Green Synthesis and Antimicrobial Evaluation of Silver Nanoparticles Using *Ocimum sanctum* (Tulsi) Leaf Extract

Omprakash Tukaram Sangule

Associate Professor, Department of Chemistry
Smt. Kasturbai Walchand College, Sangli-416416, Maharashtra, India
Email: omsangule@rediffmail.com

Abstract:

Silver nanoparticles (AgNPs) have garnered considerable interest due to their distinctive physical, chemical, and biological properties, particularly their antimicrobial potential. In the present study, an eco-friendly green synthesis method was employed using an aqueous leaf extract of *Ocimum sanctum* (Tulsi) as both a reducing and stabilizing agent for the formation of AgNPs. The successful synthesis of AgNPs was initially indicated by a visible color change from light yellow to reddish-brown. UV-Visible spectroscopy confirmed nanoparticle formation, revealing a characteristic surface plasmon resonance (SPR) peak at 377nm. Fourier-transform infrared (FTIR) spectroscopy identified functional groups, including hydroxyl, carbonyl, and amine groups, that played key roles in the reduction and capping of the nanoparticles. The synthesized AgNPs exhibited notable antimicrobial activity against selected Gram-positive and Gram-negative bacterial strains, indicating their potential application in biomedical and pharmaceutical fields.

Keywords: Silver nanoparticles, green synthesis, *Ocimum sanctum*, antimicrobial activity, FTIR spectroscopy, Ayurvedic medicine, herbal nanotechnology

1. Introduction

Nanotechnology has rapidly emerged as a transformative field within modern science, offering innovative solutions across medicine, electronics, energy, and environmental sectors [1]. Among its various components, metal nanoparticles—especially those synthesized from noble metals such as silver, gold, platinum, and palladium—have garnered significant interest [2]. Silver nanoparticles (AgNPs), in particular, have gained prominence due to their exceptional antimicrobial, catalytic, and optical properties [6]. Their versatility has led to widespread applications in biomedicine, wound healing, water purification, textiles, and cosmetics, driving a growing demand for sustainable synthesis methods [9].

Historically, the synthesis of nanoparticles has relied on physical and chemical techniques such as sol-gel processing, chemical precipitation, hydrothermal synthesis, pyrolysis, and chemical vapor deposition [5]. While these methods provide precise control over particle size and morphology, they often involve toxic reagents, high energy consumption, and sophisticated

equipment [6]. Additionally, challenges such as nanoparticle instability, polydispersity, and high production costs limit their scalability and environmental compatibility [7]. These issues have spurred the search for alternative, greener approaches to nanoparticle fabrication [8]. In this regard, plant-based synthesis—commonly referred to as "green synthesis"—has emerged as a promising alternative [9]. This method utilizes phytochemicals present in natural extracts to reduce and stabilize metal ions, offering an eco-friendly, cost-effective, and scalable solution [10]. Ocimum sanctum (commonly known as Tulsi), a revered medicinal plant in Ayurvedic medicine, has shown considerable potential in this area [11]. Widely cultivated across India, Malaysia, Australia, and parts of Africa and the Arab world, Tulsi is renowned for its broad-spectrum antimicrobial, anti-inflammatory, and therapeutic properties [12]. The plant's bioactive constituents, particularly eugenol and other essential oils, contribute to its effectiveness against a variety of pathogens [13].

The present study aims to synthesize silver nanoparticles using an aqueous leaf extract of Ocimum sanctum. This green synthesis approach not only aligns with sustainable principles but also seeks to enhance antimicrobial efficacy by combining the inherent bioactivity of Tulsi with the antimicrobial properties of AgNPs [17].

2. Experimental Details:

2.1. Materials Required:

Fresh *Ocimum sanctum* (Tulsi) leaves were collected for the green synthesis of silver nanoparticles. The materials used included Whatman No. 1 filter paper, conical flasks, a magnetic stirrer, measuring flasks, wire gauze, a laboratory burner, and sterile Petri plates. All chemicals were of analytical grade; deionized water was used throughout the experiment, and a 1 mM aqueous solution of silver nitrate ($AgNO_3$) served as the metal precursor [9, 10].

2.2. Preparation of Leaf Extract

Fresh *Ocimum sanctum* (Tulsi) leaves were collected and thoroughly rinsed with tap water to remove dust and surface contaminants. The leaves were then finely chopped, and 10 grams of this plant material were added to a beaker containing 90 cm^3 of double-distilled water. The mixture was heated and maintained at a boiling temperature for 20 minutes to facilitate the extraction of bioactive phytochemicals [11].

