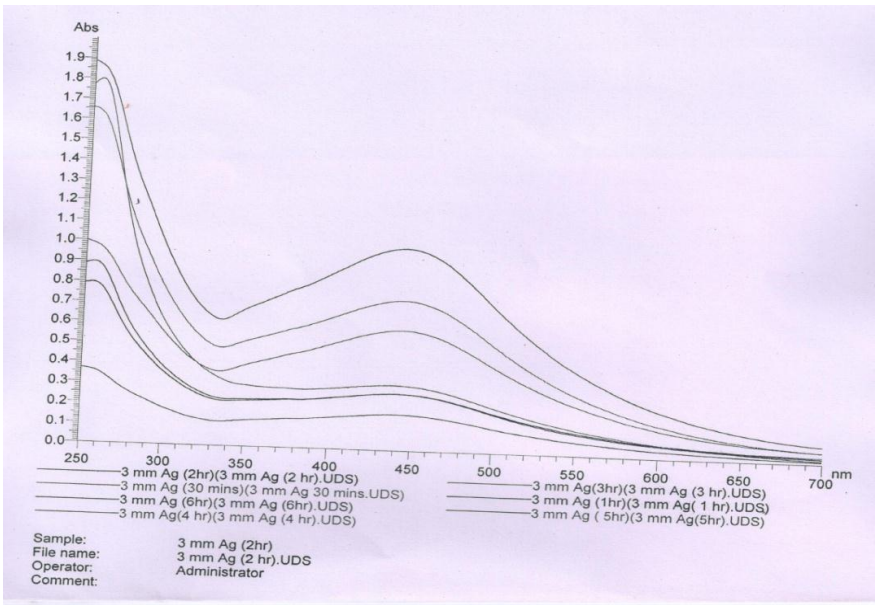


**Figure- 1: UV- Visible spectra were taken at different time period for 3mM $AgNO_3$ solution (30mins to 5hrs)**

### FTIR analysis

FTIR gives the information about functional groups present in the synthesised silver nanoparticles for understanding their transformation from simple inorganic $AgNO_3$ to

elemental silver by the action of the different phytochemicals which would act simultaneously as reducing, stabilizing and capping agent. FTIR spectrum clearly illustrates the biofabrication of silver nanoparticles mediated by the plant extracts. Figure- 2 (a,b,c) shows the FTIR spectrum of dried Melia dubia leaf extract, the silver nitrate salt and Melia dubia mediated synthesized AgNPs.In $AgNO_3$ peaks were observed at 3467cm$^{-1}$,1632cm$^{-1}$,1357.70cm$^{-1}$,653cm$^{-1}$which are associated OH stretching, C=O stretching, CH stretching, CH stretching respectively. In the Melia dubia leaf extract peak were observed at 3353.27cm$^{-1}$, 2929.33 cm$^{-1}$,2820.23cm$^{-1}$,1613.29 cm$^{-1}$, 1361.84 cm$^{-1}$, 1072.44 cm$^{-1}$, 771.14 cm$^{-1}$, 670.34 cm$^{-1}$ which are associated OH stretching, CH stretching, C=N stretching, C=N stretching, N-H stretching, CH stretching, CN stretching, C-Cl stretching. In the synthesized AgNPs from Meliadubia peaks were observed at 3425.67cm$^{-1}$, 2085.60cm$^{-1}$,1639.86cm$^{-1}$, 1119.05 cm$^{-1}$, 676.23 cm$^{-1}$ which are associated with NH stretching, C=O stretching, N-O stretching, CH2 & CH3 deformation, C-O stretching and halogen group presence. The Melia dubia plant extract shows broad peak at 3467.77cm$^{-1}$ which indicate the presence of OH group or carboxyl groups and after synthesis of AgNPs there is a shift in the broad peak to the right at 3425.67cm$^{-1}$ indicating the NH stretching. These carboxyl and amide group indicate the presence of secondary amines which is a signature marker of proteins confirming the biofabrication of the nanoparticles by the action of the protein or phytochemicals. Figure- 2 (a) to (c) clearly illustrates the biofabrication of the AgNPs by the action of the phytochemicals such as phenols, terpenoids, flavonoids and alkaloids in Melia dubia.

