Research Article

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Exploring the Anti-bacterial Potential of Orchid-derived Silver Nanoparticles

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Abstract

In this study, silver nanoparticles (AgNPs) were synthesized in an environmentally friendly manner using plant extracts from *Luisia tristis*. The formation of the nanoparticles was confirmed by a reddish-brown colour change and further characterized using ultraviolet–visible (UV–Vis), Fourier transform infrared spectrometer (FTIR), scanning electron microscope (SEM), and transmission electron microscope (TEM) techniques. The average size of the particles was found to be 16–48 nm. The antimicrobial activity of the AgNPs was evaluated against harmful bacteria and compared to the commonly used antibiotic ciprofloxacin. The AgNPs were found to be highly effective, with a 24 mm zone of inhibition against *Escherichia coli*, and more effective than ciprofloxacin. Additionally, a minimum inhibitory concentration assay was performed with a concentration of 100 mg/mL of AgNPs, which were found to effectively inhibit the growth of selected pathogens. Overall, the study demonstrates the potential for using plant-derived AgNPs as a natural and eco-friendly alternative for antimicrobial and antioxidant applications. This method is a fast, cost-effective way to generate silver nanoparticles at room temperature and may be useful in creating environmentally friendly antibacterial solutions for biomedical applications.

Keywords: silver nanoparticles (AgNPs); *Luisia tristis*; transmission electron microscope (TEM); scanning electron microscope (SEM); energy-dispersive X-ray analysis (EDAX); antimicrobial; minimum inhibitory concentration (MIC)

Introduction

Nanotechnology is an active field of research that deals with the design, synthesis, and alteration of particles in the size range of 1–100 nm [1]. These particles have unique chemical, physical, and biological properties due to their small size [2]. Nanoparticles (NPs) have the potential for solving

environmental issues [3–6]. Research on the safe production of silver nanoparticles (AgNPs) is crucial, as these particles are commonly used in consumer products such as shampoos, detergents, soaps, toothpaste, and cosmetics [7].

Noble metal nanoparticles have gained attention for their unique properties such as optical, mechanical, magnetic, electrical, and chemical capabilities compared to bulk materials [8]. Silver has long been known for its antibacterial properties and is used in various settings to prevent bacterial growth, including dental procedures, catheters, and wound dressings. Ag ions and Ag-based compounds have strong biocidal effects on microorganisms and AgNPs have potential for the detection and treatment of cancer and drug delivery [9–11]. They are also used in molecular diagnostics and photonic devices due to their optical properties and in antimicrobial coatings on textiles and healthcare devices [12]. Moreover, phytoconstituents are valuable and encouraging candidates for synthesizing green silver nanoparticles (AgNPs) which possess great potential for infectious diseases. The use of plant extracts for nanoparticle synthesis is a cost-effective, non-toxic, and environmentally friendly method [13].

The velvet orchid, *Luisia tristis* is an epiphytic or lithophytic orchid that is native to Asia, Australia, and several Western Pacific Islands. It is known for its wiry stems, cylindrical leaves, and blooming stems with up to three green blooms with a dark scarlet to dark maroon labellum. It is commonly found in the Western Ghats of India, including Tamil Nadu, Kerala, and Karnataka [14], and has a flowering season from March to August. The plant is used in traditional medicine for various ailments, such as treating fractures, boils, tumours, and abscesses in India, Nepal, and Bangladesh [15]. The paste made from crushed herbs is used to treat jaundice for ten days. The juice from the leaves is applied to chronic wounds, and worms can be eliminated with the juice. The dried plant paste mixed with ginger and turmeric is used to treat jaundice for ten days [16].

The present research aims to synthesize stable silver nanoparticles using an aqueous extract of *L. tristis* leaves via a bioreduction approach. The filtrate of *L. tristis* leaves was used to synthesize silver nanoparticles, and the properties of the resulting nanoparticles were characterized, as well as their antibacterial efficacy against a variety of bacterial strains. The novelty of this study lies in the use of an extract from the leaves of the velvet orchid, *L. tristis,* for the synthesis of silver nanoparticles as well as the evaluation of their potential as an antimicrobial agent.

Experimental

Plant material

The samples of the *L. tristis* plant were obtained from the National Orchidarium and Experimental Garden, Botanical Survey of India, Southern Regional Centre, Yercaud, Salem District, Tamil Nadu, India. As this plant is considered vulnerable by the International Union for Conservation of Nature (IUCN), the samples were collected solely for the purpose of research and not for commercial use. The novelty of this research is the use of this plant as a source for the green synthesis of silver nanoparticles.

