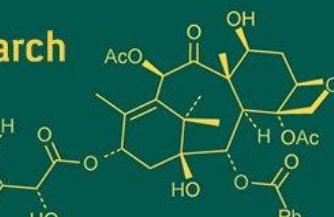
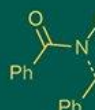


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(5): 823-827
www.biochemjournal.com
Received: 01-03-2026
Accepted: 04-04-2026

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Structural characterization and antimicrobial evaluation of *Syzygium cumini* seed biofabricated silver nanoparticles

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i5j.8538>

Abstract

The development of eco-friendly nanomaterials has attracted increasing interest due to their extensive biomedical, environmental and industrial applications. Among various nanomaterials, silver nanoparticles are particularly valued for their distinctive physicochemical characteristics and potent antimicrobial properties. The present study reports the green synthesis of silver nanoparticles using aqueous seed extract of *Syzygium cumini*, a medicinal plant traditionally recognized for its antioxidant and antidiabetic properties. Biosynthesis was carried out by treating the seed extract with 1 mM silver nitrate solution and nanoparticle formation was initially confirmed by a visible color change of the reaction mixture to dark brown. Ultraviolet-Visible spectroscopy revealed a characteristic surface plasmon resonance peak at 410 nm, confirming silver nanoparticle formation. Fourier Transform Infrared spectroscopy indicated the involvement of plant-derived phytochemicals, such as phenolic and flavonoid compounds, in the reduction and stabilization process. X-ray diffraction analysis confirmed the crystalline nature of the nanoparticles, while scanning electron microscopy demonstrated predominantly spherical particles with sizes ranging from 20 to 50 nm. Dynamic light scattering analysis revealed an average particle size of 32.6 nm with a polydispersity index of 0.212, indicating narrow size distribution. A zeta potential value of -102 mV suggested excellent stability and monodispersity of the synthesized nanoparticles. The antimicrobial activity of the biosynthesized silver nanoparticles was evaluated against selected Gram-negative and Gram-positive bacterial strains, including *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. The nanoparticles exhibited strong antibacterial activity against all tested organisms. The enhanced antimicrobial efficacy may be attributed to the synergistic effects of silver ions and bioactive phytoconstituents, resulting in disruption of bacterial cell membranes and interference with cellular metabolic processes. The findings highlight that *Syzygium cumini* seed-mediated silver nanoparticles offer a green, cost-effective, and sustainable synthesis approach with promising antimicrobial potential for biomedical and pharmaceutical applications.

Keywords: Silver nanoparticles, *Syzygium cumini*, green synthesis, antimicrobial, nanotechnology

Introduction

The rapid emergence and global dissemination of antimicrobial resistance among pathogenic microorganisms pose a serious threat to public health, necessitating the development of novel and effective antimicrobial strategies. In this context, nanotechnology has gained significant attention for its potential to offer innovative solutions, particularly through the use of metal-based nanoparticles. Nanoparticles, typically ranging from 1 to 100 nm in size, exhibit unique and enhanced physicochemical properties compared to their bulk counterparts due to their reduced dimensions and high surface-to-volume ratio (Rajput *et al.*, 2020) [8]. Among various noble metal nanoparticles, silver nanoparticles (AgNPs) have been extensively explored owing to their broad-spectrum antimicrobial activity, chemical stability, and comparatively low cytotoxicity toward mammalian cells (Ahmad *et al.*, 2016) [1].

Conventional physical and chemical methods for AgNP synthesis often require high energy input and involve toxic reagents, raising environmental and biomedical safety concerns. In contrast, green synthesis approaches using biological resources provide an eco-friendly, cost-effective, and sustainable alternative. Plant extracts are particularly advantageous, as their phytochemicals such as polyphenols, flavonoids, terpenoids, carbohydrates, and proteins act

as natural reducing, capping, and stabilizing agents. Green synthesis strategies for AgNPs broadly include use of microorganisms or their metabolites and plant-derived extracts (Fernandes *et al.*, 2023) [5].

