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Eco-Friendly Synthesis of Silver Nanoparticles Using *Rivea ornate* choisy Leaf Extract with Antimicrobial Activity Assessment

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Abstract Nanotechnology is a rapidly advancing field that involves the manipulation of materials at the nanoscale. In recent years, "green" synthesis methods have gained popularity for nanoparticle production due to their eco-friendly and sustainable nature. This study presents a green synthesis approach for the production of silver nanoparticles (AgNPs) using the leaf extract of *Rivea ornata choisy,* widely used as a medicinal plant. The objective of this study was to explore the antimicrobial potential of AgNPs synthesized from the leaves of *Rivea ornate choisy.* The formation of AgNPs was indicated by a noticeable color change in the silver nitrate solution upon addition of the plant extract. This change was further confirmed through UV-Visible spectroscopy, showing characteristic surface plasmon resonance (SPR) peaks in the range of 425–475 nm. The nanoparticles were then separated via centrifugation and subjected to further characterization. X-ray diffraction (XRD) analysis revealed distinct diffraction peaks corresponding to the (111), (200), (220), (222), and (311) planes, confirming the crystalline nature of the synthesized AgNPs. (24 nm) The antimicrobial activity of the biosynthesized AgNPs was evaluated by using the standard disc diffusion method. Antibacterial activity was tested against two Gram-positive bacteria (*Bacillus* sp. and *Staphylococcus* sp.) and three Gram-negative bacteria (*Escherichia coli*, *Pseudomonas* sp., and *Proteus* sp.). Antifungal activity was assessed against *Rhizopus* sp., *Aspergillus* sp., *Mucor* sp., *Fusarium* sp., and *Penicillium* sp. Statistical analysis revealed that the synthesized silver nanoparticles exhibited significantly higher degree of antifungal and antibacterial activity against all tested fungi and bacteria. The biosynthesized silver nanoparticles using *Rivea ornata choisy* leaf extract proved excellent antimicrobial activity. These findings highlighted the potential of *Rivea ornata*-mediated AgNPs as effective antimicrobial agents, paving the way for future applications in biomedicine and sustainable nanotechnology.

Keywords: Antimicrobial Activity, Bioreduction, Green Synthesis, *Rivea ornata choisy*, Silver Nanoparticles

Introduction

Herbalism, also known as botanical medicine, medical herbalism, herbal medicine, herbology, and phototherapy, is a traditional medicine practice utilizing plants and plant extracts. Plants have been a significant source of medicines since human civilization, and despite advancements in allopathy, they remain a major source of drugs in modern and classical Ayurvedic systems worldwide. Approximately one-third of all pharmaceuticals are plant-based, including microorganisms like fungi and bacteria (Cowan., 1999)

The majority of medicinal plants in Sri Lanka are higher plants, with a minimal representation of lower plants. The families Fabaceae, Euphorbiaceae, Solanaceae, Poaceae, Asteraceae, Malvaceae, Cucurbitaceae, Rubiaceae, and Convolvulaceae are among the most commonly utilized in traditional medicinal practices across the country (Rajakaruna et al., 2025)

Chemically prepared nanoparticles are not appropriate for medical usages due to hazardous chemicals binding on their surface (Ouda., 2014). Using biological systems such as microorganisms, plants, viruses and animal cell cultures is an alternative procedure for preparation of NPs (Singhal et al., 2011). Green synthesis methods are eco-friendly, cost-effective, and readily scaled up for large-scale synthesis, with silver nanoparticles serving as a universal antimicrobial agent (Nishibuchi et al., 2012;Mittal et al., 2014; Dua et al 2023).

Green-synthesized silver nanoparticles (AgNPs), produced using plant extracts as reducing and stabilizing agents, exhibit broad-spectrum antimicrobial activity against bacteria, fungi, and viruses due to their small size, high surface area, and unique physicochemical properties (Abbasi et al., 2016). These AgNPs are also known for their anti-inflammatory, antifungal, antibacterial, anticancer, wound healing, and photo-imaging applications (Moodley et al., 2018; Abdel-Rahmam et al., 2022). Notably, plant-mediated AgNPs have shown enhanced antibacterial efficacy against both Gram-positive and Gram-negative bacteria (Praba et al., 2015; Lakshmanan et al., 2018).

