Eco-Friendly Synthesis of Silver Nanoparticles Using *Corymbia ptychocarpa* Extracts: Characterization and Antibacterial Activity

ANTONY ADAIKALAM A [1]& ARUMUGAM M [2]

[1]Antony Adaikalam A - J. J. College of Arts and Science (Autonomous), (Affiliated to Bharathidasan University, Tiruchirappalli), J. J. Nagar, Sivapuram, Pudukkottai - 622422, Tamil Nadu, India, Mail ID- antonyadaikalama@gmail.com

[2]Dr. Arumugam M The Head of Department of Botany, J. J. College of Arts and Science (Autonomous), (Affiliated to Bharathidasan University, Tiruchirappalli), J. J. Nagar, Sivapuram, Pudukkottai - 622422, Tamil Nadu, India.

Abstract:

The increasing prevalence of antibiotic-resistant bacteria necessitates the development of alternative antimicrobial agents. Green synthesis of silver nanoparticles (AgNPs) using plant extracts offers a promising, eco-friendly approach to address this challenge. This study investigates the synthesis of AgNPs using leaf and stem extracts of *Corymbia ptychocarpa* and evaluates their antibacterial potential. The green synthesis method produced spherical AgNPs ranging from 15-40 nm, confirmed by UV-Vis spectroscopy and scanning electron microscopy. The antibacterial activity of these AgNPs was assessed against six pathogenic bacterial strains using the well diffusion method. Results demonstrated significant dose-dependent antibacterial effects, with stem-mediated AgNPs exhibiting superior efficacy compared to leaf-mediated AgNPs. The synthesized AgNPs showed particularly strong activity against Enterococcus faecalis and Proteus vulgaris, approaching the efficacy of conventional antibiotics in some cases. This research highlights the potential of *C. ptychocarpa*-derived AgNPs as effective antimicrobial agents, offering a sustainable alternative to traditional antibiotics and paving the way for further exploration of plant-based nanomaterials in combating bacterial infections.

Keywords:

Green synthesis, Silver nanoparticles, *Corymbia ptychocarpa*, SEM analysis, Antibacterial activity,

INTRODIUCTION

The green synthesis of silver nanoparticles (AgNPs) using plant extracts is a promising alternative to conventional chemical methods due to its environmental sustainability, cost-effectiveness, and reduced toxicity [1] . This eco-friendly approach has gained attention for its potential to develop novel antimicrobial agents with diverse medical applications [1]. Research shows that plant-derived AgNPs have strong antibacterial efficacy against both Gram-positive and Gram-negative bacteria, including pathogens like Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeruginosa [2][3]. Factors such as the nanoparticles' size, shape, and concentration, as well as the phytochemical composition of the plant extracts, modulate their antimicrobial activity[2].

While chemical synthesis of AgNPs remains common, it often involves hazardous chemicals [4][5]. In contrast, green-synthesized AgNPs utilize phytochemicals like flavonoids and terpenoids as reducing and capping agents, enhancing stability and

antibacterial properties[6][7]. These nanoparticles, especially those with smaller dimensions, show superior efficacy in penetrating the cell envelope of Gram-negative bacteria [2]..Additionally, green-synthesized AgNPs demonstrate quorum quenching activity, disrupting bacterial communication and inhibiting infection processes[6]. This multifaceted antibacterial mechanism positions plant-derived AgNPs as a promising platform for next-generation antimicrobial therapeutics.

The integration of nanotechnology and phytochemistry in AgNP biosynthesis marks a paradigm shift in antimicrobial research, addressing antibiotic resistance sustainably and effectively. Understanding the molecular mechanisms of these nanoparticles and optimizing their synthesis could lead to innovative, eco-friendly antimicrobial agents with broad-spectrum activity and minimal environmental impact. *Corymbia ptychocarpa*, an understudied Myrtaceae species, has gained attention due to the antimicrobial potential demonstrated by its taxonomic allies. Recent studies on related species have shown promising results. The reported significant antimicrobial activity of eucalypt kino flavonoids against Pseudomonas aeruginosa and Staphylococcus aureus [8]. The demonstrated potent antimicrobial effects of *Corymbia ficifolia* leaf extracts against both Gram-positive and Gram-negative bacteria[9]. These findings suggest that *C. ptychocarpa*may possess similar bioactive compounds with potential antimicrobial applications, warranting further investigation into its phytochemical profile and antibacterial properties.With this background the current study was aim to evaluate the antibacterial efficacy of Silver Nano particle that biologically synthesized by Leaf and stem extract of*C. ptychocarpa.*

