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Green synthesis of silver nanoparticles (AgNPs) using leaf extracts of hibiscus and night jasmine and evaluation of their antimicrobial activities

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ABSTRACT

The biosynthesis of silver nanoparticles (AgNPs) with plant-based extracts has emerged as a sustainable, cost-effective, and environmentally friendly alternative to conventional chemical synthesis processes. Indian medicinal plants serve as potential sources of natural reducing and stabilizing agents essential for AgNP formation. AgNPs were synthesized using 1 mM aqueous $AgNO_3$ and 5% leaf extracts of *Tagetes erecta* (Marigold) and *Tridax procumbens* (Tridax). The formation of AgNPs was confirmed by a visible color change and UV–Vis spectrophotometric analysis. The antimicrobial efficacy of the synthesized AgNPs was evaluated against *Escherichia coli*, *Bacillus* sp., and *Aspergillus niger*. Their potential for water disinfection was further assessed using samples collected from river, pond, and canal sources. Both Marigold- and Tridax-derived AgNPs exhibited enhanced antimicrobial activity compared to $AgNO_3$ and crude plant extracts. Marigold-mediated AgNPs demonstrated slightly higher inhibition zones against Gram-positive (*Staphylococcus aureus*, *Klebsiella pneumoniae*) and Gram-negative (*E. coli*) bacteria, as well as *A. niger*. In water disinfection studies, the synthesized AgNPs achieved over 50% bacterial growth reduction.The study highlights the strong bio-reducing capacity of plant-derived compounds in generating AgNPs with notable antimicrobial and disinfection properties. Marigold extract exhibited marginally higher efficiency, making it a promising candidate for green nanoparticle synthesis. Further investigation is required to evaluate the long-term environmental impacts, stability, and cytotoxicity of the synthesized AgNPs. The objective of this study is to establish a sustainable and eco-compatible approach for synthesizing AgNPs with potential applications in antimicrobial treatment and water purification.

KEY WORDS

Silver nanoparticles; Antimicrobial activity; UV–Vis spectrophotometric analysis; Plant-derived compound; Water disinfection

ARTICLE HISTORY

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Introduction

India has an extensive collection of medicinal plants that have been essential to ancient treatment systems like Ayurveda. These plants contain bioactive compounds with proven therapeutic and biochemical significance. In recent years, they have gained renewed attention for their role as natural reducing and stabilizing agents in nanobiotechnology. This sustainable approach aligns with global efforts toward eco-friendly and cost-effective scientific innovations.

Nanobiotechnology, an emerging field at the intersection of nanoscience and biology, enables the synthesis of nanoparticles (NPs) using biological systems. NPs, typically less than 100 nm in size, exhibit enhanced physicochemical activities such as high reactivity and surface area compared to their bulk counterparts [1]. Among noble metal nanoparticles, silver nanoparticles (AgNPs) have attracted significant attention due to their antimicrobial and catalytic properties and are widely used in pharmaceuticals, cosmetics, textiles, and water purification [2,3]. However, conventional synthesis methods often rely on toxic reagents and high energy inputs, necessitating safer and greener alternatives.

Plant-mediated synthesis of nanoparticles has emerged as an efficient and eco-compatible alternative to chemical and physical approaches. Plant phytochemicals, including flavonoids, terpenoids, and phenolics, are serve as natural reducing and stabilizing agents in this synthesis. Previous studies have demonstrated successful synthesis of AgNPs using plant species including *Acalypha indica*, Aloe vera, *Garcinia mangostana*, and *Crataegus douglasii* [4, 5]. These examples validate the potential of plants as biological factories for nanoparticle production with broad biomedical and environmental applications.

In the present study, an eco-friendly and swift microwave-assisted approach was used to synthesize AgNPs from leaf extracts of three Indian medicinal plants: *Musa balbisiana* (Banana), *Azadirachta indica* (Neem), and *Ocimum tenuiflorum* (Black Tulsi). The synthesized nanoparticles were characterized for their structural and optical properties using advanced analytical techniques. Antimicrobial efficacy was evaluated through disc diffusion assays against selected microbial strains, while phytotoxicity assessments were performed on *Vigna radiata* (Moong bean) and *Cicer arietinum* (Chickpea) to study germination and oxidative stress responses under different AgNP concentrations.

