Comparative analysis of the antibacterial activity of Silver nanoparticles synthesized from leaf extracts of *Ocimum sanctum*, *Coriandrum sativum*, *Hemidesmus indicus*, and *Tinospora cordifolia*

Mahima Golani[1], Divya Parnaik[1] & Nandini Phanse[1]

[1] PMB Gujarati Science College, Indore (M.P.), India

Correspondence: Mahima Golani, Dept of Microbiology, PMB Gujarati Science College, Indore (M.P.) – India. E-mail: go.mahi@yahoo.com

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Abstract

The synthesis of metal nanoparticles using biological resources, such as plant leaves, offers a green, cost-effective, and eco-friendly approach. In this study, 1 mM Silver nitrate solution was mixed with freshly prepared aqueous leaf extracts of each medicinal plant like, “Tulsi (*Ocimum sanctum*), Coriander (*Coriandrum sativum*), Anantmul (*Hemidesmus indicus*) and Giloy (*Tinospora cordifolia*)” in 1:4 ratios. The mixtures were then incubated at 75 °C for 25 min, forming Silver nanoparticles giving a deep brown color to the solution with Silver in a reduced colloidal form. Laser diffraction and UV-*Vis* spectroscopy further confirmed the synthesis, with the absorption peak for the nanoparticles observed between 419-438 nm. The antibacterial activity of the synthesized nanoparticles was tested against Gram-positive pathogens, including *Staphylococcus aureus* and *Staphylococcus argenteus*, as well as Gram-negative bacteria like *Escherichia coli* and *Salmonella typhi*. Comparative analysis showed that nanoparticles synthesized from Tulsi, Coriander, and Giloy extracts demonstrated superior antibacterial efficacy, highlighting their potential for use in biomedical and industrial applications such as wound healing, medical devices, and water purification.

Keywords: silver nanoparticles, *Ocimum sanctum*, *Coriandrum sativum*, *Hemidesmus indicus*, *Tinospora cordifolia*, antibacterial activity.

Análise comparativa da atividade antibacteriana de nanopartículas de Prata sintetizadas a partir dos extratos foliares de *Ocimum sanctum*, *Coriandrum sativum*, *Hemidesmus indicus* e *Tinospora cordifolia*

Resumo

A síntese de nanopartículas metálicas utilizando recursos biológicos, como folhas de plantas, oferece uma abordagem verde, econômica e ecologicamente correta. Neste estudo, uma solução de 1 mM de nitrato de Prata foi misturada com extratos de folhas recém-preparados de plantas medicinais como Tulsi (*Ocimum sanctum*), Coentro (*Coriandrum sativum*), Anantmul (*Hemidesmus indicus*) e Giloy (*Tinospora cordifolia*) em uma proporção de 1:4. As misturas foram incubadas a 75 °C por 25 min, resultando em uma coloração marrom-escura, indicando a formação de nanopartículas de prata em forma coloidal reduzida. A análise com feixe de laser e a espectroscopia UV-*Vis* confirmaram a síntese, com o pico de absorção das nanopartículas observado entre 419-438 nm. As propriedades antibacterianas das nanopartículas de Prata sintetizadas foram testadas contra bactérias patogênicas Gram-positivas, incluindo *Staphylococcus aureus* e *Staphylococcus argenteus*, bem como bactérias Gram-negativas, como *Escherichia coli* e *Salmonella typhi*. A análise comparativa revelou que as nanopartículas sintetizadas a partir dos extratos de Tulsi, Coentro e Giloy exibiram maior eficácia antibacteriana, sugerindo o potencial das nanopartículas de prata para várias aplicações biomédicas e industriais.

Palavras-chave: nanopartículas de prata, *Ocimum sanctum*, *Coriandrum sativum*, *Hemidesmus indicus*, *Tinospora cordifolia*, atividade antibacteriana.