MATERIALS AND METHODS

Sample Collections

*Corymbia ptychocarpa[10]*was collected freshly from their natural habitats from region of Pudukkottai district of Tamil Nadu, India. The morphological and taxonomical characters were observed and characters are described in technical terms.

Laboratory Preparation of Extracts from Plants

Leaves and Stem cleaned by washing under tap water and then rinsed with deionized water. The plant parts were then shadow dried for 2–3 days and ground into powder using an electric blender. Leaf and stem powder ofeach 4 g were suspendedseparately in distilled water (200 mL) and kept in a water bath at a temperature of 70–80 °C for 30 min in a beaker. The remaining extracts were filtered in a conical flask using Whatman Grade 1 Filter Paper, cooled down, and refrigerated at 4 °C for further use in the synthesis of AgNPs.

To prepare a 1mM Silver Nitrate ($AgNO_3$) solution in 500 mL of distilled water, accurately weigh 0.084935 grams of $AgNO_3$, dissolve it in a small amount of distilled water, then transfer the solution to a 500 mL volumetric flask and add distilled water up to the 500 mL mark, ensuring thorough mixing. Store the solution in a clean, labelled container, preferably in a dark place to prevent decomposition by light.

Green Synthesis of AgNPs from the Extract

The assaysolution was made by mixing AgNO3 solution, followed by Leaf and stem extracts to the ratio of 1:10 v/v, respectively, to produce a volume of 200 mL in a conical flask wrapped with aluminium foil. The mixtures were incubated at room temperature in dark until the yellow colour of the solution turned dark brown. The samples were then centrifuged at 5000 revolutions per minute for 20 min, and the supernatant was discarded. Deionized

water (5 mL) was added to the precipitate and centrifuged again with the same specifications. The process was repeated. The final precipitate was then placed in a hot air oven for 30 min at 60 °C, and the dried form was utilized in further testing.

AgNPs Characterization

UV Visible Spectroscopy:The most important technique for confirming the formation of nanoparticles is ultraviolet visible (UV Vis) spectrophotometry. AgNP formation was verified using a UV visible spectrophotometer, which monitored the band (300–700 nm) of surface plasmon resonance.

SEM analysis: The morphology of AgNPs was presented by SEM analysis. The AgNPs were homogenized by an agate mortar. Later, AgNPs were placed on the strap with carbon tape and gold plated under vacuum andAgNPs were analyzed in SE mode with a high vacuum. The appropriate voltage was applied to attain the best screen and particle size.

Antimicrobial activity

Bacterial cultures were prepared one day prior to the experiment. A single colony was picked from the plate and transferred to Brain Heart Infusion (BHI) cultures and incubated for 16–18 h at 37 °C prior to the test. On the day of the experiment bacterial cultures were diluted at 1/100 with BHI, to ensure a constant bacterial population growth. The antimicrobial activity of synthesized silver nanoparticles was evaluated against *Staphylococcus aureus* (MTCC 96), *Vibrio cholerae* (MTCC 3906), *Salmonella typhi* (MTCC 733), *Enterobacter aerogenes* (MTCC 2823), *Enterococcus faecalis* (MTCC 439) and *Proteus vulgaris* (MTCC 1771) by well diffusion method. Muller Hinton Agar plates were prepared, as per manufacturer's instructions. The test microorganisms were seeded over the MHA plates, using sterile cotton swabs, to make a lawn culture. Wells of 6 mm diameter were punched over the agar plates using a sterile puncher. The AgNP's were diluted in 40% DMSO and make it into 16 and 32 mg/ ml and loaded 100 µl using sterile pipettes. The plates were incubated at 37 °C for 24 h. Zones of clearance around the wells after the incubation period confirmed the antimicrobial activity of the respective extract. The same procedure was repeated for all the test strains. Each experiment was carried out in triplicate. The clearance zones formed around each well was measured and the average diameter of the inhibition zone was taken for evaluating the antimicrobial activity of the extracts. The percentage of inhibition was calculated by the fallowing formula: -

$$\%\ of\ Inhibition = \frac{(S-N)}{(P-N)} \times 100$$

Where S - Sample's ZoI; P -Positive control's ZoI; N - Negative control's ZoI.