The microwave-assisted synthesis yielded stable silver nanoparticles within a short duration. The biosynthesized AgNPs demonstrated significantly enhanced antimicrobial activity compared to crude leaf extracts, with Neem and Black Tulsi-derived AgNPs showing superior inhibition zones [6]. Phytotoxicity tests revealed concentration-dependent effects lower concentrations promoted seed germination, while higher doses induced oxidative stress. These findings confirm the efficiency and biological compatibility of plant-mediated AgNPs [7, 8].

The current study is limited by variable nanoparticle yield and morphology due to natural differences in plant phytochemistry. Moreover, long-term environmental impacts

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and cytotoxicity remain insufficiently explored [9]. Further standardization and large-scale studies are essential to ensure reproducibility and ecological safety.

The objective of this study is to develop an eco-friendly, sustainable, and cost-effective method for AgNP synthesis using Indian medicinal plants; to characterize their structural and antimicrobial properties; to evaluate their phytotoxic effects on legumes; and to explore their potential use in pollution remediation and agricultural applications.

Materials and Methods

Aqueous plant extract is mixed with an aqueous metal salt solution in the process of bioreduction. Nanoparticle formation occurs at room temperature, and the process generally requires minutes to a few hours to complete, producing stable nanoparticles effectively.

Preparation of the leaf extract

Two commonly available Indian medicinal plants, *Tagetes erecta* (Marigold) and *Tridax procumbens* (Tridax daisy), were selected based on their availability, cost-effectiveness, and established medicinal properties (Figure 1 and Figure 2) (Table 1). Fresh and healthy leaves were obtained locally in Bhubaneswar and Cuttack, Odisha, India. The leaves were thoroughly washed twice to eliminate dust and surface contaminants, first with tap water and subsequently with distilled water. The cleaned leaves were chopped into little pieces and allowed to air dry at room temperature. Approximately 20 g of each finely chopped leaf sample was transferred into a 250 mL Erlenmeyer flask containing 100 mL of distilled water and heated at 60°C for 1 hour. The mixtures were then filtered using Whatman No. 1 filter paper to eliminate solid residues, leaving clear aqueous extracts. The obtained filtrates were stored at 4°C for subsequent nanoparticle synthesis. Each procedure was carried out in a sterile environment to assure experimental accuracy and prevent contamination.

Table 1. Detailed description of two medicinal plants used in the present study.

Sl.no	Plant common names	Odia name	Biological name	Family	Sub family	Codes
1	Marigold	Gendu	*Tagetes erecta*	Asteraceae	Asteroideae	HR
2	Coatbuttons/ tridax daisy	Bisalya karani	*Tridax procumbens*	Asteraceae	Heliantheae	TP

Figure 1. *Tagetes erecta* (Marigold).

Figure 2. *Tridax procumbens* (Tridax daisy).

Synthesis of silver nanoparticles (AgNPs)

For the biosynthesis of silver nanoparticles, 45 mL of 1 mM aqueous silver nitrate ($AgNO_3$) solution was mixed with 5 mL of each leaf extract (*Tagetes erecta* and *Tridax procumbens*) separately in 250 mL Erlenmeyer flasks. The reduction of Ag^+ ions to Ag^0 nanoparticles was facilitated by the phytochemicals present in the plant extracts, serving as natural reducing and stabilizing agents. The reaction mixtures were continuously stirred using a magnetic stirrer under dark conditions at room temperature to prevent photoactivation of silver ions. The colour of the solution changed from colourless to brownish or reddish-brown, indicating the formation of nanoparticles, allowing for visual monitoring of the reaction's progress. Periodic sampling was carried out, and by using UV–Visible spectrophotometry in the wavelength range of 300–800 nm reaction kinetics were analyzed. The appearance of a characteristic surface plasmon resonance (SPR) peak confirmed the formation of AgNPs. Additionally, AgNP synthesis was performed using an equal mixture of both leaf extracts to assess any synergistic effects on nanoparticle formation and antimicrobial activity. The resulting colloidal suspensions were sealed and stored at 4°C for further characterization and biological studies.

