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BIOGENIC SILVER NANOPARTICLES USING *CALOPHYLLUM INOPHYLLUM* LEAF EXTRACT: SYNTHESIS, SPECTRAL ANALYSIS, AND ANTIMICROBIAL STUDIES

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ABSTRACT

Plant extracts are a cost effective, ecologically friendly, and efficient alternative for the large scale synthesis of nanoparticles. In the present study, green synthesis of silver nanoparticles (Ag NPs) by using *Calophyllum inophyllum* L. leaf extract was evaluated. The Ag NPs were characterized by Scanning Electron Microscopy (SEM), UV–Visible absorption spectroscopy and FT –IR analysis. The SEM analysis of silver nanoparticles revealed spherical shape in morphology with their sizes ranging from 70 to 100 nm. The *in vitro* antimicrobial activity of the Ag NPs synthesized by *C. inophyllum* leaf extract was studied against some human pathogenic microbes by using agar well diffusion assay. The synthesized Ag NPs exhibited good antimicrobial potential against tested bacterial and fungal pathogens. This plant leaf extract compounds can be extended to the synthesis of other metal and metal oxide nanoparticles.

Key Words: silver nanoparticles, *Calophyllum inophyllum* L., SEM analysis, antimicrobial activity.

INTRODUCTION

The medical properties of silver have been known for over 2,000 years. Since the nineteenth century, silver-based compounds have been used in many antimicrobial applications. Nanoparticles have been known to be used for numerous physical, biological, and pharmaceutical applications. Silver nanoparticles are being used as antimicrobial agents in many public places such as railway stations and elevators in China, and they are said to show good antimicrobial action.

It is a well-known fact that silver ions and silver-based compounds are highly toxic to microorganisms which include 16 major species of bacteria (Slawson *et al*., 1992; Zhao and Stevens, 1998). This aspect of silver makes it an excellent choice for multiple roles in the medical field. There are many ways depicted in various literatures to synthesize silver nanoparticles. These include physical, chemical, and biological methods.

The physical and chemical methods are numerous in number. The problem with most of the chemical and physical methods of nanosilver production is that they are extremely expensive and also involve the use of toxic, hazardous chemicals, which may pose potential environmental and biological risks. It is an unavoidable fact that the silver nanoparticles synthesized have to be handled by humans and must be available at cheaper rates for their effective utilization; thus, there is a need for an environmentally and economically feasible way to synthesize these nanoparticles.

The quest for such a method has led to the need for biomimetic production of silver nanoparticles whereby biological methods are used to synthesize the silver nanoparticles. The growing need to develop environmentally friendly and economically feasible technologies for material synthesis led to the search for biomimetic methods of synthesis (Kalishwaralal *et al*., 2008). In most cases, the chemical synthesis methods lead to some chemically toxic substances being absorbed on the surface and can hinder their usage in medical applications (Parashar*et al*., 2009). There are three major sources of synthesizing silver nanoparticles: bacteria, fungi, and plant extracts.

Calophyllum inophyllum is a low-branching and slow-growing tree of the family Guttiferae. It usually reaches 8 to 20 metres in height. The tree is believed to have diuretic properties. The gum extracted from the plant is emetic and purgative and is used for the treatment of wounds and ulcers. An infusion of gum, bark and leaves is used for sore eyes. The leaves soaked in water are applied to inflamed eyes. The leaf infusion is taken internally for heatstroke (Edeoga *et al*., 2005). The plant has anti-inflammatory and pain relieving properties. We report for the first time, the synthesis and characterization of silver nanoparticles generated by *C. inophyllum* L. aqueous leaf extract. The biologically synthesized nanoparticles were analyzed and tested against several different pathogenic microorganisms.

Synthesis and characterization of silver nanoparticles by *Calophyllum inophyllum* L. leaves

Collection of plant

The leaves of *Calophyllum inophyllum* L. were collected from Government Arts College campus, Kumbakonam, Tamil Nadu, India (Fig.1).Specimen was further confirmed with reference to Herbarium sheets available in the Rapinat Herbarium, St. Joseph College, Thiruchirappalli, Tamilnadu, India.