After boiling, the solution was allowed to cool slightly and was initially filtered through muslin cloth to remove coarse plant residues. It was then filtered through Whatman No. 1 filter paper for finer filtration. The final volume of the filtrate was adjusted to 100 cm^3 using double-distilled water. The freshly prepared aqueous extract was stored at 4°C and used later for the green synthesis of silver nanoparticles (AgNPs) and antimicrobial evaluation [12].

2.3. Synthesis of Silver Nanoparticles (AgNPs):

To initiate the synthesis of silver nanoparticles, a 1 mM aqueous solution of silver nitrate ($AgNO_3$) was prepared using double-distilled water. This solution was mixed with the prepared leaf extract in a 1:9 volume ratio (leaf extract : silver nitrate solution). The resulting mixture was continuously stirred at 800 rpm using a magnetic stirrer for approximately one hour to facilitate the reduction process.

A visual change in the solution's color from pale yellow to a reddish-brown hue confirmed the formation of silver nanoparticles, attributed to surface plasmon resonance [13]. The formation and characterization of AgNPs were further validated using UV-Visible spectroscopy and Fourier-transform infrared (FTIR) spectroscopy [14]. Change in colour is shown in Fig.1

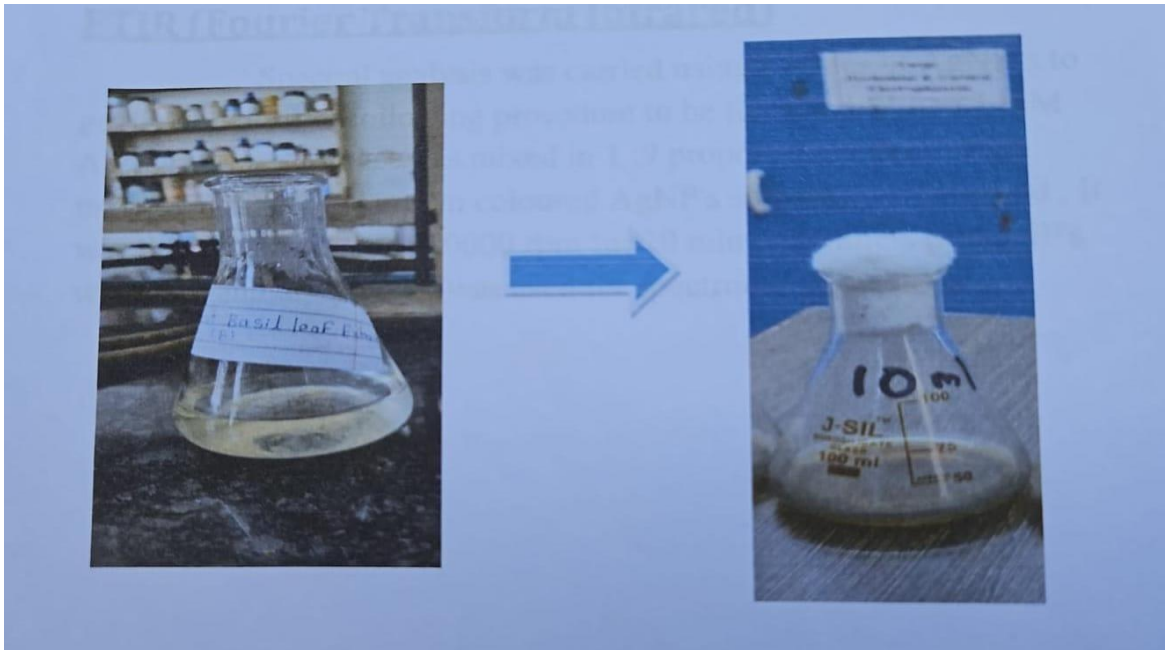

Fig.1 Change in colour from pale yellow to a reddish-brown hue.

2.4. UV-Visible Spectral Analysis:

The formation of silver nanoparticles was monitored by recording the absorption spectrum of the AgNP solution using a UV-visible spectrophotometer. The scan was conducted across a wavelength range of 200 to 800 nm, with a 1 mM deionized water solution used as a blank for baseline correction [15].