Preparation of AgNPs

Fresh samples of *L. tristis* were collected to prepare the extract. The aqueous extract was prepared by cutting and cleaning the fresh leaves, then heating them in 100 mL of sterile distilled water at 60 °C for 5 min. The extract was filtered through Whatman No. 1 filter paper and stored at 4 °C for further experiments. The experimental protocols used were based on the previously described method, with slight modifications [17].

Characterization of synthesized AgNPs

UV–Vis spectroscopy analysis

Silver nitrate was obtained from Hi-Media Laboratories Pvt. Limited in Mumbai, India, and used as received. Among the various plant extracts tested, the aqueous extract showed the most efficient synthesis of AgNPs. To create the nanoparticles, 90 mL of a 1 mmol/L solution of silver nitrate was added to 10 mL of the aqueous extract while the orbital shaker was spinning at 120 r/min and the reaction was conducted at room temperature. Throughout the experiment, necessary controls were maintained. The protocol was repeated three times at regular intervals to standardize the data. By taking aliquots (0.2 mL) of the suspension, diluting it with 2 mL of deionized water, and measuring the

UV–Vis spectra at the wavelength of 300–600 nm using an Elico microprocessor-based UV–Vis spectrophotometer, the bio-reduction of Ag^+ in aqueous solution was monitored. To identify the band pattern or any differences in the synthesis rate caused by changes in the extract concentration, UV–Vis spectra were recorded after 24 h.

Fourier transform infrared spectroscopy (FTIR) analysis

The functional groups responsible for the reduction of Ag ions and capping of the bio-reduced silver nanoparticles were identified by using FTIR. A Shimadzu IR double-beam spectrophotometer was used to record FTIR spectra via the potassium bromide (Shimadzu) pellet method in a 1:30 ratio (NPs: Shimadzu). The spectra were collected in transmittance mode with a resolution of 4 cm^{-1}. Stretching peaks were displayed as a percentage of transmittance on the *Y*-axis and wave number (cm^{-1}) on the *X*-axis. Spectra were captured in the range of 600–3 600 cm^{-1} and analyzed by removing the spectrum of pure Shimadzu.

Scanning electron microscopy (SEM) with dispersive X-Ray (EDX) analysis

A SEM analysis was performed using a ZEISS EVO-18 machine at 20 kV. A thin film of the sample was created on a carbon-coated tape by applying a small amount of the sample's dried powder to the grid. Any excess sample was removed with blotting paper, and the film on the SEM grid was then given a brief carbon coating to prevent surface charging. The surface structure of the reaction products during the biogenesis of AgNPs was determined by SEM examination.

Transmission electron microscope (TEM) with EDAX analysis

A small volume of synthesized AgNPs was dropped on a carbon-coated copper grid and left to dry. The dried sample was then placed on a specimen holder and imaged using TEM equipment at the Tamil Nadu Agricultural University, Coimbatore, India. The elemental composition of the nanoparticles was analyzed by using EDX.

Antimicrobial activity

Bacterial strains

Staphylococcus aureus (MTCC-3160), *Bacillus cereus* (MTCC-430), *Escherichia coli* (MTCC-1698), and *Pseudomonas aeruginosa* (MTCC-424) were used in the present study. These were grown on nutrient agar plates and stored on nutrient agar slants at 4 °C. The overnight culture of these microbes in nutrient broth was used for the experiment.

Antibacterial activity

The antibacterial activity of the synthesized nanoparticles was evaluated against chosen strains using the agar-well diffusion method. Overnight, bacterial cultures grown in nutrient broth were evenly spread across nutrient agar plates using a sterile cotton brush. A sterilized cork borer was used to create 6 mm-diameter wells. A 25-µg ciprofloxacin disc was impregnated with 50 µL and 100 µL of freshly prepared nanoparticles. The plates were incubated overnight at 37 °C, and the diameter of the zone of inhibition in mm was recorded and analyzed.

Evaluation of increased fold area

The inhibitory zone results from the antibiotic alone or in combination with nanoparticles were used to calculate the increase in fold area [18]. The antibacterial activity of the synthesized AgNPs was evaluated using the agar-well diffusion method against two Gram-positive (*B. subtilis* and *S. aureus*) and two Gram-negative (*P. aeruginosa* and *E. coli*) bacterial strains, with ciprofloxacin as the control.

