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Green Synthesis and Characterization of Silver Nanoparticles Using Leaf Extracts of *Clitoria Ternatea*

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ABSTRACT
Bionanotechnology has emerged up as integration between biotechnology and nanotechnology for developing biosynthetic and environmental friendly technology for synthesis of nanomaterials. Silver has been known to have effective bactericidal properties for centuries. Nowadays, silver based topical dressings have been widely used as a treatment for infection in burns, open wounds, and chronic ulcer. As the pathogenic organisms are getting evolved day by day due to mutation and gaining antibiotic resistance, an important industrial sector of nanoscience deals with the preparation and study of nanoparticles in antibacterial clothing, burn ointments, and coating for medical device. The size of nanomaterials is much smaller than that of most biological molecules and structures; therefore, nanomaterials can be useful in both in vivo and in vitro biomedical research application. The purpose of the study is to synthesize and characterize the plant mediated silver nanoparticles using Clitoria ternatea. Further investigation of the shape and size of nanoparticle was done by X-ray diffraction and scanning electron microscopic studies. A silver nanoparticle at different concentration was assessed for its antibacterial effect, against various nosocomial pathogens.
Key words:*Clitoria ternatea, Silver Nanoparticles, FTIR, UV, XRD.*

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INTRODUCTION

Nanotechnology is the fastest growing area of manufacturing in the world today and there is an increasingly frantic search for new nanomaterials and methods to make them. It has been well known that living cells are the best examples of machines that operate at the nanolevel and perform a number of jobs ranging from generation of energy to extraction of targeted materials at very high efficiency [1].
One of the fields in which nanotechnology finds extensive applications is nanomedicine, an emerging new field which is an outcome of fusion of nanotechnology and medicine. Medicine is no more physician job exclusively, the materials and devices designed at the level of nanoscale are for diagnosis, treatment, preventing diseases and traumatic injury, relieving pain and also in the overall preservation and improvement of health [2]. Nanotechnology can improve our understanding of living cells and of molecular level interactions. A number of nanoparticles based therapeutics have been approved clinically for infections, vaccines, and renal diseases [3]. Oligodynamic silver having antimicrobial efficacy extends well beyond its virotoxicity and it has lethal effects spanned across all microbial domains [4]. The application of silver nanoparticles in drug delivery, drug discovery, and new drug therapies has declared war on many dreadful diseases and they use the body natural transport pathway and natural mechanism of uptake of the drug by the diseased cells [5].
Nanoparticles can be synthesized using various approaches including chemical, physical, and biological approaches. Although chemical method of synthesis requires short period of time for synthesis of large quantity of nanoparticles, this method requires capping agents for size stabilization of the nanoparticles. Chemicals used for nanoparticles synthesis and stabilization are toxic and lead to non-eco-friendly byproducts. The need for environmental nontoxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as byproducts. Thus, there is an increasing demand for "green nanotechnology." Many biological approaches for both extracellular and intracellular nanoparticles synthesis have been reported till date using microorganisms including bacteria [6], fungi [7, 8], and plants [9–11].
Plants provide a better platform for nanoparticle synthesis as they are free from toxic chemicals and provide natural capping agents. Moreover, use of plant extracts also reduces the cost of microorganisms

isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms.

MATERIAL AND METHODS

Plant Material

Clitoria ternatea habit is a twining shrub with alternate imparipinnate leaves. About their flowers are solitary or in pairs. The classification of*Clitoria ternatea* is given in the following: family: Fabaceae; subfamily: Faboideae; genus: Clitoria; species: ternatea; botanical name: Clitoria ternatea; common name: sankupushpam.

Collection and Preparation of Plant Materials

Fresh leaves of two different plants, that is,*Clitoria ternatea* and Solanum nigrum, free from diseases were collected from Madurai and then washed thoroughly 2-3 times with tap water and once with sterile water. 20 g of fresh leaves was finely chopped and added to 100 mL of distilled water and stirred at 60°C for 1 h. After boiling, the mixture was cooled and filtered with Whatman paper number 1. Filtrate was collected.