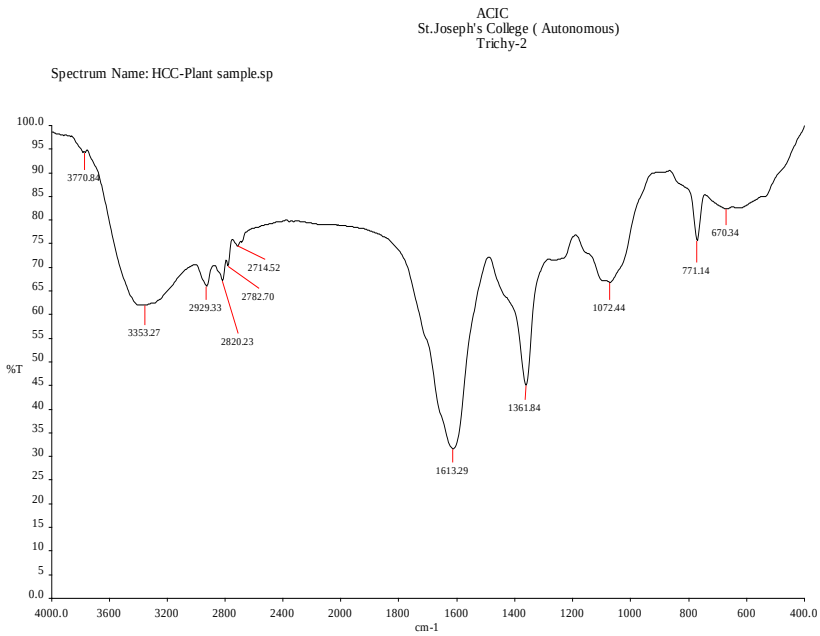


**Figure – 2a: FTIR graph of Leaf extract of Melia dubia**

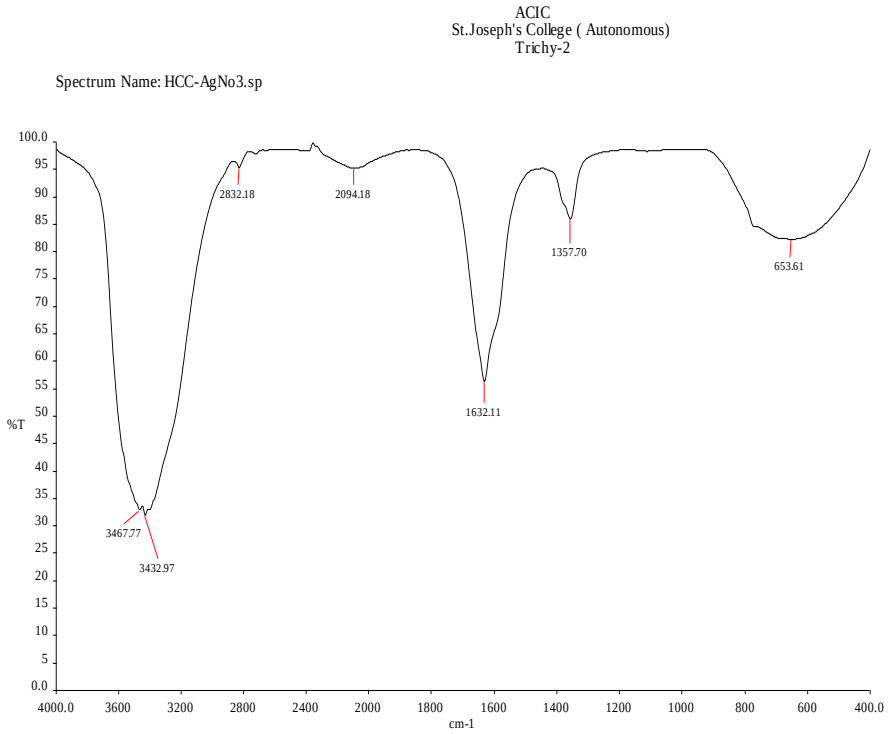


**Figure - 2b: FTIR graph of $AgNO_3$**

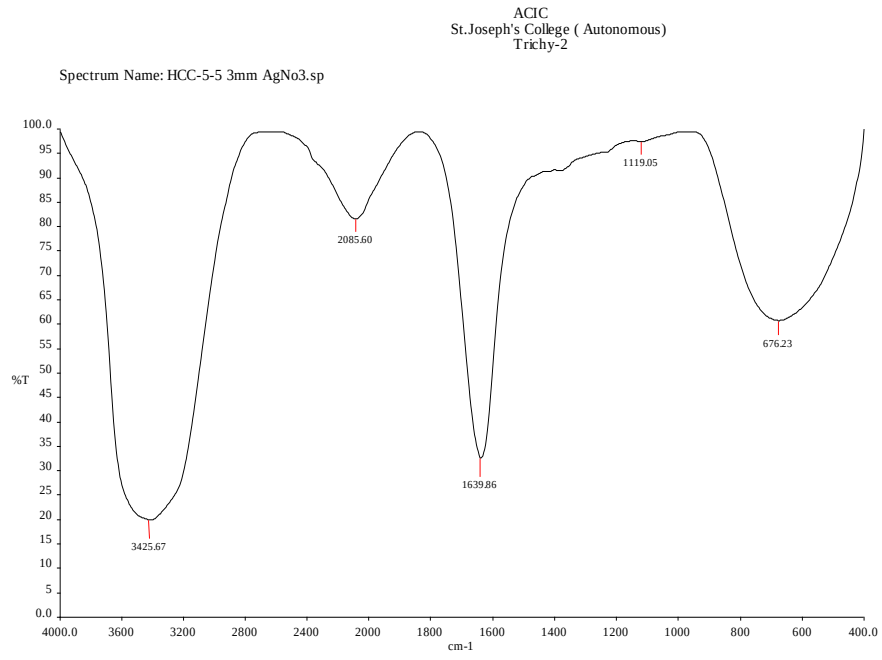


**Figure – 2c: FTIR graph of Synthesized AGNPs**

## Scanning Electron Microscopy

A scanning electron microscope was employed to analyze the shape of the silver nano particles that were synthesized by green method (Figure-3). SEM analysis shows that the