Syzygium cumini (Jamun), a member of the family Myrtaceae, is a well-known medicinal plant widely used in traditional systems of medicine for its antioxidant, antidiabetic, anti-inflammatory, and antimicrobial properties (Singh and Navneet, 2021) [12]. The seeds of *S. cumini* are rich in phenolic compounds and flavonoids, making them suitable candidates for nanoparticle synthesis. The present study focuses on the green biosynthesis of silver nanoparticles using the aqueous seed extract of *Syzygium cumini*. Furthermore, the synthesized AgNPs were characterized using advanced analytical techniques to evaluate their physicochemical properties and potential biomedical applicability.

Materials and Methods

Preparation of Aqueous Seed Extract

Fresh, fully mature *Syzygium cumini* fruits were collected and thoroughly washed under running tap water to remove adhering dust and debris. The pulp was completely removed and the seeds were rinsed twice with deionized water. The cleaned seeds were shade-dried and finely pulverized using a mechanical grinder. Ten grams of the seed powder were mixed with 100 mL of distilled water in a sterile conical flask and stirred continuously on a magnetic stirrer for 24 h at room temperature. The resulting mixture was filtered through Whatman No. 1 filter paper (Whatman Ltd., England), and the filtrate was collected and stored at 4 °C for further use.

Biosynthesis of Silver Nanoparticles

Silver nanoparticles were synthesized by adding 5 mL of freshly prepared 1 mM silver nitrate (AgNO_3) solution dropwise to 10 mL of *Syzygium cumini* seed extract at room temperature. The reaction volume was adjusted to 50 mL by the addition of an appropriate amount of deionized water. The mixture was stirred at 200 rpm for 1 h using a magnetic stirrer (Gopala, 2019) [6] and subsequently incubated in the dark overnight. The formation of silver nanoparticles was preliminarily confirmed by a visible change in the color of the reaction mixture. Following incubation, the mixture was centrifuged and the resulting pellet was washed repeatedly with deionized water to remove unreacted components. A negative control containing 5 mL of 1 mM AgNO_3 and 10 mL of distilled water, processed under identical conditions without plant extract, was maintained.

Characterization of Silver Nanoparticles

Ultraviolet-Visible spectroscopy was performed to confirm nanoparticle formation by detecting the surface plasmon resonance peak. X-ray diffraction analysis was conducted to determine the crystalline nature of the nanoparticles. Dynamic light scattering analysis was performed to determine average particle size and polydispersity index, while zeta potential measurements were carried out to assess surface charge and stability.

Antimicrobial Activity Evaluation

The antimicrobial activity of the biosynthesized silver nanoparticles was evaluated using Bauer-Kirby's disc diffusion method. The Muller Hinton Agar (M173Himedia,

India) media was sterilized at 121 °C and 15 lbs for 15 min in an autoclave. The cultured plates were prepared with a depth of about 4 mm. The cultures were transferred to the center of an agar plate independently and homogeneously speeded the culture on surface of the plate with a sterile bent-glass rod. *Syzygium cumini* seed extract along with biosynthesized AgNPSC solution were impregnated onto discs under aseptic conditions, then dried and placed onto cultured plates using sterile forceps. The plates were then incubated for 24 h at 37 °C in an incubation chamber (Sekhar *et al.*, 2018) [10]. The antimicrobial activity was evaluated by standard microbial inhibitory assays quantifying the zone of inhibition for the test organisms viz. Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) and Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*).