Before applying silver nanoparticles (AgNPs), their formation must be confirmed using various characterization methods. A preliminary indicator is the visible colour change of the solution, followed by UV-Vis spectrophotometry, which detects characteristic nanoparticle peaks typically within the 400–800 nm range due to surface plasmon resonance (SPR) (Prathna et al., 2011; Balashanmugam et al., 2013). Additionally, X-ray diffraction (XRD) is a key technique for confirming nanoparticle formation, determining crystal structure, size, crystallinity, and purity, based on Bragg's law (Mashwani et al., 2015).

Rivea ornata Choisy, commonly known as the "Christmas vine" or "ornate morning glory," is a woody climber native to southern India and Sri Lanka. Traditionally known as "Masuttai" in Tamil, various parts of this plant have been used in indigenous medicine for treating digestive disorders, inflammation, fatigue, bronchitis, and even hemorrhoids (Hwisa et al., 2013). It is also consumed as a leafy vegetable to boost hemoglobin levels and cleanse the blood. Its leaves and flowers are rich in iron and bioactive compounds, making it a promising candidate for green synthesis applications (Soujanya et al., 2021; Sudheer et al., 2021; Karunarathna et al., 2023).

Given its therapeutic potential and rich phytochemical profile, *Rivea ornata* offers a natural, sustainable source for the synthesis of AgNPs (Nahoor Meeran., 2019; Hosamani et al., 2000; Sharma et al., 2013). Its leaves contain bioactive compounds that can facilitate the reduction of silver ions and stabilize the resulting nanoparticles.

In this study, we report the eco-friendly synthesis of silver nanoparticles using *Rivea ornata* leaf extract and their structural characteristics and antimicrobial activity. Characterization techniques including UV–Vis spectroscopy and X-ray diffraction (XRD) were employed to confirm nanoparticle formation and analyze crystal structure, and particle size. The findings from this research not only validate the traditional use of *Rivea ornata* but also support its application in modern nanomedicine as a bio-resource for developing safe and effective antimicrobial agents.

Materials and methods

Preparation of plant extract

Leaves of *Rivea ornata* Choisy were collected from the Herbal Garden of the Department of Agriculture, Thirunelvallay, Sri Lanka (Figure 1). The leaves were thoroughly washed with tap water, shade-dried for 10 days, and then cut into small pieces. A 20 g portion of the dried leaves was transferred into a 500 mL beaker containing 100 mL of deionized water and boiled for 5 minutes. After allowing the mixture to cool to room temperature, it was filtered through Whatman No.1 filter paper. The obtained leaf extract was then stored at 4 °C for further use.

Figure 1: Plant of *Rivea ornata* Choisy

Synthesis of Silver nanoparticles

Silver nanoparticles were synthesized by mixing 10 mL of plant extract with 100 mL of 10 mM $AgNO_3$ solution, followed by stirring at 37 °C for 15 minutes in the dark to prevent photoactivation. A color change from colourless to yellowish-brown indicated the reduction of Ag^+ ions. The mixture was then cooled, left undisturbed for 24 hours to complete the bioreduction, and analyzed using UV-Vis spectrophotometry to confirm nanoparticle formation. The resulting colloidal solution was stored for future use.

Characterization of AgNps

The synthesized silver nanoparticles were characterized using a UV-Visible spectrophotometer (Jasco V-570 UV/VIS/NIR) over a wavelength range of 200–800 nm. The synthesized AgNPs were isolated by centrifugation at 7,500 rpm for 20 minutes (HERMLE-Z 287A) to remove free proteins and other impurities (Gurunathan., 2015). Phase purity and crystallinity were assessed through powder X-ray diffraction (XRD) using a PANalytical AERIS instrument. The XRD measurements were conducted at room temperature with Cu Kα radiation ($\lambda = 1.5408$ Å), under operational conditions of 40 kV accelerating voltage and 44 mA emission current. The crystalline size of the nanoparticles was calculated using the Scherrer equation:

$$\mathbf{D = K\lambda / (\beta \cos \theta)}$$

where D is the crystallite size, K is the Scherrer constant, λ is the X-ray wavelength, β is the full width at half maximum (FWHM) of the diffraction peak, and θ is the Bragg angle.

Determination of antimicrobial activity

Collection of Microbial cultures

The fungi *Rhizopus* sp., *Aspergillus* sp., *Mucor* sp., *Fusarium* sp., *Penicilium* sp. and the bacteria *Bacillus* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Escherichia coli, Proteus* sp. were obtained from the culture collections of the Department of Botany, University of Jaffna. Fungal cultures were maintained in Potato Dextrose Agar (PDA) medium whereas bacterial cultures were maintained in Nutrient Agar (NA) medium.

