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Green synthesis of silver nanoparticles using *Ocimum sanctum* leaves and their anti-bacterial activity.

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Abstract: In the present study, silver (Ag) nanoparticles (NPs) were synthesized from aqueous leaf extracts of *Oscimum sanctum* and investigated for antibacterial potential. The spectrum of UV–Visible of the prepared Ag-NPs showed an absorption peak at 415 nm due to the excitation of surface plasmon resonance (SPR). The result of antibacterial activities showed that biosynthesized Ag-NPs had an inhibiting activity against *E. coli* bacteria, where the inhibition zone was maximum (2.85 cm) at 25 ppm concentration and minimum zone of inhibition was (1.6 cm) at 10 ppm concentration of Ag-NPs.

Keywords: Silver nanoparticles (Ag-NPs), leaf extract, UV analysis, *Oscimum sanctum*, zone of inhibition.

1.Introduction

Nanotechnology has emerged as a transformative field, offering innovative solutions in medicine, electronics, agriculture, and environmental science. Nanoparticles (NP) which are 1-100 nanometers in size, among the various nanoparticles, **silver nanoparticles (AgNPs)** have garnered significant interest due to their exceptional antimicrobial, antioxidant, and catalytic properties. Silver is a non-toxic, safe inorganic antibacterial agent that can eliminate approximately 650 types of disease-causing microorganisms (Jeong, Yeo, & Yi, 2005). AgNPs have special properties that make them useful in areas like biomedicle (Chaloupka, K, et al.,2010) drug delivery (Prow, T.W et al 2011), water cleaning (Dankovich, T.A. and Gray, D.G. 2011), and farming (Nair, R., et al, 2010)."Silver nanoparticles can attach to bacterial cell walls, affecting their ability to function and breathe. They can also enter the cells and harm essential molecules like DNA and proteins. Moreover, their ability to release silver ions may also help kill bacteria (Pallab et al., 2008). The silver nanoparticles showed efficient antimicrobial property compared to other due to their extremely large surface area providing better contact with cell wall of microorganisms (Ibrahim, 2015). The conventional methods of AgNP synthesis often involve hazardous chemicals and energy-intensive processes. In response to growing environmental concerns, the green synthesis approach has been developed as an eco-friendly, cost-effective, and sustainable alternative. Green synthesis involves the use of biological materials—such as plant extracts, bacteria, fungi, or algae—as reducing and stabilizing agents for the fabrication of nanoparticles. This method eliminates the need for toxic chemical reducers and stabilizers, making it an environmentally benign alternative. Plant-mediated synthesis of nanoparticles offers several advantages: it is simple, scalable, and leverages the rich phytochemistry of plants to control the size and shape of the nanoparticles. There are various studies on the synthesis of nanoparticles through the green route using parts of plant extracts such as tea leaves, the stem bark of *Callicarpa maingayi*, *Terminalia chebula*, *Papaver somniferum*, and *Aloe vera*. Shinde and Patil (2024) synthesized the AgNPs from *Ficus religiose* bark extract.

Ocimum sanctum (Tulsi) is an aromatic, erect, and branched herb or subshrub, typically growing 30-75 cm tall. It features hairy stems and simple, opposite leaves that are typically green or purple, and strongly scented. The small, purplish flowers are arranged in elongated, terminal racemes. The root system is fibrous and forms the main part of the plant. Its fruit consists of subglobose nutlets that are pale brown or reddish with black marks. The plant known for its medicinal properties and used for treating cough, coryza, hay asthma, bronchial infection, worm infections, kidney stones etc. Plant is rich in bioactive compounds such as **eugenol, flavonoids, terpenoids, phenolic acids, and alkaloids**. These phytochemicals act as natural reducing agents that convert silver ions (Ag^+) into metallic silver nanoparticles (Ag^0). Additionally, these compounds also serve as capping agents, preventing agglomeration and enhancing the stability of the nanoparticles. In the present work, Ag-NPs was prepared using *Ocimum sanctum* leaf extract and studied its antibacterial activity at different concentration.

2.MATERIAL AND METHODS:

2.1 Preparation of leaf extract

For the preparation of leaf extract, fresh leaves of *Oscimum sanctum* were collected in a beaker and washed several times with sterile distilled water to clear the impurities. 10 gm washed leaves were cut into fine pieces and crushed with the help of mortar and pestle in 100 ml sterile distilled water. After grinding the aqueous extract was taken in 250 ml beaker and boiled for 10 min at 70 °C temperature. The plant extract was allowed to cool at room temperature and then filtered with whatman filter paper. The filtrate was centrifuged for 20–25 min at 10000 rpm. The final aqueous solution was collected and stored at 4 °C for nanoparticle synthesis (More and Baig, 2013).

2.2 Preparation of 0.01 M silver nitrate:

In the preparation of 0.01 M silver nitrate solution, 1.67 g of analytical grade pure silver nitrate salt was weighed using an analytic balance and was used to prepare 0.01 M of silver nitrate solution.

