

Eco-Friendly Synthesis and Physicochemical Characterization of Silver Nanoparticles Mediated by *Eugenia roxburghii*
DC. Extract with Antibiofilm Efficacy against Pathogenic Bacteria

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ABSTRACT

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The increasing resistance of bacteria to conventional antibiotics necessitates the exploration of alternative antimicrobial strategies. This study presents a green, eco-friendly synthesis of silver nanoparticles (AgNPs) using methanolic leaf extract of *Eugenia roxburghii* DC., a medicinal plant known for its rich phytochemical content. The synthesized AgNPs were characterized by UV-Visible spectroscopy, X-ray diffraction (XRD), high-resolution transmission electron microscopy (HR-TEM), and zeta potential analysis. A distinct surface plasmon resonance peak at 417 nm confirmed nanoparticle formation, while XRD and HR-TEM analyses revealed a crystalline, predominantly spherical morphology with an average particle size of 24–35 nm. The AgNPs exhibited strong antibacterial activity, particularly against *Staphylococcus aureus*, as demonstrated by disc diffusion and minimum inhibitory concentration (MIC) assays. Furthermore, the nanoparticles significantly inhibited biofilm formation, as observed on Congo Red Agar plates. These findings underscore the potential of *E. roxburghii*-mediated AgNPs as a sustainable and biocompatible alternative for combating pathogenic bacteria and biofilm-related infections. Future in vivo and toxicological studies are warranted to evaluate clinical applicability.

Keywords : Silver Nanoparticles, *Eugenia Roxburghii*, Green Synthesis, Antibacterial Activity, Biofilm Inhibition, Nanotechnology.

Introduction:

Many plants in the Myrtaceae family are known for their medicinal properties, and local communities have traditionally used them to manage conditions such as diabetes, arthritis, and hypertension (Raghavendra et al., 2013). While crude plant extracts

contain bioactive compounds, their bioavailability is often limited. This limitation can be overcome by converting these extracts into nanomaterial-based forms, which significantly enhance their therapeutic potential (Iravani, 2011).

Within this family, the genus *Eugenia* is particularly noteworthy for its bioactive compounds with pharmaceutical potential. Species of *Eugenia* are also known for producing edible fruits that are rich in vitamins and minerals (Pereira et al., 2012). One such species is *Eugenia roxburghii* DC., commonly referred to as Roxburgh's Cherry owing to the appealing taste of its fruits. This wild, fruit-bearing plant is predominantly found in the coastal and tropical regions of India and Sri Lanka. *E. roxburghii* is known to contain a variety of secondary metabolites with reported anticancer and antibacterial properties (Dash et al., 2020).

Nanoparticles (NPs)—defined as particles with dimensions in the nanometer scale—have garnered significant attention due to their high surface area and diverse applications in fields such as catalysis, optics, electronics, and particularly in antibacterial and antimicrobial therapies (Rai et al., 2009). Metallic nanoparticles, in particular, exhibit unique physical and chemical properties compared to their bulk counterparts, making them highly effective antimicrobial agents (Singh et al., 2018).

Plants and their parts have proven effective in the green synthesis of metal nanoparticles, serving as reducing and stabilizing agents (Ahmed et al., 2016). Among metallic nanoparticles, silver nanoparticles (AgNPs) are especially noteworthy due to their wide-ranging applications in medical and biotechnological sectors (Loo et al., 2018).

Conventional methods of AgNP synthesis—both physical and chemical—come with significant drawbacks, such as high production costs, use of toxic chemicals, energy-intensive processes, and irregular particle sizes (Shankar et al., 2004). In contrast, biological (green) synthesis methods offer a safer, more sustainable alternative, utilizing non-toxic phytochemicals from plants, microbes, and biopolymers. The resulting nanoparticles are not only

biocompatible but also exhibit potent biological efficacy (Khan et al., 2019).

Previous studies have reported the successful synthesis of AgNPs using various *Eugenia* species, such as *E. jambolana*, *E. caryophyllata*, and *E. uniflora*, representing antibacterial and even antidiabetic special effects (Barros et al., 2018; Fernandes et al., 2020; Prasad et al., 2021). However, to date, no study has reported the synthesis of AgNPs from *Eugenia roxburghii* DC., a wild, edible plant known for its rich phytochemical content and medicinal potential.

