BIOSYNTHESIS OF PLANT MEDIATED SILVER NANOPARTICLES USING THE LEAF EXTRACT OF *Ocimum sanctum* AND EVALUATION OF ANTIMICROBIAL ACTIVITY

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Abstract

Biological synthesis of nanoparticles has attracted attention due to this inherent advantages over the physical and chemical methods of nanoparticle synthesis. The greener way of the attributes synthesis. It is to avoid use of the hazardous chemicals and their subsequent effects. In this investigation, leaf extract of *Ocimum sanctum* has been used to develop safe, reliable and eco-friendly process for the synthesis of silver nanoparticles. This approach showed that the leaf extract of *O.sanctum* reduced the silver nitrate and ultimately to silver nanoparticles. The generated silver nanoparticles were characterized by using UV–Vis spectroscopy, Transmission electron microscopy (TEM) and Fourier transform spectroscopy (FTIR). The formation of silver nanoparticles was confirmed by UV-Vis spectrophotometer. The UV-Vis spectra showed peak at 350.45 nm. The size, morphology and concentration were characterized by Scanning Electron microscopy (SEM). TEM image showed that generated nanoparticles were spherical in shape with size range of 7-18 nm. The silver nanoparticles revealed antimicrobial activity against *Pseudomonas aeruginosa, Staphylococcus aureus* , *Candida albicans* and *Candida glabrata*, which was assessed by well diffusion method

Keywords: Silver nanoparticles, antimicrobial activity, biological synthesis, Characterization

Introduction

Nanotechnology is an important field of modern research that deals with design, synthesis, and manipulation of particles structure ranging from approximately 1-100 nm in one dimension. Remarkable growth in this up-and-coming technology has opened novel fundamental and applied frontiers, including the synthesis of nanoscale materials and exploration or utilization of their exotic physicochemical and optoelectronic properties. Nanotechnology is rapidly gaining importance in a number of areas such as health care, cosmetics, food and feed, environmental health, mechanics, optics, biomedical sciences, chemical industries, electronics, space industries, drug-gene delivery, energy science, optoelectronics, catalysis, reprography, single electron transistors, light emitters, nonlinear optical devices, and photo electrochemical applications [1,2]

Nanoparticles can be synthesized using various approaches including chemical, physical and biological. 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-ecofriendly byproducts. The need for environmental non-toxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as by products. Thus, there is an increasing demand for "green nanotechnology" [3]. Many biological approaches for both extracellular and intracellular nanoparticles synthesis have been reported till date using microorganisms including bacteria, fungi and plants [4,5] *O.sanctum* (Tulsi) is a medicinal herb abundantly found and cultured in India, Malaysia, Australia, WestAfrica and some of the Arab countries. In the present study an attempt has been made to combine the inherent antimicrobial activities of silver metal and Tulsi extract for enhanced antimicrobial

activity. Chewing of tulsi leaves also cures ulcers and infections of mouth [6]. They have exhibited many pharmacological activities such as antimicrobial activity such as antifungal activity, antiviral activity, wound healing effect, antigenotoxic effect and antioxidant activity. Recent studies show that it is also helpful in inhibiting the growth of HIV and carcinogenic cells [7].

Hence, in the present article deals with the role of *O.sanctum* was chosen in the synthesis of silver nanoparticles and the antimicrobial activity against the pathogenic microorganisms such as *Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans* and *Candida glabrata.*

Materials and Methods

Preparation of plant extract

The collected plant leaves were washed thrice in sterile distilled water to remove adhering soil particles and salts. The washed samples were shade dried for one week at room temperature. The leaves were cut in to small pieces and grinded into powder. The pure plant extract was prepared by adding 10 gm of plant powder in to 100ml of distilled water and boiled for 5 minutes. The boiled extract was filtered through Whatmann No.1 filter paper and the supernatant was used.

Synthesis of silver nanoparticles

In the typical process of synthesis of silver nanoparticles, 10 ml of pure plant extract was added into 90 ml of 1mM of silver nitrate solution in 250 ml of conical flask.The reaction mixture was kept at room temperature under mechanical stirring. The colour change was recorded and the formation of nanoparticles was monitored.

UV- Vis spectroscopy analysis of AgNPs

Synthesis of silver nanoparticles was determined by using ultraviolet–visible (UV–Vis) spectroscopy. The reduction of the Ag+ ions in solution was monitored by periodic sampling of aqueous component and measuring the UV- spectra of the solution. UV-spectra of these aliquots were monitored as a function of reaction on a spectrophotometer (UV-1800 series).