Minimum inhibitory concentration (MIC) of tested microbes

A 96-well microtiter plate was used to determine the minimum inhibitory concentration (MIC) of *B. cereus* (MTCC-430), *S. aureus* (MTCC-3160), *E. coli* (MTCC-1698), and *P. aeruginosa* (MTCC424) [19]. A 2-fold serial dilution was performed, starting with a stock extract of *L. tristis* (500 mg/mL) in column 1, row 1, and adding sterile distilled water to the subsequent rows. Bacterial culture and double-strength nutritional broth were added to each well. The plates were incubated overnight at 37 °C, and the growth of the bacteria was measured using an ELISA reader (BIO-RAD) with 2,3,5-triphenyltetrazolium Chloride Red (0.02 mg/mL) as a growth indicator. The MIC was estimated as the lowest concentration at which the tested bacterial culture does not show measurable growth.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The *in vitro* antioxidant activity of AgNPs was

evaluated using a modified DPPH radical scavenging method [20]. 0.5 mL of a 0.15 mmol/L DPPH solution was mixed with 1 mL of the extract (in methanol) and incubated at 200 °C for 30 min. The absorbance was measured at 517 nm. The common antioxidant butylated hydroxyl toluene (BHT) was used as a reference. The percentage of antioxidant activity (AA%) or scavenging activity was calculated as follows: AA% = (Control Absorbance – sample absorbance) / control absorbance ×100%

Results

Synthesis of AgNPs

The synthesis of AgNPs was performed in an environmentally friendly manner using plant extracts. The formation of the nanoparticles was confirmed by the observation of a reddish-brown colour change, which occurred within an hour of mixing the aqueous plant extract into the synthesis process. The reaction was complete after 24 h (Fig. 1).

Characterization of synthesized AgNPs

UV–Vis analysis

The formation of AgNPs was found to be comparable to that of the chemical method of production. The UV–Vis spectra peak was narrow, indicating that the nanoparticles were similar in size to what had previously been reported in the literature. The solution's UV–Vis absorption spectrum revealed the typical surface Plasmon resonance band for AgNPs in the 420–440 nm range (Fig. 2). This is consistent with the typical UV–Vis absorption peak for silver nanoparticles, which is reported to be between 400 and 500 nm.

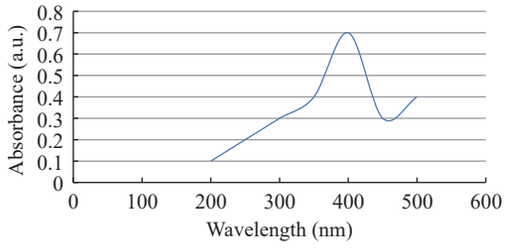


Fig. 2 UV–Vis spectroscopy analysis of AgNPs synthesized.

FTIR analysis

To identify the possible biomolecules in the leaf extract of *L. tristis* that act as capping agents for the efficient stabilization of the AgNPs, FTIR analysis was performed (Fig. 3), and the absorbance bands observed in the region of 600–3 600 cm^{-1} were 3 302, 2 129, 1 635, 1 087, 1 041, 601, 555, 470, 439, and 408 cm^{-1}. The O-H stretching (3 302 cm^{-1}), N=C=N stretching (2 129 cm^{-1}), C=C stretching (1 635 cm^{-1}), C–O stretching (1 087 cm^{-1}), CO–O–CO stretching (1 041 cm^{-1}), C–I stretching (601, 555, 470, 439, and 408 cm^{-1}) confirmed the presence of halides. The absorption peak at 3 302 cm^{-1} indicated the presence of alcohol and phenolic groups. The absorption spectra at 2 129 cm^{-1} represented the corresponding carbodiimide groups. The C=C stretching at 1 635 cm^{-1} revealed the presence of alkene (Fig. 3). The remarkable stability of the generated silver nanoparticles was enhanced by the FTIR analysis, which significantly corroborated the capping behaviour of bio-reduced AgNPs produced by *L. tristis* leaf extract.