2.5. FTIR (Fourier Transform Infrared) Analysis:

FTIR spectral analysis was performed on the powdered form of the synthesized silver nanoparticles (AgNPs). To obtain the powder, the 1 mM $AgNO_3$ solution was mixed with the leaf extract in a 1:9 ratio under continuous magnetic stirring, resulting in the formation of a

red-brown AgNP solution. The mixture was then centrifuged at 10,000 rpm for 20 minutes. The collected pellet was dried, and the resulting powder was used for FTIR analysis [16].

2.6. Antimicrobial Activity:

The antimicrobial potential of the synthesized silver nanoparticles (AgNPs) was evaluated using the standard disc diffusion method against specific bacterial strains [17]. Bacterial cultures in the logarithmic phase of growth, adjusted to the MacFarland turbidity standard (approximately 10^8 CFU/mL), were uniformly spread on Mueller-Hinton Agar (MHA) plates with a thickness of 4 mm.

Sterile filter paper discs were saturated with different test substances: silver nanoparticles, Tulsi leaf extract, and silver nitrate solution. These discs were aseptically placed on the inoculated agar surfaces using sterile forceps, ensuring proper contact. A negative control was also included, consisting of a disc moistened with sterile distilled water, to validate the experiment. The plates were incubated at 37°C for 24 hours to allow bacterial growth and interaction with the compounds. After incubation, the clear zones around the discs, known as zones of inhibition, were observed and measured to evaluate antibacterial effectiveness. To ensure reliable results, incubation times were carefully controlled, avoiding both prolonged and shortened periods [17].

3. Results and Discussion:

3.1. UV-Visible Spectroscopy

The optical properties of the biosynthesized silver nanoparticles were first examined using UV-visible spectroscopy. Absorbance readings were recorded over a spectral range of 200 to 800 nm to confirm the reduction of silver ions during the nanoparticle synthesis process. The solution derived from the *Ocimum sanctum* leaf extract exhibited a distinct absorption peak at around 377 nm. This peak is commonly associated with the surface plasmon resonance (SPR) of silver nanoparticles, providing clear evidence of their successful formation [8]. Additionally, the absorption observed between 300 and 500 nm suggests the presence of phytochemicals in the leaf extract, which likely played a crucial role in reducing the silver ions to their metallic form [10]. The spectral data also showed a trend where an increase in wavelength corresponded with a decrease in absorbance intensity. This could indicate the aggregation of nanoparticles, potentially resulting in the formation of larger silver particles within the colloidal solution [12]. The UV-visible spectrum is shown in Figure 2.

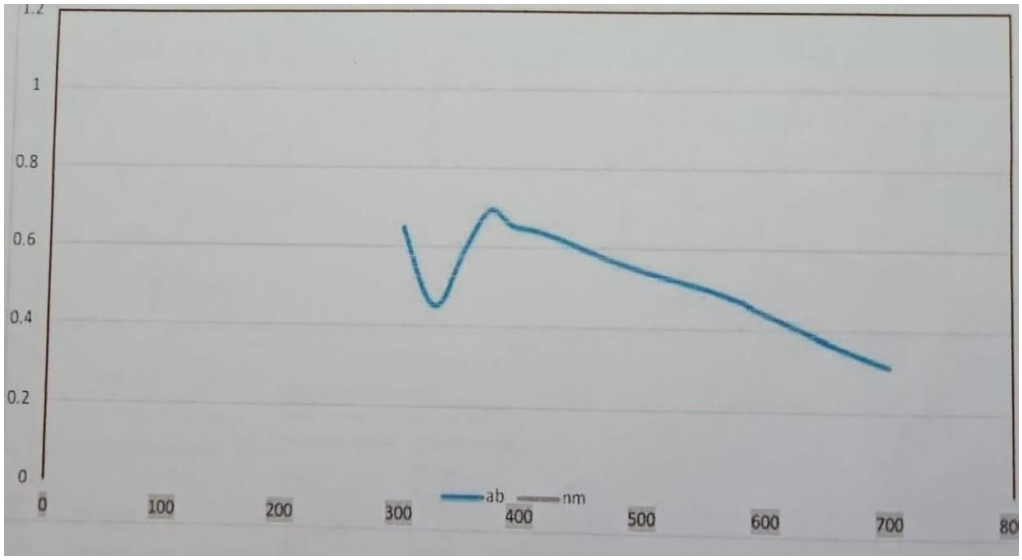

Figure 2: UV–Visible absorption spectrum of silver nanoparticles synthesized using *Ocimum sanctum* leaf extract. A distinct surface plasmon resonance (SPR) peak is observed at approximately 377 nm, confirming the formation of silver nanoparticles.

