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RESEARCH ARTICLE

Synthesis, Characterization, Antibacterial and Antifungal studies of Silver Nanoparticles from *Ocimum gratissimum L.*

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Abstract:
A vital need in the field of nanotechnology is the development of reliable and eco-friendly processes for synthesis of metallic nanoparticles. This present study revealed the green synthesis of Silver nanoparticles from the leaf extract of *Ocimum gratissimum* L. The nanoparticles synthesis was confirmed by colour change from yellow to dark brown. The Surface Plasmon absorption bands at different time intervals 10, 30 and 60 min were recorded at 421 nm, 428 nm and 439 nm respectively. Further the nanoparticles were characterized by Scanning Electron Microscopy, Energy dispersive x-ray analysis and Fourier Transform Infra Red spectroscopy. The antibacterial activity of Silver nanoparticles was evaluated against *Escherichia coli* and *Staphylococcus aureus*. The antifungal activity of Silver nanoparticles was evaluated against *Aspergillus niger*. Extracellular synthesis of Silver nanoparticles using *Ocimum gratissimum* leaf extract is conventional, eco-friendly and cost effective.

Keywords: *Ocimum gratissimum,* Silver nanoparticles, Antibacterial activity, Antifungal activity.

Introduction

Nanotechnology has grown to be an important research field with tremendous applications for society, industry and medicine. Several chemicals used in the synthesis of nanoparticles are toxic which leads to environmental pollution[1]. Therefore biological sources can be an alternative for the synthesis of nanoparticles[2, 3, 4]. Plants are the richest bioresources of drugs in traditional and modern medicine[5]. Silver nanoparticles have wide range of applications such as catalysis [6], drug delivery[7], biosensing[8, 9] and optics[10]. Biogenic path of nanoparticles synthesis using microorganisms[11-13], enzymes [14] and plant extracts[15-20] were suggested as possible eco - friendly alternatives to chemical and physical methods. Here we report an in expensive and green method for the synthesis of Silver nanoparticles by reduction process using *Ocimum gratissimum* (Figure 1), an herbaceous plant belongs to the family Lamiaceae. It has many vernacular names, the most commonly used ones are being Vriddhu tulsi in Sanskrit, Ram tulsi in Hindi, Nimma tulsi in Kannada, Lavanga tulsi in Telugu and Clove Basil in English[21]. The medicinal value of the plant lies in its phytochemical composition. The most important of these phytochemicals are alkaloids, tannins, steroids, triterpenoids, flavonoids, pentoses, hexoses, lipids, carbohydrates and phenolic compounds[22]. The essential oil of *Ocimum* consists of compounds such as euginole, methyleugenol, cis-ocimene, trans-ocimene, pinene, camphor, germacrene-D, trans-carypophyllene, farnesene, 1-bisabolene and gratissimol[23]. These essential oils are being used as pharmaceutical agents because of their antimicrobial, antifungal, insecticidal, analgesic, antimalarial, cytotoxic, Leishmanicidal activity, antioxidant, antidiarrhoeal, antimutagenic, Nematicidal activity, anti-inflammatory, antihypertensive and anticonvulsant activity[24]. Herein we report the Synthesis, characterization, antibacterial and antifungal studies of Silver nanoparticles first time from *Ocimum gratissimum* L.

2. Materials and Methods

Collection of plant material

Ocimum gratissimum leaves were collected from green house garden, Kodad, Nalagonda district, Telangana State, India. The plant was identified by the Plant systemic laboratory, Department of Botany, Kakatiya University, Warangal, Telangana State, India, and the herbarium sheet was preserved in the Department as a record. Voucher specimen accession number given to *Ocimum gratissimum* L. is **KUW1893**.

Preparation of leaf extract

Fresh plant leaves were collected and washed several times with tap water and later with deionised water. 10 grams of washed fine cut leaves along with 100 ml double distilled water were taken in 250 ml glass beaker and boiled for 5 minutes at 90^{o}C. The extract was cooled to room temperature and filtered with Whatman No.1 filter paper. The filtrate was centrifuged for 10 minutes at 10000 rpm the supernatant was collected and stored at 4^{o}C.

