Green Synthesis of Silver Nanoparticles of Ocimum *tenuiflorum L.* From Marathwada Region

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Abstract: Tulshi is worldwide used as medicinal purpose and sacred worship in temples. Tulsi is herbaceious plant belongs to family Lamiaceae. The family is rich source of secondary metabolites and chemical nutrients. It contains several vitamins and essential oils. The plants contain silver ions which is useful in chemical and nano industrial purpose. Green syenthesis of silver ions from plants is eco-friendly and cost effective. By using some characterization techniques silver nanoparticles were easily identified. Techniques like UV-Vis Spectroscopy, FTIR, X-Ray Diffractions, Dynamic light scattering, scanning electron microscope, Transmission electron microscope etc.

Keywords- Ocimum, Silver nanoparticles, Uv vis spectroscopy, FTIR, DLS, XRD etc.

I. INTRODUCTION

Ocimum tenuiflorum L., commonly known as Holy Basil or Tulsi, is a medicinal and aromatic plant belonging to the Lamiaceae (mint) family. It is native to the Indian subcontinent and widely cultivated in Southeast Asia. Key Information: Botanical Name: *Ocimum tenuiflorum L.* (synonym: *Ocimum sanctum L.*) Common Names: Holy Basil, Tulsi (India), Thai Holy Basil Family: Lamiaceae (Mint family) Native Region: India, Southeast Asia Plant Type: Perennial herb or small shrub Plant Characteristics: Leaves: Green or purple, oval-shaped, slightly serrated, and aromatic. Flowers: Small, purplish or pinkish, arranged in terminal spikes. Stem: Slightly hairy, woody at the base. Height: Grows up to 30–60 cm (12–24 inches). Aroma: Strong, clove-like fragrance. Medicinal & Health Benefits: Adaptogenic properties: Helps the body adapt to stress. Immunity booster: Rich in antioxidants and anti-inflammatory compounds. Respiratory health: Used for colds, coughs, and asthma. Antimicrobial: Effective against bacteria, viruses, and fungi. Digestive aid: Supports gut health and relieves bloating. Culinary & Traditional Uses: Tea & Infusions: Used in herbal teas for relaxation and immunity. Ayurveda: Key ingredient in Ayurvedic medicine for detoxification. Religious Significance: Considered sacred in Hinduism and often grown in homes and temples.

Photograph- 1. *Ocimum tenuiflorum L.* plant 2. *Ocimum tenuiflorum L.* inflorescences.

