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Biosynthesis and Characterization of Silver Nanoparticles from Leaf Extracts of *Ocimum sanctum* and Its Anti-Microbial Activities

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Abstract

The field of nanotechnology is one of the most active researches nowadays in modern material science and technology. Eco-friendly methods of green mediated synthesis of nanoparticles are the present research in the limb of nanotechnology. The aim of the present study was to synthesize silver nanoparticles from 1mM $AgNO_3$ solution through aqueous leaf extract of *Ocimum sanctum* reducing as well as capping agent. Silver nanoparticle which has promising antimicrobial properties. Green synthesised silver nanoparticles showed zone of inhibition against isolated gram positive (*Bacillus* species and *Staphylococcus* species) and gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacterial and fungal strain (*Fusarium* species, *Penicillium* species and *Aspergillus species*). The nanoparticles were characterized by Scanning Electron Microscopy. Different reducing and capping agent was characterised by various phytochemical tests.

Keywords
Nanoparticles, *Ocimum sanctum,* Antimicrobial activity

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INTRODUCTION

The field of nanotechnology is one of the most active researches nowadays in modern material science and technology. Nanoparticles are fundamental building blocks of nanotechnology. The most important and distinct property of nanoparticles is their exhibit larger surface area to volume ratio [1]. Physical and chemical methods are more popular for nanoparticle synthesis but the use of toxic compounds limits their application [2] and also not economically feasible one. An array of physical, chemical and biological methods has been used for synthesis of noble metal nanoparticles of particular shape and size for various applications, but they remain expensive and involve the use of hazardous chemicals [3].

An eco-friendly green mediated synthesis of inorganic nanoparticle is a fast-growing research in the limb of nanotechnology [4]. The techniques for obtaining nanoparticles using naturally occurring reagents such as sugars, biodegradable polymers (chitosan, etc.), plant extracts, and microorganisms as reductants and capping agents could be considered attractive for nanotechnology [5]. Greener synthesis of nanoparticles also provides advancement over other methods as they are simple, one step, cost-effective, environment friendly and relatively reproducible and often results in more stable materials [6]. Medicinal plants contain some organic compounds which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [7].

Ocimum sanctum, a wild herbaceous plant is very common in all tropical countries, including India. The stems are slender and often reddish in color, covered with yellowish bristly hairs especially in the younger parts. The leaves are oppositely arranged, lanceolate and are usually greenish or reddish, underneath measuring about 5 cm long. The stem and leaves produce white or milky juice when cut [8].

Recently, Singhal *et al*., in 2011, synthesized silver nanoparticles using *Ocimum sanctum* leaf extract which showed significant antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* [9].

MATERIALS AND METHODS

Sample collection

The leaves of Tulsi plant was collected from local areas of Valsad District, Gujarat state, India. The fresh leaves were collected in polyethylene zipper bags, later washed two times with distilled water. The plants materials were thoroughly washed with distilled water and weight were determined.

Extraction Method

Tulsi leaf extract was prepared with 10 g of fresh leaves taken in separate beakers. It was thoroughly washed with tap water and then with distilled water for at least 2 times and cut into small pieces. The chopped leaves were boiled in 100ml of distilled water for 15 minutes. The leaf broth was then cooled and filtered. Filter by whatmann No.1 filter paper. It was then stored at 4°C after covering the beaker with aluminium foil for further use. The obtained leaf extracts which appeared light green in colour was stored at 4°C for further use [9].

Synthesis of Sliver Nanoparticles

Stock solution was prepared by dissolving 1mM sliver nitrate and volume made up to 100 ml with distilled water. 5ml of Tulsi leaf extract was added to 95 ml of 1mM $AgNO_3$ solution and allowed to react at room temperature in dark for 24 hours. After 24 hours, the reduction of Ag+ to Ag^o was confirmed by the colour change of solution from colourless to brown. After that extract was stored at 4^{o}C for further use [9].

Characterization of silver nanoparticles

Determination of phytochemicals: Chemical tests were carried out on the aqueous extracts to identify the constituents using standard procedures as described by [10].