Preparation of 1 mM $AgNO_3$ solution

Accurate concentration of 1 mM $AgNO_3$ (Merck India Ltd) was prepared by dissolving 0.169 gm $AgNO_3$ in 1000 ml double distilled water and stored in Amber coloured bottle to avoid auto oxidation of Silver.

Bio synthesis of Silver nanoparticles

In the single step green synthesis, 5 ml of leaf extract was added to 95 ml of 1 mM aqueous $AgNO_3$ solution and heated up to 90^{o}C for 5 minutes, the immediate colour change indicate the formation of Silver nanoparticles. The Silver nanoparticles solution thus obtained was purified by repeated centrifugation at 10000 rpm for 15 minutes. The supernatant was transferred to a clean dry beaker for further settlement of particles and repeated centrifugation was carried using cooling microfuge to get dried and purified Silver nanoparticles. The particles obtained were used for further characterization.

3. Characterization

UV –Visible spectra analysis

Synthesized silver nanoparticles were initially characterized by taking small aliquot of sample in to UV –Visible spectrophotometer absorption spectra at 300-700 nm using Shimadzu UV -1800 Spectrophotometer.

SEM analysis

Scanning Electron Microscopic (SEM) analysis was carried by using Zeiss, EV-18 model.

EDS Analysis

Energy Dispersive X-ray analysis (EDS) was carried on Zeiss, EV-18 model. The peaks obtained from EDS were analyzed for the element composition of the sample.

FTIR- Spectroscopy

Fourier-transform infra red spectroscopy Bruker Tensor 27 model was used for the analysis of the reduced Silver. The spectrum was recorded in mid-IR region of 400-4000 cm^{-1} with 16 scan speed, using attenuated total reflectance (ATR) technique.

Preliminary screening of Phytochemicals

About 10 grams of washed fine cut leaves were soaked in 100 ml of deionised water and boiled for 5 minutes to obtain crude extract. Phytochemical screening was carried employing standard procedures.

4. Results and Discussion

The present study emphasizes the use of *Ocimum gratissimum* for the Synthesis of Silver nanoparticles with potent antibacterial and antifungal effects. Extract from this plant may act as reducing and capping agent in Silver nanoparticles synthesis. Studies have indicated that biomolecules like proteins, phenols, and flavonoids not only play a role in reducing the ions to the nano size, but also play an important role in the capping of the nanoparticles[25, 26]. The nanoparticles were preliminarily characterized by UV-Visible Spectroscopy, which is proved to be a very useful technique for the analysis of nanoparticles. As the leaf extract was mixed with the aqueous solution of the Silver ion complex it was changed from yellow to brown colour due to excitation of the surface plasma vibrations indicating the formation of the Silver nanoparticles[27]. UV-Visible Spectrograph of Silver nanoparticles has been recorded as a function of time by using quartz cuvette with distilled water as the reference. The reaction between 95 ml Silver Nitrate solution and 5 ml leaf extract was carried at 90^{o}C, the colour change was observed at different time intervals 10, 30, 60 min (Figure 2).The UV absorption spectrum is recorded at 421 nm, 428 nm and 439nm respectively.
The SEM images exhibit the formation of porous surface with spherical nanoparticles and are clearly distinguishable in 55.08- 77.90 nm size (Figure 3).

The EDS spectra revealed the purity of the material and the complete chemical composition of synthesized Silver nanoparticles. In the present synthesis EDS analysis (Figure 4) shows 99% of Silver indicating the purity of the synthesized sample.

