Research Article

Effect of Various Physico-chemical Factors on Synthesis of Biogenic Silver Nanoparticles Using Leaf Extract of *Cananga odorata* (Lam.) Hook.f. & Thomson. and its Anti-bacterial effect

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ABSTRACT

The present study is undertaken to explore the biosynthesis of silver nanoparticles using leaf extract of *Cananga odorata* (Lam.) Hook's. & Thomson. The physico-chemical factors used in the experiments are quantity of the leaf extract, incubation temperature and pH. The visual observation of the reaction mixture showed change in the colour from pale yellow to deep brown indicating synthesis of silver nanoparticles. The UV-vis absorption peaks shifted to shorter wavelengths as the quantity of the leaf extract, incubation temperature and pH was increased. FTIR analysis shows that the secondary metabolites functional groups of *C .odorata* leaf extract such as CH_3 group, ketones, aldehydes, carboxylic acids and amines bring about the fabrication of silver nanoparticles. EDS analysis supports the presence of the elemental silver, which is depicted in the graph. Zeta potential values of leaf extract quantity, incubation temperature and pH indicated that the capping molecules on the surface of AgNPs are negatively charged and the nanoparticles are moderately stable. The HRTEM micrographs showed that the size of AgNPs range from 100-10nm. Moreover the synthesized AgNPs were found to show good anti-microbial activity against *S.aureus* and *S.typhi*. The significance of employing higher plants in green nanotechnology includes less time consumption, easy to handle, economically viable, large scale production and excludes the usage of any toxic external reducing and stabilizing agents.

Keywords: *Cananga odorata,* silver nanoparticles, FTIR, HRTEM.

INTRODUCTION

The synthesis of metal nanoparticles (NPs) is a growing area of research in material science since they exhibit unique size and shape dependent characteristics different from those of bulk metals.[1,2] Use of microorganisms viz. algae, fungi, and bacteria for synthesis of nanoparticles was reported by several workers.[3] The use of green plants for the synthesis of nanoparticles laid the foundation for environmentally benign green nanotechnology and this method could be advantageous over other biological processes by eliminating the elaborate process of maintaining cell culture.[4] Recently, reports have shown that silver nanoparticles have been synthesized using various plant species such as *Ficus benghalensis, Piper longum, Cymbopogon citratus, Millingtonia hortensis, Iresine herbstii, Tinospora cordifolia, Alternanthera sessilis,* etc.[5-11] Among the other noble metal nanoparticles, silver is of particular interest due to its unique properties, such as catalytic activity, chemical stability, excellent electrical conductivity, etc. Employing higher plants in green nanotechnology involves large scale production, it is ecofriendly and excludes the usage of any external reducing and stabilizing agents.

The present investigation is undertaken to study a novel approach for the synthesis of silver nanoparticles using leaf extract of the plant *Cananga odorata* (Lam.) Hook.f. &Thomson. which belongs to family Annonaceae. *C. odorata*, also known as ylang-ylang, thrives well in tropical environments. The essential oil obtained from ylang-ylang is widely used in cosmetics and aroma therapy. It is believed to an aphrodisiac, relieves high blood pressure and normalize sebum secretion for skin problems. One of the most popular uses of *C. odorata* medicinally is in the circulatory and nervous system. The plant lowers the user's blood pressure and also slows down a rapid heartbeat, which could either be due to stress, anger, or anxiety.

MATERIALS AND METHODS

Materials

Silver nitrate ($AgNO_3$) was purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India.

Cananga odorata Leaf extract preparation

Fresh and healthy leaves of *C.odorata* were collected from the Botanical garden in Karnatak University campus, Dharwad, Karnataka, India. The leaves were thoroughly washed in the deionized water to remove any dust and dried. 5 gm of the dried and incised leaves were boiled in 100mL of deionized water for 10 minutes on water bath at 50°C. The cell walls in the leaves rupture due to boiling and release the inner cellular material into the solution. Thereafter, the resulting extract was cooled down to room temperature and filtered with whatman no.1 filter paper to obtain the filtrate. The filtrate was collected and stored in refrigerator at 4°C for further use.