3.2. FTIR Analysis

The FTIR spectrum of silver nanoparticles (AgNPs) synthesized using basil (*Ocimum sanctum*) leaf extract exhibited characteristic peaks at 471, 600, 1122, 1383, 1623, 2924, and 3426 cm^{-1}. These absorption bands correspond to various functional groups: the peak at 471 cm^{-1} is attributed to polysulfide (S–S) stretching, while the one at 600 cm^{-1} is associated with disulfide (S–S) stretching. The band at 1122 cm^{-1} indicates C–O stretching of cyclic ethers, and the peak at 1383 cm^{-1} reflects –OH stretching vibrations of phenolic or tertiary alcohol groups. The absorption at 1623 cm^{-1} may be attributed to C=N stretching vibrations, possibly from imines or related organic nitrogenous compounds. The peaks at 2924 cm^{-1} and 3426 cm^{-1} correspond to asymmetric C–H stretching of methylene groups and hydrogen-bonded –OH stretching, respectively [11]. Medium-intensity bands observed in the spectrum are also due to –C–C– skeletal vibrations, as well as C–OH and C–O–C stretching vibrations. These spectral features confirm the presence of phytochemicals such as phenols, alcohols, ethers, and nitrates in the basil extract, which contribute significantly to both the reduction of Ag^{+} ions to metallic silver (Ag^{0}) and the stabilization of the resulting nanoparticles. Thus, the functional groups identified in the FTIR analysis play a vital role in the green synthesis and stabilization of AgNPs [13]. FTIR spectra is shown in Fig.3