Statistical analysis

Statistical analysis was performed using SPSS version 21 software. The experimental data, obtained from three replicates for each experiment, were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was conducted to determine significant differences among groups. Post hoc comparisons were carried out using Tukey's Honestly Significant Difference (HSD) test to identify specific differences between group means. A p-value < 0.05 was considered statistically significant for all analyses.

RESULT AND DISCUSSION:

AgNP's Characterization:

The successful synthesis of silver nanoparticles (AgNPs) from *Corymbia ptychocarpa* leaf and stem extracts was confirmed by a distinct color change in the reaction mixture from yellow to dark brown, which is a well-established indicator of AgNP formation attributed to the surface plasmon resonance (SPR) phenomenon characteristic of metal nanoparticles [11]. This color transition results from the reduction of silver ions (Ag+) to metallic silver (Ag0) nanoparticles by biomolecules in the plant extracts, which act as both reducing and capping agents [6]. The intensity of the brown colour often correlates with the concentration and size of the AgNPs formed, with darker brown suggesting a higher yield or larger particles [12].

UV Visible Spectroscopy:

The UV-Vis spectra confirmed the successful synthesis of AgNPs from both *Corymbia ptychocarpa* leaf and stem extracts. Leaf-mediated AgNPs showed a SPR peak at 483.5 nm with an absorbance of 2.371, while stem-mediated AgNPs exhibited a peak at 440 nm with an absorbance of 2.51. These distinct SPR peaks indicate AgNP formation and suggest typical spherical AgNP sizes of 10-100 nm [6]. The variation in peak wavelengths implies potential differences in particle size or shape between leaf and stem-mediated AgNPs, with stem-derived particles possibly being smaller or more uniform [12]. High absorbance values indicate a good nanoparticle yield from both extracts, with the stem extract showing a slightly higher absorbance, suggesting a potentially higher concentration or better reducing capacity.

