



Green synthesis, characterization and antibacterial activity of silver nanoparticles using *Curcuma pseudomontana*

T Parvathi¹, M Sathish¹, P K Siva¹, R Bhuvaneswari², M Arumugam^{1*}

¹ Department of Botany, J.J College of Arts and Science (Autonomous), Pudukkottai, Affiliated to Bharathidasan University, Thiruchirappalli, Tamil Nadu, India

² Department of Physics, Government Arts College for Women (Autonomous), Pudukkottai Affiliated to Bharathidasan University, Thiruchirappalli, Tamil Nadu, India

Abstract

The rapid biological synthesis of silver nanoparticles using *Curcuma pseudomontana* leaves extract provides eco-friendly, simple and efficient route for synthesis of benign nanoparticles. The size were bigger as the nanoparticles were surrounded by a thin layer of protein and metabolites such as terpenoids having functional groups of amines, alcohols, ketones, aldehydes, etc., which were found from the characterization using UV-Vis spectrophotometer, SEM, XRD, and FTIR techniques. The size of the nanoparticles in different concentrations was also different which depend on the reduction of metal ions.

Keywords: silver nanoparticles, *Curcuma pseudomontana*, green synthesis, antibacterial activity

Introduction

Due to swift industrialization and urbanization our environment is undergo huge smash up and a large amount of perilous and superfluous chemical, gases or substances are released. Nanotechnology applications are highly suitable for biological molecules, because of their exclusive properties. Metals nanoparticles have a high specific surface area and a high fraction of surface atoms (Amarendra *et al.*, 2010) [1]. Nanoparticles can be synthesized using various methods including chemical, physical, and biological. Chemicals used for nanoparticles synthesis are toxic and non-eco friendly byproduct. Plant provide a better platform for green nanoparticles synthesis as they are free from chemicals as well as provide natural capping agent. Since, there are several experimental performed on the synthesis of silver nanoparticles using medicinal plants such as *Oryza sativa*, *Helianthus annuus*, *Saccharum officinarum*, *Sorghum bicolor*, *Zea mays*, *Basella alba*, *Magnolia kobus*, *medicago sativa* (Alfalfa), *Cinamomum camphora*, and *Geranium sp* (Ankamwar *et al.*, 2005; Iravani *et al.*, 2014; Cassandra *et al.*, 2014) [2, 8, 4]. The bacteriocidal properties of silver nanoparticles are expressed by release of silver ions from the particles, which exhibit the antimicrobial activity. Nanotechnology is rapidly growing field and there importance in a number of such as health care, cosmetic, food and feed, environment health, mechanics, optics, biomedical sciences, chemical industries, electronics, space industries, drug-gene delivery, energy science, optoelectronics, catalysis, reprography, single electron transistors, light emitter, nonlinear optical devices and photo electrochemical applications (Yaqoob Sundas Bahar *et al.*, 2020) [13]. Nanomaterials are seen as solution in the field of solar energy conversion, catalysis, medicine and water treatment. Generally the nanoparticles are designed with surface modifications tailored to meet the needs of specific applications. The enormous diversity of the nanoparticles arising from their wide range of active chemical compounds

shape and morphologies (Ibrahim Khan *et al.*, 2019) [7]. The medium in which the particles are present, the state of dispersion of the particles and most importantly, the numerous possible surface modification the nanoparticles can be subjected to make this an important active field of science now-a-day. Nanoparticles can be broadly grouped into two, namely, organic nanoparticles which including carbon nanoparticles (Fullerenes) which, some of the inorganic nanoparticles include nanoparticles (Jeevanandam *et al.*, 2018) [9]. Inorganic nanomaterial have been widely used for cellular delivery due to their versatile features like wide availability, rich functionality, good compatibility, capability of targeted drug delivery and controlled release of drug. The presence of surfactants comprising functionalities for interaction with particle surface can stabilize particle growth and protect particles from sedimentation, agglomeration or losing their surface properties (Burdette and Frossard, 2021) [3]. Biological methods can be used to synthesize silver nanoparticles without the use of any toxic and expensive chemical substances. It was suggested that the phytochemicals are involved directly in the reduction of the ions and formations of silver nanoparticles. Biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction / oxidation. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis not required, high pressure, energy, temperature and toxic chemicals (Deepali *et al.*, 2019) [5]. One of the substances used in nanoformulation is silver (nanosilver). Due to its antimicrobial properties, silver has been used in filters to purify the drinking and clean swimming pool water. Silver nanoparticles are mostly smaller than 100nm and consist of about 20 - 15,000 silver atoms. Still, the remarkable strong antimicrobial activity is the major direction for development nana-silver product. Example are food packaging materials and food supplements, odour-resistant textiles, electronics,

household appliances, cosmetics, and medical advices, water disinfectants and room sprays.