- **Test for tannins:** About 2 ml of the aqueous extract was stirred with 2 ml of distilled water and few drops of $FeCl_3$ solution were added. The formation of a green precipitate was an indication for the presence of tannins.
- **Test for saponins:** 5ml of aqueous extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication for the presence of saponins.
- **Test for flavonoids:** To 1 ml of aqueous extract was added 1 ml of 10% lead acetate solution. The formation of a yellow precipitate was taken as a positive test for flavonoids.
- **Test for terpenoids:** 2 ml of the organic extract was dissolved in 2 ml of chloroform. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. A greyish colour indicates the presence of terpenoids.

Determination of SEM analysis

The nanoparticles which we have synthesized are well dispersed. We can pellet it down at 7000 rpm for 15 minutes for 2 times. Whatever pellet we were get, just resuspended it in 50% acetone in milli Q water and repeat the centrifugation and dry it in desiccator. That's how we were get crystalline silver nanoparticles.

SEM- EDX Analysis was carried out in instrument JSM 6390 with acceleration voltage 20 kV. SEM reveals information about the sample including external morphology, chemical composition and crystalline structure and orientation of materials making up the sample. SEM provides detailed high-resolution images of the sample by rastering a focused electron beam across the surface and detecting secondary or back

scattered electron signal. The EDX spectrum of the silver nanoparticles was performed to confirm the presence of elemental silver signal and provides quantitative compositional information [11].

Determination of antimicrobial activity

- **Test microorganisms:** The microbial strain studied were gram positive (*Bacillus* and *Staphylococcus* species) and gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacterial and fungal strain (*Fusarium* species*, Penicillium* species and *Aspergillus* species). These strains were obtained from Laboratory of Microbiology Department of Dolat Usha Institute of Applied Sciences and Dhiru Sarla Institute of Management & Commerce, Valsad. Microorganisms were maintained on nutrient agar slants at 4°C and sub-cultured every month.
- **Preparation of inoculums:** Each organism was recovered for testing by sub-culturing on fresh Nutrient agar and Sabouraud dextrose agar medium. A loopful of inoculum of each bacteria and fungi were suspended in nutrient broth and incubated overnight at 37°C and were used for further studies.
- **Antibacterial activity:** Antimicrobial activity of aqueous extract was determined by using agar well diffusion method [12]. In this technique about 15ml of sterile melted and cool nutrient agar was used and to it 1ml of inoculums were added, mixed well and poured into sterile petri dishes. Agar plates were allowed to solidify at room temperature. Further plates were divided into three parts and with the help of sterile cup-borer 6mm three well were made at the centre of different parts in plate. The various samples were carefully placed in the wells and further were subjected to diffusion for 1 hour at 4°C. The plates were then incubated at 37°C for 24 hours for antimicrobial activity study. Next day the plates were observed for microbial zone of inhibition around the environmental samples present in wells. The zones were measured with the help of zone meter.

Antifungal activity:

Antifungal activities of the synthesized silver nanoparticles were determined, using the agar well diffusion assay method (NCCLS, 2000). Approximately 20 ml of molten and cooled media (SDA) was poured in sterilized Petri dishes. The plates were left overnight at room temperature to check for any contamination to appear. The fungal test organisms were grown in dextrose broth for 24 h. A 100 ml Sabouraud dextrose broth culture of each fungal organism was used to prepare fungal lawns. Agar wells of 5 mm diameter were prepared with the help of a sterilized stainless-steel cork borer. Three wells were prepared in the agar plates. The wells were loaded with 50µl of silver nanoparticles, 50µl of leaf extract and 50µl of silver nitrate. The plates containing the fungal and silver nanoparticles were incubated at 37°C. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the wells [13]. The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter.

RESULTS AND DISCUSSION

Biosynthesis of silver nanoparticles from Tulsi plant extracts.

In this study, silver nanoparticles were synthesized using Tulsi leaf extract under static condition. It is well known that silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. As the extract was mixed in the aqueous solution of the silver ion complex, it started to change the colour from colourless to yellowish brown due to reduction of silver ion which indicated formation of silver nanoparticles (Figure 1).