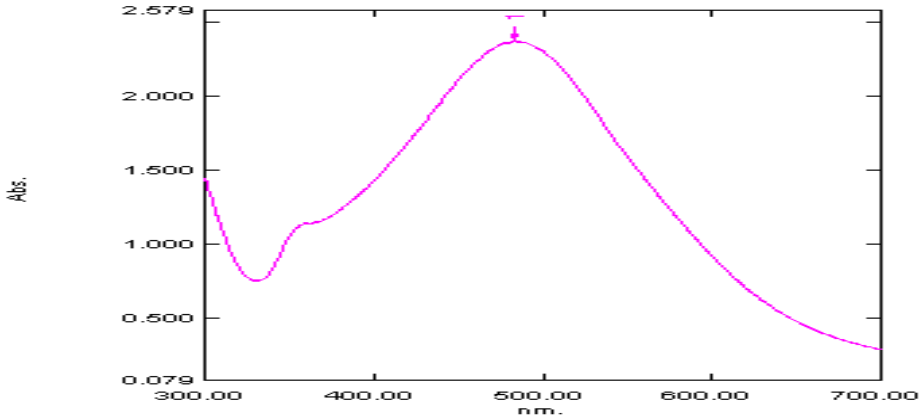

Fig.1. UV-Vis spectrum of AgNPs from both *Corymbia ptychocarpa* leaf extracts

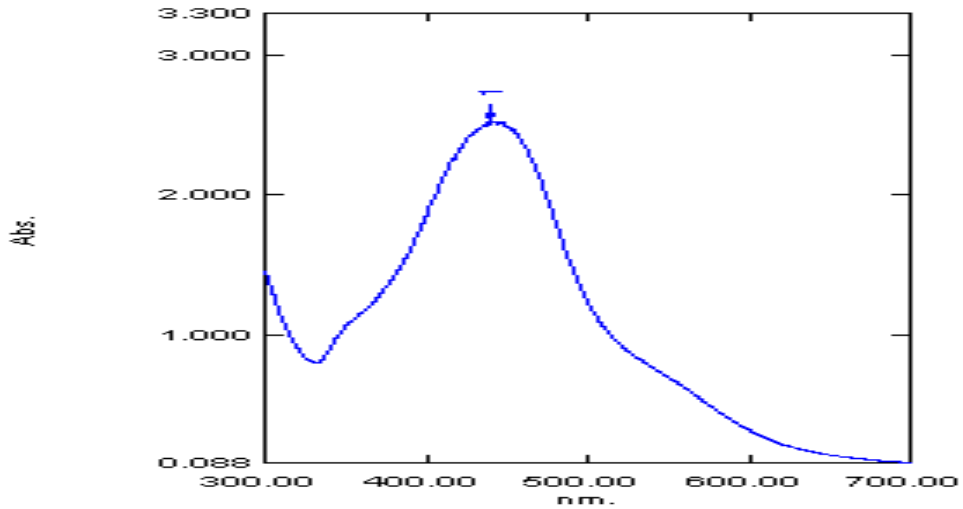

Fig.2. UV-Vis spectrum of AgNPs from both *Corymbia ptychocarpa* Stem extracts

SEM analysis.

The scanning electron microscopy (SEM) analysis confirms the successful synthesis of silver nanoparticles (AgNPs) from *Corymbia ptychocarpa* leaf and stem extracts. The SEM micrographs reveal the presence of nanostructures, validating the results obtained from UV-Vis spectroscopy.

SEM image of Leaf-mediated AgNPsshows particles with a predominantly spherical morphology, whilestem-mediated AgNPsdisplays more irregular, quasi-spherical shapes. This morphological difference aligns with the variation observed in the UV-Vis spectra peaks.

Both images demonstrate that the synthesized AgNPs are well-dispersed, indicating effective capping and stabilization by the plant extract components. The particles in both samples appear to be in the nanoscale range, with sizes roughly estimated between 20-100 nm based on the scale bar provided (200 nm).

The stem-mediated AgNPs seem to have a slightly larger average size and more varied shapes compared to the leaf-mediated ones (fig 3), which could explain the difference in their respective UV-Vis absorption peaks.

These SEM results provide visual confirmation of nanoparticle formation and offer insights into their morphology and distribution. The observed differences between leaf and stem-mediated AgNPs suggest that the choice of plant part influences the nanoparticle characteristics, likely due to variations in the biomolecules present in each extract[13][6].

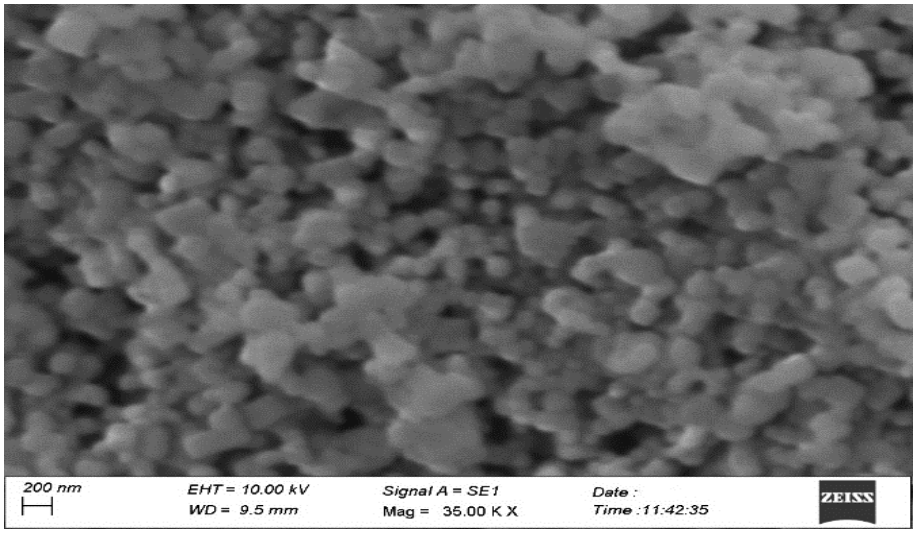

Fig 3. The scanning electron microscopy (SEM) analysis of silver nanoparticles (AgNPs) from *Corymbia ptychocarpa* stem extract.