SEM analysis with EDX Analysis

The reduced form of silver nitrate underwent scanning electron microscopic examination. It was crystal clear that the heat-dried silver particles from the bio-reduced colloidal suspension showed the well-dispersed AgNPs (Fig. 4) revealed a star and cuboid shape, and they were found to be monodisperse. The average size of the particles was 16–48 nm, with some particles measuring less than 50 nm. A few

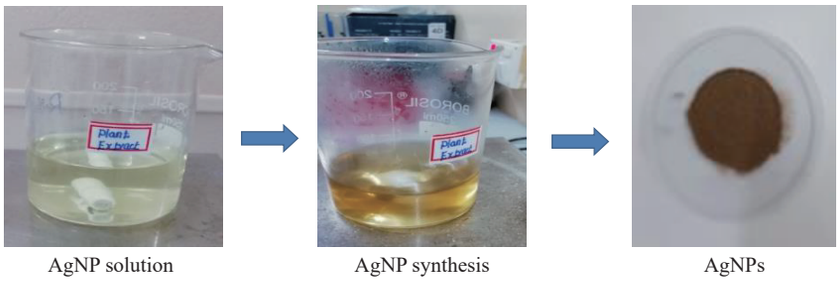


Fig. 1 Before (left) and after (right) adding plant extract solutions to a silver nitrate solution.

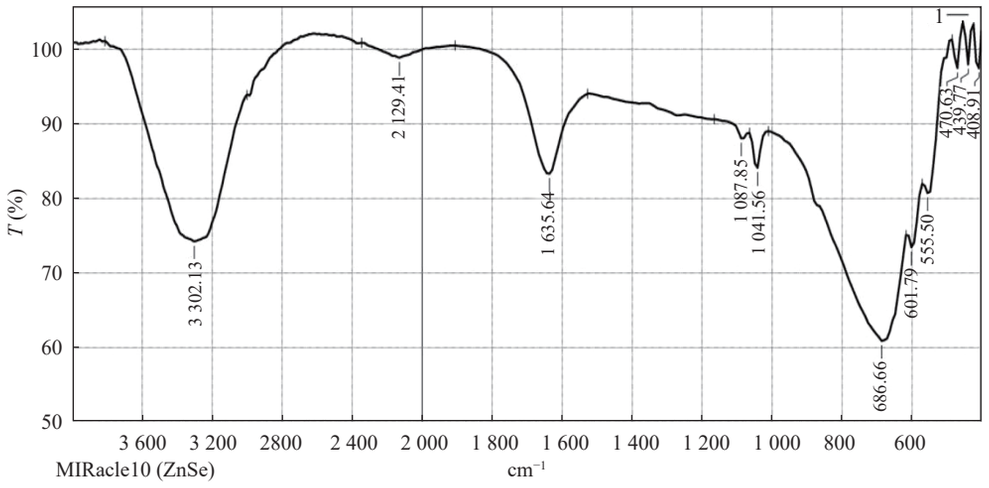
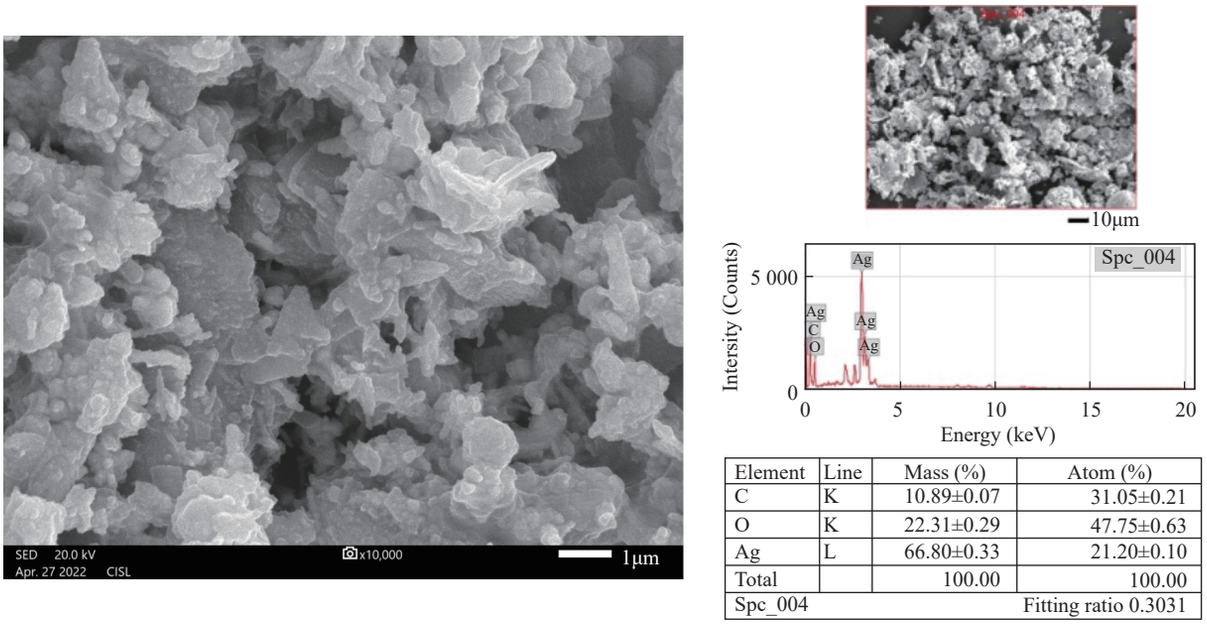