Synthesis of AgNPs with varying $AgNO_3$ and leaf extract concentrations

To improve the synthesis conditions, silver nanoparticles have been prepared at various volume ratios of silver nitrate solution to leaf extract. The reactions were carried out with varied proportions: 1:9 (5 mL leaf extract added to 45 mL of 1 mM $AgNO_3$ solution), 1:4 (10 mL leaf extract added to 40 mL $AgNO_3$), and 3:7 (15 mL leaf extract added to 35 mL $AgNO_3$). Each mixture was thoroughly stirred under identical experimental conditions to evaluate the effect of extract concentration on nanoparticle formation, reaction rate, and stability. The resulting solutions were analyzed for visual color change and spectrophotometric response to determine the optimal ratio for efficient AgNP synthesis (Figure 3).

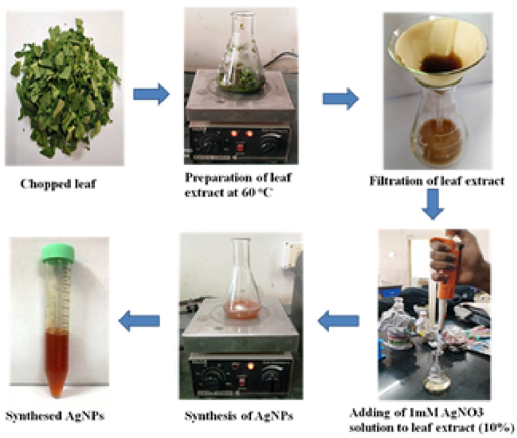

Figure 3. The Process in synthesis of AgNPs from leaf extracts.

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UV–Visible spectral analysis

For UV–Visible spectroscopic analysis, 1 mL of the synthesized AgNP colloidal solution was diluted tenfold with deionized water to ensure optimal absorbance readings. By using a UV-Visible spectrophotometer (Cary, VARIAN) with a wavelength range of 300-800nm the diluted samples were analyzed. The distinctive surface plasmon resonance (SPR) peak found in this range corroborated the silver nanoparticles' production and optical characteristics.

Evaluation of antimicrobial activity

The antibacterial and antifungal activities of the raw leaf extracts and their corresponding AgNPs were evaluated and compared against five bacterial strains Bacillus subtilis (Gram-positive), Escherichia coli (Gram-negative), Klebsiella pneumoniae (Gram-negative), Salmonella typhimurium (Gram-negative), and Staphylococcus aureus (Gram-positive) along with one fungal strain, Aspergillus niger, all procured from the American Type Culture Collection (ATCC) (Table 2). The antimicrobial activity was assessed using the well-diffusion method. Using sterile cotton swabs, nutrient agar plates were prepared and evenly inoculated with the corresponding microbial cultures. Wells were then bored into the agar using a sterile corer, and 50 µL of each synthesized AgNP suspension was carefully added into the wells. For 24 to 48 hours, the plates were incubated at 37°C. Following incubation, the zones of inhibition around each well were evaluated to assess antimicrobial effectiveness. The produced AgNPs were compared to conventional antibiotics, ampicillin (10 µg) and penicillin (10 µg), to assess their antibacterial potency.

Table 2: Description of bacterial and fungal strains used for antimicrobial test.