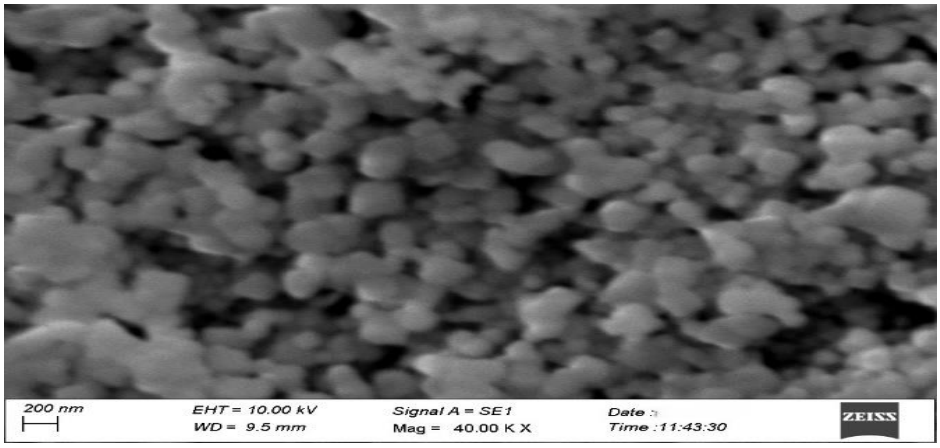

Fig 4. The scanning electron microscopy (SEM) analysis of silver nanoparticles (AgNPs) from *Corymbia ptychocarpa* leaf extract.

Antibacterial Activity:

The antibacterial efficacy of silver nanoparticles (AgNPs) synthesized from *Corymbia ptychocarpa* leaf and stem extracts was evaluated against six pathogenic bacterial strains.

Table 1 summarizes the zone of inhibition (ZoI) measurements and percentage inhibition for each treatment.

S.No	Indictor strains	Samples	Concentration (mg)	Mean and SE	F	Percentage of Inhibition
1	*Methicillin-resistant Staphylococcus aureus (MRSA)*	L	1.6	8.00 ± 0.57 ^{bcde}	162.554	38.46
			3.2	9.33 ± 0.33 ^{acde}		44.85
		S	1.6	13.1 ± 0.33 ^{abe}		62.98
			3.2	14.1 ± 0.44 ^{abcd}		67.78
		P	0.125	20.8 ± 0.16abcd		100
2	*Vibrio cholera*	L	1.6	10.5 ± 0.28^{cde}	160.660	47.51
			3.2	10.8 ± 0.44^{cde}		48.86
		S	1.6	15.6 ± 0.16^{abe}		70.58
			3.2	14.6 ± 0.44^{abe}		66.06
		P	0.125	22.1 ± 0.44^{abcd}		100
3	*Salmonella typhi*	L	1.6	13.3 ± 0.88^{bcde}	94.436	57.57
			3.2	11.6 ± 0.33^{acde}		50.21
		S	1.6	18.8 ± 0.16^{abde}		81.38
			3.2	21.1 ± 0.44^{abce}		91.34
		P	0.125	23.1 ± 0.44^{abcd}		100
4	*Enterobacter aerogenes*	L	1.6	8.66 ± 0.33^{bcde}	175.041	47.32
			3.2	10.3 ± 0.33^{acde}		45.17
		S	1.6	18.5 ± 0.28^{abde}		44.15
			3.2	18.3 ± 0.60^{abce}		80.26
		P	0.125	22.8 ± 0.60^{abcd}		100
5	*Enterococcus faecalis*	L	1.6	15.1 ± 0.16^{cde}	143.650	75.12
			3.2	15.8 ± 0.16^{cde}		78.60
		S	1.6	20.6 ± 0.16^{ab}		99.50