One major focus of contemporary antimicrobial research is on biofilm-forming bacteria. Biofilms are structured communities of bacteria encapsulated in an extracellular polymeric matrix that adheres to surfaces (Costerton et al., 1999). These biofilms contribute to phenotypic and genetic changes in the bacteria, significantly enhancing their resistance to antibiotics, extreme pH, oxidative stress, and high salt conditions (Hoiby et al., 2010). Biofilms are especially problematic in medical contexts, commonly forming on implants, catheters, and other devices, thereby increasing bacterial pathogenicity (Donlan & Costerton, 2002).

Although AgNPs have been synthesized from other plants such as *Azadirachta indica* (Neem), *Aloe vera*, *Embllica officinalis* (Amla), and *Cinnamomum camphora* (Song & Kim, 2009), no such research has been reported using *E. roxburghii*. In our previous work, we observed that leaf extracts of *E. roxburghii* exhibited strong antimicrobial activity (Das et al., 2022). Building on this, the present study aims to synthesize silver nanoparticles from *E. roxburghii* leaf extract and evaluate their efficacy, particularly against biofilm-forming *Staphylococcus aureus*.

Given the enhanced antimicrobial properties of nanoparticles over crude plant extracts, this investigation seeks to assess the potential of *E. roxburghii*-derived AgNPs in inhibiting bacterial

biofilms, offering a promising natural alternative for combating microbial infections.

Materials and Methods:

Plant Extract Preparation
Fresh, in fine fettle leaves of *Eugenia roxburghii* had been collected, authenticated (Voucher No. SOAU/CBT/2020/ER/01), and processed under institutional guidelines. Twenty-five grams of leaf tissue were ground with liquid nitrogen and extracted in 250 mL methanol. The extract was filtered (Whatman No. 1) and stored at -20°C .

Green Synthesis of AgNPs
AgNPs were synthesized by mixing 20 mL of leaf extract with 100 mL of 0.1 mM AgNO_3 (5:1 ratio) and incubating at 37°C , 300 rpm for 48 h. Creation of AgNPs was specified by a colour change to yellowish-brown and observed by UV-Vis spectroscopy.

Characterization

UV-Vis spectra were recorded (200–700 nm, Hitachi UH5300). XRD analysis (Rigaku Ultima IV, $\text{Cu-K}\alpha$, $\lambda = 0.1541\text{ nm}$) was conducted over $2\theta = 20^{\circ}$ – 80° . HR-TEM (JEOL JEM 2100, 200 kV) was used to assess morphology and size.

Antibacterial and Biofilm Activity

Disc diffusion assays were conducted against *E. coli*, *P. aeruginosa*, *V. cholerae*, and *S. aureus* using AgNP concentrations (120–240 $\mu\text{g/mL}$). MIC against *S. aureus* was determined via OD600 monitoring in nutrient broth. Anti-biofilm activity was evaluated on Congo Red Agar with and without AgNPs (0.065 g/mL), incubated at 34°C for 72 h.

Observation and Results:

UV-Visible Spectrophotometric Analysis

The initial confirmation of silver nanoparticle (AgNP) synthesis using *Eugenia roxburghii* leaf extract was performed using UV-Visible spectrophotometry. A

distinct colour change was observed in the reaction mixture, transitioning from green to yellowish-brown, indicating the formation of silver nanoparticles. The absorbance of the solution was monitored over a period of one week. A characteristic surface plasmon resonance (SPR) peak for AgNPs appeared at 417 nm, confirming nanoparticle formation (Fig. 1). The peak intensity remained stable over subsequent days, suggesting that the synthesized nanoparticles were stable over time with no significant change in absorbance.

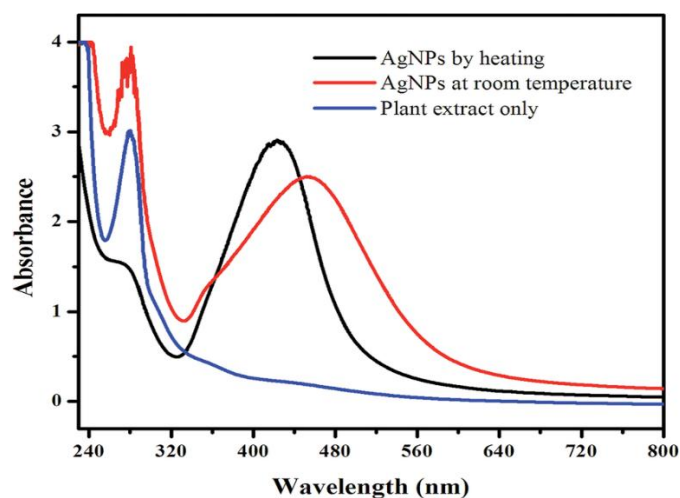


Figure 1. UV-Vis preoccupation spectrum of synthesized AgNPs from *E. roxburghii* leaf extract.