Name of the Microorganism	Sources	Gram staining
Bacillus subtilis	ATCC11774	Positive
Escherichia coli	ATCC25922	Negative
Klebsiella pneumonia	ATCC BAA-1705	Negative
Salmonella typhimurium	ATCC-14028	Negative
Staphylococcus aureus	ATCC-29213	Positive
Asperigillus niger	ATCC-6275	----

Disinfection of contaminated water using synthesized AgNPs

The water disinfection potential of the synthesized AgNPs was evaluated against three locally collected water samples from a river, pond, and canal. The assessment was performed using both the well-diffusion and broth-dilution methods. In the well-diffusion assay, 0.5 mL of each water sample was spread on nutrient agar plates, and 50 µL of each AgNP solution was added to individual wells. The inhibitory zones were identified during a 24-hour incubation period at 37°C. In the broth-dilution procedure, 0.1 mL of each water sample was added to test tubes containing 10 mL nutritional broth, followed by 1 mL of the mixed AgNP solution. Control tubes without AgNPs were also prepared. After incubation (37°C, 24–48 h), optical density (OD) was measured at 600 nm to determine microbial reduction efficiency.

Results and discussions

Synthesis of silver nanoparticles

During the synthesis of silver nanoparticles (AgNPs) using different plant leaf extracts, a distinct color transformation was observed following the addition of silver nitrate ($AgNO_3$), as illustrated in Figures 4 and 5. Surface plasmon resonance (SPR) resulting from the excitation of free electrons in the AgNPs is responsible for the reaction mixture's brown hue. As the leaf extracts were gradually added to the aqueous $AgNO_3$ solution, the color shifted from faint yellow to yellowish brown, then reddish brown, and eventually to a stable colloidal brown, indicating nanoparticle production. In this study, the AgNPs synthesized using *Tagetes erecta* (Marigold) and *Tridax procumbens* (Tridax) exhibited a final reddish-brown coloration, indicative of complete reduction of Ag^+ ions to Ag^0 nanoparticles. These results are consistent with earlier findings, validating the successful biosynthesis of AgNPs [10].

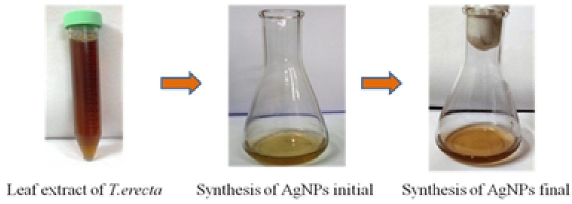

Figure 4. Synthesis of silver nanoparticles from *T.erecta*.

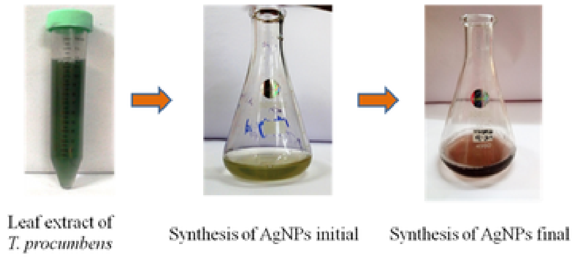

Figure 5. Synthesis of silver nanoparticles from T.procumbens.

UV–Vis spectrophotometric characterization of AgNPs

The UV–Vis spectra analysis of *T. erecta* and *T. procumbens* obtained after the completion of the reaction mediated by various leaf extracts are presented in Figures 6 and 7. The absorption maxima of the synthesized silver nanoparticles (AgNPs) were observed in the range of 445–455 nm, corresponding to the characteristic surface plasmon resonance (SPR) of AgNPs. Among all extracts, the neem (*Azadirachta indica*) leaf extract exhibited the most rapid bioreduction of Ag^+ ions, followed by *Jatropha curcas* and *Ocimum tenuiflorum* (tulsi), as indicated by sharp and well-defined absorption peaks. In contrast, other leaf extracts showed relatively broader peaks, suggesting slower reduction rates and the formation of polydispersed nanoparticles. The spectra also confirmed that nanoparticle formation occurred rapidly within the first 15 minutes, and the resulting colloidal AgNPs remained stable for over 24 hours post-synthesis, aligning with earlier findings [11].

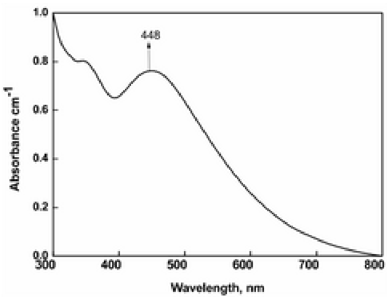

Figure 6. UV-Vis spectral analysis of *T. erecta* leaf extract mediated AgNPs.