Determination of antifungal activity by Disc diffusion method

Fungal spore suspension [x* 10^6 number of spores/mL] of 0.1mL was spread separately on the entire surface of the PDA plate with the help of a sterile spreader. 9 mm diameter of sterile discs were placed on the medium aseptically. Then 150 µL of test solutions, standard (1 mg of Mancozeb/10 mL of sterile water) and control (pure solvents of leaf extract, deionized water and silver nitrate solution [10mM]) were eluted into each disc separately with the help of a sterile micropipette. All the plates were incubated at room temperature for 4-5 days and the zone of inhibition around the disc was measured after 72 hours. Each of the experiment was repeated thrice and the mean value was obtained. The above procedure was repeated for each of the fungus.

Determination of antibacterial activity by Disc diffusion method

Antibacterial activity of *Rivea ornata choisy* extracts was determined using agar disc diffusion method. 0.1 mL of each standardized bacterial suspension with 0.5M McFarland standard was spread on the entire surface of the NA plate uniformly with the help of a sterile spreader. 9 mm diameter sterile discs were placed on the medium aseptically. Then 150µL of test solutions, standard (100ppm streptomycin) and control (pure solvents of leaf extract, deionized water and silver nitrate solution [10mM]) were eluted into each disc separately with the help of a sterile micropipette. All the plates were incubated at 37°C for 24 hours and the zone of inhibition around the disc was measured. Each of the experiment was repeated thrice and the mean value was obtained. The above procedure was repeated for each of the bacterium (Balouiri et al., 2016, Parekh et al., 2007, Saboo et al., 2014)

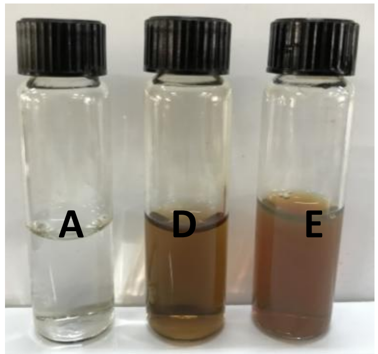
Statistical Analysis

All experimental data were analyzed statistically by using Minitab 17 Statistical Software.

Results and Discussion

Colour change

After addition of *Rivea ornata choisy* extract to the aqueous solution of $AgNO_3$of 10 mM concentration, the mixture showed a gradual change in color at room temperature with yellowish- brown colour and the colour intensified after 24 hours (Figure 2).



A - AgNO3
D - leaf extract(F-LE)
E -AgNPs

Figure 2: Colour change of leaf extract after addition of $AgNO_3$ solution.

Characterization of silver nanoparticles (AgNPs)

UV-Vis spectroscopy analysis

The reduction of silver ion to silver nanoparticle was reflected in spectral data obtained by using a UV-Vis spectrophotometer. It shows an absorbance peak at 450 nm, which is specific for silver nanoparticles (Figure 3).