FT-IR analysis of AgNPs

Possible functional group involved in the synthesis and stabilization of silver NPs was studied by FTIR spectroscopy. The FTIR was recorded in the range of 500-4500 cm^{-1} and the various modes of vibrations were identified and assigned to determine the different functional groups present in the leaf extract of *O. sanctum.*

Scanning Electron Microscopy(SEM)

SEM analysis was done using JEOL JSM-6610 LV SEM machine. Thin films of the sample was prepared on a carbon coated platinum grid by adjusting the dropping of a very small amount of the sample on the grid and the extra solution was removed using a blotting paper. The film on the SEM grid was dried under a mercury lamp for 5 min. The thin film on grid was examined using Scanning Electron Microscope.

Transmission Electron Microscopy(TEM)

TEM analysis of the silver nanoparticles were made by coating them over copper grids and micrographs were obtained using CM 200 FEG Phillips transmission electron microscope. The morphology of nanoparticle was determined. The average particle size was calculated.

Antimicrobial Activity

Pathogens

Staphylococcus aureus NCIM 5021, Pseudomonas aeruginosa NCIM 5029, Candida albicans NCIM 3471and *Candida glabrata* NCIM 3236 *were* obtained from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India-411008.

Well diffusion method

The antibacterial potentialities of the nanoparticles were determined by agar well diffusion method using Mueller Hinton agar medium. The pathogens were activated by inoculating a loopful of the strain in the nutrient broth (20 ml) in a 100 ml Erlenmeyer flask and incubated at 37°C on a rotary shaker for 24 h. Then 0.1 ml of fresh inoculum (containing around 1-2 × 10^6CFU / ml as per McFarland standards) was spread onto the surface of sterile Mueller Hinton agar using a sterilized glass spreader. Wells were made on the seeded plates with the help of a sterilized cork borer (8mm Hi-Media). The collected different

samples were further dissolved in sterile water. The diluted samples (100µl of 10mg/ml concentration) were dispended into the well and the plates were incubated aerobically at 37°C for bacteria. In the same way a positive control wells were made with only Ampilicilin (10µg). The entire microbial assay was carried out under strict aseptic conditions. The zones of inhibition (mm) of the samples were examined after 24 h [8].

Anticandidal activity

Opportunistic pathogenic yeast *C. albicans,* and *C. glabrata* inocula were prepared from 18 h cultures. Yeast inocula were spread on Petri dishes containing Sabouraud dextrose agar. Wells were made on the seeded plates with the help of a sterilized cork borer (8mm Hi-Media). The samples were dissolved in sterile water. The prepared samples were dispended into the well and the plates were incubated aerobically at 37°C for fungus and for 24-42 hrs. In the same way a positive control was made with only miconazole (30µg) respectively. The zone of inhibition (ZOI) was measured after incubation [9].

Results and Discussion

Visible Observation of silver nanoparticles

Colour change from light pale to brown was observed on mixing of leaves extract of *Ocimum sanctum* with 3 mM silver nitrate solution (Fig.1). The visible colour change indicates the formation of silver nanoparticles. This could be due to the as a result of reduction of $AgNO_3$ and stimulation of surface plasmon resonance. No precipitation was observed. The colour change was stable even after completion of the reaction.

Figure 1: Synthesis of Silver Nanoparticles

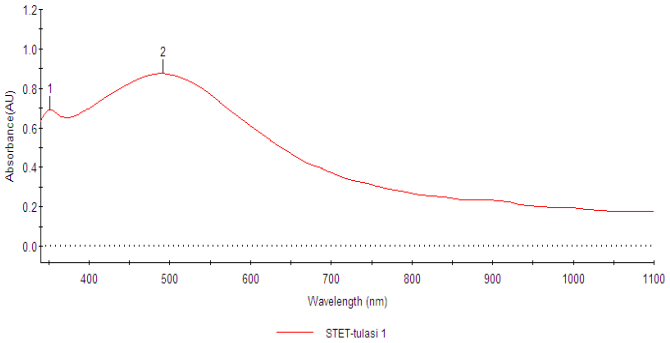
UV-Vis Spectrophotometer analysis

The UV-Vis spectroscopy is one of the most widely used simple and sensitive technique for the analysis of nanoparticle synthesis. The colour exhibited by the samples is due to the excitation of electrons of the transition metals which affects the absorbance in the ultraviolet region. The silver nanoparticles synthesized by *O.sanctum* leaf extract were confirmed by using UV-Vis spectrophotometer, which showed the peak at 350.45 nm that corresponds to the absorbance of silver nanoparticles (Table 1 and Figure 2).

