RESEARCH ARTICLE

BIOLOGICAL SYNTHESIS OF SILVER NANOPARTICLES FROM *COLEUS AROMATICUS* LEAVES AND VALIDATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY

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Abstract:

The green synthesis of silver nanoparticles (AgNPs) has gained significant attention due to its eco-friendly, cost-effective, and scalable approach. In this study, *Coleus aromaticus* leaf extracts, both boiled and squeezed, were utilized to synthesize AgNPs from silver nitrate ($AgNO_3$) solutions. The efficiency of nanoparticle formation was analyzed using UV-visible spectroscopy and transmission electron microscopy. Antimicrobial assays, including the agar cup and disc diffusion methods, were performed against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* to evaluate antibacterial and antifungal efficacy. The synthesized nanoparticles demonstrated strong antibacterial activity, with *S. aureus* exhibiting a lower minimum inhibitory concentration (MIC) than *E. coli*. However, limited antifungal activity was observed against *C. albicans*. Notably, nanoparticles synthesized using the boiled extract showed superior antimicrobial efficacy compared to the squeezed extract. This study highlights the potential of *Coleus aromaticus*-derived AgNPs as a sustainable antimicrobial agent, paving the way for future biomedical and industrial applications.

Keywords: Silver Nanoparticles, Green Synthesis, *Coleus aromaticus*, Herbal Antimicrobial Agents.

Introduction:

Nanoparticles are microscopic structures ranging from 1 to 100 nanometers (nm) in diameter and have diverse applications in drug delivery, biosensing, environmental sanitation, and nanomedicine (Zhang *et al.*, 2016). Among metallic nanoparticles, silver nanoparticles (AgNPs) have garnered significant interest due to their remarkable antimicrobial, antioxidant, and anti-inflammatory properties. These attributes make them valuable for use in medical diagnostics, wastewater treatment, food preservation, and pharmaceutical formulations (Sharma *et al.*, 2009).

Traditional physical and chemical methods for synthesizing AgNPs often involve high costs, toxic reagents, and energy-intensive processes. In contrast, biological or "green" synthesis offers a sustainable alternative, leveraging plant extracts, microorganisms, or enzymes to facilitate nanoparticle formation under mild conditions (Sondi & Salopek-Sondi, 2004). This approach enhances biocompatibility, reduces environmental hazards, and ensures better stability of nanoparticles.

In this study, *Coleus aromaticus*, a medicinal herb known for its antimicrobial and therapeutic properties, was used to biosynthesize AgNPs. Despite its traditional applications in treating ailments such as respiratory infections, indigestion, and inflammation, its potential for nanoparticle-mediated antimicrobial activity remains underexplored (Varadarajan *et al.*, 2022). The study aimed to validate the antimicrobial and antioxidant efficacy of AgNPs synthesized using *C. aromaticus* leaf extracts. Antimicrobial assays against *Escherichia coli* (Gram-negative bacteria), *Staphylococcus aureus* (Gram-positive bacteria), and *Candida albicans* (fungus) were performed to assess the efficacy of the synthesized nanoparticles (Li *et al.*, 2010). Additionally, UV-visible spectroscopy and transmission electron microscopy (TEM) were used to characterize the nanoparticles.

This research highlights the feasibility of using *C. aromaticus*-mediated silver nanoparticles as an eco-friendly antimicrobial agent, with potential applications in biomedicine and industry.

Materials and Methods

Preparation of *Coleus aromaticus* Leaf Extract

The leaves of *C. aromaticus* were first rinsed under running tap water and then washed with distilled water to remove surface contaminants. Two types of extracts were prepared: squeezed extract and boiled extract.

1. **Squeezed Extract:** Fresh leaves were passed over a flame briefly to soften their surface and then manually squeezed to obtain the extract.
2. **Boiled Extract:** Five grams of washed leaves were finely chopped and boiled in 100 mL of distilled water for 10 minutes. The solution was cooled, filtered using Whatman No. 1 filter paper, and stored in a sterile screw-cap bottle at 4°C until further use.