			3.2	20.0 ± 0.28^{abe}		99.50
		P	0.125	20.1 ± 0.33^{abd}		100
6	*Proteus vulgaris*	L	1.6	14.6 ± 0.60^{cde}	69.904	67.90
			3.2	15.5 ± 0.28^{cde}		72.09
		S	1.6	20.0 ± 0.28^{abe}		93.02
			3.2	20.8 ± 0.33^{ab}		96.74
		P	0.125	21.5 ± 0.28^{abc}		100
L = Leaf-mediated AgNPs, S = Stem-mediated AgNPs, P = Positive control. Mean values with different superscript letters (a-e) are significantly different ($p < 0.05$). dF = 4,1; p = < 0.001.						

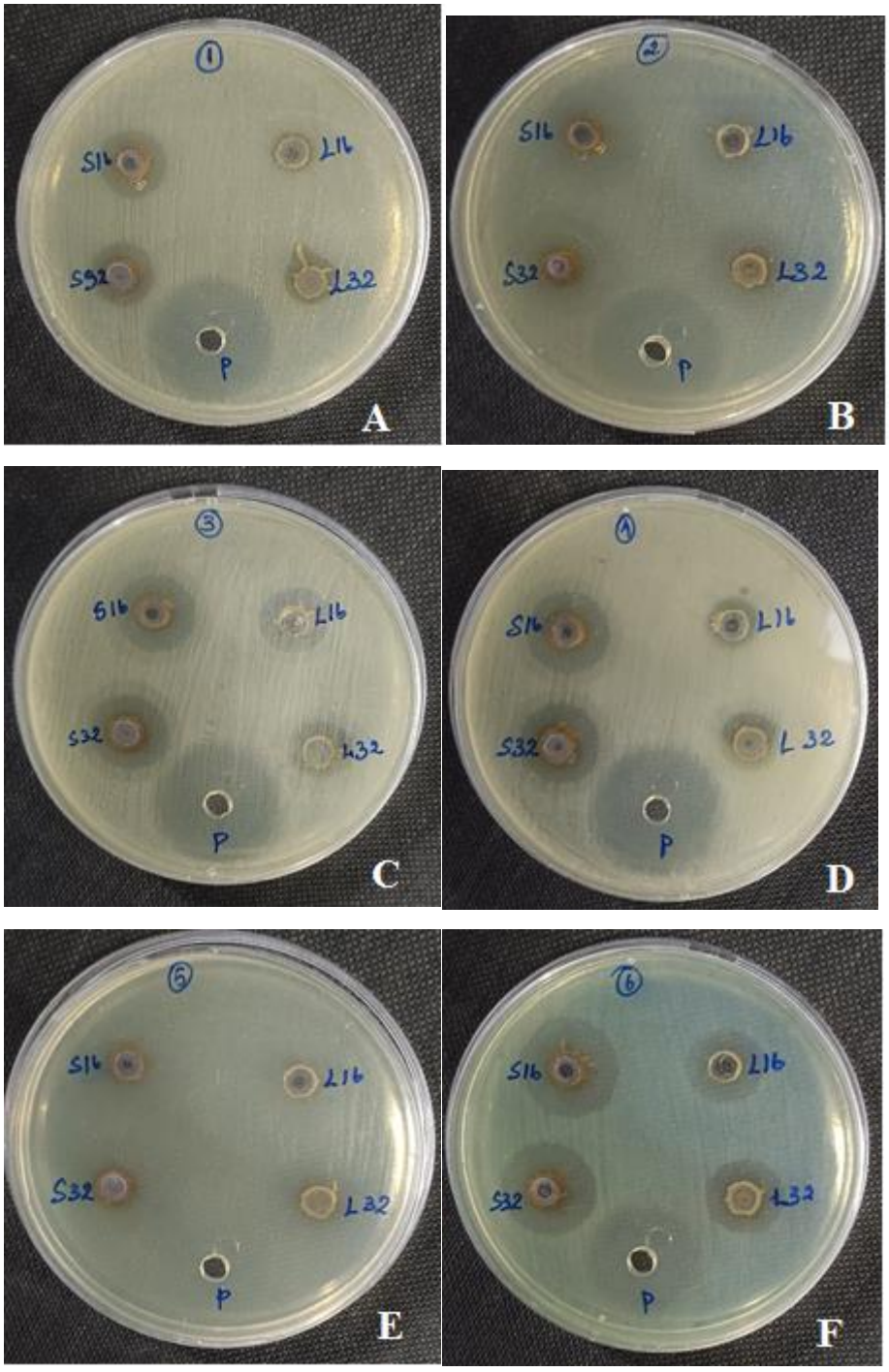

Fig 5. Assessment of antibacterial potential of AgNPs against human infections pathogens using well diffusion assay with various concentration [**A**].*Methicillin-resistant Staphylococcus aureus (MRSA)* [**B**]. *Vibrio cholera.* [**C**]. *Salmonella Typhi.*

[**D**]. *Enterobacteraerogenes*. [**E**]. *Enterococcus faecalis*. [**F**].*Proteus vulgaris*.

Dose-Dependent Inhibition: Both leaf and stem-mediated AgNPs exhibited dose-dependent antibacterial activity, with higher concentrations (3.2 mg) generally showing greater inhibition than lower concentrations (1.6 mg). This observation is consistent with previous studies on AgNP antimicrobial activity [14] and can be attributed to the increased nanoparticle density at higher concentrations, leading to enhanced interaction with bacterial cells.

Differential Efficacy of Leaf vs. Stem-Mediated AgNPs: Stem-mediated AgNPs consistently demonstrated superior antibacterial activity compared to leaf-mediated AgNPs across all tested strains. For instance, against *S. aureus*, stem-mediated AgNPs at 3.2 mg showed 67.78% inhibition, while leaf-mediated AgNPs at the same concentration exhibited only 44.85% inhibition. This disparity in efficacy may be attributed to differences in nanoparticle characteristics, as suggested by our UV-Vis spectroscopy and SEM analyses. The variation in phytochemical composition between leaf and stem extracts likely influences the size, shape, and surface properties of the resulting AgNPs, factors known to affect their antimicrobial potency [15].