1. Introduction

Silver nanoparticles synthesized by plant extracts have garnered significant interest due to their potential applications in various fields, particularly in biomedicine. These nanoparticles are synthesized through a green synthesis method using plant extracts, which offers several advantages over conventional chemical methods, such as cost-effectiveness, eco-friendliness, and scalability.

One of the most intriguing properties of Silver nanoparticles synthesized by plants is their potent antibacterial activity. These nanoparticles have shown significant antibacterial activity against a wide range of bacterial strains, including both Gram-positive and Gram-negative bacteria. This antibacterial activity is primarily attributed to the small size of nanoparticles, which facilitates their interaction with bacterial cell membranes, leading to increased permeability and ultimately cell death. Additionally, Silver nanoparticles can interfere with bacterial enzymes and DNA, further contributing to their antibacterial effects.

The rise of antibiotic resistance has heightened the focus on utilizing plant materials as medicinal sources for diverse human ailments (Prajwala et al., 2018). This shift underscores the urgency of finding alternative treatments amid growing concerns over the efficacy of existing drugs against infectious diseases. Silver nanoparticles have been synthesized from the leaves of various plants including *Azadirachta indica* (Neem), *Ocimum sanctum* (Holy basil), Ficus benghalensis (Banyan), and *Tinospora cordifolia* (Giloy) (Jain et al., 2017).

Phytochemicals, such as alkaloids, flavonoids, and saponins, are known for their role in reducing metal ions during nanoparticle synthesis. Plants like Tulsi, Coriander, and Anantmool contain high concentrations of these compounds, which contribute to both the synthesis of nanoparticles and their antimicrobial effects (Dhama et al., 2014; Sandhu et al., 2013).

Ocimum sanctum, also known as holy basil or Tulsi, is a member of the Labiateae family. The leaf extract specifically contains flavonoids, alkaloids, saponins, tannins, phenols, anthocyanins, and terpenoids (Pattanayak et al., 2010; Jaggi et al., 2003). In some regions of India, the Tulsi plant is used in medical applications, such as the treatment of colds, coughs, fevers, and urinary tract infections (Dey et al., 2021).

Coriandrum sativum, commonly known as coriander, is a culinary plant from the family Umbelliferae (Apiaceae). It is widely cultivated in India, Russia, Asia, and Middle Eastern countries. Coriander leaf may be used to increase the content of antioxidants (Senthil et al., 2008). *C. sativum* contains various phytochemicals, including flavonoids, phenols, carbohydrates, proteins, cardiac glycosides, tannins, terpenoids, alkaloids, minerals, and vitamins like calcium, phosphorus, iron, carotene, and niacin. Various parts of *C. sativum* can be used for biomedical applications, including anti-bacterial and anti-cancer activities (Sathiyavati et al., 2010).

Hemidesmus indicus is locally known as Anantmool and Indian Sarsaparilla. It is an aromatic, long-rooted plant that belongs to the Apocynaceae family. The medicinal plant is used against a variety of diseases due to the presence of various phytochemicals like hemidesmol, resin, glucoside, tannin, and resin. The plant parts, roots, and rhizomes have been utilized for hundreds of years in Ayurvedic medication for relieving countless diseases. Many reported studies highlighted the potential pharmacological properties of *H. indicus* like anti-cataractous, antidiarrhoeal, anti-cancerous, anti-diabetics, anti-venom, and anti-angiogenic (Thakur et al., 2021). *Tinospora cordifolia* (Giloy), a prominent medicinal plant from the Menispermaceae family, produces diverse classes of pharmacologically active compounds. It has been used to treat various diseases, including jaundice, anemia, skin diseases, allergic conditions, diabetes, inflammation, and urinary disorders (Debnath et al., 2014). *T. cordifolia* holds a prominent place in Ayurveda as a Rasayana herb and is known as a "rejuvenating herb" due to its medicinal properties (Prajwala et al., 2019). It exhibits antimicrobial properties, with its root, stem, and leaf extracts showing activity against pathogenic microorganisms (Agarwal et al., 2019).