Biosynthesis of Silver Nanoparticles (AgNPs)

A **1 mM $AgNO_3$ stock solution** was prepared, and different concentrations (0.1, 0.5, and 1.0 mM) were used for nanoparticle synthesis. For each reaction, 3 mL of squeezed extract and 5 mL of boiled extract were separately mixed with $AgNO_3$ solutions. The reaction mixtures were kept in the dark at room temperature for 24 hours to allow complete biosynthesis. The formation of AgNPs was confirmed by observing color changes from colorless to pale yellow (squeezed extract) or reddish-brown (boiled extract). The synthesized nanoparticles were then double-filtered using Whatman No. 1 filter paper and stored at 4°C until further analysis.

Characterization of Silver Nanoparticles

UV-Visible Spectroscopy

The synthesis of AgNPs was monitored by UV-visible spectrophotometry (Shimadzu UV-1800) in the wavelength range of 200–600 nm, with distilled water as a blank. The characteristic surface plasmon resonance (SPR) peak of AgNPs was recorded to confirm nanoparticle formation (Zhang *et al.*, 2016).

Transmission Electron Microscopy (TEM)

For size and morphological analysis, AgNPs were centrifuged at 10,000 rpm for 15 minutes, and the resulting pellet was resuspended in 2 mL of distilled water. The samples were drop-cast onto a carbon-coated copper grid and analyzed using TEM (JEOL JEM-2100) (Sharma *et al.*, 2009).

Antimicrobial Assays

Agar Well Diffusion Method

The antimicrobial activity of the synthesized AgNPs was assessed using the agar well diffusion method against Gram-negative *Escherichia coli*, Gram-positive *Staphylococcus aureus*, and fungal *Candida albicans*. Mueller-Hinton agar and Sabouraud dextrose agar were prepared for bacterial and fungal cultures, respectively (Varadarajan *et al.*, 2022).

Disc Diffusion Method

Sterile discs (6 mm) were impregnated with AgNP solutions of different concentrations and placed on freshly swabbed agar plates. Pre-diffusion was allowed for 5 minutes, followed by 24-hour incubation at 37°C. The diameter of inhibition zones was recorded (Sondi & Salopek-Sondi, 2004).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC were determined following Clinical and Laboratory Standards Institute (CLSI) guidelines (2012).

1. **MIC Determination:** A **serial dilution** of AgNPs (0.5 µg/mL – 200 µg/mL) was prepared in nutrient broth (for E. coli) and Mueller-Hinton broth (for S. aureus). 0.1 mL of bacterial suspension (10^8 CFU/mL) was added to each tube and incubated at 37°C for 24 hours. The MIC was identified as the lowest concentration showing no visible turbidity.
2. **MBC Determination:** A loopful of broth from MIC tubes showing no growth was plated onto nutrient agar. Plates were incubated at 37°C for 24 hours, and the lowest concentration with no bacterial colony formation was recorded as the MBC.

Antioxidant Assay by DPPH

The antioxidant activity of *Coleus aromaticus* leaf extracts (boiled and squeezed) and synthesized silver nanoparticles (AgNPs) was assessed using the DPPH free radical scavenging assay, following the method of Deepak (2021) with slight modifications. A 0.1 mM DPPH solution was prepared in methanol, and 200 µL of each sample at varying concentrations (10–200 µg/mL) was mixed with 2.8 mL of DPPH solution, vortexed, and incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800), with methanol as a blank and DPPH solution without a sample as the control.

The **DPPH scavenging activity (%)** was calculated using the formula:

$$\left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100,$$

Acontrol and Asample represent the absorbance of the control and test sample, respectively.

Results

Biosynthesis of Silver Nanoparticles

The synthesis of silver nanoparticles (AgNPs) using Coleus aromaticus leaf extract was visually confirmed by a color change in the reaction mixtures. The squeezed extract changed from colorless to pale yellow, while the boiled extract exhibited a reddish-brown hue (Figure 1). This indicates the reduction of Ag^+ ions to Ag^0 nanoparticles, with the boiled extract demonstrating a stronger reduction potential, likely due to the increased availability of active phytochemicals (Baskaran & Ratha Bai, 2013).

Moreover, AgNPs synthesized from aged extracts (10 days post-extraction) exhibited darker coloration, indicating enhanced nanoparticle formation. This agrees with studies showing that prolonged exposure increases extract stability and enhances its reducing capacity (Chakraborty *et al.*, 2022). A kinetic study on green synthesis using C. aromaticus (2013) reported a similar gradual nanoparticle formation trend, reinforcing the role of bioactive compounds in the reduction process.