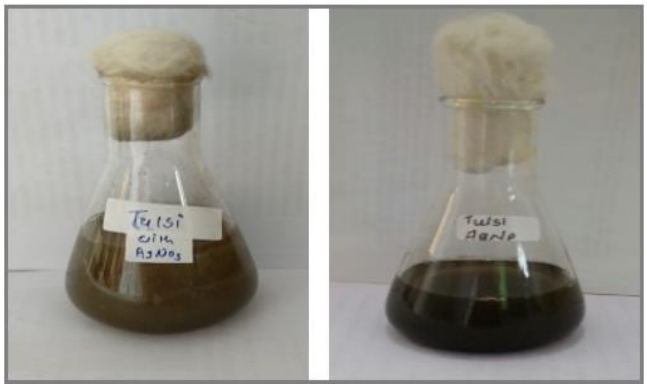

Figure 1: Synthesis of silver nanoparticles from Green Tulsi and Purple Tulsi leaves Extracts

Phytochemical analysis of Green and Purple Tulsi leaf extracts

The phytochemical characteristics of the medicinal plants investigated that are summarized in Table 1.

Table 1: Phytochemical screening from Tulsi plant.

Phytochemicals	Observation	Green Tulsi	Purple Tulsi
Flavonoid	Yellow color precipitate	+	+
Tanin	Green color precipitate	-	+
Terpenoid	Greyish color ring	+	+
Saponin	Presence of foam	-	+

The results revealed that the medicinally active constituents such as flavonoid, tannin, terpenoids and saponins were present in Purple Tulsi leaf extract. While tannin and saponins were absent in Green Tulsi leaf extract.

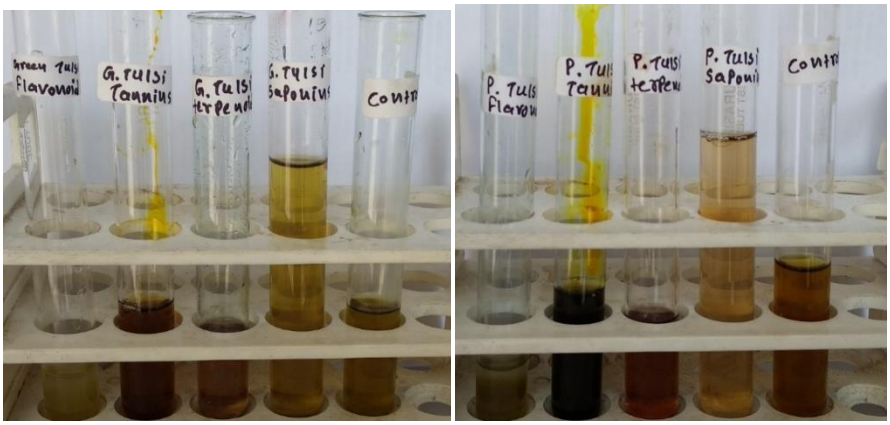

Figure 2: Phytochemical tests of green and purple Tulsi leaf extract

SEM analysis of silver nanoparticles from Tulsi leaf extract

Scanning electron microscopy provided the morphology and size details of the silver nanoparticles. It was identified that shapes of silver nanoparticles appeared like spherical shapes with rough surface. This indicates that the mono dispersive and crystalline silver nanoparticles are obtained. The elemental analysis of the silver nanoparticles was studied using Energy-dispersive microanalysis (**Figure 3**).