Results

The aqueous seed extract of *Syzygium cumini* effectively mediated the green synthesis of silver nanoparticles (AgNPs), as indicated by a characteristic visual colour change in the reaction mixture. Upon the addition of *Syzygium cumini* seed extract (SCSE), which exhibited a reddish-brown colour, to 1 mM silver nitrate (AgNO_3) solution (initially colourless and transparent), the reaction mixture gradually transformed to a dark brownish-black colour during incubation at room temperature (Fig 1). This visible colour change is a preliminary indicator of AgNP formation, attributed to the excitation of surface plasmon resonance (SPR) resulting from the reduction of Ag^+ ions. After completion of the reaction, the biosynthesized silver nanoparticles (AgNP-SC) exhibited a uniform and stable dark brown-black coloration, indicating successful reduction and stabilization of nanoparticles by phytoconstituents present in the seed extract.

Ultraviolet-Visible spectroscopic analysis further confirmed the formation of AgNPs by displaying a distinct and well-defined SPR absorption peak at 410 nm, which is characteristic of silver nanoparticles (Fig 2). The XRD pattern depicts four intense peaks at 38.15°, 46.19°, 64.45°, and 77.72° among 2 θ values ranging from 30 to 80 (Fig 3). These peaks are analogous to diffraction (111), (200), (220) and (311) lattice planes of a face centred cubic unit cell structures of silver and are in agreement with JCPDS no. 04-0783 (Banerjee and Narendhirakannan, 2011). X-ray diffraction analysis confirmed the crystalline structure of the synthesized nanoparticles. Dynamic Light Scattering analysis of silver nanoparticles (AgNPs) synthesized using aqueous extracts of *Syzygium cumini* seeds profiles a wide size distribution, with the majority of particles falling within the 10-90 nm range. The results of DLS analysis are shown in Table 1. The average hydrodynamic diameter was approximately 42.2 nm, with a polydispersity index (PDI) of 0.169, indicating a fairly monodisperse population. The zeta potential value of -102 mV indicated high stability.

The antimicrobial activity of *Syzygium cumini* seed extract and the biosynthesized silver nanoparticles was evaluated against selected Gram-positive and Gram-negative bacterial strains using the agar diffusion method. The results demonstrated a concentration-dependent inhibitory effect against all tested microorganisms.

The *Syzygium cumini* seed extract exhibited moderate antibacterial activity against *Staphylococcus aureus*, producing inhibition zones of 13 mm at 100 μL and 9 mm at

25 μ L. In comparison, the AgNPSC showed enhanced activity, with inhibition zones increasing to 17 mm at 100 μ L and 10 mm at 25 μ L. This represents a 30.77% increase in antibacterial efficacy at the higher concentration, indicating a clear improvement upon nanoparticle synthesis. Against *Streptococcus pyogenes*, the seed extract produced inhibition zones of 12 mm and 8 mm at 100 μ L and 25 μ L,

respectively (Table 2). The corresponding AgNPs demonstrated superior antibacterial activity, with inhibition zones of 14 mm at 100 μ L and 9 mm at 25 μ L. Although the enhancement was comparatively lower than that observed for other strains, AgNPs still exhibited a notable increase in antibacterial potency (16.67%) over the crude extract.

Table 1: Comparative antibacterial activity of *Syzygium cumini* seed extract and biofabricated silver nanoparticles (AgNPs) against selected bacterial Strains

Test Compound	Concentration (μ g/mL)	Zone of Inhibition (mm) against different bacteria			
		<i>S. aureus</i>	<i>S. pyogens</i>	<i>E. coli</i>	<i>K. pneumonia</i>
<i>Syzygium cumini</i> seed aqueous extract	100	13	12	15	13
	50	9	9	12	11
	25	9	8	9	9
	12.5	6	6	7	8
<i>Syzygium cumini</i> fabricated AgNPs	100	17	14	19	18
	50	13	12	16	15
	25	10	9	13	13
	12.5	8	7	10	10

The seed extract showed appreciable activity against *Escherichia coli*, with inhibition zones of 15 mm at 100 μ L and 9 mm at 25 μ L. However, treatment with biosynthesized silver nanoparticles significantly enhanced the antibacterial response, producing zones of inhibition of 19 mm and 13 mm at the respective concentrations. This corresponds to a 26.67% increase at 100 μ L and highlights the higher sensitivity of Gram-negative bacteria to AgNPs.