Fig. 3 FTIR analysis of AgNPs synthesized from *L. tristis.*



Element	Line	Mass (%)	Atom (%)
C	K	10.89±0.07	31.05±0.21
O	K	22.31±0.29	47.75±0.63
Ag	L	66.80±0.33	21.20±0.10
Total		100.00	100.00
Spc_004			Fitting ratio 0.3031

Fig. 4 SEM micrograph (100 and 50 nm) of AgNPs synthesized from *Luisia tristis*.

agglomerated particles were also observed, as indicated by the spectral shift.

TEM with EDAX Analysis

A TEM image provided clear insight of the structure of the synthesized AgNPs. The microscopy data revealed that the nanoparticles were polydispersed with a distinct shape and size. The majority of the particles were spherical in shape, but some were ellipsoidal. Their sizes ranged from 16.5 to 48 nm, with an average of 33.00 nm and no visible aggregation (Fig. 5). TEM confirmed the presence of AgNPs with precise size and shape.

SEM & TEM EDX analysis

EDX analysis provided a qualitative status of components involved in the creation of nanoparticles, confirming the reduction of silver ions into silver elements and demonstrating elemental silver as the predominant constituent. The graph from the EDX study, which verified the elemental components of silver, shows the presence of elemental silver [21, 22]. The reports of Ref. [23] were found to be supportive of these findings.

Antibacterial analysis

The use of nanoparticles as an alternative to traditional antibiotics is being studied due to the overuse of antibiotics and the growing problem of antibiotic resistance. In this study, we tested the inhibitory action of the synthesized AgNPs against harmful bacteria and evaluated their potency by measuring the presence of inhibition zones. It is important to note that the susceptibility of different types of bacteria to nanoparticles can vary. However, our results showed that the AgNPs we synthesized