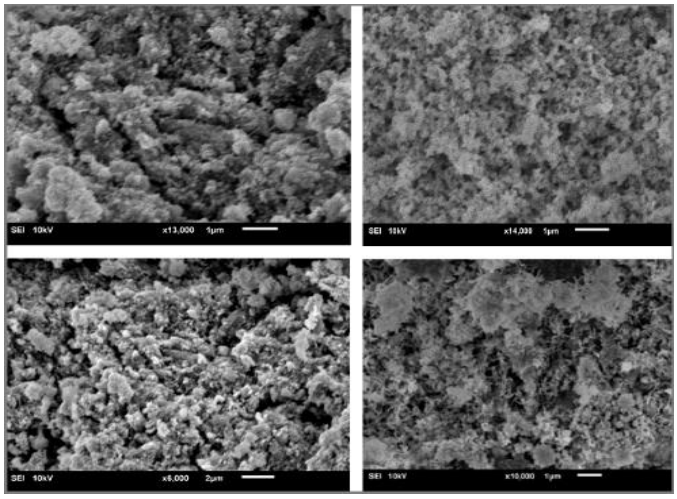

Figure 3: SEM images of silver nanoparticle synthesized from Tulsi leaf

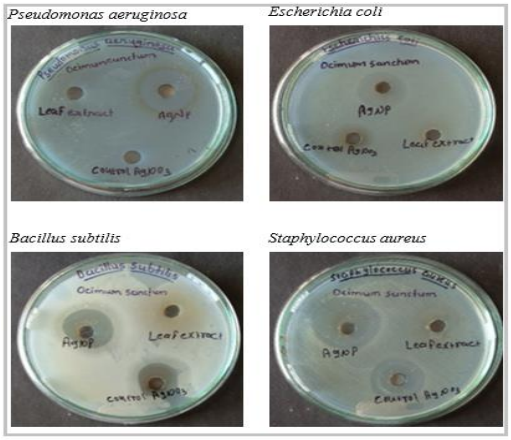

Figure 4: Antibacterial activity of AgNPs from Purple Tulsi leaf

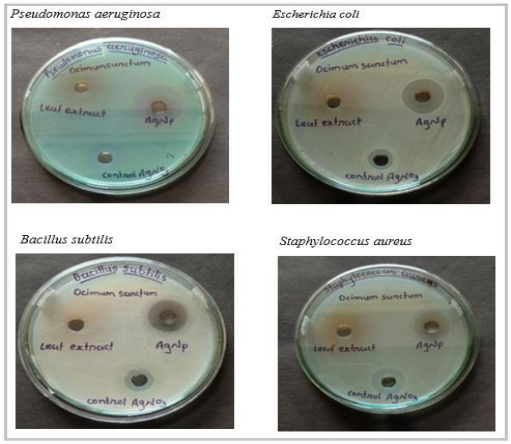

Figure 5: Antibacterial activity ofAgNPs from Green Tulsi leaf.

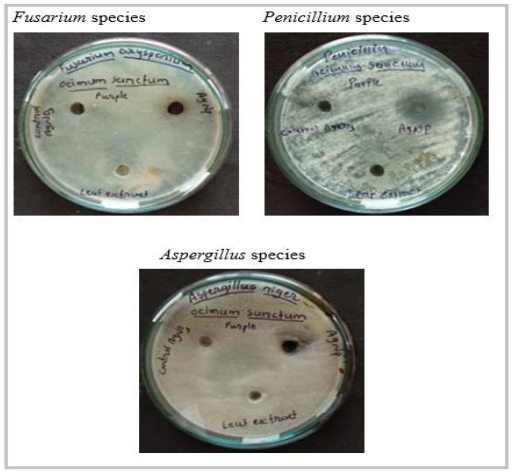

Figure 6: Antifungal activity of AgNPs from Purple Tulsi leaf

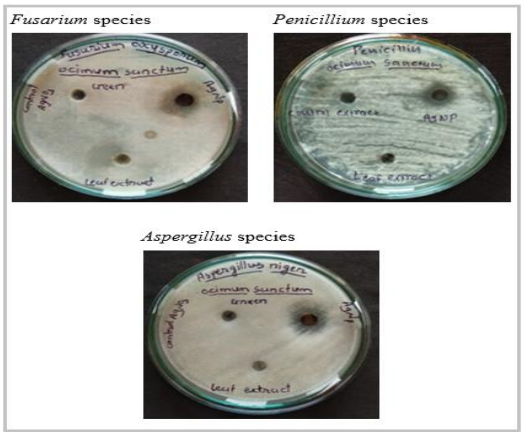

Figure 7: Antifungal activity of AgNPs from Green Tulsi leaf

The analysis revealed highest proportion of silver in the nanoparticles followed by carbon, silicon, oxygen. The SEM image showed relatively spherical shape nanoparticles. Similar phenomenon was also reported by Bauer *et* al., in1966 [13].

Anti-Bacterial Activity of Tulsi plant.

Results of anti-bacterial activity were shown in Table 2.

Table 2: Anti-Bacterial Activity of purple Tulsi leaf extract

Bacterial strain	Leaf extract (mm)	Silver nitrate (mm)	Silver nanoparticles (mm)
Pseudomonas aeruginosa	-	-	25
Escherichia coli	-	19	21
Bacillus subtilis	-	18	23
Staphylococcus aureus	-	17	30

Zone of inhibition were observed with the purple leaf extract, silver nitrate (control) and silver nanoparticles against the two Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). The anti-bacterial activity of silver nanoparticles showed more inhibition than that of plant extracts (control). For silver nanoparticles, zone of inhibition was found to be 25 mm, 21 mm, 23 mm and 30 mm against *Pseudomonas aeruginosa, Escherichia coli, Bacillus* species *Staphylococcus aureus* respectively. The highest zone of inhibition has reported by the synthesized silver nanoparticles against *Staphylococcus aureus*. On the other hand, plant extract did not exhibit any antibacterial activity.