The FTIR spectrum of Silver nanoparticles is presented in Figure5. The band at 3369cm^{-1} is assigned to the O-H stretching of H-bonded alcohols and phenols. The band at 3023 cm^{-1}is assigned to C-H stretching of aromatic rings. The band at 2971 cm^{-1}related to O-H stretching of carboxylic acids. The long sharp peak at 1740 cm^{-1}represents the C=O bond of esters, carboxylic acids and carbonyl compounds. The band at 1559 cm^{-1} corresponds to the C=C of aromatic ring. The strong sharp bands at 1371 cm^{-1} are related to the C-N stretching of aromatic

amine group. The medium band at 1222cm^{-1}represents the C-N amines and amides group. Where as in the region 1090cm^{-1}are corresponding to the C-C stretching of alcohols, carboxylic acids, ethers and esters are binding to Silver to form Silver nanoparticles are confirmed.

Phytochemical screening was carried employing standard procedures[28-30]. Natural chemical groups such as amino acids, proteins, carbohydrates, flavonoids, sterols, terpenoids and phenolic compounds were identified (Table No. 1). Tharanathan *et al* reported the presence of alkaloids, tannins, steroids, triterpenoids, flavonoids, pentoses, hexoses, lipids, carbohydrates and phenolic compounds in *Ocimum gratissimum*[22]. FT-IR predicts the molecular configuration of different functional groups present in the extract. Considerable absorption peaks are found at 3369 cm^{-1}, 3023 cm^{-1}, 2971cm^{-1}, 1740 cm^{-1}, 1559 cm^{-1}, 1371 cm^{-1}, 1222 cm^{-1}, 1090 cm^{-1} respectively (Figure 5). In the present study, the peaks are more characteristic of eugenol, methyleugenol and gratissimol.

Antibacterial activity

The biologically synthesized Silver nanoparticles exhibited excellent antibacterial activity against the bacterial pathogens *Escherichia coli* and *Staphylococci auerus* (Figure6). It has been reported that antibacterial effects were size and dose dependent and were more pronounced against Gram-negative bacteria than Gram-positive bacteria[31, 32]. The zone of inhibition is higher in the case of *E.coli* followed by *Staphylococci* when compared with standard streptomycin. The zone of inhibition was measured with transparent ruler in millimeter and compared with the standard antibiotic streptomycin. The experiments were repeated thrice and mean values of inhibition zone diameters were considered.

Antifungal activity

The Silver nanoparticles from *Ocimum gratissimum* posses moderate antifungal activity against *Aspergillus niger* (Figure 7). The leaves of *O. gratissimum* are used in the treatment of fungal infections and as a disinfectant[33]. Experiments were performed in triplicate and mean value of zone of inhibition was considered.

5. Conclusions

The bio reduced Silver nanoparticles were characterized using UV-Vis, SEM, FTIR techniques. The formation of Silver nanoparticles was confirmed by the colour change within 30 minutes. UV-Visible Spectra which were recorded after the completion of the reaction at different time intervals 10, 30, 60 min at 90^{o}C temperature. The UV absorption spectrum was recorded at 427 nm, 429 nm and 432 nm respectively. The preliminary phytochemical analysis of leaf extract revealed the presence of amino acids, carbohydrates, flavonoids, sterols, terpenoids, proteins, and phenolic compounds. FT-IR predicts the molecular configuration of different functional groups present in the extract. The SEM image shows the formation of porous surface with spherical nanoparticles of sizes between 55.08 to 77.90 nm.

Silver nanoparticles synthesized from leaf extract exhibited antibacterial activity against *Escherichia coli* and *Staphylococci auerus* bacterial strains. Further, *O. gratissimum* exhibited strong effect against the tested fungus *Aspergillus niger.* Thus it was proved from this study that the Silver nanoparticles synthesized from leaf extract seem to be having promising antibacterial and antifungal activity. Plant extract being very eco-friendly and cost effective can be used for the large scale synthesis of Silver nanoparticles in nanotechnology processing industries.