Sample Preparation

10g of fresh leaves of *Calophyllum inophyllum* L. were taken and thoroughly rinsed with deionized water and cut into small pieces. The chopped leaves were boiled in 75mL of deionized water for 3 minutes. The leaf broth was then cooled and filtered yielding 50mL of broth. It was stored in a refrigerator.

Synthesis of silver nanoparticles

Silver nitrate ($AgNO_3$) was obtained from Sigma Aldrich and used as received without further purification. 5ml leaf broth was added to 100ml of 1 mM, 2mM and 3mM silver nitrate and allowed to react at ambient conditions. The observed colour change of reaction mixture from transparent yellow to dark brown indicates the formation of silver nanoparticles. The suspension of silver nanoparticles was allowed to settle and the excess liquid was removed. The particles were then rinsed to remove any organic residue and resuspended in 95% ethanol for further characterization.

Scanning Electron Microscopy (SEM) analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. 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 were allowed to dry by putting it under a mercury lamp for 5 min.

Ultraviolet-Visible (UV-Vis) Spectroscopy analysis of silver nanoparticles

The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 2 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).

Fourier Transform Infrared Spectroscopy (FT –IR) analysis of silver nanoparticles

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 5000 rpm for 10 minutes and the resulting suspension was redispersed in 10 mL sterile distilled water. The centrifuging and redispersing process was repeated three times. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FT - IR (Bruker model, TENSOR 37).

Antimicrobial activity of silver nanoparticles synthesized by *Calophyllum inophyllum* L.

The antimicrobial activities of silver nanoparticles synthesized by *Calophyllum inophyllum* L. extract were carried out by agar well diffusion method (Perez *et al*., 1990). Totally ten human pathogenic microorganisms namely five bacterial strains such as, *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi*, *Staphylococcus aureus* and *Vibrio cholera* and five fungal strains such as, *Aspergillus flavus, A. fumigatus, A. terreus, Candida albicans* and *Fusarium moniliforme* were selected for the present investigation. The young microbial inoculums was prepared and used during the research period.

Screening of Antimicrobial Activity

The petriplates were washed and placed in an autoclave for sterilization. After sterilization, nutrient agar medium and PDA medium (Himedia, Mumbai) were poured into each sterile petriplate and allowed to solidify in a laminar air flow chamber. After solidification, using a sterile cotton swabs, fresh microbial cultures was spread over the plate by spread plate technique.

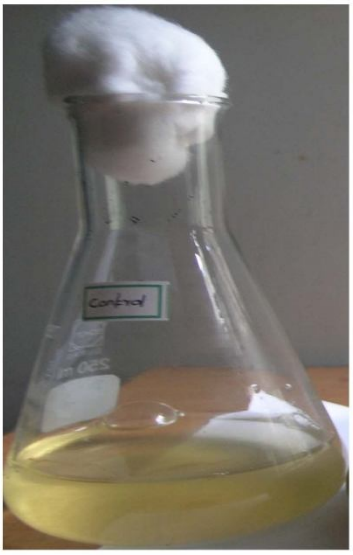
One well of 5mm size made into the agar plates with the help of sterile cork borer, the wells were loaded with 200µl of silver nanoparticles synthesized extracts of the plant leaves. All the plates were incubated at 37^0C for 24 hrs (bacteria) and 28^oC for 48-72 hrs (fungi). After incubation, the plates were observed for formation of clear inhibition zone around the well indicated the presence of antimicrobial activity. The zone of inhibition was calculated by measuring the diameters of the inhibition zone around the well.