The green synthesis of Silver nanoparticles (AgNPs) can potentially eliminate the adverse effects associated with chemical agents, making nanoparticles more compatible with eco-friendly approaches. Additionally, the synthesized AgNPs enhance the therapeutic efficacy and medicinal value of *T. cordifolia* (Singh et al., 2014). These plants have long been used for their therapeutic properties, and their extracts are now being explored for the green synthesis of Silver nanoparticles with enhanced antibacterial efficacy.

This study explores the synthesis of Silver nanoparticles from medicinal plant extracts and evaluates their antibacterial efficacy, potentially contributing to new, eco-friendly alternatives in treating bacterial infections.

2. Materials and Methods

2.1 Sample collection

During the rainy season, fresh leaf samples of *Ocimum sanctum*, *Coriandrum sativum*, *Hemidesmus indicus*, and *Tinospora cordifolia* were collected from healthy plants at the Botanical Garden of P.M.B. Gujarati Science College, Indore (M.P.), India.

2.2 Preparation of leaf extracts

Fresh leaves of *O. sanctum*, *C. sativum*, *H. indicus,* and *T. cordifolia* were collected and washed thoroughly with water to remove any dust, followed by rinsing with de-ionized water. 20 grams of each washed sample of leaf were cut into fine pieces, transferred into 250 mL beakers, and boiled in 100 mL de-ionized water for 10 min. After boiling, extracts were allowed to cool to room temperature. They were then filtered through Whatman filter paper No.5 (Song et al., 2009). The extracts were then used as reducing and stabilizing agents for $AgNO_3$.The remaining extracts were stored at 4 °C for further usage.

2.3 Preparation of 1 mM $AgNO_3$ solution

A 1 mM $AgNO_3$ solution was prepared by dissolving the 0.169 g of Silver nitrate in 1 L of de-ionized water and storing the solution in a dark brown bottle to prevent self-oxidation of Silver nitrate.

2.4 Green synthesis of Silver nanoparticles

To initiate the synthesis of Silver nanoparticles, 80 mL of 1 mM $AgNO_3$ solution was mixed with 20 mL of freshly prepared leaf extracts in separate flasks, achieving a 1:4 dilution. Mixtures were kept in a shaking water bath (Remi water bath shaker, RSB-12) at 75 °C for 25 min (Prajwala et al., 2021; Singh et al., 2014). The color change from pale yellow to dark brown colloidal solution indicated the formation of Silver nanoparticles (AgNPs). The colloidal solutions were centrifuged in a cooling centrifuge machine (Remi C-24) to separate the nanoparticles from the supernatant (Ashraf et al., 2019). The resulting nanoparticles were further characterized.

2.5 Laser beam analysis

Synthesized nanoparticles were preliminarily characterized by observing light scattering, a result of the Tyndall effect when a laser beam was passed through the sample.

2.6 UV-Visible spectroscopy analysis

The bio-reduction of Ag ions into Ag^0 was monitored by UV-*Vis* spectroscopy of the reaction mixtures. An aliquot of 0.1 mL of the sample was diluted with 2 mL of de-ionized water, and the spectrum was recorded within the range of 300-600 nm using a UV-*Vis* spectrophotometer (Model - UV 1800 Shimadzu, Japan).

2.7 Antibacterial study of Silver nanoparticles

The antibacterial activity of the Silver nanoparticles was tested against Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus argenteus*) and Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*) using agar well diffusion method on Mueller-Hinton Agar Medium plates. The 24-hour pure cultures of the bacteria were spread on Mueller-Hinton Agar Medium plates. Leaf extracts, de-ionized water, and $AgNO_3$ were used as controls, along with Silver nanoparticles. 0.08 mL of each sample was added to 6 mm wells and incubated at 37 °C for 24 h. The zone of inhibition (in mm) was measured after incubation.