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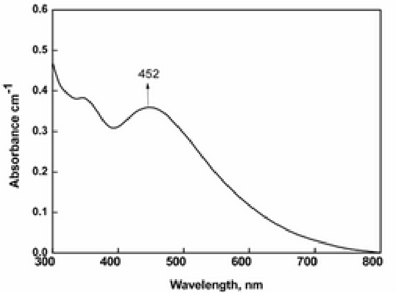

Figure 7.UV-Vis spectral analysis of *Tridax procumbens* leaf extract mediated AgNPs.

Antibacterial property analysis

The antibacterial properties of the synthesized silver nanoparticles (AgNPs) were examined, and the results are presented in Tables 3–9. The measured inhibition zone diameters (in mm) demonstrated that all AgNPs exhibited significantly higher antibacterial properties compared to their respective crude leaf extracts. Among the various AgNPs, those synthesized using *Jatropha curcas*, *Tagetes erecta*, and *Calotropis gigantea* (giant milkweed) displayed the most pronounced antimicrobial efficacy, while *Azadirachta indica* (neem), *Tridax procumbens*, and *Ocimum tenuiflorum* (black tulsi) showed comparatively moderate activity (Table 3). These findings are consistent with previous reports highlighting the strong antibacterial potential of AgNPs [12].

The AgNPs demonstrated substantial inhibitory effects against both Gram-positive (*Staphylococcus aureus*, *Klebsiella pneumoniae*) and Gram-negative (*Escherichia coli*) bacteria, as well as the fungus Aspergillus niger. In contrast, the crude plant extracts showed minimal antimicrobial activity. Furthermore, AgNPs synthesized at leaf extract to $AgNO_3$ ratios of 2:8 and 3:7 exhibited slightly higher antibacterial efficiency than those prepared at a 1:9 ratio (Table 4-8). Interestingly, the combined AgNPs derived from mixed leaf extracts also displayed strong antimicrobial effects, with the 1:9 ratio showing marginally superior activity (Table 9) (Figure 8 and Figure 9).

Table 3. Antimicrobial properties of two different leaf extrats (raw/original) and with AgNPs.

	B.subtilis	***S.typhiium***	***S.aureus***	***K.pneumoniae***	***E.coli***	***A.niger***
	Diameter of inhibition zone presented in mm					
TE-O	0	3.0	2.0	1.0	0	1.0
TE-AgNPs	1.0	2.0	4.0	3.0	9.0	3.0
TP-O	0	0	0	0	0	0
TP-AgNP	0	0	4.0	4.0	0	5.0

Table 4. Antibacterial activity of two AgNPs against *Bacillus subtilis.*

Silver nanoparticles	**Diameter of inhibition zone presented in mm**		
	Leaf extract: silver nitrate solution ratio		
	1:9	2:8	3:7
TE-AgNPs	1.0	6.0	7.0
TP-AgNPs	0	7.0	7.0

Table 5. Antibacterial activity of two AgNPs against *S. typhimurium.*

Silver nanoparticles	**Diameter of inhibition zone presented in mm**		
	Ratio of leaf extract: silver nitrate solution		
	1:9	2:8	3:7
TE-AgNPs	2.0	3.0	4.0
TP-AgNPs	1.0	4.0	3.0

Table 6. Antibacterial activity of two AgNPs against *Staphylococcus aureus.*

Silver nanoparticles	**Diameter of inhibition zone presented in mm**		
	Leaf extract: silver nitrate solution ratio		
	1:9	2:8	3:7
TE-AgNPs	4.0	5.0	7.0
TP-AgNPs	4.0	6.0	5.0

Table 7. Antibacterial activity of two AgNPs against *K.pneumoniae.*

Silver nanoparticles	**Diameter of inhibition zone presented in mm**		
	Leaf extract: silver nitrate solution ratio		
	1:9	2:8	3:7
TE-AgNPs	3.0	4.0	3.0
TP-AgNPs	4.0	3.0	3.0

Table 8. Antibacterial activity of two AgNPs against *E. coli.*

Silver nanoparticles	**Diameter of inhibition zone presented in mm**		
	Leaf extract: silver nitrate solution ratio		
	1:9	2:8	3:7
TE-AgNPs	9.0	5.0	4.0
TP-AgNPs	0	5.0	5.0

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Table 9. Antifungal activity of two AgNPs against A. niger.

