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Green Synthesis of Plant Mediated Silver Nano Particles and Evaluation of their Antimicrobial Activities

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ABSTRACT

There is an increasing commercial demand for nanoparticles due to their wide applicability in various areas such as electronics; catalysis, chemistry, energy, and medicine. Nanoparticles are prepared by a variety of chemical methods which are not environmentally friendly. We report a rapid and convenient method to reductively prepare silver nanoparticles using silver nitrate. In this report, we use aqueous extracts from plants, namely *Morinda tinctoria*, *Michelia champaca*. The extracts template the reductive preparation of silver nanoparticles which are found to be < 100 nm in size. The synthesized compounds are characterized by FT-IR, UV-VIS and XRD data. Green synthesized silver nanoparticles showed antibacterial activity against two Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and two Gram-negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli* and antifungal activity against *Candida albicans*, *Aspergillus niger* and *Cryptococcus neoformans*. The synthesized nanoparticles were found to be highly toxic against different multi drug resistant human pathogens.

Keywords: *Morinda tinctoria*, *Michelia champaca*. silver nanoparticles

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INTRODUCTION

Green chemistry is a design, development, implementation of chemical products and processes to reduce or eliminate the use and generation of substances hazardous to human health and environment. Nanotechnology is mainly concerned with synthesis of nano particles of variable sizes, shapes and chemical compositions and their potential use for human benefits. Nano particles are often referred to as clusters, nano spheres, nano rods and nano cups are just a few of the shapes at the small end of the size ranges from 1 to 100nm. Over the past few years, the synthesis of metal nano particles is an important topic of research in modern material science due to their distinctive potential applications in the field of electronic¹, magnetic², optoelectronic³, information storage⁴ and drug delivery⁵ Nanoparticles exhibit a number of special properties relative to bulk material and often have unique visible properties because they are small enough to confine their electrons and produce quantum effects⁶ Medicinal herbs have curative properties due to the presence of various complex chemical substance of different composition, which are found as secondary plant metabolite in one or more parts of these plants. These plant metabolites according to their composition are grouped as alkaloids, glycosides, corticosteroids, essential oils etc. In recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco friendliness. Ag is currently used to control bacterial growth in a variety of application, including dental work, catheters, and burn wounds. Many synthetic procedures for silver nano particles are available, but a narrow and controlled size preparation seems to be difficult to obtain because that is depend on the concentration of reacting chemical and controlled reaction environment.

MATERIALS AND METHODS:

Plant material and synthesis of Ag nanoparticles

The leaves of *Morinda tinctoria Roxb.* were collected from GITAM University campus Visakhapatnam, Andhra pradesh, India. The specimen voucher of the plant material was deposited at Botany Department Herbarium, Andhra University, Visakhapatnam, Andhra pradesh, India. The herbarium voucher number is A. U/ B. D. H/ 21106. *Michelia champaca* is a member of the family Magnoliaceae. The flowers of *Michelia champaca* Linn. were collected from Lawsons Bay Colony, Visakhapatnam, Andhrapradesh, India. The specimen voucher of the plant material was deposited at Botany Department Herbarium, Andhra University, Visakhapatnam, Andhrapradesh, India. The herbarium voucher number is A. U. (B. D. H) 21117. The leaves of *Morinda tinctoria Roxb* and flowers of *Michelia champaca Linn* were air dried for 10 days. The leaves and flowers

were ground separately to a fine powder. To 10 ml of the plant extract 90mol of 1 mM silver nitrate was added and centrifuged at 18.000 rpm for 25min. The collected pellets were stored at -4°C . The supernatant was heated at 50°C to 95°C . A change in the colour of solution was observed during the heating process.

UV-VIS Spectra analysis:

The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-VIS spectrophotometer UV-2450 (Shimadzu).

X-ray diffraction studies:

The formation and quality of compounds were checked by X-ray diffraction (XRD) spectrum (Figure-2). The XRD pattern was measured by drop coated films of AgNO_3 on glass plate and employed with X-ray diffractometer (INEL X-ray diffractometer) of characteristic $\text{Co-K}\alpha_1$ radiation ($\lambda = 1.78 \text{ \AA}$) in the range of 20° to 90° at a scan rate of $0.05^{\circ}/\text{min}$ with the time constant of 2 sec..The XRD pattern showed three intense peaks in the whole spectrum of 2θ value ranging from 10 to 80. Average size of the particles synthesized was 15nm with size range 10 to 50nm with cubic and hexagonal shape (Table-1 and Table 2).