3. Results

3.1 Synthesis of Silver nanoparticles

When the filtrate of leaf extracts of *O. sanctum, C. sativum, H. indicus,* and *T. cordifolia* (Figure 1) was mixed with 1mM Silver nitrate solution, a gradual change in the color of solution was observed as a result of the reduction of Silver ions (Ag^+) that leads to the formation of Silver nanoparticles. The change in color from pale yellow to dark brown (Figure 2) indicates the formation of Silver nanoparticles. The intensity of color and the duration of changes observed in the color of the mixture may vary depending upon the inherent properties of leaf extracts being used. The apparent excitation of plasmon resonance is the cause of the change in color of the solution to dark brown (Phanjom et al., 2012).

The Silver ion changes its state from Ag^+ to Ag^0 by getting reduced with the help of the reducing and catalytic activity of leaf extracts. Silver ions become supersaturated and the neutral Silver ions aggregate into the mixture in the form of Silver nanoparticles.

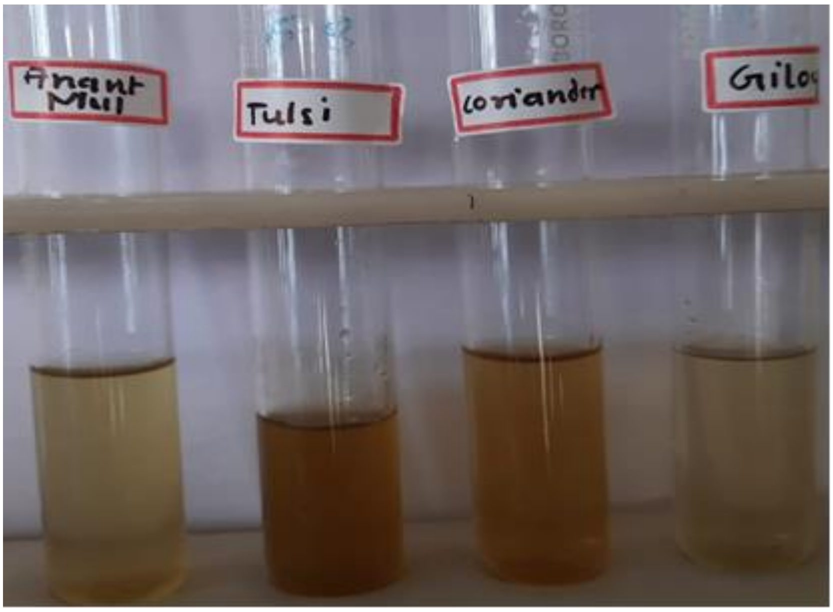

Figure 1. Leaf extracts of selected plants. Source: Authors, 2024.