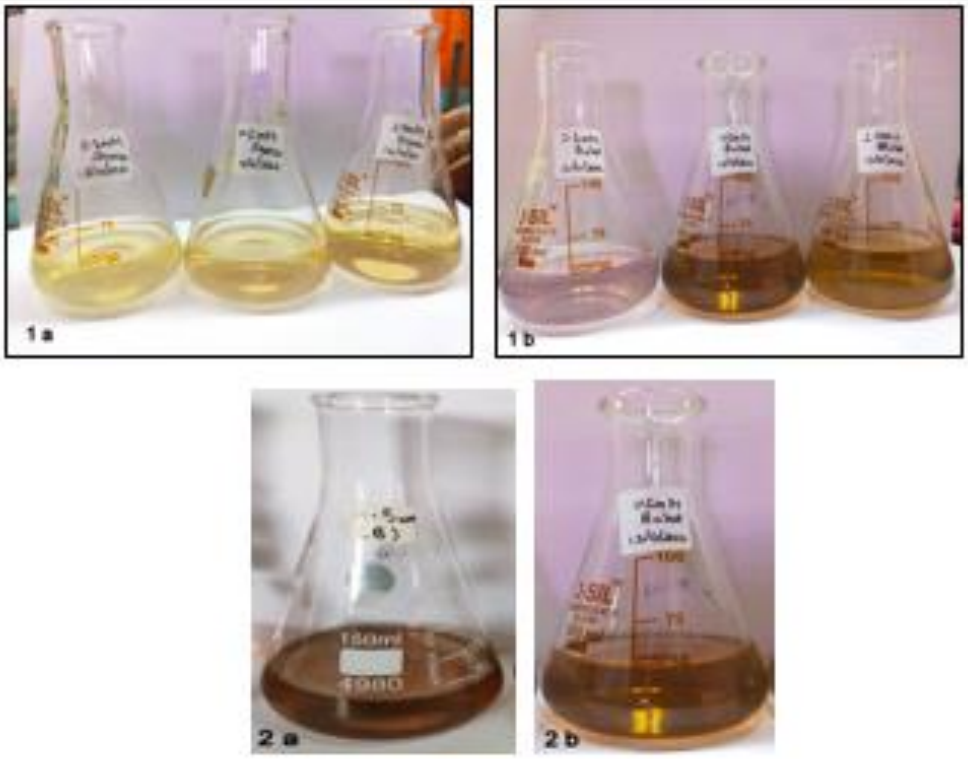

Figure 1- Nanoparticles from (a) squeezed and (b)boiled extract

Figure 2- Nanoparticles synthesised at varying time using the same boiled extract at 0.5mM concentration

UV-Visible Spectroscopy Analysis

UV-visible spectroscopy confirmed AgNP synthesis by detecting surface plasmon resonance (SPR) peaks (Figure 2). The AgNPs synthesized using boiled extracts (0.5 and 1.0 mM $AgNO_3$) exhibited strong absorption peaks around 400 nm, a characteristic wavelength for silver nanoparticles (Zhang *et al.*, 2016). Meanwhile, AgNPs synthesized from squeezed extracts showed peaks closer to 300 nm, suggesting smaller and less stable nanoparticles (Baskaran & Ratha Bai, 2013).

These findings align with a study on AgNP synthesis from C. aromaticus leaves, which reported optimal nanoparticle formation between 395–420 nm, depending on extract concentration and reaction time (Advances in Applied Science Research, 2013).

Transmission Electron Microscopy (TEM) Analysis

TEM analysis revealed that the synthesized AgNPs were irregular in shape, with a predominant size distribution of 70–80 nm, and a smaller fraction measuring 30–40 nm (Figure 3). Studies using Coleus aromaticus leaf extracts have reported similar size variations, emphasizing the need for process optimization to control nanoparticle size uniformity (Chakraborty *et al.*, 2022; Alharbi *et al.*, 2022).