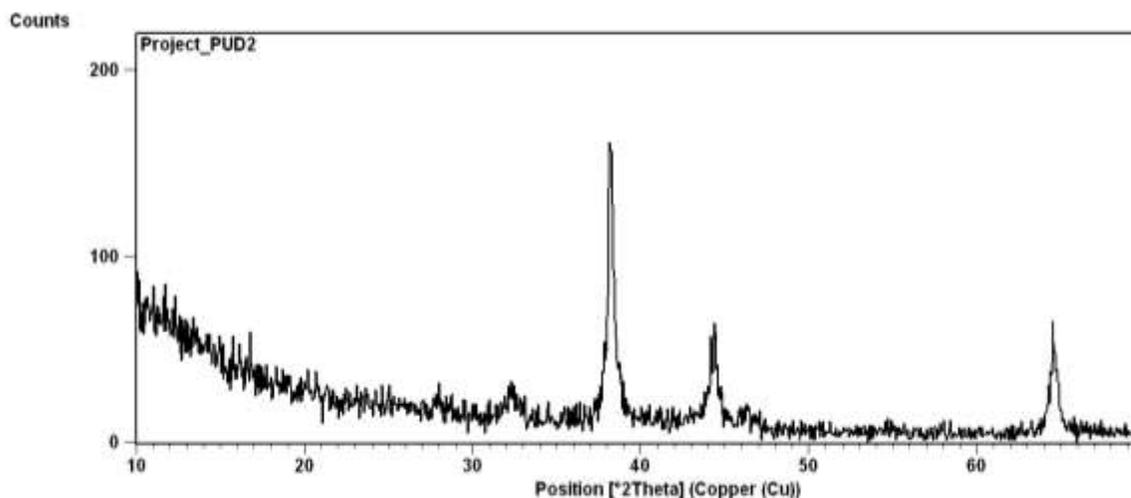


Figure 2 XRD spectra of silver nanoparticles synthesized by treating 10% *M. tinctoria* leaf extract with 1mM silver nitrate solution

Table 1: XRD pattern of silver nanoparticles synthesized by treating 10% *M. champaka* flower extract with 1mM silver nitrate solution

S.No	2θvalue	Plane	element	Phase
1	38.114	111	Ag	Cubic
2	64.526	110	Ag	Hexagonal
3	67.570	112	Ag	Hexagonal

FTIR spectral analysis:

The bio-reduced silver nitrate solution was centrifuged at 10,000 rpm for 15 min and the dried samples were ground with KBr pellets used for FTIR measurements. The spectrum was recorded in the range of 4000 - 400 cm^{-1} using Thermo Nicolet Nexus 670 spectrometer in the diffuse reflectance mode operating at resolution of 4 cm^{-1} .

RESULTS AND DISCUSSION:

UV-VIS analysis:

The synthesized Ag nanoparticles were confirmed by visual observation. The color was changed from watery into yellowish brown due to reduction of silver ions. The synthesized Ag nanoparticle using *M.tinctoria* and *M.champaca* plant extracts were detected by UV-VIS spectrophotometer at various nm. Absorption spectra of silver nanoparticles formed in the reaction mixture at different nm. i.e. 340, 380, 420, 460, 500, 540, 580 and 620nm, the particle has increasingly sharp absorbance maximum peak at 380nm (Figure-1) for *M.champaca* flower extract and 420nm (Figure-1) for *M.tinctoria* leaf extract and gradually decreased while nanometer increased.