Nanotechnology is a fast-expanding field that focuses on creating and describing nanoparticles with sizes between one and one hundred nanometers. Due to their unique qualities and uses in biomedicine, medication delivery, cosmetics, electronics, optics, energy, and bioremediation, nanoparticles generate research interest worldwide (Abbasi et al., 2020; Velsankar et al., 2020). Nanoparticles can be produced via chemical, physical, or biological methods, each with unique benefits and limits (Khalil et al., 2021; Cai et al., 2021; Kumari et al., 2023). Among these technologies, the biological production of nanoparticles has significant benefits, mainly due to their simplicity, environmental friendliness. Hazardous and costly compounds are needed to decrease, stabilize, and cover precursor salts (Salem & Fouda, 2020). Green synthesis is gaining popularity among academics as a cost-effective and efficient technique (Mruthunjayappa et al., 2022). There is an increasing interest in using biological approaches to produce eco-friendly nanoparticles. (Akther et al., 2021). Biogenic synthesis of NPs using plants, there has significant role of phytochemicals. These phytochemicals serve as stabilizing and reducing agents. Flavonoids, phenolics, and other compounds have converted silver ions into silver nanoparticles. These phytochemicals reduce silver ions, which results in the formation of stable metal nanoparticles, and this process is the basis for the synthesis of AgNPs from plant extracts. AgNPs are primarily known for their chemical stability, electrical conductivity, and antibacterial activity, making them highly desirable for various uses in industries like environmental remediation, agriculture, and health. Due to their affordability, environmental friendliness, and scalability for large-scale production, green synthesis methods using plant extracts have drawn much attention. These approaches offer a prospective substitute for conventional chemical and physical nanoparticle synthesis methods (Islam, 2021; Shaikh et al., 2021; Pradeep et al., 2021). The unique biological applications of nanomaterials such as antibacterial, anti-inflammatory, and antioxidant properties, are making them more attractive. Considering the vital role of antioxidants in preventing diseases linked to oxidative stress, researchers are now interested in the antioxidant capabilities of these green synthesized nanoparticles. (Pradeep et al., 2021; Lite et al., 2023; Liu et al., 2023). Plant-based silver nanoparticles have been discovered to show powerful antioxidant properties. According to several studies (Flieger et al., 2021; Mayegowda et al., 2023), these characteristics aid in mitigating oxidative stress-related ailments, including cardiovascular illnesses, autoimmune diseases, and some cancers. Nanoparticles are known for their distinct physicochemical characteristics, including shape, surface charge, and active surface groups that can interact with pathogens, green-produced AgNPs have been shown to show antibacterial activity (Singh et al., 2020). Vigorous antibacterial activity, low human toxicity, and numerous in vitro and in vivo applications are among the advantages of silver nanoparticles (Wang et al., 2017). AgNPs, due to their small size and large surface area, can readily penetrate bacterial cell walls. Silver nanoparticles (AgNPs) produced by green synthesis have demonstrated noteworthy antibacterial effectiveness against Gram-negative and Gram-positive bacteria, including those resistant to multiple drugs. Since silver nanoparticles (AgNPs) significantly inhibit Grampositive and Gram-negative bacteria and have a decreased propensity to get antibacterial resistance, they have found widespread application in various antimicrobial research domains.

(Cao et al., 2019; Abdellatif et al., 2021).

II. MATERIALS AND METHOD

Silver nanoparticles (AgNPs) can be synthesized and isolated from *Ocimum tenuiflorum L.* (holy basil) through a green synthesis method. This process involves using plant extracts as reducing and stabilizing agents to convert silver ions into nanoparticles.

1. Collection of Plant- *Ocimum tenuiflorum L.* species collected from Soneri Mahal Dr.

Babasaheb Ambedkar Marathwada University Campus Chatrapati Sambhajinagar Maharashtra. Collected species washed through tap water two times and disttiled water with one time then it dried under shade drying to make fine powder form.

2. Preparation of Plant Extract

Collected fresh *Ocimum tenuiflorum L.* leaves grinded mechanically in mixer to form fine powder. Extract was prepared for ulta-sonication method about 10 g of leaf powder in 100 mL of distilled water sonicated for 15–20 minutes. Extract was filtered using Whatman No. 1 filter paper and stored it at 4°C for further synthesis.

3. Synthesis of Silver Nanoparticles

Freshly prepared extract was added in prepared 1 mM silver nitrate ($AgNO_3$) solution. Then by using magnetic stirrer continuously stirr. After that recorded effect of time spectra 3 hrs interval upto 24 hrs. Observed that the color of solution changed from light yellow to brown shown visually indicate the formation of silver nanoparticles (AgNPs).

4. Isolation and Purification

Synthesised AgNPs solutions centrifuged the at 10,000–15,000 rpm for 15–30 minutes. Discarded the supernatant and washed the pellet with distilled water and ethanol to remove impurities. Dried the nanoparticles under vacuum or freeze-dry them for further characterization.

5. Characterization of *Ocimum tenuiflorum L.* by using AgNPs

Pure silver nanoparticles were succesfully used for characterizations techniques. Characterization of the synthesized silver nanoparticles (AgNPs) was performed using UVvisible spectroscopy, which confirmed the reduction of silver ions to nanoparticles through the analysis of the UV-visible spectra of the solutions. An ELISA Plate reader (Thermo Scientific MultiskanSky Plate Reader) was used to measure absorbance across wavelengths ranging from 300 to 700 nm. The particle size and zeta-potential of the AgNPs were figured out using a Malvern Zeta sizer Ver. 7.11 analyser. Fourier Transform Infrared (FTIR) spectroscopy was employed to analyse the functional groups present in the samples, using a wavenumber range of 4000 to 400 cm-1, with samples prepared on KBr pellets. An X-ray diffractometer was used to examine the structural characteristics of the synthesized AgNPs. The data were collected over a 2θ range. The AgNPs were subjected to air drying on a glass slide at ambient temperature before the XRD measurement. The crystallite domain size was calculated using the Scherrer formula based on the width of the XRD peaks.