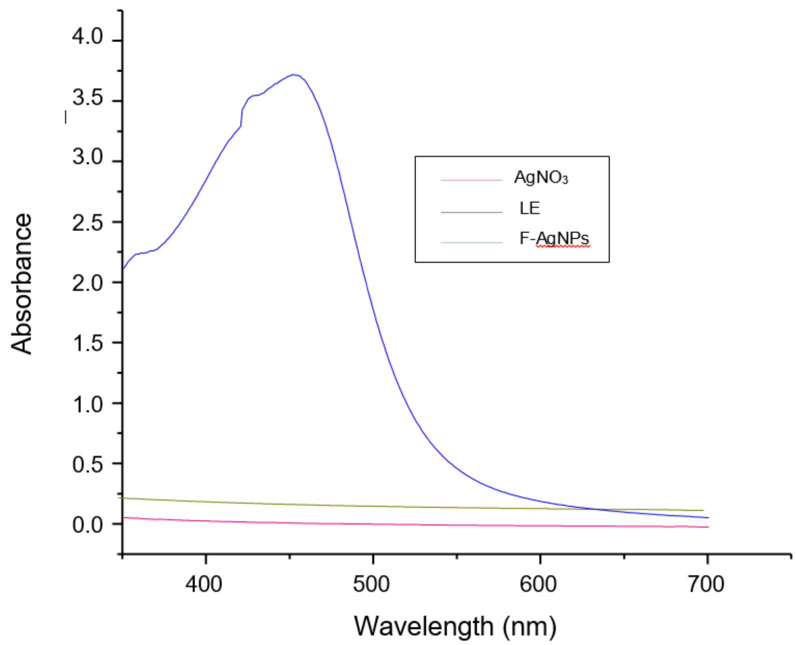


Figure 3: UV spectrum of the synthesized silver nanoparticles

X-ray diffraction analysis

The XRD pattern exhibited distinct diffraction peaks at 2θ values corresponding to the (111), (200), (220), and (311) planes (Figure 4), confirming the face-centered cubic (FCC) crystalline structure of the silver nanoparticles (de Jesús Ruíz-Baltazar et al., 2017). Additional peaks observed in the pattern can be attributed to residual organic compounds from the plant extract. These signals suggest the presence of plant-derived metabolites crystallized on the nanoparticle surfaces, supporting the involvement of phytochemical constituents in nanoparticle formation (Mashwani et al., 2015)

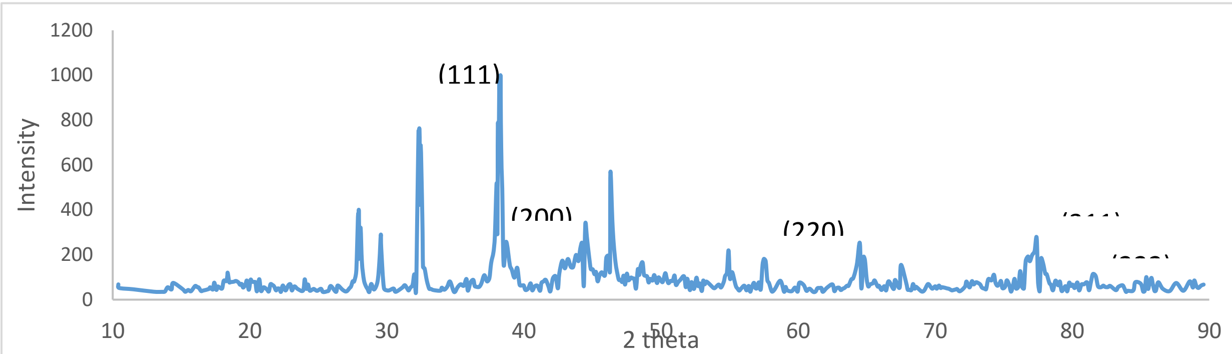


Figure 4: XRD Spectrum of synthesized AgNps

Results of antimicrobial activity

Antifungal assay

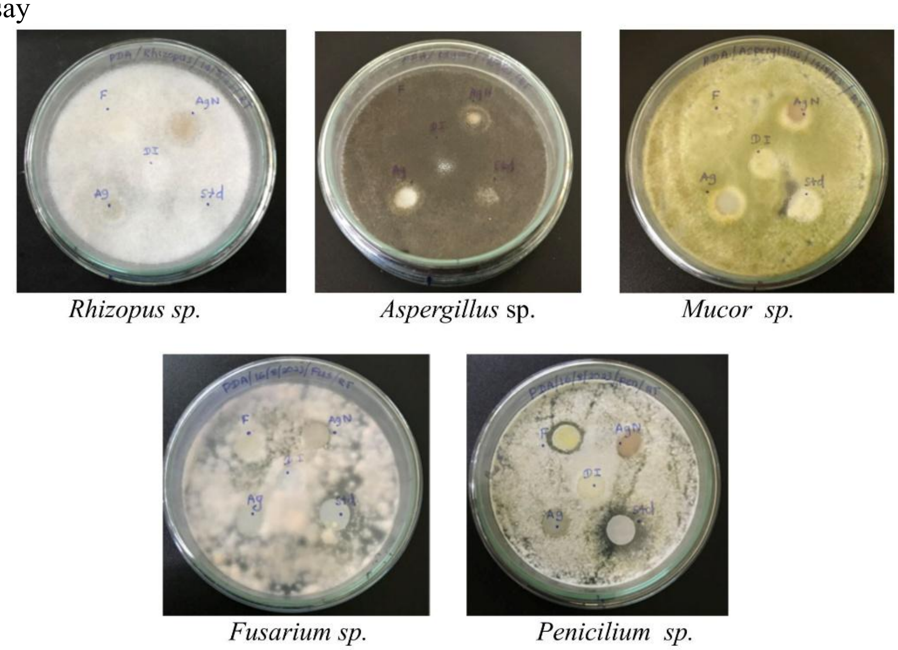


Figure 5: Effects of different treatments of *Rivea ornata* Choisy leaf against fungal cultures (LE/F - leaf extract: AgN - leaf of AgNPs: DI - deionized water: Ag - $AgNO_3$ solution (10mM): Std – standard)