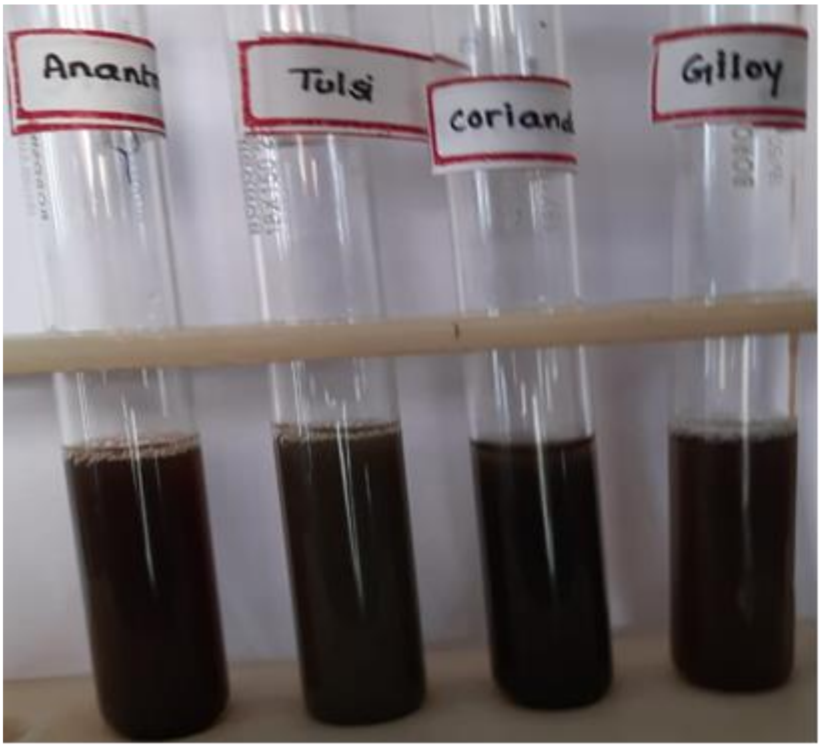

Figure 2. Synthesized Silver nanoparticles. Source: Authors, 2024.

3.2 Laser beam analysis

A preliminary analysis of the synthesized Silver nanoparticles was performed by passing a laser beam through the sample. On passing a laser beam through a colloidal solution of Silver nanoparticles, the path of the incident light became visible due to the scattering of light, a phenomenon known as the Tyndall effect, which occurs when light interacts with colloidal particles (Figure 3).

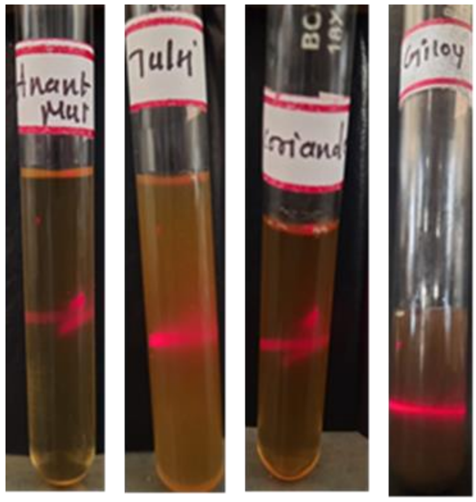

Figure 3. Colloidal solution of Silver nanoparticles showing Tyndall effect for a laser beam. Source: Authors, 2024.

3.3 UV-Visible spectrum analysis

UV–visible spectroscopy is a useful technique for confirming the synthesis of metallic Silver nanoparticles. Our samples of synthesized nanoparticles were analyzed in the UV–Visible spectroscopy. The maximum absorption peak for *T. cordifolia* (Giloy) was observed at 419 nm (Figure 4), for *O. sanctum* (Tulsi) at 438 nm (Figure 5), for *C. sativum* (Coriander) at 425 nm and for the *H. indicus* (Anantmul), an absorption peak was observed at 430nm. The reduction of Silver ions and surface Plasmon resonance is the cause of the absorption peak observed (Daisy et al., 2012). The absorption peak of the colloidal solution of silver nanoparticles was observed between 400-450nm (Begum et al., 2019). Surface Plasmon resonance occurs due to the excitation of surface plasmons on the outer surface of Silver nanoparticles, which is responsible for the observed absorption peak (Pradhan et al., 2013). The Plasmon absorbance of Silver nanoparticles is responsible for the UV-visible absorption peak observed between 417-425 nm (Okafor et al., 2013).