Silver nanoparticles	Diameter of inhibition zone presented in mm		
	Leaf extract: silver nitrate solution ratio		
	1:9	2:8	3:7
TE-AgNPs	3.0	4.0	0
TP-AgNPs	4.0	0	4.0

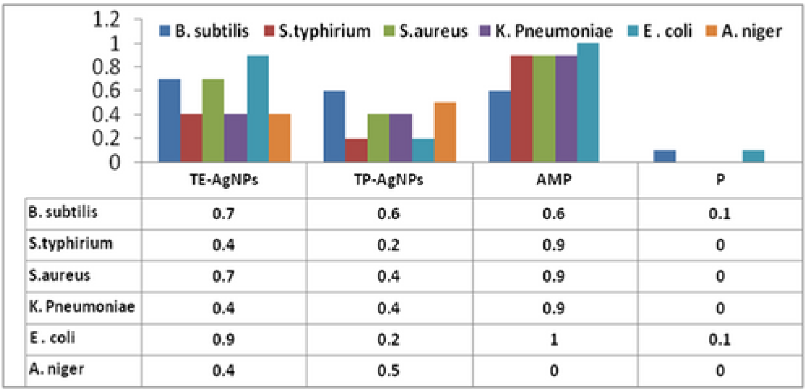

Figure 8. Comparison of antimicrobial activity between synthesised two AgNPs and commercial antibiotics (Ampicillin and Peniciliin).