Strain-Specific Susceptibility: The antibacterial activity of the synthesized AgNPs varied significantly among the tested bacterial strains ($p < 0.001$). *E. faecalis* demonstrated the highest sensitivity, with stem-mediated AgNPs achieving 99.50% inhibition at both 1.6 mg and 3.2 mg concentrations. Conversely, *S. aureus* exhibited the lowest susceptibility, particularly to leaf-mediated AgNPs (38.46-44.85% inhibition). This variability in bacterial response aligns with previous findings[16]and underscores the importance of considering strain-specific susceptibilities in the development of AgNP-based antimicrobial therapies.

Comparative Efficacy with Conventional Antibiotic: While the positive control (P) at 0.125 mg consistently achieved 100% inhibition across all strains, stem-mediated AgNPs at 3.2 mg approached comparable efficacy for certain bacteria, notably *E. faecalis* (99.50%) and *P. vulgaris* (96.74%). This high relative efficacy suggests the potential of green-synthesized AgNPs as alternative or complementary antibacterial agents, particularly in cases of antibiotic resistance.

Mechanistic Considerations: The observed antibacterial activity of AgNPs can be attributed to multiple mechanisms, including disruption of bacterial cell membranes, generation of reactive oxygen species, and interference with DNA replication [12]. The enhanced efficacy of stem-mediated AgNPs may result from a more optimal combination of these mechanisms, possibly due to the unique phytochemical profile of *C. ptychocarpa*stem extracts influencing nanoparticle formation and properties.

Conclusion

The green synthesis of silver nanoparticles (AgNPs) using *Corymbia ptychocarpa* leaf and stem extracts represents an eco-friendly and efficient approach to nanoparticle synthesis, offering substantial antibacterial activity against a range of pathogenic bacteria. The synthesized AgNPs, characterized by their spherical shape and size distribution (15-40 nm), exhibited significant dose-dependent antibacterial efficacy, with pronounced activity against both Gram-positive and Gram-negative bacterial strains.