6. Acknowledgements

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Figure 1: *Ocimum gratissimum L.*plant

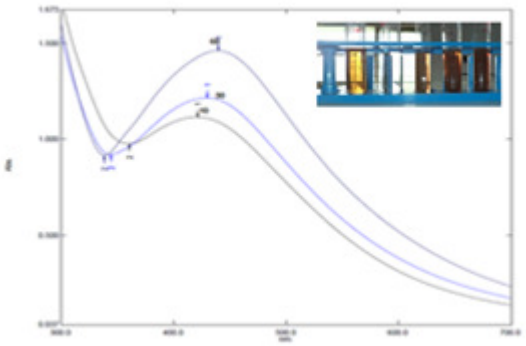


Figure 2:UV-Visible absorption spectrum of *Ocimum gratissimum*

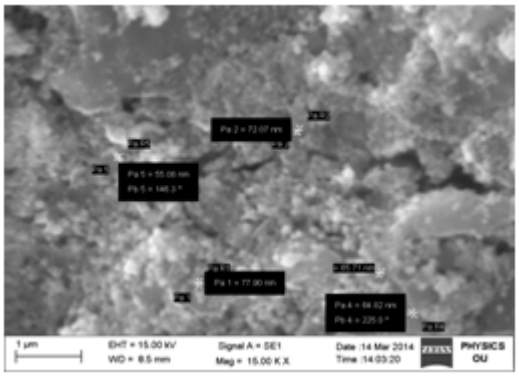


Figure 3: SEM image showing the size of silver nanoparticles of *Ocimum gratissimum*

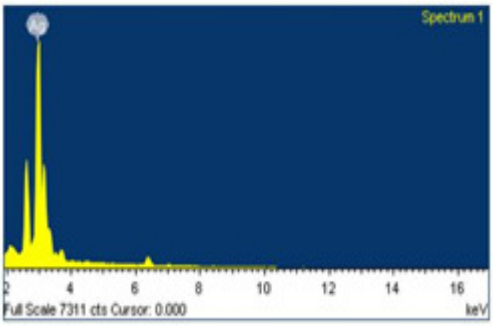


Figure 4: EDS image of silver nanoparticles produced from *Ocimum gratissimum*

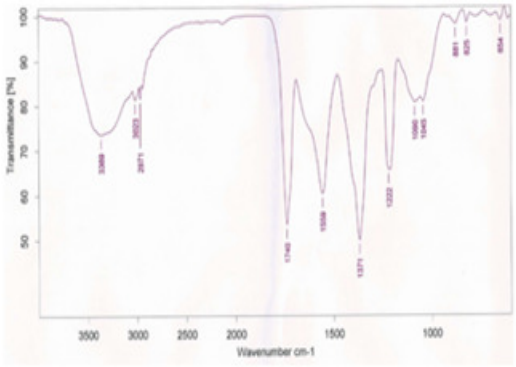
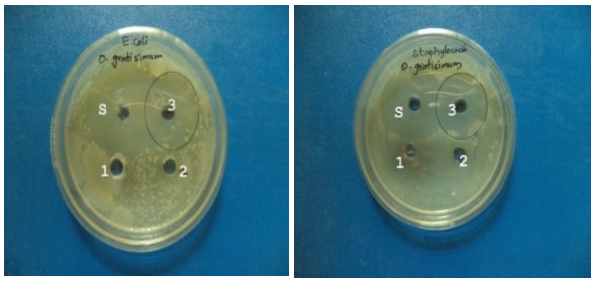


Figure 5: FTIR-spectrum of bio synthesized silver nanoparticles formed by *Ocimum gratissimum*.



(A) **(B)**

Figure 6:Zone of inhibition of Silver nanoparticles against bacterial pathogens
(A)*E. coli*. (B) *S. aureus*

Figure 7: Zone of inhibition of Silver nanoparticles against Plant fungus *A. niger.*

Table No.1 Preliminary Screening of Phytochemicals

S. No	Phytoconstituents	Leaf
1	Alkaloids	Present
2	Carbohydrates	Present
3	Steroids	Present
4	Protein and amino acids	Present
5	Tannins	Absent
6	Phenolic compounds	Present
7	Flavonoids	Present
8	Gums and mucilage	Absent
9	Glycosides	Absent
10	Saponins	Absent
11	Terpenoid	Present

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