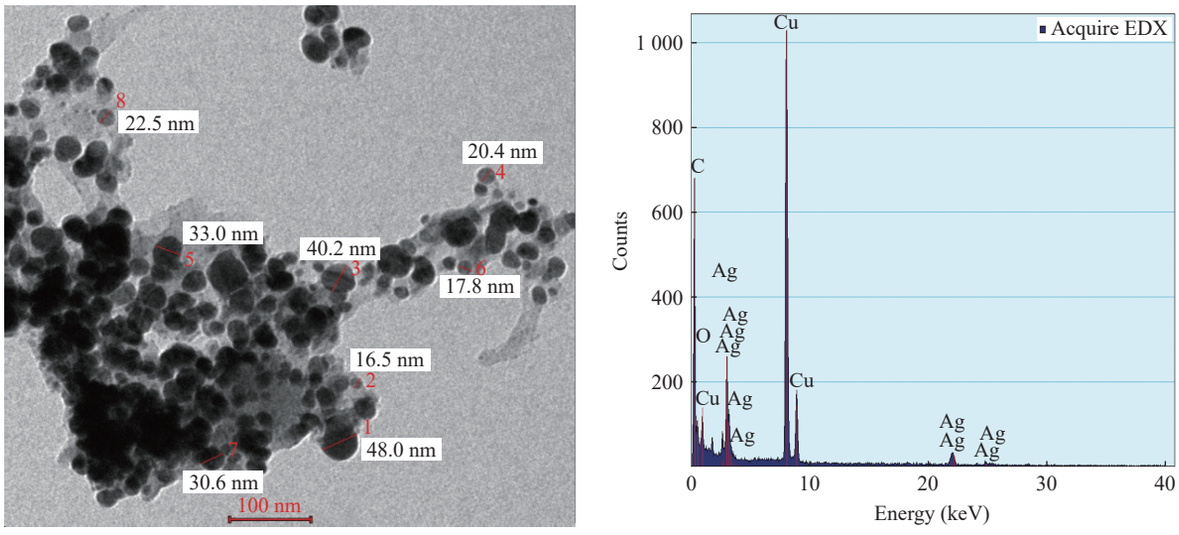
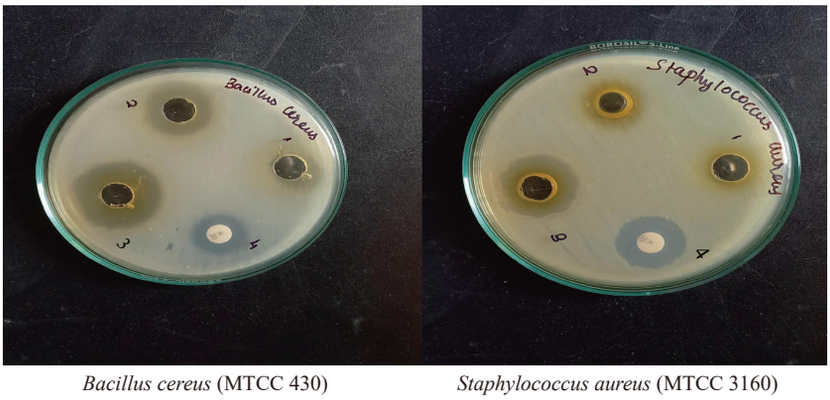


Fig. 5 TEM with EDX micrograph (100nm and 50nm) of AgNPs synthesized by *Luisia tristis*

had outstanding efficacy against bacterial pathogens. Specifically, the AgNPs were found to be highly effective against *Escherichia coli*, with a 24 mm zone of inhibition among the strains examined. We also tested the susceptibility of Gram-positive and Gram-negative bacterial strains to our synthesized AgNPs (Fig. 6). The results indicated that both groups of bacteria were susceptible to AgNPs, showing that the nanoparticles have a positive effect on inhibiting both types of bacteria. Additionally, our AgNPs were found to be more effective than the commonly used antibiotic ciprofloxacin. The results are shown in the table below (Table 1), which compares the antibacterial activities of AgNPs, $AgNO_3$, leaf extract, and standard ciprofloxacin.

Minimum inhibitory concentration assay and DPPH assay

To evaluate the effectiveness of the synthesized AgNPs in controlling microbial pathogens, a MIC assay was performed. The MIC values were determined for selected pathogens, including *B.*



Bacillus cereus (MTCC 430) *Staphylococcus aureus* (MTCC 3160)

Escherichia coli (MTCC 1698) *Pseudomonas aeruginosa* (MTCC 424)

Fig. 6 The zone of inhibition for antibacterial activity for (1) the synthesized AgNPs, (2) $AgNO_3$, (3) leaf extract, and (4) standard ciprofloxacin.

cereus, S. aureus, E. coli, and *P. aeruginosa*. The results showed that a concentration of 500 mg/mL of AgNPs was sufficient to inhibit the growth of these pathogens (Table 2). Among all the nanoparticles tested, those synthesized using *L. tristis* leaf extract had the strongest inhibitory action against human infections at very low concentrations. The antioxidant activity of the amount of DPPH scavenged in the samples increased in a dose-dependent manner (Table 3).

Table 1 Showing zone of inhibitions found in *B. cereus* (MTCC 430), *S. aureus* (MTCC 3160), *Escherichia coli* (MTCC 1698), and *P. aeruginosa* (MTCC424)

S.No.	Test organism	Zone of inhibition in diameter(mm)			
		Control	50 µL	100 µL	Standard disc (ciprofloxacin) 25 µg
1	*B. cereus* (MTCC 430),	00	30	35	25
2	*S. aureus* (MTCC 3160)	00	21	34	20
3	*E. coli* (MTCC 1698)	00	23	25	25
4	*P. aeruginosa* (MTCC424).	00	28	34	20

Table 2 Minimum inhibitory concentration (MIC) of *L. tristis* AgNPs against pathogens

Concentration inmg/0.5 mL	*B. cereus* (MTCC 430)	*S. aureus*(MTCC 3160)	*E. coli* (MTCC 1698)	*P. aeruginosa*(MTCC424).
10	++	--	++	++
30	++	++	++	++
50	++	++	--	++

Table 3 Antioxidant activity characterization of the aqueous and AgNP extracts of *L. tristis*.