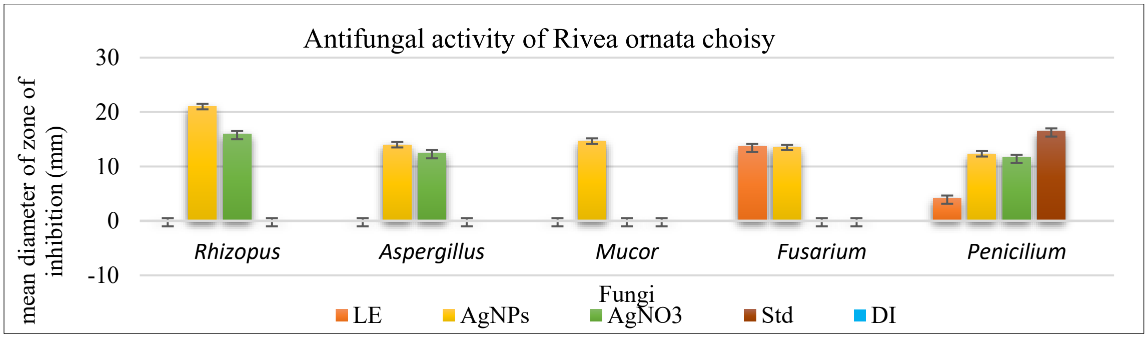
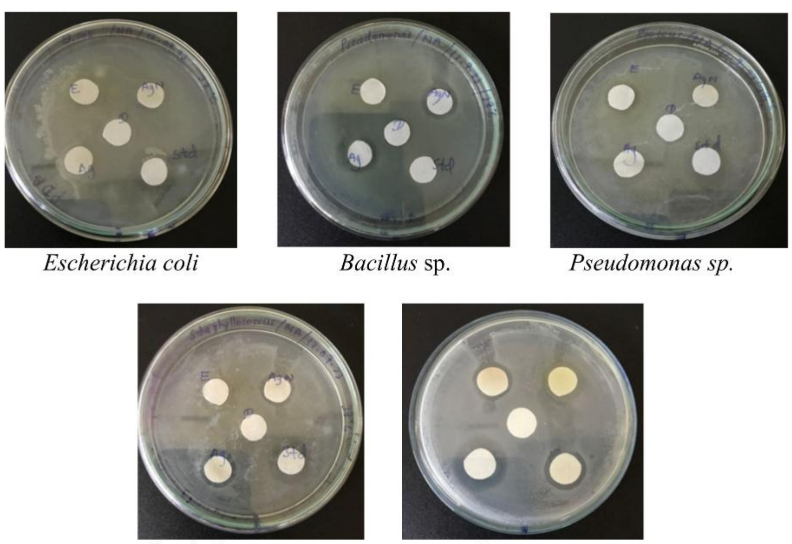


Figure 6: Inhibition zones of different treatments of *Rivea ornata choisy* leaf against fungal cultures (LE/F - leaf extract: AgN - leaf of AgNPs: DI - deionized water: Ag - $AgNO_3$ solution (10mM): Std – standard)

This study was conducted to find out the antifungal activity of AgNPs synthesized using leaf extract of of *Rivea ornata choisy* against the tested fungi *Aspergillus* sp., *Mucor* sp., *Rhizopus* sp., *Penicilium* sp., and *Fusarium* sp. The growth of all fungi was observed after 72 hours of incubation (Figure 5). It was also observed that the diameter of the zone of inhibition decreased with an increasing incubation period due to the instability of the biomolecules "in vitro" exhibited the antimicrobial activity. Different treatments had different effects on the growth of fungi

A statistical analysis was done for the comparison of antifungal activity with different treatments. A one-way ANOVA showed that there was a significant difference among treatments ($\alpha = 0.05$). Antifungal activity was high against *Rhizopus* sp. for the synthesized AgNPs compared to other fungal species. Only the leaf extract showed the antifungal activity against *Penicilium* sp. and *Fusarium* sp. Tukeys pairwise comparisons (grouping information using the Tukey method and 95% confidence) showed that the synthesized silver nanoparticles exhibited the highest antifungal activity than other treatments. Deionized water did not show antifungal activity against all tested fungi (Figure 6). The degree of inhibition depends on the type of fungi, spore concentration, types of antifungal compounds, and their concentrations.