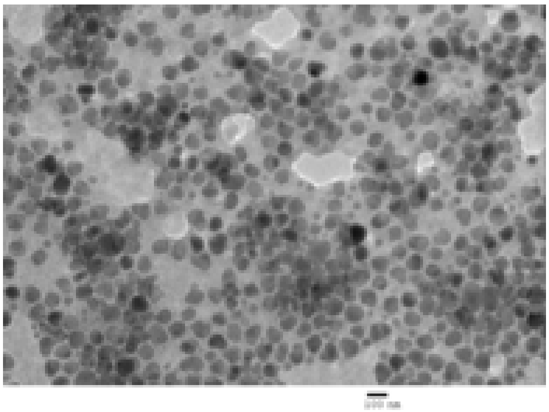

Figure 3 – Result of TEM

Concentration of AgNO3	Peak wavelength	
	Boiled extract	Squeezed extract
0.1	275.5	315
0.5	451.5	286
1.0	446.5	300

Table : 2- Result of UV visible spectroscopy

Antimicrobial Activity of AgNPs

The antimicrobial efficacy of AgNPs was assessed using agar well diffusion and disc diffusion methods. The results demonstrated strong antibacterial activity against both Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive) (Figures 4 & 5). Inhibition zones increased with nanoparticle concentration, confirming a dose-dependent antimicrobial effect (Baskaran & Ratha Bai, 2013; Chakraborty *et al.*, 2022).

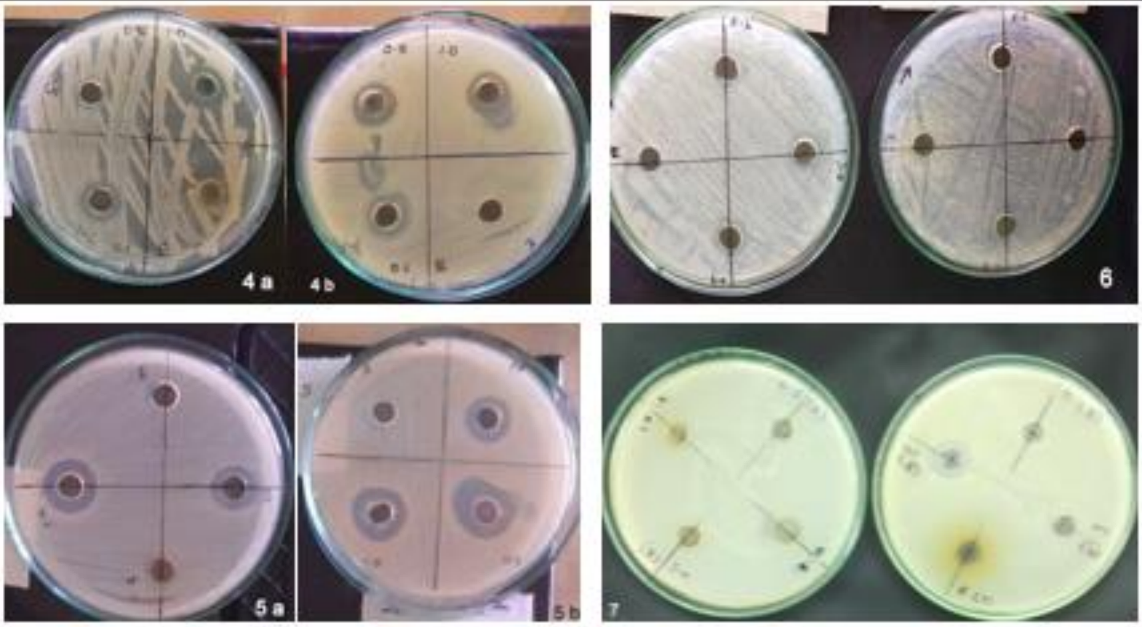

Figure 4 – agar cup of E. coli with nanoparticles from (a)squeezed and (b) boiled extract. Figure 5 – agar cup of *S. aureus* with nanoparticles from (a)squeezed and (b) boiled extract. Figure 6 – agar cup of Candida with nanoparticles from (a)squeezed and (b) boiled extract. Figure 7- comparison of effect of nanoparticles from squeezed and boiled extract on *E. coli.*

However, the synthesized AgNPs exhibited limited antifungal activity against Candida albicans, suggesting that fungicidal efficacy may require additional functionalization (Figure 6). A similar study found that silver nanoparticles required polyphenol coating or chitosan modification to enhance antifungal effects (Alharbi *et al.*, 2022).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC values were determined to evaluate the minimum effective concentration of AgNPs against bacterial strains and were found to be 50 µg/mL for *E.coli*, and 25 µg/mL for *S. aureus*. The MBC was determined to be 50 µg/mL for both *E. coli* and *S. aureus*. The lower MIC value for S. aureus (25 µg/mL) suggests greater susceptibility of Gram-positive bacteria due to the absence of an outer membrane, facilitating easier AgNP penetration (Sondi & Salopek-Sondi, 2004).