Physicochemical factors used to study the effect on the reaction mixture

The effect of different leaf extract quantity (5.0, 6.0, 7.0 and 8.0 ml), incubation temperature (20°, 30°, 40° and 50°C) and pH (pH5.0, pH6.0 and pH7.0) on nanoparticle synthesis were investigated. Characterization was done using UV-Visible Spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Energy Dispersive Spectroscopy (EDS), zeta potential measurements, and High Resolution Transmission Electron Microscope (HR-TEM).

Anti-bacterial activity

The bacteria used for analysis were *Staphylococcus aureus* (Gram +ve) and *Salmonella typhi* (Gram -ve). The media used was peptone-10 g, NaCl-10 g, and Yeast extract 5g, Agar 20g in 1000 ml of distilled water.

The stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for 18 hrs.

The wells were made in the prepared agar plates of the above media.

The 18 h old cultures (100 µl, 10-4 cfu) were inoculated in each plate and spread evenly on the plate.

The test compound (20, 40, 60, 80, 100µl) and antibiotic at different concentrations were filled in the wells after 20 min.

The experiments were conducted by well diffusion method. At 37°C for 24h all the plates were incubated and the diameter of inhibition zone was measured in mm.

RESULTS AND DISCUSSION

Effect of leaf extract quantity

The experiments were conducted to study the effect of leaf extract quantity on synthesis of silver nanoparticles.

Samples were prepared by using 5.0mL, 6.0mL, 7.0 mL, and 8.0 mL of the leaf extract quantity respectively.

UV-vis spectroscopy observation peaks of leaf extract quantity at 5, 6, 7and 8ml occurred at 461nm, 459nm, 417nm and 415nm (Fig. 1).

Based on the observation of UV–VIS spectra the sharpness of the absorption peak is dependent on the quantity of leaf extract, thus being sharper with increase in the quantity of leaf extract.

Similar results were observed with the bark extract of *Cinnamomum zeylanicum* and with the leaves of *Cinnamon camphora*.[12,13]

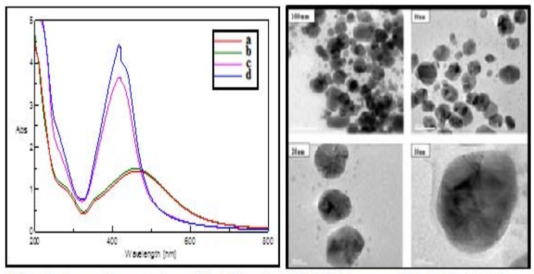
HRTEM micrographs showed that the size of AgNPs range from 100-50nm for 5 and 6ml and 20-10nm for 7 and 8ml of leaf extract quantity.

Micrograph also clearly shows that there is no agglomeration (Fig. 1).

Furthermore, it demonstrates that the AgNPs are spherical in shape.

A thin layer of organic material surrounds the nanoparticles which appear to be characteristic of AgNPs prepared with leaf extract.

These results depict that the quantity of leaf extract plays a key factor in determining the sizes of nanoparticles.



UV-vis absorption spectra of AgNPs at different leaf extract quantity (*Cananga odorata*). a) 5ml b) 6ml c)7ml d) 8ml

HRTEM images of AgNPs at different leaf extract quantity (*Cananga odorata*). a) 5ml & 6ml – 100 - 50nm b) 7ml & 8ml - 20 - 5nm.

Fig. 1

Zeta potential values of -15.4, -15.7, -16.6 and -17.1 for 5-8ml of leaf extract quantity indicates that the capping molecules on the surface of AgNPs are negatively charged and the nanoparticles are moderately stable. The interesting fact is that when the addition of leaf extract quantity was increased, more nucleation cites were induced in the colloidal solution. It is evident from the above results that the organic compounds/molecules present in the leaf extract might have been responsible for the capping and stabilization of the nanoparticles during their formation.

Effect of incubation temperature

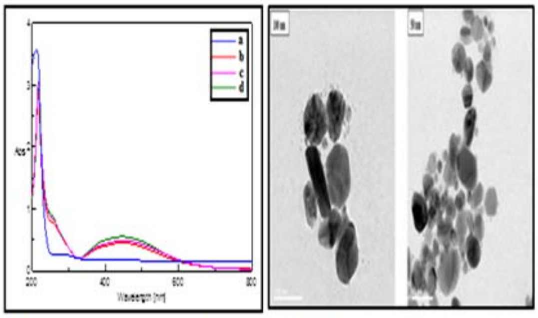
The effect of various temperatures on the formation of silver nanoparticles was investigated. UV-vis spectroscopy observation peaks of incubation temperature at 20, 30, 40 and 50°C occurred at 456 nm, 449nm, 447nm and 446nm (Fig. 2). Little variation in the wavelength of SPR band was seen when the reaction temperature was increased from 40 to 50°C. According to UV-vis spectroscopy observation, as the reaction temperature was increased from 20°C to 50°C, the SPR bands shifted to shorter wavelengths indicating a decrease in the particle size.