$$D = 0.9\ \lambda / \beta \cos \theta$$

The equation for diffraction involves λ (x-ray wavelength) = 0.15406 nm, β (full width at half maximum), and θ (diffraction angle).

III. RESULTS AND DISCUSSION

Synthesis of Silver Nanoparticles

The reduction of Ag+ to Ag0 nanoforms, which indicates the formation of silver nanoparticles, caused by the aqueous leaf extract of *O. tenuiflorum L.* in combination with 0.01 mM AgNO3 solution to gradually change in colour after two hours, the colour of the reaction mixture turned yellow to reddish brown defines the synthesis AgNPs (Hemlata et al., 2020). The reduction of silver nitrate is caused by phytoconstituents such as alkaloids, phenols, flavonoids, and terpenoids. Plant metabolites have functional groups such as hydroxyl, carbonyl, ketone, esters, and amines (Rajan et al., 2015). In addition to proteins and other phytonutrients (Prabakar et al., 2013). The primary factors lowering the stabilizing and topping agents that contribute to the formation of nanomaterials are phenols, carbohydrates (Rautela et al., 2019), flavonoids (Raghunandan et al., 2010), essential oils, alkaloids, terpenes (Wang et al., 2017), and heterocyclic compounds such as saponins (Geethalakshmi & Sarada, 2012). Several approaches were applied to validate AgNP synthesis, including UV-visible spectroscopy further, Fourier transform infrared spectroscopy, X-ray diffraction, Dynamic Light Scattering, and scanning electron microscopy.

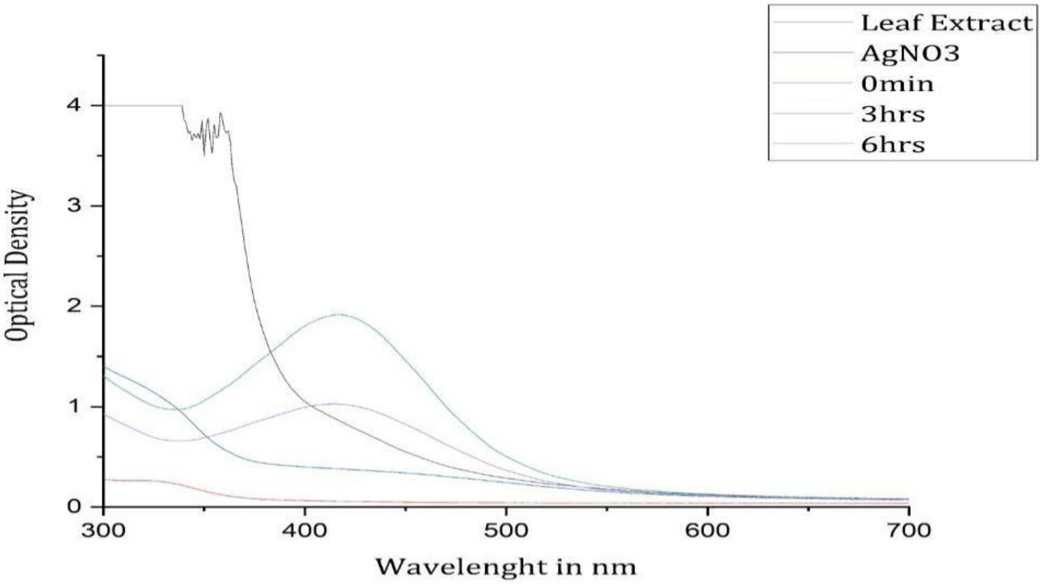

Fig.1 UV-Vis analysis of AgNPs syenthesised from *Ocimum tenuiflorum L.*leaves extract.