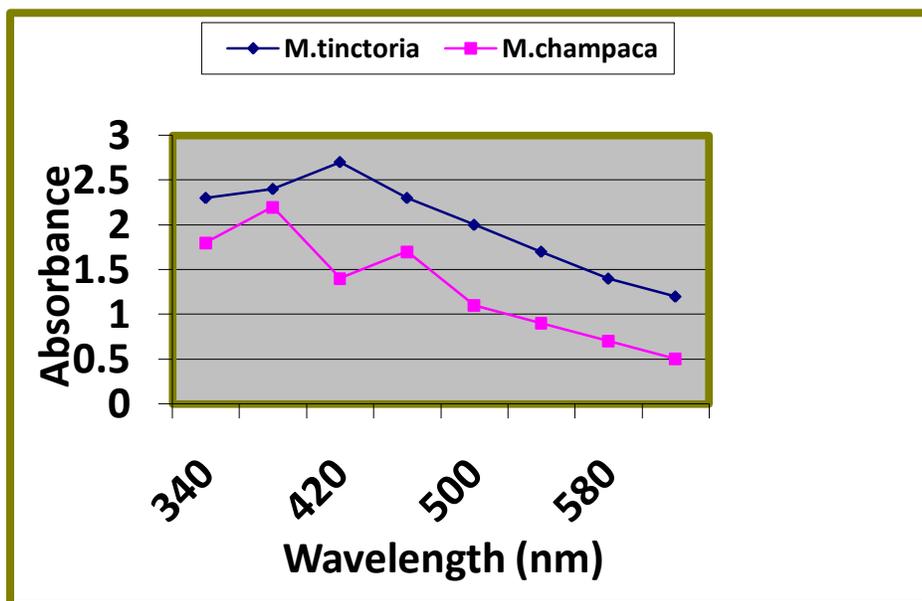


Figure 1: UV–VIS spectra of silver nanoparticles synthesized by *M. champaca* flower extract and *M.tinctoria* leaves extract

FT IR Analysis:

FTIR spectrum of Ag nanoparticles synthesized from *M.champaca* extracts were carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the metal

nanoparticles synthesized by leaf broth. The Peaks near 3440 cm⁻¹, 2924cm⁻¹ and 2854-1 assigned to OH stretching and aldehydic C-H stretching respectively.

Anti Bacterial

Assay:

The antibacterial assay includes two Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and gram negative bacteria: *Salmonella typhi*, *Shigella dysentery* which were obtained from the Department of Biotechnology, GITAM University, Visakhapatnam. Ampicillin was used as a positive control while DMSO served as a negative control. Agar well diffusion method^{7,8} was used to determine the antibacterial efficacy of the synthesized compounds. Bacterial inoculum (105CFU/ml) was spread on Muller Hilton agar. After the inoculum dried, 6mm diameter wells were made in the agar plate with a sterile cork borer. The synthesized silver nano particles dissolved in DMSO were added to the wells at a concentration of 10mg/ml. The petriplates were incubated at 37°C for 24 hrs. The zones of inhibition were measured in mm to estimate the potency of the test compounds. The investigation of antibacterial screening data revealed that the synthesized nano particles showed moderate to good bacterial inhibition. The observed zones of inhibition are presented in Table 3.

Table 2: XRD pattern of silver nanoparticles synthesized by treating 10% *M.tinctoria* leaf extract with 1mM silver nitrate solution

No	θ value	Plane	element	phase
1	38.112	111	Ag	Cubic
2	63.526	110	Ag	Hexagonal
3	65.227	112	Ag	Hexagonal

Table 3: Antibacterial Effect of Synthesized silver nanoparticles from *M.tinctoria* leaf extracts and *M.Champaca* flower extracts Diameter of the zone of inhibition (in mm)

S.No.	Name of the Organism	<i>M.tinctoria</i> leaf extract synthesized nanoparticles	<i>M.champaca</i> flower extract synthesized nanoparticles	Ampicilin
	Gram +ve			
1	<i>Stapylococcus aureus</i>	12	15	16
2	<i>Bacillus substilis</i>	16	14	16
	Gram -ve			
3	<i>Pseudomonas aeruginosa</i> ,	12	13	-
4	<i>Escherichia coli</i>	14	15	-

Antifungal studies:

Fungal culture was inoculated on potato dextrose agar. Media were poured into each sterilized Petri dish. Media plates were inoculated with liquid cultures homogenously by spread plate

method. The MIC values are represented in Table 4 Griseofulvinis taken as standard.

Table 4: Antifungal activity of Synthesized silver nanoparticles from *M tinctoria* leaf extracts and *M. Champaca* flower extracts (MIC values μ gi/ml)

S.No.	Name of the Organism	<i>M. tinctoria</i> leaf extract synthesized nanoparticles	<i>M. champaca</i> flower extract synthesized nanoparticles	Griseofulvin
1	<i>C.albicans</i>	500	500	500
2	<i>A.niger</i>	200	500	500
3	<i>C.neoformans</i>	>1000	>1000	>1000

CONCLUSION:

The bio- reduction of aqueous Ag⁺ ions by the leaf and flower extracts of the plant has been demonstrated. The reduction of the metal ions through leaf extracts leading to the formation of silver nanoparticles of fairly well defined dimensions. In the present study we found that leaves and flowers were good source for the synthesis of silver nanoparticles with an advantage of economic viability and to obtain smaller particle size. This study demonstrated the possibility of use of biologically synthesized silver nanoparticles Synthesized nanoparticles can be used as bactericidal agents and also in the field of medicine..

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