X-Ray Diffraction (XRD) Analysis

The crystal-like nature of the synthesized AgNPs was analysed using X-ray diffraction (XRD) (Fig. 2). The average particle size was estimated using the Debye-Scherrer equation:

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

Where:

- D = average particle diameter
- k = shape factor (0.9)
- λ = X-ray wavelength (0.1541 nm)
- β = full width at half maximum (FWHM)
- θ = Bragg diffraction angle

Based on the XRD pattern, the calculated average crystallite size of the AgNPs was approximately 35 nm. The lattice parameters were determined as $a = b = c = 0.4086$ nm, which is in close agreement with the standard lattice constant of silver (0.4073 nm)

reported in literature.

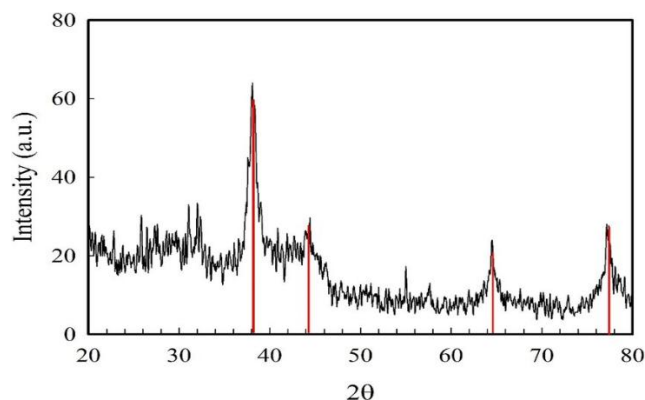


Figure 2. XRD pattern of AgNPs synthesized from *E. roxburghii* leaf extract

High-Resolution Transmission Electron Microscopy (HR-TEM)

To further examine the morphology and size distribution of the synthesized nanoparticles, high-resolution transmission electron microscopy (HR-TEM) was performed. A carbon-coated copper grid was prepared by placing a drop of the colloidal AgNP solution on it and allowing it to dry at room temperature. HR-TEM micrographs and selected area electron diffraction (SAED) patterns. The AgNPs were observed to be predominantly spherical in shape. The particle sizes ranged from 19 to 39 nm, with an average diameter of approximately 24 nm.

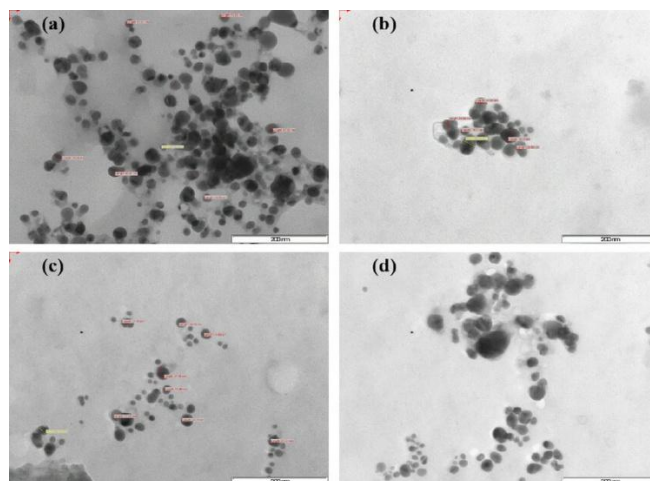


Figure 3. a) TEM micrographs image of the synthesized AgNPs, (b) HR-TEM spitting image of AgNPs, (c) SAED spitting image of AgNPs, (d) TEM image of different size.

Characterization by Zeta Potential Analysis

The surface charge of the synthesized silver nanoparticles was evaluated using Zeta potential analysis, which measures the electrostatic potential between the dispersion medium and the nanoparticle surface. This property is crucial for understanding the stability of colloidal suspensions. As shown in Figure 3, the zeta potential of the *Eugenia roxburghii*-mediated AgNPs was recorded as -38.7 mV, indicating a high degree of stability due to strong electrostatic repulsion between particles, which prevents aggregation.