Antioxidant Activity by DPPH Assay

The DPPH free radical scavenging assay was conducted to evaluate the antioxidant potential of *Coleus aromaticus* extracts (boiled and squeezed) and silver nanoparticles (AgNPs) at different concentrations (10–200 µg/mL). The results demonstrated a concentration-dependent increase in radical scavenging activity, indicating that higher concentrations exhibited greater antioxidant potential.

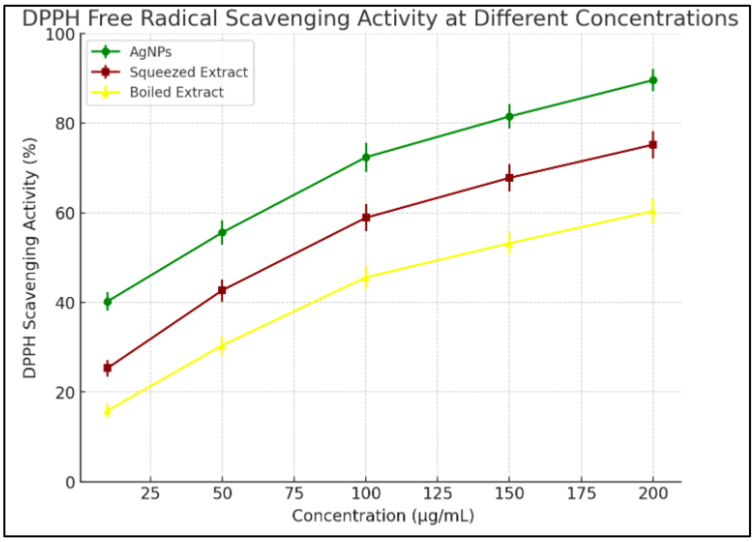

Fig 5: The graph illustrates the DPPH free radical scavenging activity of *Coleus aromaticus* extracts (boiled and squeezed) and silver nanoparticles (AgNPs) at different concentrations (10–200 µg/mL), showing a concentration-dependent increase in antioxidant potential.

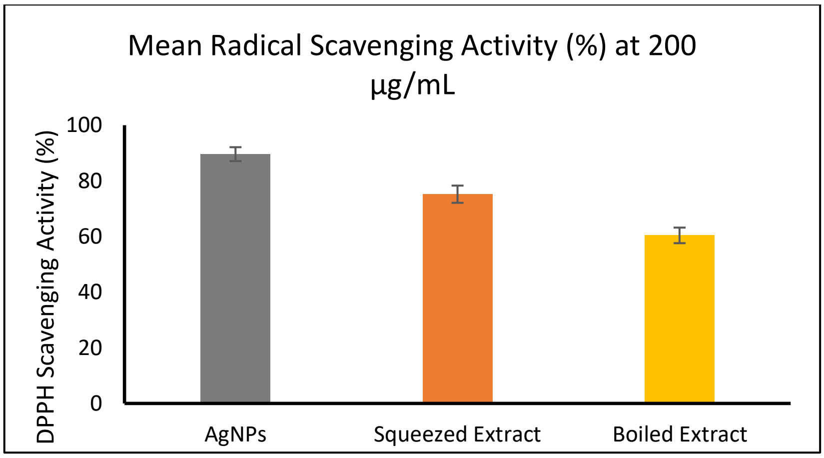

Figure 5: The bar graph compares the DPPH free radical scavenging activity of *Coleus aromaticus* extracts (boiled and squeezed) and silver nanoparticles (AgNPs) at 200 µg/mL, highlighting AgNPs as the most effective antioxidant.

Among the samples, AgNPs exhibited the highest DPPH scavenging activity, followed by the squeezed extract, while the boiled extract showed the lowest activity. At 200 µg/mL, AgNPs achieved 89.6% radical scavenging, whereas the squeezed extract showed 75.2%, and the boiled extract exhibited 60.4% scavenging activity. The superior antioxidant performance of AgNPs suggests a synergistic effect between plant-derived phytochemicals and nanoscale silver, enhancing electron transfer and free radical neutralization (Riaz *et al.,* 2020).