RESULT AND DISCUSSION

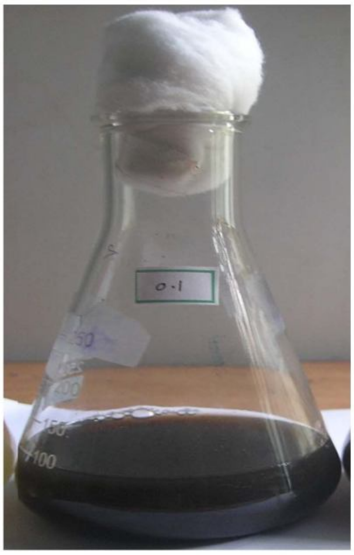
Green synthesis of silver nanoparticles from *Calophyllum inophyllum* L. was studied. As the leaf extract was mixed in the aqueous solution of the silver ion complex, it started to change the color from yellowish to brown due to reduction of silver ion which indicated formation of silver nanoparticles (Plate - 1). The appearance of a brown color confirms the existence of silver nanoparticles in the solution. Similarly, the synthesis of silver nanoparticles using plant leaves such as *Acalypha indica* (Krishnaraj *et al*., 2010); *Chenopodium album* (Dwivedi and Gopal, 2010); *Sesuvium portulacastrum* (Nabikhan *et al*., 2010); *Murraya koenigii* (curry) (Christensen *et al*., 2011); *Ocimum sanctum* (Tulsi) (Singhal *et al*., 2011); *Garcinia mangostana* (mangosteen) (Veerasamy *et al*., 2011); *Stevia rebaudiana* (Yilmaz *et al*., 2011); *Nicotiana tobaccum* (Parasad *et al*., 2011); *Ocimum tenuiflorum*, *Solanum trilobatum*, *Syzygium cumini*, *Centella asiatica*, and *Citrus sinensis* (Logeswari *et al*., 2012); *Arbutus unedo* (Kouvaris *et al*., 2012);*Ficus benghalensis* (Saxena *et al*, 2012); mulberry (Awwad and Salem, 2012) and *Olea europaea* (Awwad *et al*., 2012) has been reported in various part of the world.

Fig.1. Photograph of *Calophyllum inophyllum* Leaf

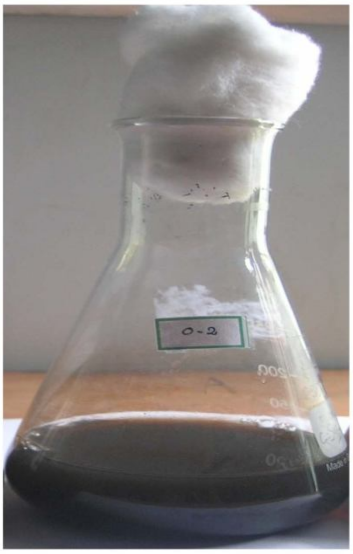
Plate 1. Green synthesis of silver nanoparticles by using leaf extract of *Calophyllum inophyllum* L.