2.3 Synthesis of silver nanoparticles:

In the green synthesis of silver nanoparticles, 100 ml of the *Oscimum sanctum* leaf extract was added to 200 ml of AgNO3 (0.01M) solution and a spontaneous reaction was allowed. The solution was then subjected to heating and stirring at 80°C. As the reaction proceeded, it was observed that the colour change was from a clear visible solution to a dark brown solution which indicated the formation of silver nanoparticles (Khan et al. 2018). The mechanism of reduction was determined
using UV-Visible spectroscopy and then silver nanoparticles were collected after centrifugation at 2500 rpm and drying in an oven at 50°C.

2.4 Assessment of antimicrobial assay

Escherichia coli (E. coli) was chosen to evaluate the antimicrobial activity of silver nanoparticles. A pure culture of E. coli was obtained, and nutrient broth was prepared. A loopful of inoculum was taken from an LB agar plate and used to inoculate 10 mL of the nutrient broth, which was then incubated overnight at 37°C. The spread plate method was employed for culturing, and wells were created using a gel puncture technique. Different concentrations of silver nanoparticles were prepared using a standard 200 ppm solution in acetone, which was made by dissolving 02 gm of silver nanoparticles in 1000 mL of acetone. A 20 µL sample from each concentration (10 ppm, 20 ppm, and 30 ppm) was loaded into each well. The plates were then incubated for 24 hours at 29°C. The zone of inhibition was measured after the overnight incubation period.

2.5 Spectral analysis of synthesized nanoparticles: Synthesized nanoparticles will be confirmed by spectral analysis.

UV - Visible Absorption spectroscopy analysis- The absorption spectra were measured with a UV-Visible Spectrophotometer. It is the most important and simple technique to confirm the formation of nanoparticles. The maximum synthesis of Ag+ was shown where maximum peak was found (Bhimba et al. 2014).

3. Result and discussion:

3.1 Visual Examination: Figures 1 show the change in colour of reaction mixture ($AgNO_3$ solution and plant bark extract) with respect to the time. Colour changes are observed in the period from 20 min, from colorless to faint yellow, indicating the formation of (AgNPs) silver nanoparticles. As time proceeds, faint, yellow-colored solution becomes dark brown at 150 min, which is due to the increasing concentration of AgNPs as well as the growth of the number of particles. There is no significant change beyond 150 min; therefore, indicating the completion of the reduction reaction.

3.2 UV Spectroscopic Analysis- The samples were observed under UV- visual spectrophotometer for its maximum absorbance and wavelength to confirm the reduction of silver nitrate. The maximum peak was found to be 415 nm indicates the presence of silver nanoparticles that are synthesized by Oscimum sanctum leaf extract.

3.3 Antimicrobial activity- Silver nanoparticles, renowned for their antimicrobial properties, are widely used across various industries, including healthcare, medicine, textile coatings, food storage, dye reduction, wound dressing, antiseptic creams, and numerous environmental applications.

We have examined *Oscimum sanctum* leaf extract mediated silver nanoparticles as possible antibacterial agents. The plant extract and those mediated silver nanoparticles were immediately tested for antimicrobial activities showing the zones of inhibition. Based on the zone of inhibition produced, synthesized silver nanoparticles prove to exhibit good antibacterial activity against E. coli. On the other hand, control and plant extract alone did not exhibit any antibacterial activity. Although, it is to be presumed that the leaves extract of the plant used possess the antibacterial activities and must be reflected through greater inhibition zone but it alone shows very low activity due to its medium of extraction as well as lower concentration during experimentation. The results of antibacterial activities of prepared silver nanoparticles are given in Table 1.

Table 1. The antibacterial activity of AgNPs against *E. coli.*

	Component	Zone of Inhibition (in cm)		
		Replica 1	Replica 2	Mean
	Control	0	0	0
	Leaf Extract	0	0	0
	10 ppm	1.7	1.5	1.6
	15 ppm	1.8	1.9	1.85
	25 ppm	3.0	2.7	2.85

The data pertaining to the antibacterial activity of silver nanoparticles against *E. coli* bacteria indicates that the minimum zone of inhibition of *E. coli* (1.6 cm) was recorded at 10 ppm concentration of silver nanoparticles while this zone was found maximum (2.85 cm) at 25 ppm concentration of AgNPs. The zone of inhibition caused by 15 ppm concentration of nanoparticles ranges in between. Thus the maximum zone of inhibition of 2.85 cm was reported at 25 ppm concentration.

4. Conclusion:

This study describes an easy and simple method for the biosynthesis of silver nanoparticles (AgNPs) from *Ocimum sanctum* leaf extract and attempt was made to characterize biosynthesized nanoparticles and assessed their antibacterial potential at different concentration of Ag-NPs. As the concentration of Ag-NPs increases, antibacterial activity of silver nanoparticle also increased.

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