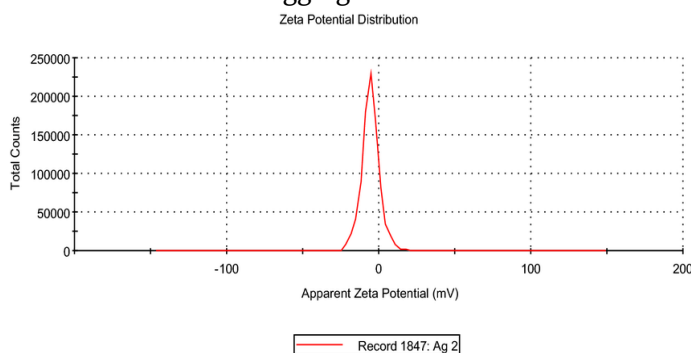


Figure 4. Zeta potential of synthesized AgNPs.

Antibacterial Activity Assessment

The antibacterial activity of the synthesized AgNPs was assessed using the disc diffusion method and minimum inhibitory concentration (MIC) assay. Among all tested bacterial strains, the AgNPs exhibited maximum inhibition against *Staphylococcus aureus* (Figure 4). Consequently, *S. aureus* was selected for further MIC analysis.

In the MIC assess, a continuous upsurge in absorbance was perceived at an absorption of 120 µg/mL, suggesting bacterial growth. However, at 240 µg/mL, a steady decline in absorbance was recorded, indicating effective inhibition of bacterial growth (Figure 4). Other tested concentrations did not show significant changes in absorbance, confirming 240 µg/mL as the most effective inhibitory dose.

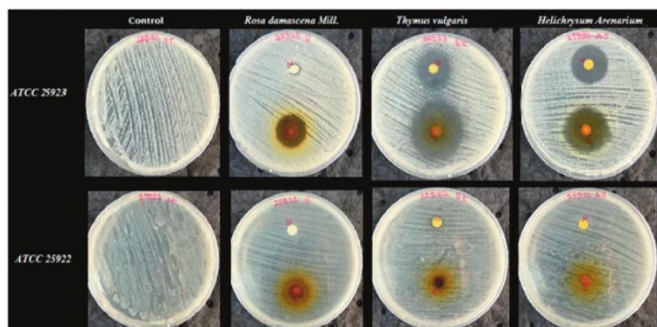


Figure 5. Antibacterial activity of the synthesized AgNPs

Effect on Biofilm Formation

The impact of the synthesized AgNPs on biofilm formation was examined using Congo Red Agar (CRA) plates. In the control group (CRA plate without AgNPs), bacteria developed a distinct color change, signifying active biofilm formation. In contrast, no color change was observed in CRA plates treated with AgNPs, indicating a significant inhibition of biofilm production by the nanoparticles (Figure 5). This confirms the potential of *E. roxburghii*-derived AgNPs in preventing biofilm-associated bacterial infections.

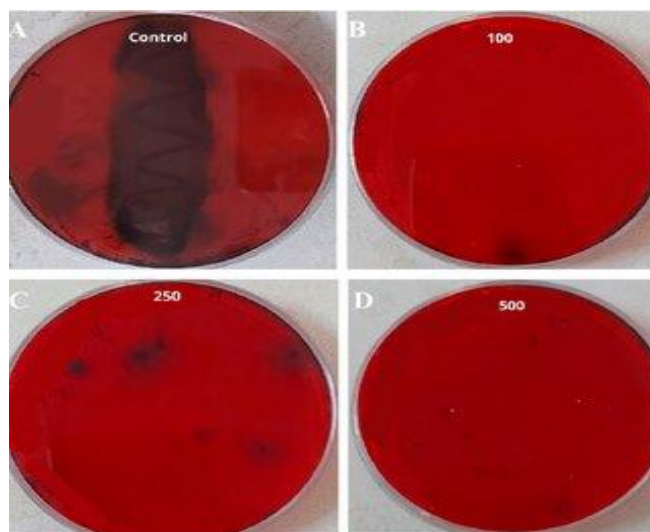


Figure 6. Effect of AgNPs on biofilm production by *S. aureus* on Congo Red Agar plates.