This data agrees with HRTEM results. HRTEM micrograph reveals that the sizes of AgNPs range from 100-50 nm at 20°C - 50°C respectively (Fig. 2). Andreescu *et al.*, reported a rapid synthesis rate of silver nanoparticles at higher temperatures.[14] Zeta potential values of -16.4, -16.7, -17.4 and -18.4 at 20, 30, 40 and 50°C indicates that the capping molecules were mainly composed of negatively charged groups which were responsible for moderate stability of nanoparticles.

Furthermore, experiments on the synthesis of silver nanoparticles in lemon verbena extracts (*Lippia citriodora*) demonstrated that increase the reaction temperature is responsible for increase in the efficiency of the silver ion reduction.[15]

Elevation in the reaction temperature causes the blocking of the secondary reduction process, due to which the reduction rate increases and thus most of the silver ions are consumed in the formation of nuclei.

Additionally, Sathis kumar *et al.,* reported an increase in surface plasmon resonance with an increase in temperature, confirming the positive correlation between the yield of the nanoparticles and the temperature.[16]



UV-vis absorption spectra of AgNPs at different incubation temperature (*Cananga odorata* leaf). a) 20 deg.C b)30 deg.C c) 40 deg.C d) 50 deg.C.

HRTEM images of AgNPs at different incubation temperature (*Cananga odorata* leaf). a) 20deg.C to 50deg.C – 100 - 50nm.

Fig. 2

Effect of pH: The experiments were conducted to study the effect on the synthesis of silver nanoparticles by varying the pH (pH5, 6 and pH7).

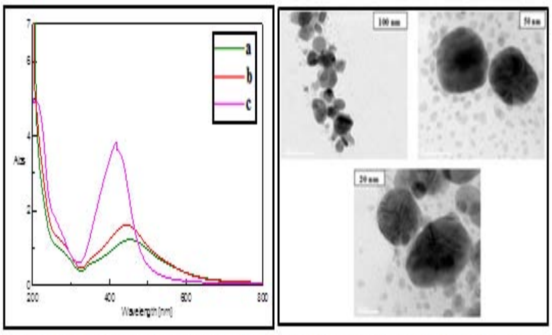
The UV–vis spectra at pH 5.0, pH6.0 and pH7.0 has shown characteristic absorption bands at 454 nm, 445nm and 418 nm respectively (Fig.3).

In general, at higher pH the absorption peaks became narrower and shift to shorter wavelength, which is due to decrease in the size of the silver nanoparticles.[17-19]

The morphology and size of the synthesized silver nanoparticles were obtained from HRTEM images (Fig. 3.)

The particles formed were spherical in shape and have a large surface area.

The HRTEM micrograph of the synthesized silver nanoparticles at pH5 and pH6 range in sizes from 100-50nm and 50-20nm at pH7 respectively (Fig. 3).



UV-vis absorption spectra of AgNPs at different pH (*Cananga odorata* leaf). a) pH5 b) pH6 c) pH7.

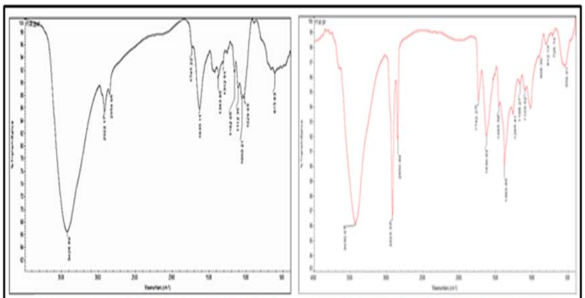
HRTEM images of AgNPs at different pH (*Cananga odorata* leaf). a) pH5 & pH6 - 100-50nm b) pH7 - 20nm.