The moderate activity of the squeezed extract could be attributed to the higher retention of polyphenols and flavonoids, known to be potent antioxidants (Kumaran & Karunakaran, 2006). The relatively lower activity of the boiled extract may be due to the degradation of heat-sensitive bioactive compounds during the extraction process (Om Prakash Rout *et al.,* 2011).

Discussion

The antibacterial properties observed in this study align with previous research on silver nanoparticle synthesis using *Coleus* species. Baskaran & Ratha Bai (2013) reported that AgNPs derived from *Coleus forskohlii* root extracts exhibited similar inhibition against both Gram-positive and Gram-negative bacteria, supporting the findings of this study.

A study by Chakraborty, *et al.* (2022) highlighted the efficacy of AgNPs synthesized from *Coleus forskohlii* tissue cultures against multidrug-resistant (MDR) bacteria, reinforcing the potential biomedical applications of *Coleus*-derived nanoparticles. The stronger antibacterial response against *S. aureus* observed in both studies suggests that peptidoglycan-layer interactions enhance AgNP penetration into Gram-positive bacteria.

The kinetic study on *C. aromaticus* (2013) provided insights into the time-dependent formation of nanoparticles, showing that AgNP synthesis reaches an optimal stage after 24–48 hours. This aligns with the current study, where maximum SPR intensity was observed at 24 hours, indicating complete reduction of silver ions.

Unlike its antibacterial effects, AgNPs synthesized from *C. aromaticus* demonstrated weak antifungal activity against *C. albicans*. This is consistent with studies suggesting that fungal cell walls require higher nanoparticle concentrations or functionalization to disrupt their structure (Martínez-Gutierrez *et al.,* 2010). A study by Alharbi, *et al.* (2022) suggested that coating AgNPs with polyphenols or chitosan enhances their antifungal efficacy, a strategy worth exploring in future research.

The antioxidant properties observed in this study align with previous research on *Coleus aromaticus* and its free radical scavenging potential. Kumaran & Karunakaran (2006) reported strong antioxidant activity in *C. aromaticus* aqueous extracts due to the presence of phenolics, flavonoids, and rosmarinic acid, which play a crucial role in DPPH radical neutralization. This supports the moderate activity observed in the squeezed extract, where a higher concentration of these phytochemicals was likely retained.

The higher scavenging ability of AgNPs correlates with findings by Riaz, *et al.* (2020), who demonstrated that green-synthesized AgNPs exhibit superior antioxidant activity due to their high surface area, enhanced electron transfer ability, and phytochemical coating. The study highlighted that AgNPs capped with plant-derived biomolecules showed enhanced radical-scavenging potential compared to plant extracts alone, a trend that was observed in the current study as well.Additionally, Kunjan, *et al.* (2024) reported that metallic nanoparticles, particularly iron oxide and silver nanoparticles synthesized using *Coleus amboinicus*, displayed enhanced free radical scavenging compared to plant extracts alone. This is consistent with the high DPPH scavenging activity seen in AgNPs synthesized from *C. aromaticus* in this study.

Conversely, the lower antioxidant potential of the boiled extract could be explained by Om Prakash Rout, *et al.* (2011), who found that heat-sensitive antioxidants degrade upon prolonged boiling, leading to reduced radical-scavenging efficacy. This suggests that mild extraction methods (such as squeezing or cold maceration) may be preferable for retaining antioxidant properties.

Conclusion

This study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using *Coleus aromaticus* leaf extract, highlighting its eco-friendly and cost-effective approach. The boiled extract exhibited higher reducing potential, leading to better nanoparticle formation. AgNPs showed strong antibacterial activity, with *S. aureus* being more susceptible than *E. coli*, but exhibited limited antifungal efficacy against *Candida albicans*, suggesting the need for further modifications. The DPPH assay revealed AgNPs had the highest antioxidant activity, followed by the squeezed extract, while the boiled extract showed the lowest scavenging ability, likely due to thermal degradation of bioactive compounds. These findings reinforce the biomedical potential of *C. aromaticus*-derived AgNPs in antimicrobial coatings, wound healing, drug formulations, and antioxidant therapies, warranting further optimization and cytotoxicity assessments for broader pharmaceutical applications.

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