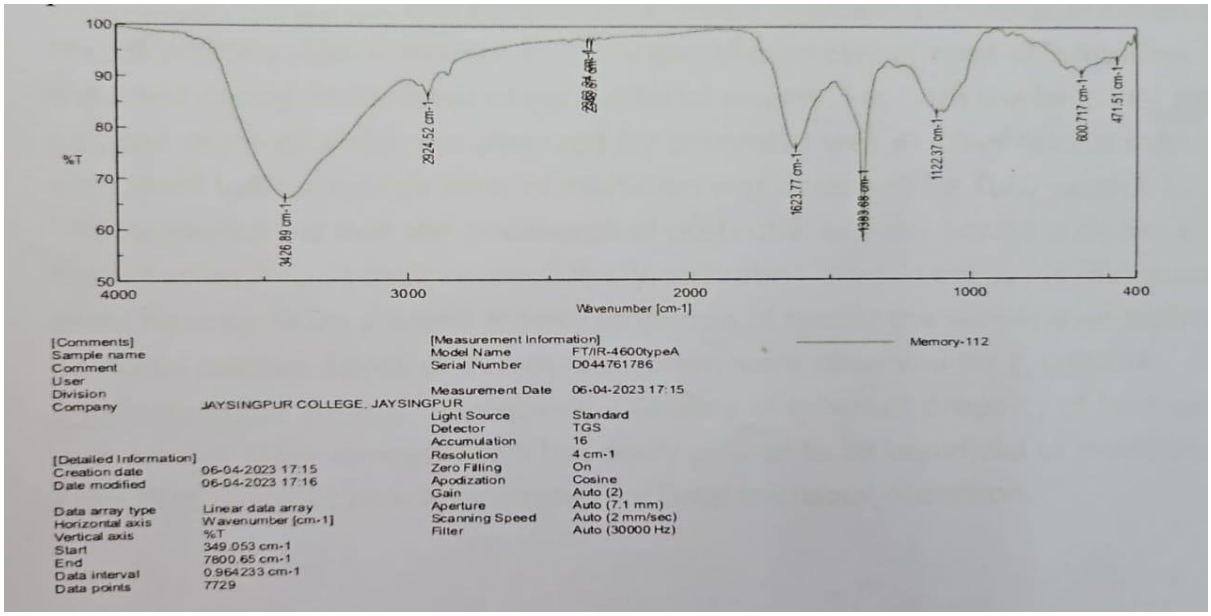

Fig.3-FTIR spectra

3.3. Microbiological Characteristics of Test Organisms

Escherichia coli (*E. coli*) is a Gram-negative bacterium characterized by a thin peptidoglycan layer that does not retain crystal violet during Gram staining. Therefore, it appears pink when counterstained with Safranin. Morphologically, *E. coli* presents as small, pink rods and is catalase-positive, producing the enzyme catalase that catalyzes the decomposition of hydrogen peroxide into water and oxygen [10]. In contrast, *Staphylococcus aureus* is a Gram-positive bacterium with a thick peptidoglycan cell wall that retains crystal violet, appearing purple-blue under the microscope. It typically forms clusters and is both catalase-positive and coagulase-positive. *S. aureus* is a common pathogen responsible for a variety of infections, including skin infections, pneumonia, and abscesses [12].

3.4. Antimicrobial Activity:

Silver is widely recognized for its potent antibacterial properties. Similarly, *Ocimum sanctum* (Tulsi) extract has been valued in traditional medicine and modern applications for its antimicrobial effects, largely attributed to its essential oil constituents and other phytochemicals. In this study, silver nanoparticles (AgNPs) synthesized using Tulsi extract were evaluated for their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The antibacterial efficacy was assessed by measuring the diameters of the inhibition zones using the disc diffusion method [14]. No inhibition was observed in the control group (distilled water) or with silver nitrate solution alone. Tulsi extract alone produced a moderate inhibition zone, reflecting its inherent antimicrobial activity. Notably, the AgNPs synthesized with Tulsi extract exhibited significantly enhanced antibacterial activity, with inhibition zones measuring 11 mm for *E. coli* and 10 mm for *Staphylococcus aureus*. These results suggest that the synergistic interaction between Tulsi phytochemicals and silver nanoparticles enhances the

antimicrobial potential, allowing for a reduced effective dose of silver and offering a safer, more efficient method of microbial control.

Comparison of antimicrobial activity in terms of zone of inhibition against *E. coli* and *S. aureus* is shown in Table1

Table 1: Diameter of Zones of Inhibition (mm) by Disc Diffusion Method

Components	***E. coli***	***Staphylococcus aureus***
Distilled water (control)	NZ	NZ
Silver nitrate solution	NZ	NZ
Tulsi extract	8	8
AgNPs	11	10

[NZ means No Zone of Inhibition]

Above Table summarizes the results of the antibacterial activity against *E. coli* and *Staphylococcus aureus*. The control (Distilled water) and silver nitrate solution showed no inhibition zones, indicating a lack of antibacterial activity under test conditions. In contrast, Tulsi extract exhibited a moderate inhibitory effect, while the biosynthesized AgNPs demonstrated significantly higher antibacterial activity. These findings clearly support the enhanced antimicrobial performance of silver nanoparticles when synthesized via green methods using plant extracts [14].

4. Conclusion:

This study presents a compelling demonstration of an eco-friendly and efficient approach to synthesizing silver nanoparticles (AgNPs) using the aqueous leaf extract of *Ocimum sanctum* (Tulsi). The FTIR spectral analysis confirmed the presence of key functional groups—such as phenols, alcohols, ethers, and nitrates—indicating the active role of phytochemicals in both reducing silver ions and stabilizing the formed nanoparticles. These findings validate the biochemical competence of Tulsi extract as a dual-functioning agent in nanoparticle synthesis [8]. The antimicrobial evaluation revealed a notable enhancement in antibacterial efficacy when silver was combined with Tulsi extract. While the extract alone showed modest inhibition zones against *Escherichia coli* and *Staphylococcus aureus*, the biosynthesized AgNPs exhibited significantly larger zones of inhibition (11 mm and 10 mm respectively). This indicates a synergistic interaction between Tulsi phytochemicals and silver nanoparticles, enhancing their antimicrobial potential and supporting the use of reduced silver concentrations for effective microbial control [13].

By integrating the bioactive potential of a time-honored medicinal plant with the advancements of nanotechnology, this work underscores a sustainable alternative to conventional nanoparticle

synthesis methods. The AgNPs derived from Tulsi not only offer environmental and economic advantages but also hold promising applications in medical therapeutics, food safety, and cosmetics. Future research can expand on this foundation to investigate long-term stability, cytotoxicity, and broad-spectrum activity of these green-synthesized nanomaterials, further cementing their place in interdisciplinary scientific and industrial applications [15].

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