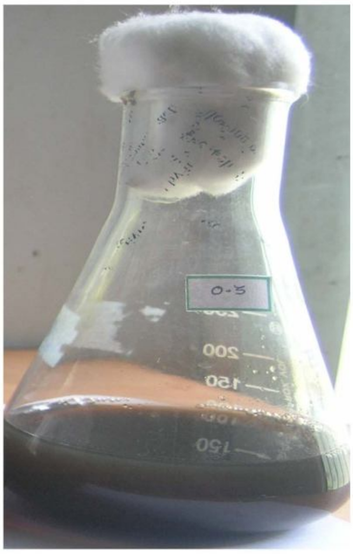
Control



1 mM $AgNO_3$



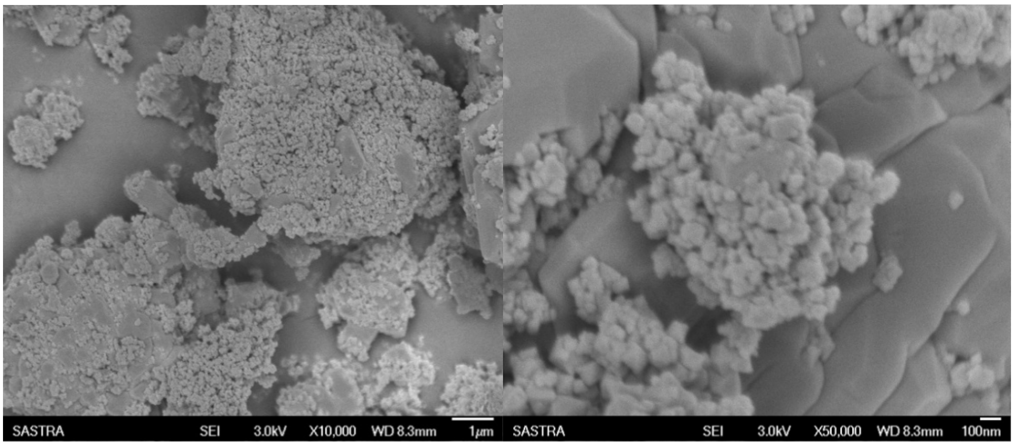
2 mM $AgNO_3$



3 mM $AgNO_3$

The silver nanoparticles were characterized using SEM, UV–Visible absorption spectroscopy and FT –IR analysis. The SEM images of silver nanoparticles synthesized by *Calophyllum inophyllum* L. were showed in plate - 2. This image shows individual silver nanoparticles as well as a number of aggregates. The silver nanoparticles are predominantly spherical in morphology with their sizes ranging from 70 to 100 nm. The findings of the present investigations have accordance with Gopinath *et al*. (2013) who reported the SEM image silver nanoparticles revealed the spherical shaped with the average size of about 20- 50nm.

Plate – 2. SEM images of silver nanoparticles synthesized by *Calophyllum inophyllum* L.



The ultraviolet-visible spectra of the silver nanoparticles showed strong peaks at 430 - 440 nm range, which indicated the presence of silver nanoparticles (Fig. 2).

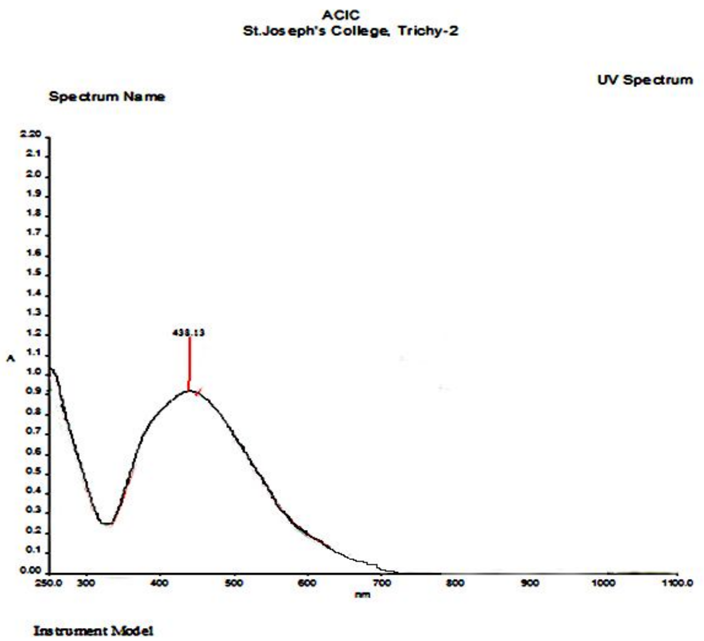


Fig.2. UV – Visible spectrum of silver nanoparticles synthesized by

***Calophyllum inophyllum* L.**

FT- IR analysis was used to characterize the nature of capping ligands that stabilizes the silver nanoparticles formed by the bioreduction process. The FT- IR data revealed that the stabilizing agents were presented in the plant extracts. Absorbance bands were observed in the region of 1000-3500 cm^{-1}. They were 3421.14, 2068.19 and 1638.39 cm^{-1}(Fig.3). These absorbance bands are known to be associated with the stretching vibrations for N-H- stretching vibrations, secondary, free, two bands , and N-H bending vibrations primary respectively.