plant has tremendous capability to synthesize silver nano particles which were roughly spherical in shape.

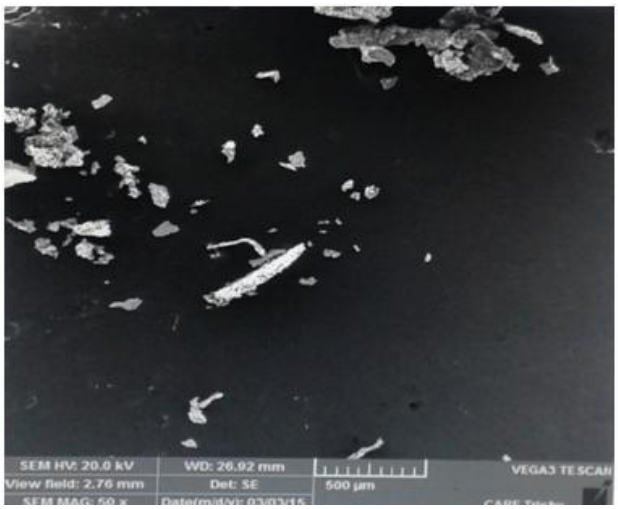


**Figure-3: SEM image of Meliadubia**

**XRD Analysis**

XRD analysis is used to determine the phase distribution, crystallinity and purity of the synthesised nanoparticles particles. Fig 4 shows the XRD patterns of Meliadubia . With reference to the JCPDS data file No. 04-0783 it was concluded that the nanoparticles were crystalline in nature having approximate spherical shape with size equivalent to 21.7nm with no impurities.