Fig. 3

The zeta potential values are -21.7, -22.9 and -24.1 at pH5, 6 and pH7 indicating that the capping molecules were mainly negatively charged groups and are responsible for good stability of nanoparticles. Dubey *et al.,* reported that silver nanoparticles demonstrate a lower zeta potential value at strongly acidic pH compared to alkaline pH solutions, which show higher stability.[20] Andreescu *et al.,* reported that an increase in pH results in higher absolute value of the negative zeta potential, due to which highly dispersed nanoparticles are formed.[14] Recently, Veerasamy *et al.,* while working on *Mangosteen* leaf extract reported that at low pH aggregation of silver nanoparticles is favoured over the nucleation.[21] It is seen that at higher pH, a large number of functional groups are present which facilitates a higher number of Ag(I) to bind and subsequently result in high yield of nanoparticles.

Characterization of the synthesized silver nanoparticles

Fourier Transform Infrared Spectroscopy (FT-IR)



FT-IR spectra of *Cananga odorata* leaf

FT-IR spectra of silver nanoparticles

Fig. 4

FTIR measurement was carried out to identify the potential biomolecules present in *C. odorata* leaf which are responsible for the bioreduction and capping of synthesized silver nanoparticles. The FTIR spectra of untreated leaf extract and silver nanoparticles solution are shown (Fig. 4). There was a shift in the following peaks: 2854.95-2850.89, 1745.22-1742.27, 1635.11-1630.83, 1312.51-1265.41, 1112.35-1103.52, 1029.93-906.30, cm−1(Table.1). Shift in the peak 2854.95-2850.89 cm−1 is due to the asymmetric stretching of the CH3 group. Another shift in the peak from 1745.22 to1742.27 cm−1 is because of C=O stretching modes of the carbonyl functional group in ketones, aldehydes and carboxylic acids. A shift is observed from 1635.11 to 1630.83cm^{-1} due to amide I and amide II linkages of the proteins. A shift in this peak (from 1312.51-1265.41cm−1) indicated the possible involvement of C–O–H bending of carboxylic acids or in-plane O−H bending vibrations in nanoparticle synthesis. The vibration shifts of 1112.35-1103.52cm-1 and 1029.93-906.30cm-1 is probably involved in C−N or C−O stretching vibrations and C–N stretching vibrations of aliphatic amines. The biological components interact with metal ions and bring about their reduction.[22,23] The results show that reduction of silver ions to silver nanoparticles by *C. odorata* leaf extracts was done by the metabolite functional groups such as CH_3 group, ketones, aldehydes, carboxylic acids and amines (Table 1).

Table 1: FTIR measurements showing leaf extract and Silver nanoparticles

Sl.No	Absorption peak cm-1 of leaf extract	Absorption peak cm-1 of Silver nanoparticles	Functional groups
1.	2854.95	2850.89	Asymmetric stretching of the CH_3 group.
2.	1745.22	1742.27	C=O stretching modes of the carbonyl functional group in ketones, aldehydes and carboxylic acids.
3.	1635.11	1630.83	Amide I and amide II linkages of the proteins.
4.	1312.51	1265.41	C–O–H bending of carboxylic acids or in-plane O–H bending vibrations.
5.	1112.35	1103.52	C–N or C–O stretching vibrations.
6.	1029.93	906.30	C–N stretching vibrations of aliphatic amines.

Table 2: Zone of inhibition (mm) obtained by well method.

S. No	Components	Zone Of Inhibition (mm)	
		S.aureus	***S.typhi***
1	Gentamycin	13mm,18mm, 21mm, 25mm, 27mm and 34mm at 25µg, 50 µg, 100 µg, 200 µg, 400 µg and 800 µg	2mm,13mm,16mm,21mm,25mm,27mm at 25µg, 50 µg, 100 µg, 200 µg, 400 µg and 800 µg
2	Plant extract	_	_
3	Silver nanoparticles	3mm, 9mm, 12mm and 13mm at 40µl, 60µl, 80µl and 100µl	6mm, 11mm, 11mm and 12mm at 40µl, 60µl, 80µl and 100µl

Energy Dispersive X-ray Analysis (EDS)