1. UV-visible spectroscopy.

In this investigation, as shown in Figure 1, the spectrum between 300 and 600 nm, and the solution was scanned within this range. The UV-Vis. spectrum of AgNPs revealed an intense surface plasmon resonance (SPR) peak around 435 nm. The intensity of the SPR band rose with reaction time, showing that AgNPs were successfully synthesized. After one week, the stability of the synthesized AgNPs was evaluated, and it was discovered that there was a minor increase in absorption. UV-visible spectroscopy is the principal confirmatory tool for studying nanoparticle production. The presence of AgNPs is shown by the color of the reaction mixture gradually changing from pale green towards yellowish-brown (Giri et al.,2022). Absorbance peak at 390 to 470 nm, ascribed to the silver nanoparticles. The broad spectrum of peaks shows that Ag nanoparticles were distributed uniformly (Sigamoney et al., 2016).

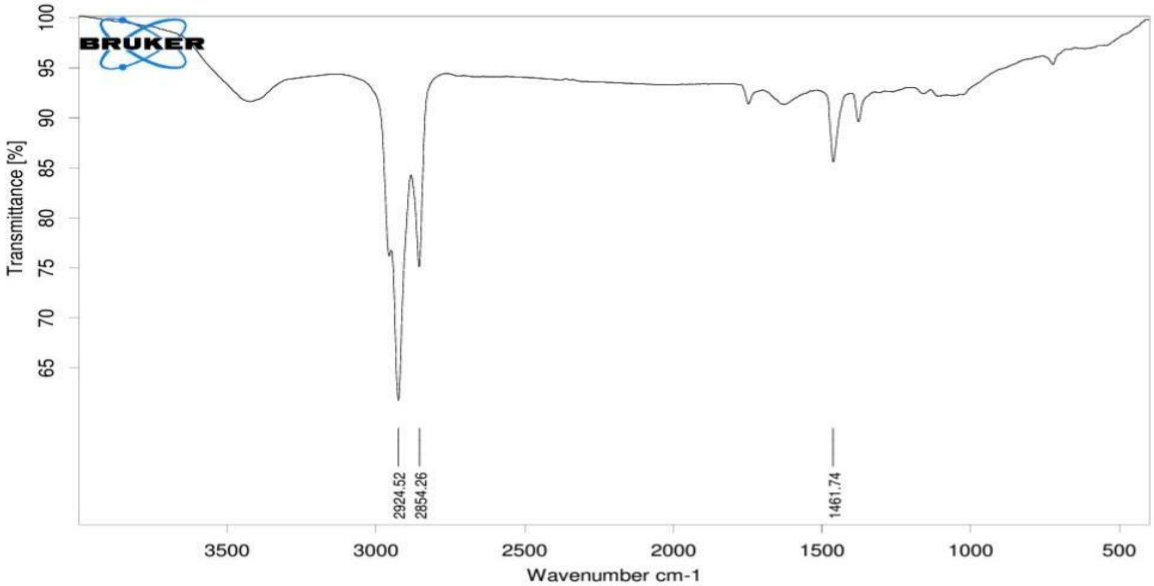

Fig.2 FTIR analysis of AgNPs syenthesised from *Ocimum tenuiflorum L.* leaves extract.