The results of anti-bacterial activity were shown in Table 3.

Table 3: Antibacterial activity of AgNPs from Green Tulsi leaf.

Bacterial strain	Leaf extract (mm)	Silver nitrate (mm)	Silver nanoparticles (mm)
Pseudomonas aeruginosa	-	16	25
Escherichia coli	-	13	21
Bacillus subtilis	-	14	19
Staphylococcus aureus	-	17	24

Zone of inhibition was observed with the green leaf extract, silver nitrate as a control and silver nanoparticles against the two Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). The anti-bacterial activity of silver nanoparticles showed more activity than that of plant extracts. For silver nanoparticles from Green Tulsi leaf extract the zone of inhibition was found to be 25 mm for *Pseudomonas aeruginosa*, 21 mm for *Escherichia coli,* 19 mm for Bacillus species and 24 mm for *Staphylococcus aureus*.

Based on the zone of inhibition produced, synthesized silver nanoparticles prove to exhibit good antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. On the other hand, plant extract did not exhibit any antibacterial activity.

Anti-fungal Activity of Tulsi leaf

The results of anti-fungal activity were show in Table 4.

Table 4: Anti-Fungal Activity of purple Tulsi leaf extract

Fungal strain	Leaf extract (mm)	Silver nitrate (mm)	Silver nanoparticles (mm)
Fusarium species	-	-	-
Penicillium species	-	-	13
Aspergillus species	-	-	11

Zone of inhibition was observed with the Purple leaf extract, silver nitrate as a control and silver nanoparticles against fungal strain (*Fusarium* species*, Penicillium* species and *Aspergillus* species). The anti-fungal activity of silver nanoparticles showed more activity than that of plant extracts. For silver nanoparticles from Purple Tulsi leaf extract the zone of inhibition was found to be 13 mm for *Penicillium* species, 11 mm for *Aspergillus* species and no zone were obtained for *Fusarium* species. The highest zone of inhibition has reported by the synthesized silver nanoparticles against *Penicillium* species. On the other hand, plant extract did not exhibit any antifungal activity.

The results of anti-fungal activity were show in Table 5.

Table 5: Anti-Fungal activity of AgNPs from Green Tulsi leaf.

Fungal strain	Leaf extract (mm)	Silver nitrate (mm)	Silver nanoparticles (mm)
Fusarium species	-	-	14
Penicilliums pecies	-	-	13
Aspergillus species	-	-	14

Zone of inhibition was observed with the crude extract, silver nitrate as a control and silver nanoparticles against fungal strain (*Fusarium* species*, Penicillium* species and *Aspergillus* species). The anti-fungal activity of silver nanoparticles showed more activity than that of plant extracts. For silver nanoparticles from Green Tulsi leaf extract the zone of inhibition was found to be 14 mm for *Fusarium* species, 13 mm for *Penicillium* species, 14 mm for *Aspergillus* species.

The highest zone of inhibition has reported by the synthesized silver nanoparticles against *Fusarium* species. On the other hand, plant extract did not exhibit any antifungal activity.

CONCLUSION

A simple, fast and economical biological procedure was introduced to synthesize silver nanoparticles by using *Ocimum sanctum* L. leaf extract. The extract of *Ocimum sanctum* L. has a reducing and capping agent that can identified by phytochemical test. The spectroscopic characterization of silver particles by SEM supports the stability of the biosynthesized nanoparticles.

The antibacterial and antifungal activities of the nanoparticles have been evaluated. Our current study revealed that the SNPs of purple Tulsi have more effective against microorganisms than green Tulsi. The synthesized SNPs showed significant antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, whereas less activity against *Escherichia coli* was observed. The SNPs of green Tulsi has more effective against fungi than purple Tulsi. The antifungal activity of SNPs showed significant activity against *Fusarium oxysporum* and *Aspergillus niger.* Whereas less activity was observed against *Penicillium* species.

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