Table 1: UV-Vis peak value of synthesis of silver nanoparticles using leaf extract of *O.sanctum*

WAVELENGTH(NM)	ABSORBANCE(AU)
350.45	0.6906
490.55	0.8753

Figure 2: UV-Vis spectrophotometer analysis of silver nanoparticles generated in the presence of the leaf extract of *Ocimum sanctum*



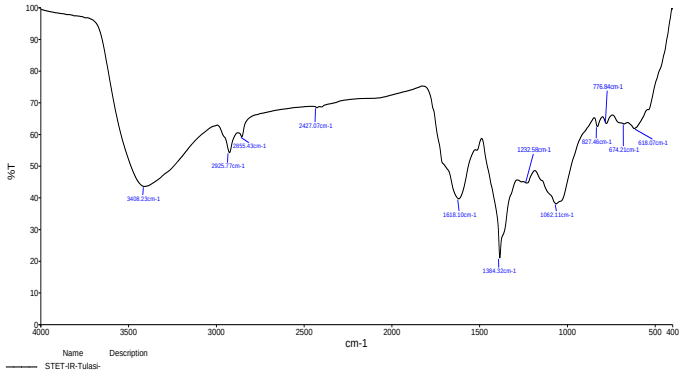
FT-IR

FT-IR analysis of silver nanoparticles synthesized by using leaves extract of *Ocimum sanctum* showed that the nanoparticle possessed definite surface morphology. FTIR spectrum revealed prominent bands at 3408.23 cm-1, 2925.77 cm-1, 2855.43 cm-1, 1618.10 cm-1, 1384.32 cm-1, 1232.58 cm-1, 1062.11 cm-1 and 776.84cm-1, 674.21 cm-1, 618.07 cm-1. These groups could be responsible for synthesis and stabilization of silver nanoparticles (Table 2 and Figure 3).

Table 2: FT-IR peak value of synthesis on silver nanoparticles of leaf extract of *ocimum sanctum*

S. NO.	GROUP OF FREQUENCY (CM^{-1}) OF THE SAMPLE	BOND	FUNCTIONAL ASSIGNMENT
1.	3408.23 CM^{-1}	N-H	Amine
2.	2925.77 CM^{-1}	-C-H(stretch)	Alkanes
3.	2855.43 CM^{-1}	-C-H (stretch)	Alkanes
4.	2427.07 CM^{-1}	UNKNOWN	-
5.	1618.10 CM^{-1}	C=C	Alkene
6.	1384.32 CM^{-1}	CH_3 (bend)	Alkanes
7.	1232.58 CM^{-1}	C-O-C (stretch)	Alcohols
8.	1062.11 CM^{-1}	C-O-C (stretch)	Alcohols
9.	827.46 CM^{-1}	UNKNOWN	-
10.	776.84 CM^{-1}	C-Cl	Alkyl Halide
11.	674.21 CM^{-1}	C-Cl	Alkyl Halide
12.	618.07 CM^{-1}	UNKNOWN	-

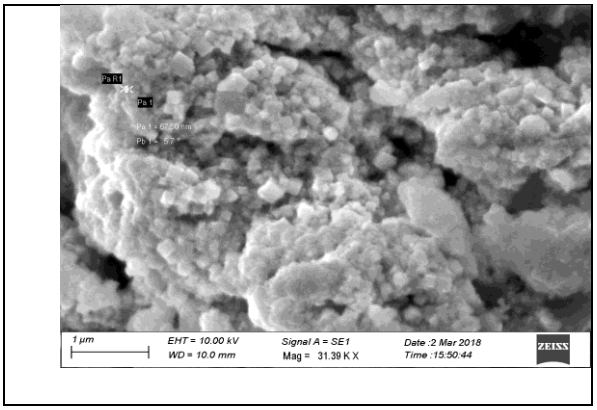
Figure 3: FT-IR analysis of generated silver nanoparticles by *Ocimum sanctum leaves* extract



Scanning Electron Microscopy (SEM) Analysis

Figure 4 shows representative SEM images recorded from drop-coated films of the silver nanoparticles synthesized by treating silver nitrate solution with leaves extract of *O.sanctum*. The silver nanoparticles formed were predominantly tubular and cubical with uniform shape and the similar phenomenon was reported by Chandran et *al.*(2006)[10].