2. FTIR analysis

This study used FT-IR spectra to analyze the molecular vibrations, aura of biomolecules, and favourable functional groups to assess the effectiveness of the synthesis and stabilization of AgNPs using *O. tenuiflorum L*. leaf extract. Fig. 2. shows the FT-IR analysis for AgNPs, the *O. tenuiflorum L*. leaf extract reveals distinct bands at specific wavenumbers that suggest the existence of different functional groups. Identified were bands at 546.41 cm−1, corresponding to the stretching of C–Br in a halo compound, the bending of C=C in alkenes, and the stretching of C–Cl in another halo compound. In addition, bands were seen at 743.31 cm−1 showing the bending of C–H bonds, and bands at 1015.85 cm−1 standing for the stretching vibrations of CF bonds in a fluorine compound. In addition, the bands seen at 1308.56 cm−1 corresponded to the stretching vibrations of a Sulfone compound with S=O bonds. Various signals were detected at specific wavenumbers, showing diverse types of molecular vibrations. These included C–F stretching and O–H bending for alcohol, N–O stretching for nitro compound, C=C stretching for conjugated alkene, C-H bending for aromatic compound, and C–H bond vibration for atmospheric CO2. Additionally, significant signals were observed for stretching vibrations of O–H, C–H, and N–H bonds. There were noticeable bands at 546.41 cm−1 in the spectrum, indicating the occurrence of C–Cl stretching vibrations in a halo compound (Kumar & Yadav, 2008; Geethalakshmi & Sarada, 2012; Rajan et al., 2015; Sigamoney et al., 2016; Liaqat et al., 2022; Sarvalkar et al., 2023). Overview of the characteristic bands and their corresponding assignments, which can aid in comprehending and interpreting the FT-IR spectra findings for AgNPs and *O. tenuiflorum L*.leaf extract. FT-IR analysis provided essential information about molecular structure and functional groups role in synthesizing and stabilizing AgNPs.

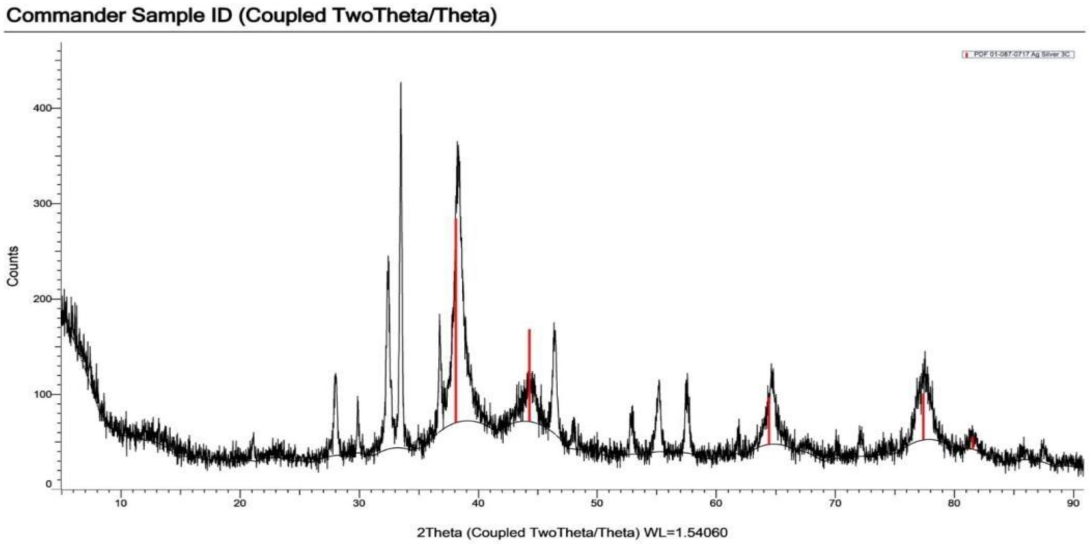

Fig.3 XRD analysis of AgNPs syenthesised from *Ocimum tenuiflorum L*. leaves extract.

3. X-Ray Diffraction analysis

Biogenic nanoparticles crystalline structure was verified by X-ray crystallography. This experimental methodology utilised a Bruker X-ray diffractometer, employing Cu Kα radiation with a specific wavelength of 0.15418 nm. The investigation utilizing X-ray diffraction was performed over a scanning range of 2θ = 10◦ to 90◦, with a scanning step size of 0.02◦. As illustrated in Figure 3. The X-ray diffraction (XRD) pattern of the synthesized AgNPs reveals multiple peaks, prominently featuring five significant peaks located at 38.09◦, 44.16◦, 64.63◦, 77.23◦, and 81.51◦. The identified peaks in the data align with the specific planes of silver's face-centered cubic (fcc) crystal structure. The application of the Scherrer formula revealed that the crystallite domain size is quantified at 16.38 nm. The synthesized AgNPs display crystalline and cubic structures.