Synthesis of Silver Nanoparticles

0.1 M of aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 5 mL of leaf extract of*Clitoria ternatea* and *Solanum nigrum* was added to 45 mL of 0.1 M $AgNO_3$ solution for bioreduction process at room temperature.

UV-VISIBLE ABSORBANCE SPECTROSCOPY

UV-Visible spectroscopy analysis was carried out on a Systronic UV-Visible absorption spectrophotometer 117 with a resolution of ±1 nm between 200 and 1000 nm processing a scanning speed of 200 nm/min. Equal amounts of the suspension (0.5 mL) were taken and analysed at room temperature. The progress of the reaction between metal ions and the leaf extract was monitored by UV-Visible spectra of silver nanoparticles in aqueous solution with different wavelength in nanometers from 340 to 800 nm. The reduction of silver ions and formation of silver nanoparticles occurred within an hour of reaction. Control was maintained by using $AgNO_3$.

FOURIER TRANSFORMS INFRARED SPECTROSCOPY (FTIR)

For FTIR measurements, the synthesized silver nanoparticles solution was centrifuged at 10000 rpm for 30 minutes. The pellet was washed thrice with 5 mL of deionised water to get rid of the free proteins or enzymes that are not capping the silver nanoparticles. The pellet was dried by using vacuum drier. It was analysed by FTIR.

X-RAY DIFFRACTION

A thin film of the silver nanoparticle was made by dipping a glass plate in a solution and carried out for X-ray diffraction studies. The crystalline silver nanoparticle was calculated from the width of the XRD peaks and the average size of the nanoparticles can be estimated using the Debye-Scherrer equation:

SCANNING ELECTRON MICROSCOPY

The pellet was subjected for SEM analysis. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid; extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry for analysis.

RESULTS

UV-Visible Absorbance Spectroscopy

The reduction of silver nitrate using the plant leaf extract was viewed by the colour change in the reaction solutions (Fig.1). In UV-V is spectra recorded for the reaction solution of reduced silver nitrate by leaf extract of*Clitoria ternatea* . The maximum absorbance peak was seen at 440 nm for Clitoria ternatea, respectively.

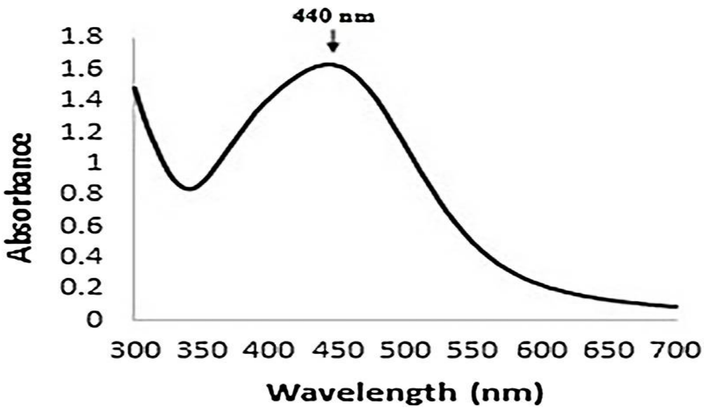

Fig.1 UV-VIS spectroscopy for silver nanoparticles synthesised usingClitoria ternatea leaves extracts.

Fourier Transforms Infrared Spectroscopy (FTIR)