Concentration (mg/mL)	Types of extract
	Methanol (%)
1	11.1 ± 0.41
2	24.2 ± 0.16
3	40.5 ± 0.24
4	50.3 ± 0.20
5	66.6 ± 0.32

Discussion

The UV–Vis spectroscopic analysis of the synthesized colloidal solution of AgNPs was conducted using a quartz cuvette with water as a reference. The results showed a reduction of Ag ions in the aqueous solution of the silver complex during the reaction with the ingredients present in the *L. tristis* plant leaf extract. The absorbance maximum was found to be at 420 nm, indicating the formation of spherical AgNPs or anisotropic particles. These particle's appearance and ratio can change over time, as seen in many studies. However, the UV–Vis spectra for the leaf extract alone revealed no absorption in the spectral range between 300 and 600 nm, which is consistent with previous findings obtained with the leaf extract alone [24].

The FTIR analysis revealed the presence of various functional groups in the leaf extract of *L. tristis*, which were responsible for the reduction of metal ions and the efficient stabilization of the synthesized AgNPs. The absorbance bands observed in the region of 3 600–424 cm^{-1}, including CO–O–CO stretching, C–Br stretching, and C–I stretching, confirmed the presence of halides. The absorption peaks at 3 718, 3 240, and 2 893 cm^{-1} indicated the presence of alcohol, phenolic groups, and carboxylic acid groups, respectively. The C=O stretching at 1 604 cm^{-1} revealed the presence of amides. The SEM analysis of the synthesized AgNPs showed that they were uniformly distributed and had a relatively spherical shape with a diameter range of 16–48 nm. The SEM and TEM analyses of the synthesized AgNPs revealed that they were uniformly distributed and had a relatively spherical shape with a diameter range of 16–48 nm. This is consistent with the findings of Bauer et al. [25], who also reported similar nanoparticle morphology. TEM images were collected from drop-coated films of AgNPs to further characterize the particles, their sizes, and distribution. The majority of the synthesized AgNPs were

spherical with an average size of 16–48 nm, which is in line with the variable nanoparticle morphology observed in other studies [26, 27].

The results showed that the AgNPs synthesized from the *L. tristis* exhibited strong activity against a variety of bacterial species. This antimicrobial and MIC activity of silver may be enhanced by the presence of high levels of secondary metabolites such as polyphenols, flavonoids, and tannins, as reported in previous studies [28, 29]. Additionally, the present study also demonstrated the potential antibacterial activities of the AgNPs synthesized from the *L. tristis* extract. This may be due to the denaturation of the bacterial cell wall, which leads to the blocking of bacterial respiration, destabilization of the outer membrane, and depletion of intracellular ATP, as reported in previous studies [30]. Overall, this study highlights the potential of *L. tristis* as a natural source for the synthesis of AgNPs with strong antibacterial properties.

Conclusion

The goal of this study was to investigate the synthesis and antibacterial properties of AgNPs using the leaves of *L. tristis*. Through the use of cost-effective and eco-friendly methods, the silver nanoparticles were successfully produced and characterized using various techniques such as UV–Vis spectrophotometry, FTIR, SEM, and TEM. Results from antimicrobial and MIC screening tests showed that the AgNPs had strong inhibitory effects against harmful bacteria. These findings suggest that silver nanoparticles produced naturally using plant extracts like *L. tristis* may have potential applications in the medical field due to their potenttial antibacterial properties.

CRediT Author Statement

Conceptualization: K. Saravanan and L. Prabha. Methodology: K. Saravanan, A. Subramanian, and K. Chidambaram. Formal analysis: A. Kalirajan, N. Veeraiyan, and K. Saravanan. Investigation: K. Saravanan and A. Subramanian; Data curation: K. Abdhul, L. Prabha. C. Ramalakshmi, and S. Karthik. Writing-original draft preparation: K. Seventhilingam, K. Chidambaram, C. Ramalakshmi, and A. Subramanian. Writing-review and editing: L. Prabha and K. Chidambaram. Project administration: A. Subramanian. Supervision: L. Prabha. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare that no competing interest exists.

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