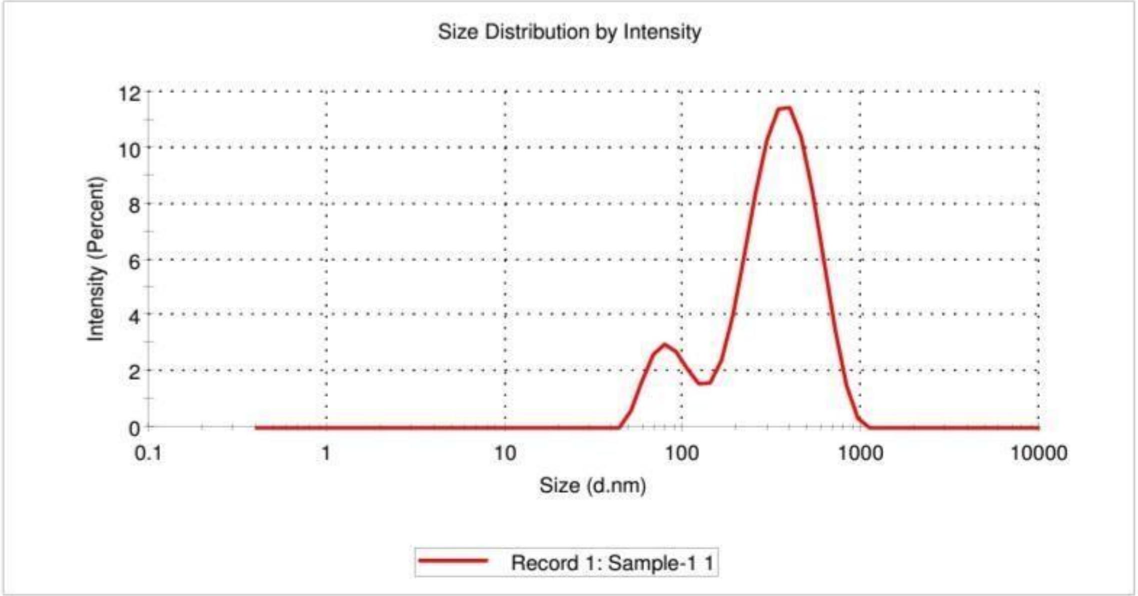

Fig.4 DLS analysis of AgNPs syenthesised from *Ocimum tenuiflorum L.* leaves extract.

4. Dynamic Light Scattering Analysis

By employing the DLS method, we could analyse the hydrodynamic particle size distribution and surface charge (ζ-potential) of the biosynthesized AgNPs. According to the average size of the AgNPs generated in this work using *O. tenuiflorum L*. extract was 297.9 nm. The diameter of the particle is figured out by using the concepts of light scattering and Brownian motion to the measuring of particle sizes in solution (Liaqat et al., 2022). The hydrodynamic size of AgNPs is influenced by the hydration layer that is present on their surface. Furthermore, the plant components in the extract of leaves can have an impact on the particle size. Nanoparticle diameters less than 150–300 nm was proper for cellular absorption (Hemlata et al., 2020; Murugan et al., 2023).

IV. CONCLUSION

*O. tenuiflorum L.*leaf extract was used in the study to report the biosynthesis of AgNPs at room temperature. Utilizing various methods, such as UV-visible spectroscopy, dynamic light scattering, Transmission electron microsc ope, Scanning electrom microscope, FT-IR spectroscopy, and XRD, the AgNPs showed stable production. The AgNPs demonstrated strong antioxidant capabilities and the ability to scavenge free radicals. AgNPs have the potential to be a promising drug for biomedical applications due to their antioxidant, anti-inflammatory, and ability to suppress bacterial growth. This might open new opportunities for the advancement of therapeutics to treat many kinds of ailments.

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