FTIR measurements were carried out to identify the possible biomolecules responsible for the capping and efficient stabilization of the silver nanoparticles synthesized by the plant extracts. Figures 3 show the leaf broth of*Clitoria ternatea* and *Solanum nigrum*, respectively. Absorbance bands of*Clitoria ternatea* were observed at 3317.34 cm^{-1} assigned to O–H (s) stretch, 3317.34 cm^{-1} assigned to O–H (s) stretch, 1614.31 cm^{-1} assigned to C=C aromatic stretch, 1394.44 cm^{-1} assigned to C–H alkenes stretch, 1191.93 cm^{-1} assigned to C–N amines stretch, and 752.19 cm^{-1} and 655.75 cm^{-1} assigned to C–H alkenes stretch. Absorbance bands of *Solanum nigrum* were observed at 3317.34 cm^{-1} assigned to O–H (s) stretch, 2933.88 cm^{-1} assigned to C–H (s) stretch, 1606.59 cm^{-1} assigned to C=C aromatic stretch, 1394.44 cm^{-1} assigned to C–H alkenes stretch, 1191.93 cm^{-1} assigned to C–N amines stretch, 1122.49 cm^{-1} assigned to C–N amines stretch, and 752.19 cm^{-1} and 655.75 cm^{-1} assigned to C–H alkenes stretch.

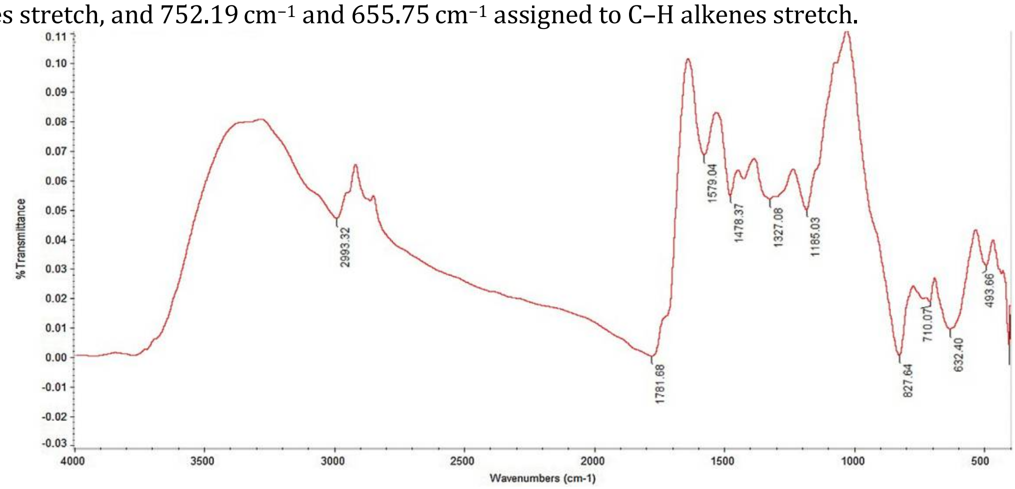

Fig. 3 FTIR spectrum of silver nanoparticles synthesized by using the leaf extract of*Clitoria ternatea.*

X-Ray Diffraction

The silver oxide molecules formed are necessarily subjected to XRD analysis for the measurement of size of these particles. Figures 4 show the XRD pattern obtained for the silver nanoparticles synthesized using the leaf extract of*Clitoria ternatea* and Solanum nigrum. The intense peak of nanoparticles 28, 33, 38, 44, 46, 55, 58, 65, and 77 in *Clitoria ternatea* appeared which are indexed as crystalline silver. The sharpening of the peaks clearly indicates that the particles are the spherical nanoparticles. The average size of the silver nanoparticles is estimated by using Debye Scherrer's formula. The average of silver nanoparticles synthesized by *Clitoria ternatea* is 20 nm and average of silver nanoparticles.