Antibacterial assay



Escherichia coli *Bacillus* sp. *Pseudomonas sp.*

Staphylococcus sp. *Proteus sp.*

Figure 7: Effects of different treatments of *Rivea ornata choisy* leaf against bacterial cultures (LE/F - leaf extract: AgN - leaf of AgNPs: DI - deionized water: Ag - $AgNO_3$ solution (10mM): Std – standard)

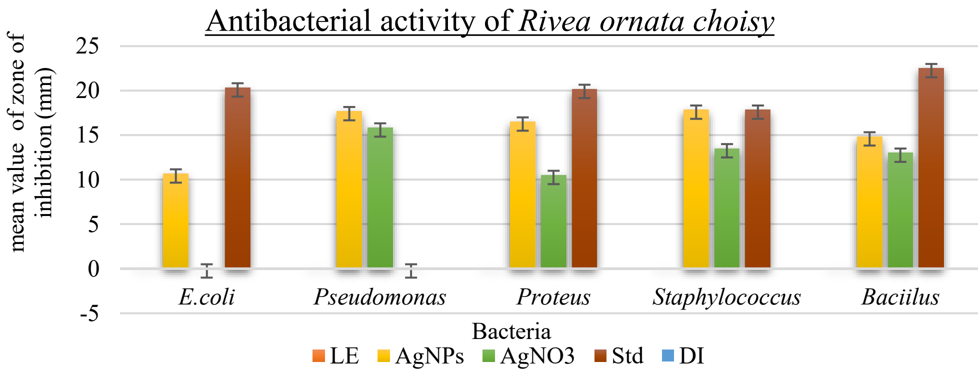


Figure 8: Inhibition zones of different treatments of *Rivea ornata choisy* leaf against bacterial cultures. (LE/F - leaf extract: AgN - leaf of AgNPs: DI - deionized water: Ag - $AgNO_3$ solution (10mM): Std – standard)

The present study was to find out the degree of antibacterial activity of synthesized AgNPs of leaves of *Rivea ornata choisy* against the tested bacteria *Escherichia coli*, *Bacillus* sp., *Pseudomonas* sp., *Proteus* sp. and *Staphylococcus* sp.(Figure 7).

Here, 100 ppm streptomycin antibiotic was used as a standard in order to assess the degree of effectiveness of the samples. The standard streptomycin exhibited the highest antibacterial activity compared to the other treatments except *Pseudomonas* sp. Deionized water and leaf extracts did not show any antibacterial activity.

A statistical analysis was done for the comparison of antibacterial activity with different treatments. One-way ANOVA showed that there was a significant difference among treatments ($\alpha = 0.05$). Silver nanoparticles exhibited the higher degree of antibacterial activity than leaf extract and control. By Tukey pairwise comparisons, there was no significant difference between the synthesized silver nanoparticles and the standard, showed the highest degree antibacterial activity (Figure 8). The degree of inhibition depends on the type of bacteria, bacterial concentration, growth conditions, types of antibacterial compounds and their concentrations.

Conclusion

The study aimed to synthesize silver nanoparticles (AgNPs) using an eco-friendly method with *Rivea ornata Choisy* leaf extract as both reducing and capping agent. The formation of AgNPs was indicated by a yellowish-brown colour change and confirmed by UV-Vis spectroscopy (absorption at 425–475 nm) and XRD analysis, which showed effective stabilization. Synthesized silver nanoparticles exhibited significantly higher degree of antifungal and antibacterial activity against all tested fungi and bacteria. The biosynthesized silver nanoparticles using *Rivea ornata choisy* leaf extract proved excellent antimicrobial activity. These stable, biosynthesized AgNPs show promise for use in agriculture, catalysis, biomedical, and pharmaceutical applications.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Author contributions

JPJ: Conceptualization, Supervision, Resources, Methodology review and editing
ACT: Resources, Supervision, Methodology, review and editing
MA: Investigation and Software
MHFH: Methodology, Data curation, Writing–original draft

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