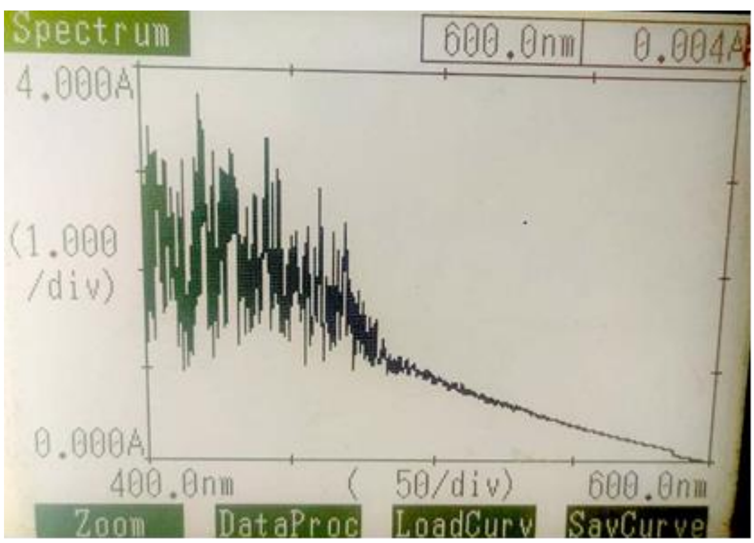

Figure 4. Absorption peak for *Tinospora cordifolia* (Giloy). Source: Authors, 2024.

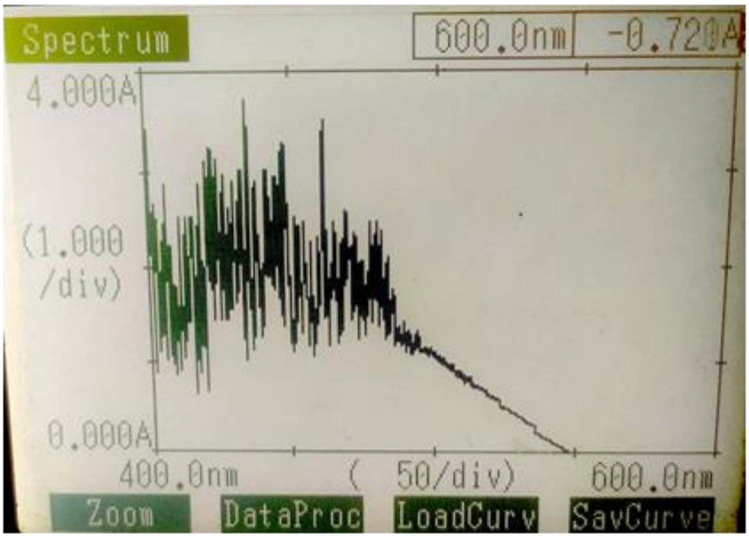

Figure 5. The absorption peak of *Ocimum sanctum* (Tulsi). Source: Authors, 2024.

3.4 Antibacterial study of Silver nanoparticles

The phyto-assisted synthesized Silver nanoparticles exhibited inhibitory effects on different Gram-positive pathogenic bacteria - Staphylococcus aureus, Staphylococcus argenteus, and Gram-negative bacteria i.e. *E. coli*, *S. typhi*. The zones of inhibition were observed around the well-containing samples of Silver nanoparticles synthesized from different samples of plants. No zone of clearance or inhibition was observed for leaf extracts and de-ionized water, which were used as controls in testing the antibacterial activity of the synthesized Silver nanoparticles. This suggests that the leaf extracts and water did not inhibit the growth of the respective pathogenic bacteria.

The nanoparticles synthesized from some plant samples were found to be more effective against the pathogenic bacteria compared to Silver nitrate. The zones of inhibition observed for different plant samples against various pathogenic bacteria are shown in (Table 1 and Figure 6).

Table 1. Zone of inhibition of Silver nanoparticles against various pathogens.

Name of organisms	Diameter of Zone of Inhibition (in mm)				
	Silver Nitrate	Tulsi	Coriander	Anantmul	Giloy
Staphylococcus aureus	19	20	20	19	20
Staphylococcus argenteus	20	21	21	20	20
Salmonella typhi	25	26	27	25	27
Escherichia coli	19	22	21	19	20

Source: Authors, 2024.