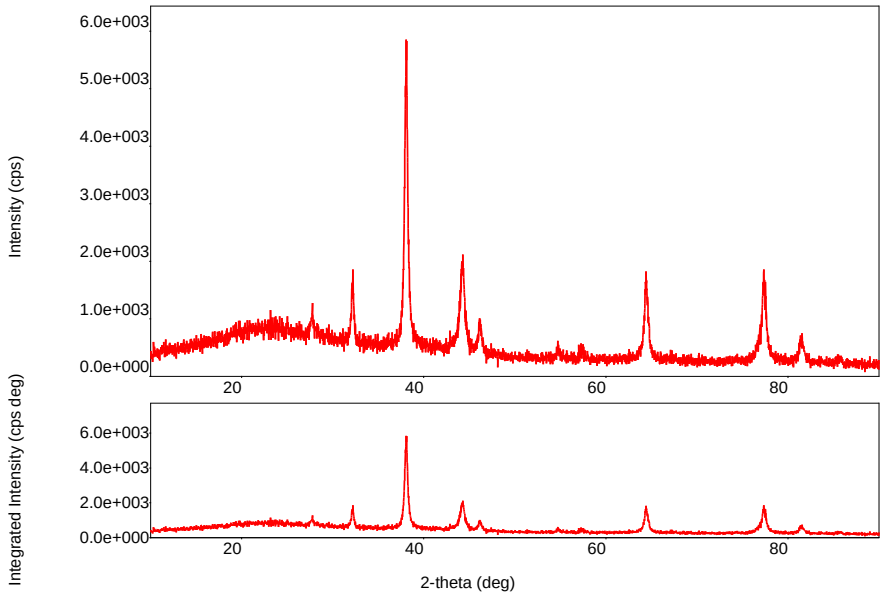


**Figure- 4: XRD pattern of Meliadubia**

**Antibacterial Activity**

Antibacterial activity of the synthesized AgNPs was tested against four clinically important pathogens Vibrio cholerae, Escherichia coli, staphylococcus aureus and Klebsiella pneumonia by following the procedure of sondiet al. which showed promising antibacterial activity against all the pathogens.

| PATHOGENS | Aqueous Extract added and Zone of inhibition (mm/ml) | | | | |
|---|---|---|---|---|---|
| | 25 µl | 50 µl | 75 µl | 100 µl | Control |
| Staphylococcus aureus | 15 | 17 | 18 | 20 | 20 |
| E.coli | 15 | 17 | 20 | 22 | 20 |
| Klebsiellapneumoniae | 12 | 15 | 18 | 21 | 28 |
| Vibrio cholera | 15 | 17 | 20 | 22 | 25 |

## CONCLUSION

Silver nanoparticles (AgNPs) were successfully obtained from bioreduction of silver nitrate solutions using Meliadubia leaf extract. Owing to varying properties of this plant species, AgNPs obtained from them also varied in size. AgNPs have been appropriately characterized using UV-vis spectroscopy, SEM and XRD analysis. FTIR analysis revealed the efficient capping and stabilization properties of these AgNPs. The XRD patterns confirmed the purity, phase composition and nature of the synthesised nanoparticles. The particles also exhibited good antimicrobial activity against K. pneumonia, S.aureus, V. choleraeand E.coli. Hence, due to their benign and stable nature and antimicrobial property, these AgNPs may be well utilized in industrial and remedial purposes. However, plant uptake and utilization of AgNPs require more detailed research on many issues like uptake potential of various species, process of uptake and translocation and the activities of the AgNPs at the cellular and molecular levels.

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