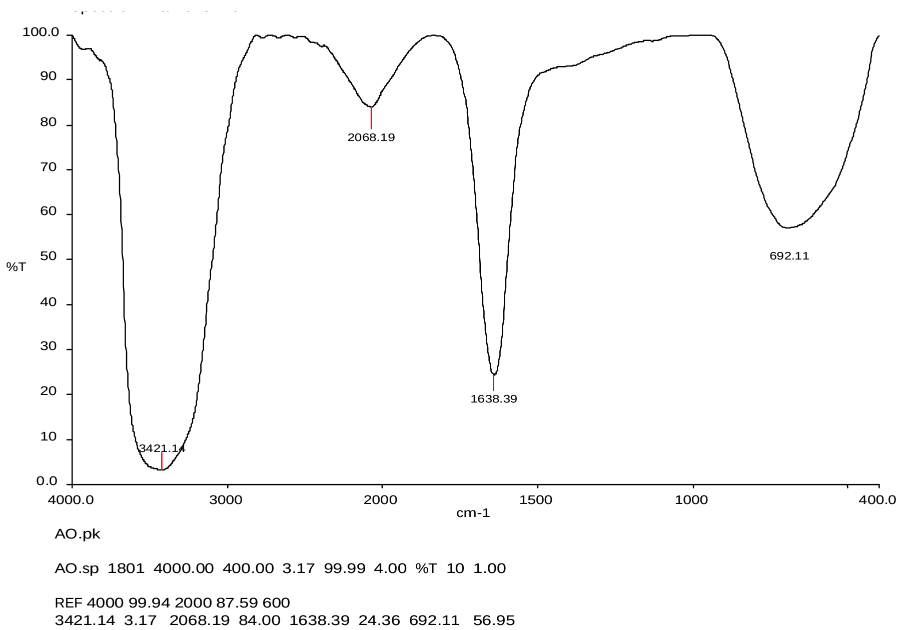


Fig.3. FT – IR Spectroscopy analysis of silver nanoparticles synthesized by

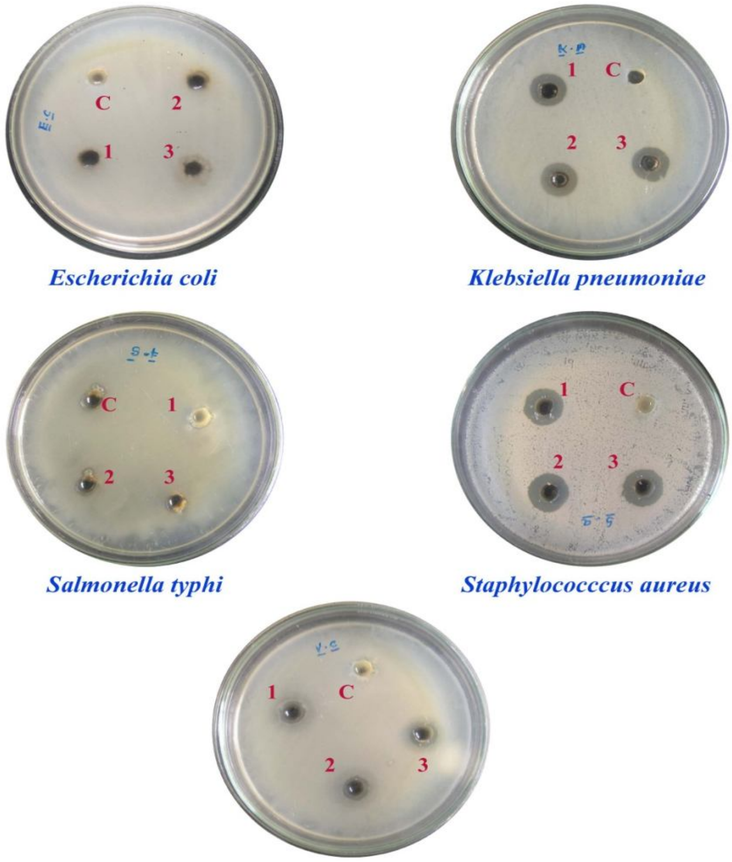
***Calophyllum inophyllum* L.**

In vitro antibacterial efficacy of silver nanoparticles synthesized by *Calophyllum inophyllum* L. was investigated by agar well diffusion method. The highest antibacterial activity was observed against *Staphylococcus aureus* (17 mm) followed by *Klebsiella pneumoniae* (16 mm), *Escherichia coli* and *Vibrio cholerae* (10 mm) at 3mM concentration(Table 1; Plate 3). Recently, Gopinath *et al*. (2013) reported that the Ag NPs synthesized by leaf extract of *Pterocarpus santalinus* exhibited good antibacterial potential against gram-positive and gram-negative bacterial strains.