Materials and Methods

Preparation of plant extract

Free leaves of *Curcuma pseudomontana* were collected and washed three to four times with water to remove the dust particles and then shade dried to remove the residual moisture and grinded. Finally grinded powders are stored at brown bottles until their further use. Then plant extract was prepared by mixing 1% of plant extract with deionized water in a 250ml of conical flask. The mixture was incubated for 30min and the subjected to centrifuge for 30min at room temperature with 5000rpm. The supernatant was separated and filtered with whatman filter paper with the help of vacuum filter. Then the solution was used for the reduction of silver ion Ag^+ to silver nanoparticles (Ag).

Synthesis of silver nanoparticles

Four different concentration ratios of plant and metal ions were prepared for nanometal formation. 0.17% of 1mM $AgNO_3$ metal ion was added in the prepared plant extract. The bio reduction metals or nanoparticles formation was measured in UV-Vis spectro photometer at 100 to 800nm.

Characterization of Silver Nanoparticles

UV-Vis analysis

After the addition of $AgNO_3$ to the plant extract. The spectra were taken in different time intervals up to 24hrs of $AgNO_3$ addition.

FTIR analysis

The-active chemical compounds of the synthesized silver nanoparticles was studied by FTIR spectrometer for FTIR study. The solution was dried at 75°C and the dried powders were characterized in the range 4000 - 400 cm^{-1} using KBr pellet method.

XRD analysis

The phase variety and particles sizes of synthesized silver nanoparticles was determined by X-ray diffraction spectroscopy. The synthesized silver nanoparticles were studied with CuK α radiation at voltage of 30 kV and current of 20 MA with scan rate of 0.03°/s. Different phase present in the synthesized sample software with search and match facility. The particle size of the prepared sample were determined Scherrer's equation as follows $D = 0.9\lambda / \beta \cos\theta$. Where D is the crystal size, λ is the wavelength of X-ray, θ is the Bragg's angle in radians and B is the full width half maximum of the peak in radians.

SEM analysis

The morphological features of synthesized silver nanoparticles from *T. bracteata* plant extract was studied by Scanning Electron Microscope. After 24 hrs of the addition of $AgNO_3$. The SEM slides were prepared by making a smear of the solution on slides. A thin layer of platinum was coated to make the samples conduction. Then the samples were characterized in the at an accelerating voltage of 20 KV.

Antimicrobial activity test

The antimicrobial activity of synthesized nanoparticles was studied by disc diffusion method measurements against

some pathogenic bacteria like *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*. Required amount of agar medium was prepared.

Result and Discussion

Uv-Vis. spectroscopy

Reduction of silver ions into silver nanoparticles by plant extract was observed as a result of the colour changes. The colour changes due to the Surface Plasmon Resonance phenomenon (SPR). The sharp peak of silver nanoparticles was observed around 440nm. The intensity of absorption of the metal ions occurs rapidly; more than 90% reduction of Ag^+ ions is complete within 4 Hrs. After addition of the metal ions to the plant extract on the behalf of UV-Vis data it was cleared that *C.pseudomontana* reduces metal ions (Vijay Kumar *et al.*, 2016)^[12].

SEM analysis

Scanning Electron Microscopic study provides the morphology and size details of the green synthesized silver nanoparticles. Comparison of experimental result indicate that the diameter of green nanoparticles in the solution have sizes of several nm in case of 30:1, 60:1, and 120:1 ratios where as in 60:1 ratio the size is several nm. The size of prepared nanoparticles was more than the size of nanoparticle which should be i.e. between 1-100 nm. The size was more than the desired size as a result of the protein which were bound in the surface of the nanoparticles (Harekrishna and Ibrahim, 2015)^[6].

FTIR analysis

Fourier Transform Infra Red measurement was carried out to identify the biomolecules for capping and efficient stabilization of the green synthesized nanoparticles. The FTIR spectrum of silver nanoparticles in case both of 60:1 and 120:1 ratios showed the band between 3490-3500 cm^{-1} corresponds to O-H stretching H-bonded alcohols and phenols.

The peak found around 1500-1550 cm^{-1} showed a stretch for C-H bond peak around 1450-1500 cm^{-1} showed the bond stretch for N-H. From the analysis of FTIR studies we conformed that the carbonyl groups from the amino acid residues and proteins has the stronger ability to bind metals indicating that the protein could possibly from the metals nanoparticles to prevent agglomeration and there by stabilize the medium. Carbonyl groups proved that flavanones or terpenoids absorbed on the surface of metal nanoparticles (Preetha Devaraj *et al.*, 2013)^[11]. The presence of reducing sugars in the solution could be responsible for the reducing metal ions and formation of the corresponding metal nanoparticles. These issues can be addressed once the various fractions of the plant leaves extract separated, indentified and individually assayed for reduction of the metal ions. This rather elaborate study is currently underway.

XRD analysis

XRD spectrum showed distinct diffraction peak around 38°, which are indexed by the (100) of the Face Centered Cubic (FCC) silver particles. Independent crystallization of the capping agents was ruled out due to the process of centrifugation and redispersion of the pilled in millipore water after nanoparticles formation as a part of purification process. Broader peaks signify smaller particle size and

reflect the effects due to experimental conditions on the nucleation and the crystal nuclei.