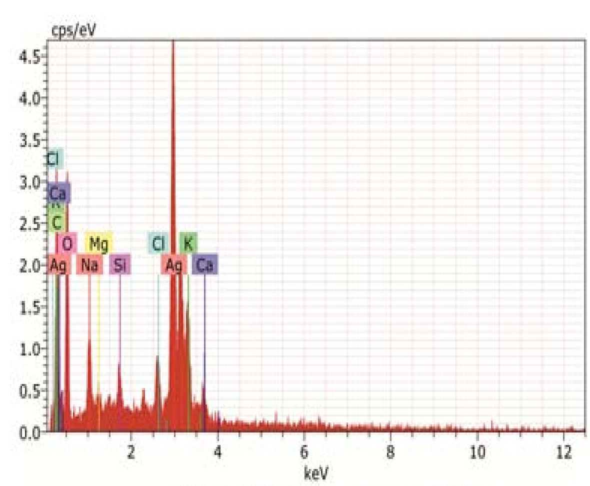


FIG. 5. EDS of silver nanoparticles

Generally metallic silver nanoparticles show typical absorption peak at 3 keV approximately due to surface plasmon resonance.[24-26] The X-ray counts are displayed in the vertical axis, while the energy in keV is displayed in horizontal axis. EDS analysis supports the presence of elemental silver, which is depicted in the graph (Fig. 5). The major emission energy lines of silver (Ag) agree with the peaks in the spectrum, which confirms the presence of silver. Strong signal energy peaks in the range of 2–4 keV signals for carbon, oxygen, chloride, calcium, magnesium, potassium, silicon and sodium, which may be due to the other biomolecules capping the nanoparticles. Among the different signals, the C and K signals were probably due to X-ray emission from proteins/enzymes/carbohydrates present in the *C. odorata* leaf extract.

Anti-bacterial analysis

The antimicrobial analysis of silver nanoparticles was carried out against both gram positive (*S. aureus*)and gram negative (*S.typhi*) bacteria and these synthesized nanoparticles exhibited good activity with both the bacteria (Table 2 and Fig 6.). The aqueous extract of *C. odorata* did not show any zone of inhibition. (Table 2 and Fig 6).

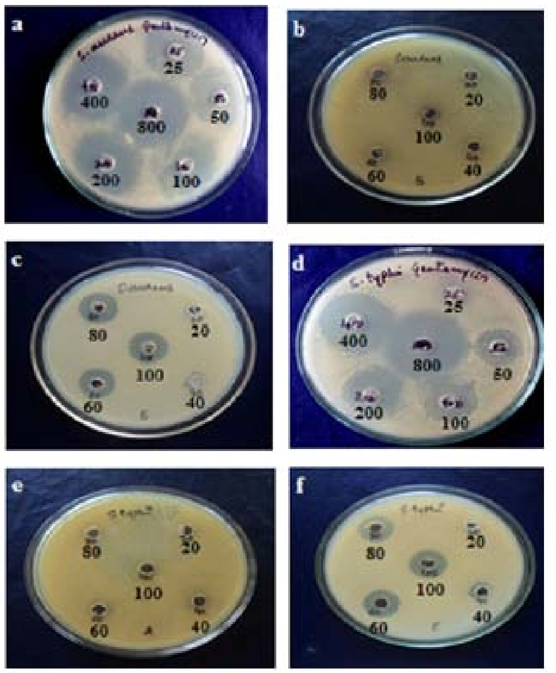


Fig. 6. Antimicrobial activities of silver nanoparticles synthesized against A. *S.aureus* (a. *Gentamycin*. b.Leaf extract c. AgNPs) B. *S.typhi* (d. Gentamycin. e. Leaf extract f.AgNPs).

CONCLUSION

Biosynthesis of silver nanoparticles using *C. odorata* leaf extract were investigated. The physico-chemical parameters on the biosynthesis of silver nanoparticles play a crucial role. The UV-vis, and HRTEM nanoparticles observations showed that there was a decrease in the nanoparticle size with increase in quantity of leaf extract, temperature and pH. Zeta potential data showed that the capping molecules were negatively charged and were moderately stable. FTIR analysis shows that the secondary metabolites functional groups of *C .odorata* leaf extract such as CH_3 group, ketones, aldehydes, carboxylic acids and amines brings about the fabrication of silver nanoparticles. EDS analysis supports the presence of the elemental silver, which is depicted in the graph. The study of AgNPs on pathogens, *S.aureus* and *S.typhi* opens the door for an efficient antibacterial activity. The above study demonstrates a rapid green chemistry approach for the synthesis of silver nanoparticles by using *C. odorata* leaf extract, which provides an eco-friendly, cost effective, simple, and an efficient way for the fabrication of silver nanoparticles.

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