Table 1.Antibacterial activity of synthesized silver nanoparticles extract from *Calophyllum inophyllum* L.

S. No.	Test Organisms (Bacterial pathogens)	Zone of inhibition (diameter in mm) Different concentration of silver nanoparticles			
		Control	1mM	2 mM	3 mM
1.	*Escherichia coli*	-	-	8	10
2.	*Klebsiella pneumoniae*	-	12	14	16
3.	*Salmonella typhi*	-	-	-	-
4.	*Staphylococcus aureus*	-	15	16	17
5.	*Vibrio cholerae*	-	9	9	10

Plate 3. Antibacterial activity of silver nanoparticles synthesised by using leaf extract of *Calophyllum inophyllum* L.



Vibrio cholerae

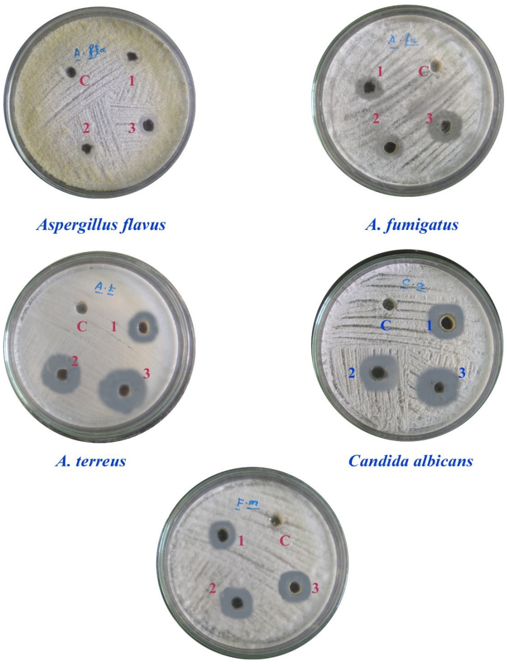
C - Control, 1 - 1 mM $AgNO_3$, 2 - 2 mM $AgNO_3$, 3 - 3 mM $AgNO_3$

The silver nanoparticles synthesized by *Calophyllum inophyllum* L. showed effective antifungal activity against *Aspergillus fumigatus, A. terreus*, *Candida albicans*, moderate activity against *Fusarium moniliforme* and least activity against *A. flavus* (Table 2; Plate 4). Similarly Pasupuleti *et al*. (2013) reported that the Ag NPs synthesized by *Rhinacanthus nasutus* leaf extract showed highest activity against the *A. flavus* and *A. niger*.

Table 2.Antifungal activity of synthesized silver nanoparticles extract from *Calophyllum inophyllum* L.

S. No.	Test Organisms (Fungal pathogens)	Zone of inhibition (diameter in mm) Different concentration of silver nanoparticles			
		Control	1mM	2 mM	3 mM
1.	*Aspergillus flavus*	-	10	11	12
2.	*A. fumigatus*	-	13	14	18
3.	*A. terreus*	-	10	12	19
4.	*Candida albicans*	-	12	15	19
5.	*Fusarium moniliforme*	-	11	14	15

Plate 4. Antifungal activity of silver nanoparticles synthesised by using leaf extract of *Calophyllum inophyllum* L.



Aspergillus flavus *A. fumigatus* *A. terreus* *Candida albicans* *Fusarium moniliforme*

C - Control, 1 - 0.1 mM $AgNO_3$, 2 - 0.2 mM $AgNO_3$, 3 - 0.3mM $AgNO_3$

The overall investigation could be concluded that *Calophyllum inophyllum* L. is a good candidate for synthesis of silver nanoparticles. The antimicrobial screening demonstrated that the synthesized Ag NPs had a high inhibitory effect on pathogenic bacteria as well as fungi. This green synthesized method is rapid, facile, convenient, less time consuming, environmentally safe, and can be applied in a variety of existing applications. This plant leaf extract compounds can be extended to the synthesis of other metal and metal oxide nanoparticles.

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