Klebsiella pneumoniae exhibited moderate susceptibility to the seed extract, with inhibition zones of 13 mm at 100 μ L and 9 mm at 25 μ L. In contrast, the silver nanoparticles showed the highest antibacterial enhancement against this strain, yielding inhibition zones of 18 mm and 13 mm at 100 μ L and 25 μ L, respectively. The increase in activity (38.46%) suggests strong nanoparticle-bacterial interactions and pronounced efficacy against this Gram-negative pathogen.

Discussion

The successful biosynthesis of silver nanoparticles using *Syzygium cumini* seed extract demonstrates the efficiency of plant-mediated green synthesis methods. The presence of bioactive phytochemicals plays a crucial role in reducing silver ions and stabilizing the formed nanoparticles. The nanoscale size, spherical morphology, and high stability of the synthesized nanoparticles enhance their interaction with bacterial cell membranes. The observed antimicrobial activity against both Gram-negative and Gram-positive bacteria may be attributed to the combined effects of silver ions and phytoconstituents, leading to membrane disruption,

protein denaturation, and inhibition of essential metabolic pathways.

This transformation is attributed to the surface plasmon resonance (SPR) phenomenon, which arises due to the excitation of conduction electrons on the surface of silver nanoparticles. The intensity and depth of the brown coloration increased over time, suggesting progressive nucleation and growth of nanoparticles as a result of a decrease in the concentration of reduced silver ions in the aqueous medium. These results are consistent with the results of Khane *et al.* (2022). The results of visual confirmation are in line with the findings reported by Atale *et al.* (2017) [2] and Sahu *et al.* (2019) [9] during synthesis of silver nanoparticles from *Syzygium cumini* seed extract.

The rapid onset of color change reflects the strong reducing potential of *S. cumini* phytochemicals particularly flavonoids, tannins and phenolic compounds which facilitate the reduction of Ag^+ ions to elemental silver (Ag^0). The stable brown coloration persisted for several days, indicating effective capping and stabilization by biomolecules present in the extract (Kavital *et al.* 2024) [7]. These results approved the elementary composition of nanoparticles being silver. The sharp and strong peaks depict the high crystalline nature of synthesized silver nanoparticles. The results reported by Chakravarty *et al.* (2022) are in close conformity with the results obtained in our present study. Overall, the biosynthesized silver nanoparticles exhibited greater antibacterial efficacy than the crude seed extract at both tested concentrations, with comparatively higher inhibitory activity observed against Gram-negative bacteria.

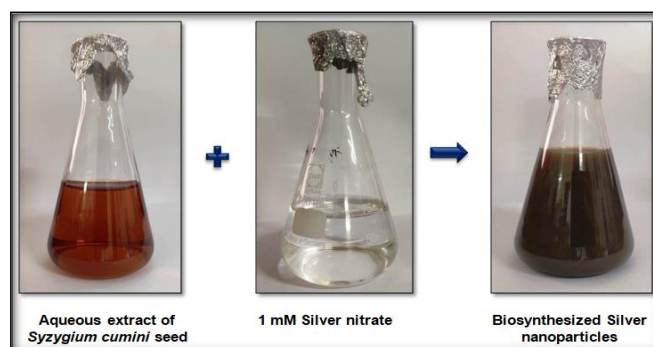


Fig 1: Visual confirmation of the biosynthesis of silver nanoparticles (AgNPs) using *Syzygium cumini* seed extract and silver nitrate solution