Antibacterial activity

Antibacterial activity of the silver nanoparticles was tested by disc diffusion method against gram positive and negative pathogenic bacteria. Silver nanoparticles synthesized by *C. pseudomontana* extract have been found to passes highest

antimicrobial activity against *S. aureus*, *B. subtilis*, *S. aeruginosa* and *E. coli* when compared to control antibacterial. This differential antibacterial activity of silver nanoparticles can be attributed to their differential size and shape; the antimicrobial activity increases with decreasing size of the silver nanoparticles (Loo Yuet Ying *et al.*, 2018) [10].

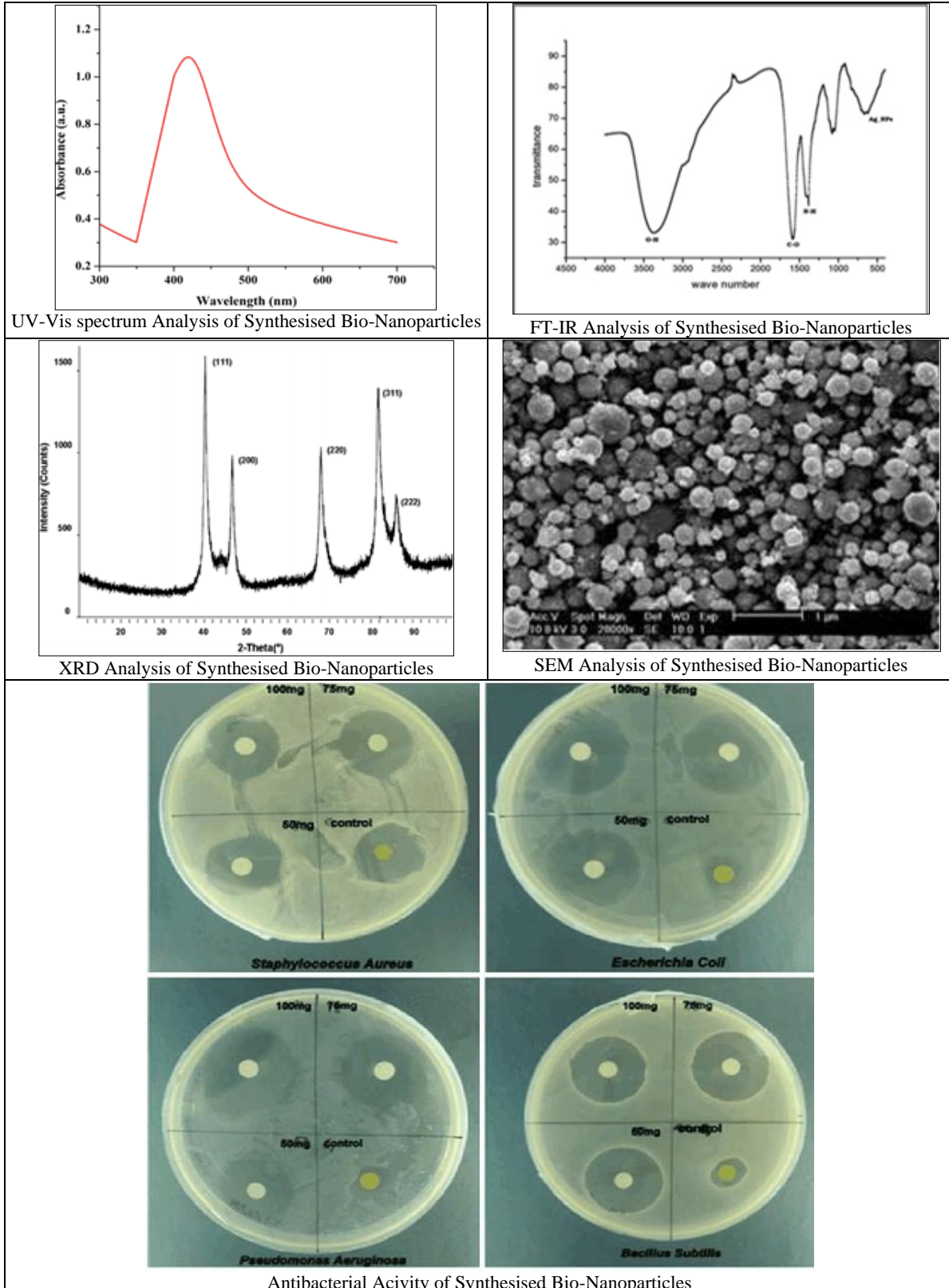


Fig 1

Summary

It was concluded that plant mediated synthesis of green silver nanoparticles possess potential antimicrobial application. The use of silver nanoparticles in drug delivery systems might be the future thrust in the field of medicine. Hence, it was demonstrated that, this green synthesized nanoparticles as a novel therapeutic agent, will be useful in many biomedical applications.

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