Discussion:

Nitrate reductase (NR) is a vital enzyme involved in nitrogen assimilation across various plant species, catalyzing the conversion of nitrate (NO_3^-) to nitrite (NO_2^-) within the cytoplasm of plant cells (Huang et al., 2016). This enzyme has also been implicated in the **bioreduction of silver ions** to silver nanoparticles (AgNPs) via an **NADPH-dependent pathway** (Bai et al., 2014). When exposed out to silver ions, nitrate reductase eases their reduction, allowing nanoparticle development.

Several related studies have reported similar findings in *Eugenia* and *Syzygium* species. For example, AgNPs synthesized from *Syzygium jambolana* exhibited a UV-Vis absorbance peak at **460 nm**, with **TEM-derived particle sizes ranging from 6 to 23 nm** (Kumar et al., 2018). Similarly, in *Syzygium cumini*, a UV-Vis peak was noted at **~450 nm**, and XRD analysis showed particle sizes around **3.5 nm** (Sathishkumar et al., 2013). In the case of *Eugenia uniflora*, the absorbance peak was observed at **440 nm**, with particles ranging from **25 to 50 nm** (Franzolin et al., 2022).

In this present study, a **colour modification** in the mixture of reaction was observed upon combining *E. roxburghii* excerpt with silver nitrate, representative

the reduction of silver ions likely owing to **Surface Plasmon Resonance (SPR)** excitation. UV–Vis spectral analysis confirmed this with a characteristic **SPR peak at 417 nm**, suggesting successful and stable nanoparticle formation (Giri et al., 2022).

The XRD pattern of the synthesized AgNPs revealed a **face-centered cubic (FCC)** structure (Giri et al., 2022). The diffractogram exhibited characteristic **Bragg reflections** corresponding to the (111), (200), (220), and (311) planes of metallic silver, consistent with JCPDS No. 89-3722. The **average crystal-like magnitude** was calculated to be approximately **35.2 nm**. An additional diffraction peak at $2\theta \approx 28^\circ$ likely corresponds to the **crystallization of bio-organic phases** on the nanoparticle surface.

High-resolution transmission electron microscopy (HR-TEM) long-established the **morphology and magnitude distribution** of the AgNPs. The images revealed **spherical-shaped particles** with **some degree of agglomeration**, possibly suggesting future sedimentation. The particle sizes ranged from **19 to 39 nm**, with an **average size of 24 nm**. The **Selected Area Electron particles Diffraction (SAED)** arrangement obtained by pointing the electron beam upright to one nanoparticle confirmed its **crystalline nature**. A detailed HR-TEM image of a single particle revealed a **well-defined spherical morphology**.

These results collectively confirm the successful **green synthesis of silver nanoparticles using *Eugenia roxburghii* leaf extract**, with strong evidence from UV–Vis, XRD, and HR-TEM analyses supporting their structure, testability, and morphology.

Conclusion:

The biosynthesis of silver nanoparticles (AgNPs) using *Eugenia roxburghii* leaf extract was successfully achieved and characterized. The formation of AgNPs was confirmed by a distinct absorption peak at 417 nm using UV–Vis spectroscopy, while XRD analysis

confirmed their crystalline nature. High-resolution TEM revealed spherical nanoparticles with uniform distribution, supporting the effective synthesis approach.

The synthesized AgNPs exhibited significant antimicrobial activity, particularly in inhibiting biofilm formation by *Staphylococcus aureus*. Given the growing concern over biofilm-associated infections and antibiotic resistance, these findings highlight the potential of *E. roxburghii*-derived nanoparticles as promising alternatives to conventional antimicrobials (Rai et al., 2009; Casterton et al., 1999).

However, to advance toward clinical applications, comprehensive in vivo studies are essential to assess the nanoparticles' efficacy and safety. Toxicological evaluations in animal models and human cell lines will be crucial to ensure biocompatibility and eliminate the risk of adverse effects (Loo et al., 2018; Ahmed et al., 2016). If proven safe, such green-synthesized AgNPs could be explored further for treating various human ailments, including diabetes, arthritis, and hypertension, which local communities already associate with *E. roxburghii* use (Dash et al., 2020; Das et al., 2022).

Moreover, this study opens avenues for synthesizing other metal-based nanoparticles from *E. roxburghii*, potentially enhancing its medicinal applications. The work demonstrates a sustainable and eco-friendly route to nanoparticle synthesis, contributing both to nanotechnology and herbal medicine development.

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