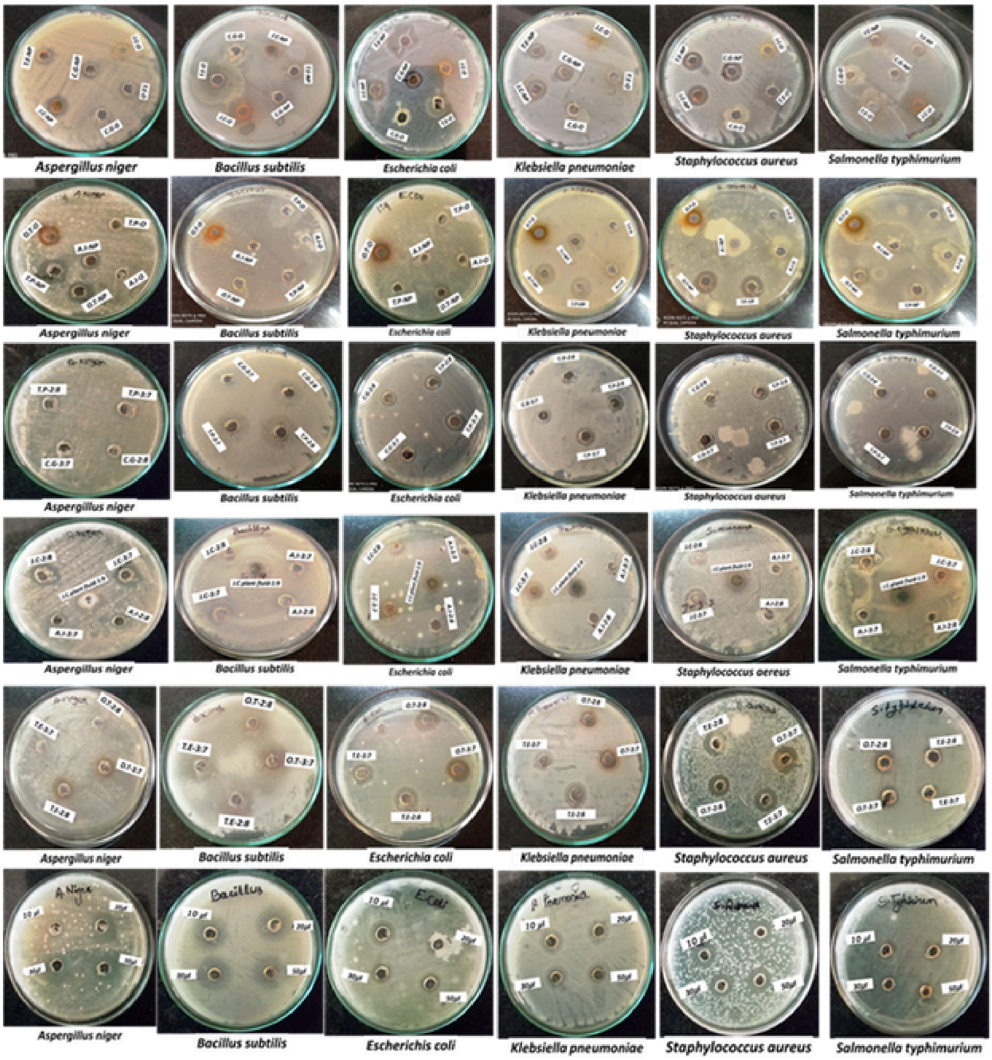

Figure 9. Evaluation of antimicrobial activities of synthesised two AgNPs from two leave extracts through well diffusion method.

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Discussions

The synthesized silver nanoparticles (AgNPs) using leaf extracts of *Tagetes erecta* and *Tridax procumbens* demonstrated significant antimicrobial efficacy against selected bacterial and fungal strains [13]. The formation of AgNPs was confirmed through observable color changes of the reaction mixture and characteristic absorption peaks between 445–455 nm in UV-Vis spectrophotometric analysis, corresponding to surface plasmon resonance of AgNPs. Among the two plant-mediated AgNPs, T. erecta exhibited higher antimicrobial activity compared to *T. procumbens.*

The synthesized AgNPs showed maximum inhibitory effects against *Staphylococcus aureus* (Gram-positive), *Klebsiella pneumoniae* and *Escherichia coli* (Gram-negative), as well as the fungal strain *Aspergillus niger*. Furthermore, when evaluated for water disinfection, the AgNPs effectively reduced over 50% of the bacterial load in contaminated water samples, demonstrating their potential as eco-friendly nanomaterials for antimicrobial and water purification applications [14, 15].

Conclusions

The present study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using the aqueous leaf extracts of *Tagetes erecta* and *Tridax procumbens* through a simple, cost-effective, and eco-friendly bioreduction process. The formation of AgNPs was visually confirmed by a distinct color change in the reaction mixture and spectrophotometrically verified through absorption peaks between 445–455 nm, characteristic of surface plasmon resonance. The variation in phytochemical composition between the two plant species influenced both the particle size and antimicrobial potential of the synthesized AgNPs.

The AgNPs derived from *T. erecta* exhibited superior antimicrobial activity compared to those synthesized from T. *procumbens*, indicating that plant-specific bioactive compounds play a crucial role in nanoparticle stabilization and bioreduction efficiency. The AgNPs showed remarkable antibacterial and antifungal activity, particularly against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Aspergillus niger*, demonstrating their broad-spectrum antimicrobial potential.

Moreover, the synthesized AgNPs exhibited efficient water disinfection capability by reducing more than 50% of the microbial population in contaminated water samples. This highlights their potential utility as an environmentally benign alternative to conventional chemical disinfectants.

Overall, the study confirms that *T. erecta* and *T. procumbens*-mediated AgNPs possess significant antimicrobial and water purification potential. These findings contribute to the growing field of green nanotechnology, emphasizing the sustainable use of plant-based reducing agents for nanoparticle synthesis. Future research should focus on optimizing synthesis parameters, assessing cytotoxicity, and exploring large-scale applications in healthcare, environmental remediation, and water treatment systems.

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