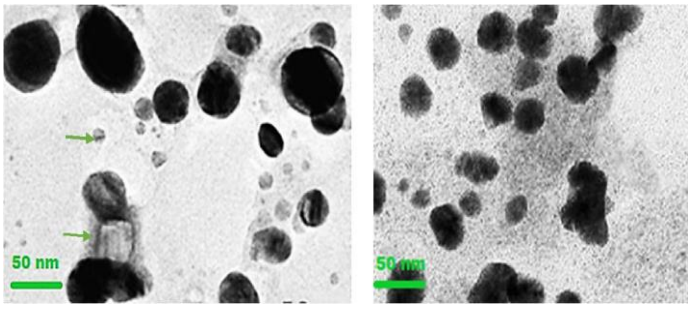
Figure 4: SEM image of synthesis of silver nanoparticles from leaf extract of Ocimum *sanctum*



Transmission electron microscopy (TEM) analysis

The unique morphology and size distribution of the prepared nanoparticles were elucidated by using Transmission electron microscopy. Figure-5 exhibits the TEM micrograph of the prepared silver nanoparticles at magnification 14 nm. It was observed that the generated silver nanoparticles were spherical in shape with size range from 7 to 18 nm.

Figure 5: TEM image of synthesis on silver nanoparticles from leaf extract of *Ocimum sanctum*



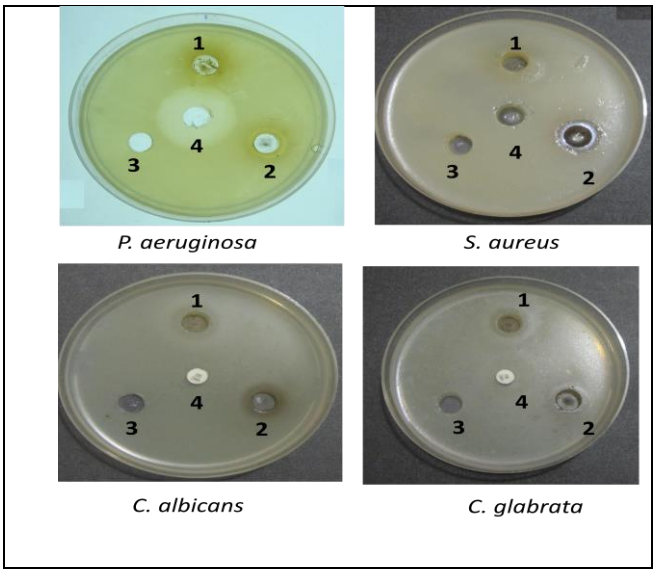
Antimicrobial activity

The antimicrobial activity of the silver nanoparticles was evaluated against pathogenic microorganisms such as *Pseudomonas aeruginosa*,staphylococcus *aureus, Candida albicans, and Candida glabrata* using well diffusion method. The antimicrobial activity was evaluated by measuring the zone of inhibition. Results in Table 3 and plate 1 clearly indicate the greater zone of inhibition with increasing concentration of silver nanoparticles. It has been observed that the generated silver nanoparticles were able to inhibit growth of *P.aeruginosa, S.aureus* and *C.albicans* and *C. glabrata*. However, as compared to *Pseudomonas aeruginosa, Staphylococcus aureus* greater inhibition was observed in fungal isolate *Candida albicans, Candida glabrata* at both the concentrations (10µg and 30µg). The results indicate that silver nanoparticles synthesized from leaf extract of *O.sanctum* showed potential antimicrobial activity against the *C. albicans*.

Table 3: Antimicrobial activity of *Ocimum sanctum* AgNPs on Pathogenic Microbes

Pathogens	Zone of inhibition (mm)			
	Ocimum sanctum		Standard antibiotic	
	Plant sample (10 µg)	AgNPs (100 µg)	Ampilicilin (10 µg)	Miconazole (30 µg)
Bacteria strain				
P. aeruginosa	9.5±0.5	11.3±1.6	25.1±1	NA
S. aureus	8.8±0.7	10.5±1.3	13.5±0.5	NA
Fungal strain				
C. albicans	8±1	11±1	NA	7.8±0.7
C. glabrata	7.8±0.7	11.3±1.15	NA	7.5±0.8

Plate 1: Antimicrobial activity of Sample *Ocimum sanctum* and its Silver Nanoparticles



1. Sample 2. Nano particle 3. Control well 4. Positive control

Conclusion

The green approach for the synthesis of AgNps and CuONPs using plant material as reducing and capping agent has many advantages such as ease with which the process can be scaled up, economic viability, environmently benign and renewable and there is no need to use high pressure, energy, temperature and toxic chemicals. It is concluded that the green synthesized AgNPs using the leaf extract of *O.sanctum* has antimicrobial potential and it can be used effectively as a bactericidal and fungicidal agent.

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