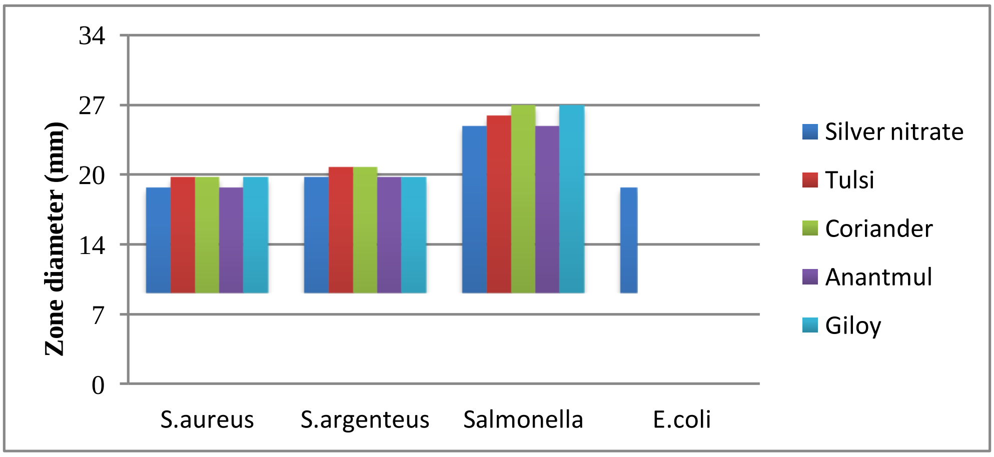

Figure 6. Comparative analysis of zone of inhibition of Silver nanoparticles against various pathogens. Source: Authors, 2024.

In the case of *S. aureus*, nanoparticles synthesized from Tulsi, Giloy, and Coriander were found to be more effective than Silver nitrate solution while leaf extracts of all selected plants have shown no effect as an antibacterial agent (Figure 7). In the case of *S. argenteus*, Tulsi and Coriander were more effective (Figure 8), while for *S. typhi*, Tulsi, Coriander, and Giloy were found to be more effective than Silver nitrate out of Coriander and Giloy found to be most effective showing antibacterial effect. For *E. coli*, Tulsi, Coriander, and Giloy were found to be more effective than Silver nitrate out of which Tulsi was the most effective (Figure 9). Silver nanoparticles synthesized from Anantmul were found to be least effective for the pathogenic organisms.

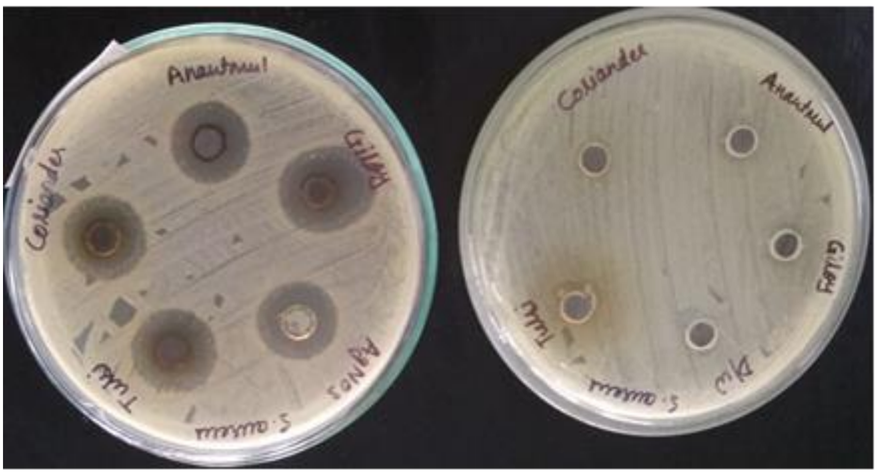

Figure 7. Silver nanoparticles as synthesized by different samples showing inhibitory effect on *S. aureus* while leaf extracts having no effect on the pathogenic bacteria. Source: Authors, 2024.