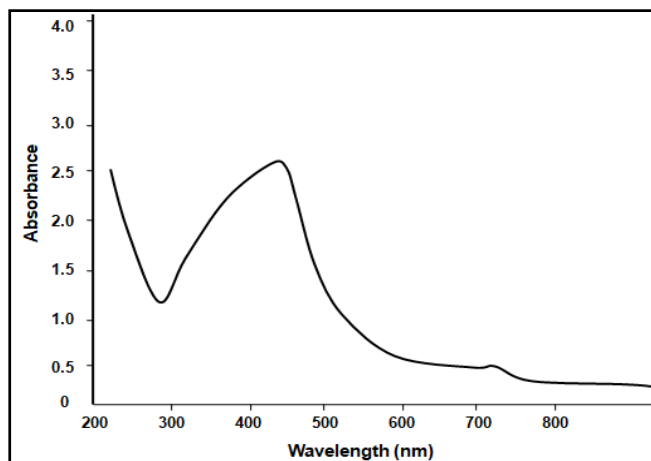


Fig 2: UV-Visible spectroscopic analysis of *Syzygium cumini* seed extract biosynthesized silver nanoparticles (AgNPs)

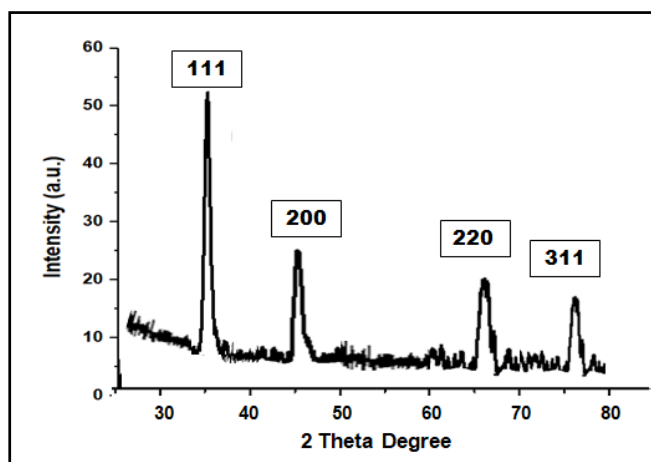


Fig 3: X-ray Diffraction (XRD) Pattern of biosynthesized silver nanoparticles (AgNPs) derived from *Syzygium cumini* seed extract

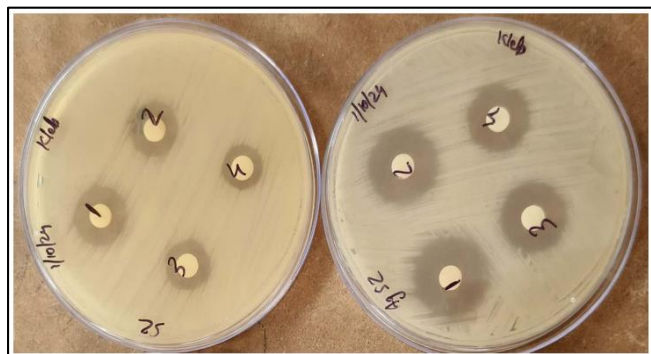


Fig 4: Microbial inhibitory assays showing comparative zone of inhibition of *Syzygium cumini* seed extract and its biofabricated silver nanoparticles (AgNPs) against selected bacterial Strains

Conclusion

The quest for a safer technique to manufacture AgNPs has received significant interest for several years. Multiple characterization approaches were used to examine the morphology of AgNPs produced from plants, which indicated variability in size, crystallinity, and form distribution, this is both a challenge and a limitation. The present study establishes an eco-friendly and cost-effective method for the biosynthesis of stable silver nanoparticles using aqueous seed extract of *Syzygium cumini*. The synthesized nanoparticles exhibited favorable physicochemical characteristics and significant

antimicrobial activity against pathogenic bacteria. These findings suggest that *Syzygium cumini*-mediated silver nanoparticles hold promising potential for applications in biomedical and pharmaceutical fields.

Acknowledgments

The authors gratefully acknowledge the facilities and support provided by the College of Veterinary Science and Animal Husbandry, Mhow, and Nanaji Deshmukh Veterinary Science University, Jabalpur.

Conflict of Interest

The authors declare no conflict of interest related to this study.

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