These findings underscore the potential of *C. ptychocarpa*-derived AgNPs as effective antimicrobial agents, paving the way for their application in various medical and environmental contexts. Further research is warranted to elucidate the underlying mechanisms of action and to explore the potential synergistic effects of combining AgNPs with conventional antibiotics, aiming to enhance the therapeutic efficacy and mitigate the growing issue of antibiotic resistance.

Reference:

1. Al-Maqtari, M. A., Alattab, B. M., &Qaid, A. A. (2023). Biosynthesis of Silver Based Nanoparticles Using Plant Leaves Extracts and Their Antibacterial Activities. *Sana'a University Journal of Applied Sciences and Technology*, *1*(4).
2. Chatterjee, M., Pal, A., Halder, B., Pooja, S.G., Samanta, P., &Saha, N. (2022). Plant based synthesis of silver nanoparticles and their effects on microorganisms.
3. Farjeen, M. S., Divya, S. K., Jeyadoss, T., & Kumar, M. A. (2014). Quorum quenching and antibacterial activity of silver nanoparticles synthesized from medicinal plants against methicillin-resistant Staphylococcus aureus (MRSA). *Int. J. Pharm. Pharm. Sci.*, *6*, 2-6.
4. Song, J.Y., Kim, B.S. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess BiosystEng 32, 79–84 (2009). https://doi.org/10.1007/s00449-008-0224-6.
5. Lee, S., & Jun, B. (2019). Silver Nanoparticles: Synthesis and Application for Nanomedicine. International Journal of Molecular Sciences, 20. https://doi.org/10.3390/ijms20040865.
6. Ahmed, S., Ahmad, M., Swami, B. L., &Ikram, S. (2016). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. Journal of advanced research, 7(1), 17-28.
7. Vaghela, H., Shah, R., &Parmar, K. (2017). Biogenic synthesis of silver nanoparticles using Bauhinia variegata bark extract and its antibacterial efficacy. International Journal of Nanomaterials and Chemistry, 3(2), 45-9.
8. Nobakht, M., Trueman, S., Wallace, H., Brooks, P., Streeter, K., &Katouli, M. (2017). Antibacterial Properties of Flavonoids from Kino of the Eucalypt Tree, Corymbia torelliana. Plants, 6. https://doi.org/10.3390/plants6030039.
9. Dezsi, Ș., Bădărău, A., Bischin, C., Vodnar, D., Silaghi-Dumitrescu, R., Gheldiu, A., Mocan, A., &Vlase, L. (2015). Antimicrobial and Antioxidant Activities and Phenolic Profile of Eucalyptus globulusLabill. and Corymbia ficifolia.
10. F. Muell., K.D. Hill & L.A.S. Johnson Leaves. Molecules, 20, 4720 - 4734. https://doi.org/10.3390/molecules20034720.
11. Sharma, V. K., Yngard, R. A., & Lin, Y. (2009). Silver nanoparticles: green synthesis and their antimicrobial activities. Advances in colloid and interface science, 145(1-2), 83-96.
12. Prabhu, S., &Poulose, E. K. (2012). Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. International nano letters, 2, 1-10.
13. Iravani, S., Korbekandi, H., Mirmohammadi, S. V., &Zolfaghari, B. (2014). Synthesis of silver nanoparticles: chemical, physical and biological methods. Research in pharmaceutical sciences, 9(6), 385-406.
14. Rai, M., Yadav, A., &Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. Biotechnology advances, 27(1), 76-83.

15. Kuppusamy, P., Yusoff, M. M., Maniam, G. P., &Govindan, N. (2016). Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications–An updated report. Saudi Pharmaceutical Journal, 24(4), 473-484.
16. Franci, G., Falanga, A., Galdiero, S., Palomba, L., Rai, M., Morelli, G., &Galdiero, M. (2015). Silver nanoparticles as potential antibacterial agents. Molecules, 20(5), 8856-8874.