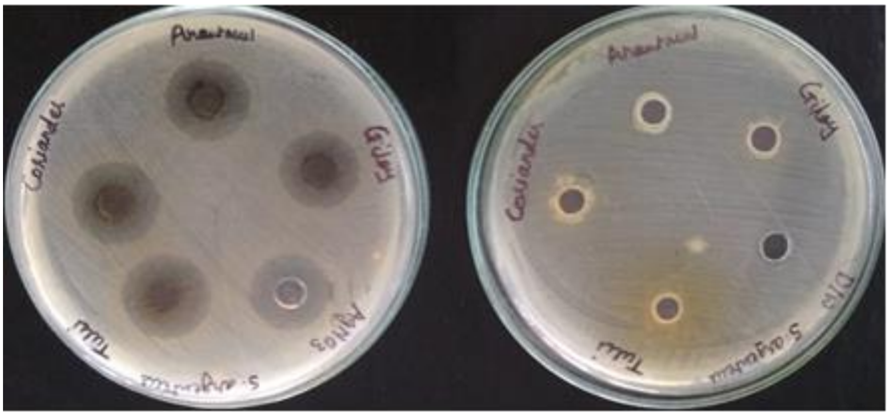

Figure 8. Silver nanoparticles as synthesized by different samples showing inhibitory effect on *S. argenteus* while leaf extracts having no effect on the pathogenic bacteria. Source: Authors, 2024.

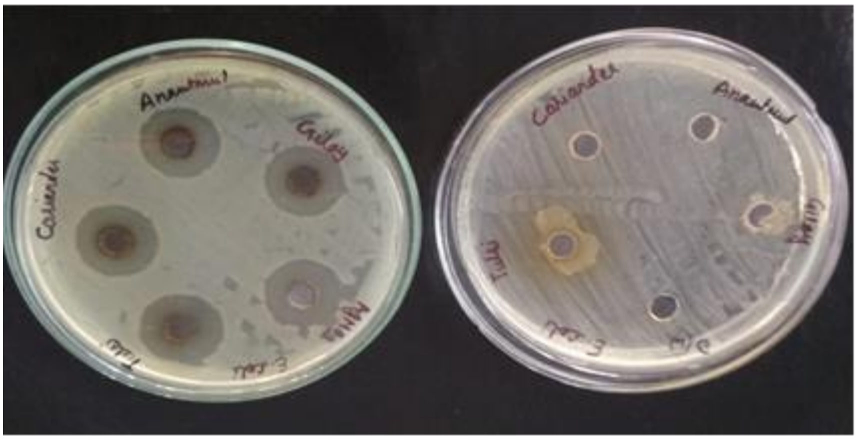

Figure 9. Silver nanoparticles as synthesized by samples showing inhibitory effect on *E.coli*. Source: Authors, 2024.

Our results are by Phanse et al. (2022; 2023) who reported antibacterial activities of Silver nanoparticles synthesized from the leaf extracts of *C. sativum* (Phanse et al., 2022) and *T. cordifolia* (Giloy) (Phanse et al., 2023) against *S. aureus* and *S. typhi*. Similar results were obtained by Tailor et al. (2020) and Jain et al. (2017) who reported antibacterial activities of Silver nanoparticles synthesized from the leaf extracts of Tulsi against *E. coli* sp. (Tailor et al., 2020; Jain et al., 2017).

4. Conclusions

In conclusion, Silver nanoparticles synthesized from plant extracts hold significant promise as novel antibacterial agents, with potential applications in healthcare, environmental remediation, and other fields requiring effective antimicrobial solutions. The comparative analysis showed that nanoparticles synthesized from Tulsi, Coriander, and Giloy extracts demonstrated superior antibacterial efficacy, highlighting their potential for various biomedical and industrial applications. These nanoparticles could be utilized in developing new drugs and treatments for infections caused by pathogens such as *Staphylococcus aureus*, *Staphylococcus argenteus*, *Escherichia coli*, and *Salmonella typhi*.

6. Acknowledgments

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7. Authors' Contributions

Mahima Golani: performed the experiments. *Divya Parnaik*: contributed in the drafting of the manuscript and graphical abstract. *Nandini Phanse*: contributed to the drafting of the manuscript and graphical abstract.

8. Conflicts of Interest

No conflicts of interest.

9. Ethics Approval

Not applicable.

10. References

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