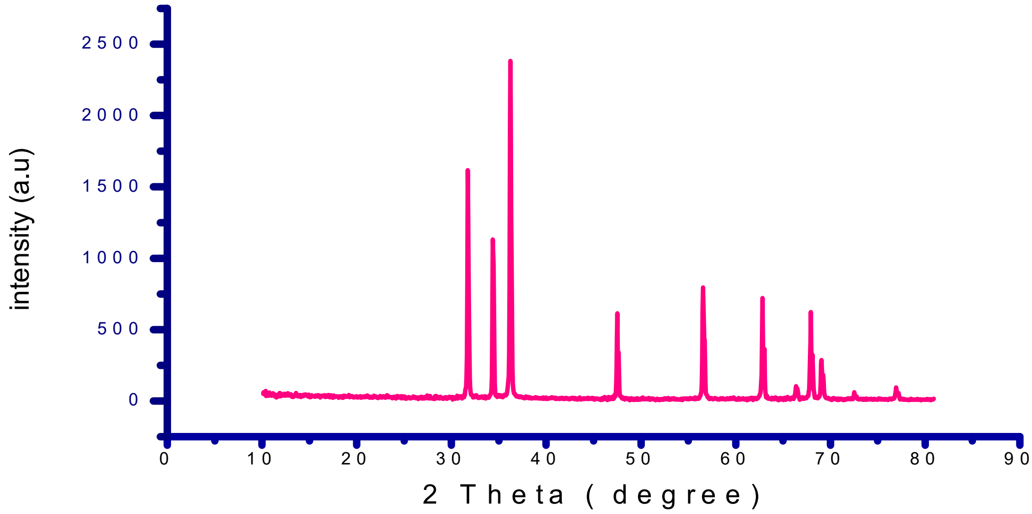

Fig. 4 XRD pattern of silver nanoparticles synthesized using*Clitoria ternatea* leaf extract

DISCUSSION

The formation of silver nanoparticles using plant leaf extract of*Clitoria ternatea* was viewed by the colour change from colourless to yellowish brown. Similarly, Sastry et al. reported that the silver nanoparticles exhibited striking colours, from light yellow to brown. Further, Shankar et al. reported that silver nanoparticles exhibited yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. By using UV-Visible spectrum the maximum absorbance peak for*Clitoria ternatea* and *Solanum nigrum* was seen at 420 and 440 nm, respectively. Similarly, Prasad and Elumalai [3reported that absorption spectra of silver nanoparticles formed in the reaction media have absorbance peak at 430–440 nm. Veerasamy et al. reported that absorption spectra of silver nanoparticles formed in the reaction media have absorbance peak at 438 nm. Different parameters were optimized including temperature, pH, and time which had been identified as factors affecting the yields of silver nanoparticles. Gilaki reported that pH, strength of elements, plant sources, time and incubation temperature nanoparticles synthesis reaction mixture, the synthesis methods, it is possible to create a wide range of different nanoparticles. FTIR analysis confirmed that the bioreduction of Ag^+ ions to silver nanoparticles is due to the reduction by capping material of plant extract. Similarly, Gole et al. reported that proteins present in the extract can bind to silver nanoparticles through either free amino or carboxyl groups in the proteins. Prasad et al. [13] reported that the carboxyl (–C=O), hydroxyl (–OH), and amine (–NH) groups of leaf extracts are mainly involved in fabrication of silver nanoparticles. The average of silver nanoparticles is synthesized by*Clitoria ternatea* 20 nm and average of silver nanoparticles is synthesized by *Solanum nigrum*. The observed result was in accordance with the results of Raut et al. (2009), where the XRD studies reveal that silver nanoparticles are polydispersed and ranged in size from 10 to 50 nm with an average size of 27 nm. reported that the silver nanoparticles synthesized from Gelidiella acerosa extract of XRD pattern reveal that the average size of the silver nanoparticles was 23 nm.

CONCLUSION

A critical need in the field of nanotechnology is the development of a reliable and eco-friendly process for synthesis of metallic nanoparticles. Nanoparticles are being viewed as fundamental building blocks of nanotechnology. Silver nanoparticles play a profound role in the field of biology and medicine due to their attractive physiochemical properties. In the present study, we have demonstrated that use of a natural, low cost biological reducing agent and*Clitoria ternatea* and *Solanum nigrum* leaves extracts can produce metal nanostructures, through efficient green nanochemistry methodology, avoiding the presence of toxic solvents and waste. The biosynthesized silver nanoparticles using *Clitoria ternatea* and *Solanum nigrum* leaves extract proved to be excellent against nosocomial pathogens and also compared to the fact that the silver nanoparticles of *Clitoria ternatea* showed higher activity than the silver nanoparticles of *Solanum nigrum* against nosocomial pathogens.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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