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Antibacterial, Antioxidant and Antidaibetic Properties of Copper Nanoparticles Synthesised Using Syzygium Cumini Leaves

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Abstract

Copper nanoparticles (CuNPs) were successfully synthesized using the aqueous extract of *Syzygium cumini* (Jamun) leaves through a green synthesis approach. The synthesized nanoparticles were characterized and evaluated for their biological activities. Antibacterial activity was assessed against both Gram-positive and Gram-negative bacteria using the agar well diffusion method. The antioxidant potential of nanoparticles was determined using DPPH radical scavenging assays, while their antidiabetic activity was evaluated through in vitro α-amylase and α-glucosidase inhibition assays. Results demonstrated that the biosynthesized CuNPs exhibited significant antibacterial, antioxidant, and antidiabetic properties, indicating their potential as a safe and eco-friendly bioactive material for pharmaceutical and biomedical applications. The study highlights the use of Jamun leaves as a sustainable and effective reducing and stabilizing agent in the green synthesis of copper nanoparticles.

Keywords- Nanobiotechnology, antioxidant, antidiabetic, green nano materials.

INTRODUCTION

Syzygium genus, includes about 1200 species, is considered as the largest genus in the myrtle family (Myrtaceae) with some species economically important and widely cultivated for their edible fruits and medicinal properties [1]**.** In various regions of India, it is also referred to as Ram jamun, Indian black cherry, and black plum [2]**.** *Syzygium cumini* Linn is an evergreen tropical plant. It belongs to the family Myrtaceae and is native of India. Plants are highly valuable and have been used by people for thousands of years as a medicine to cure many diseases. It is popularly known as the Indian blackberry. The plants are widely distributed throughout tropical and subtropical regions of India. Jamun is harvested prior to monsoon season and its harvesting time is short and limits for 30 – 40 days Leaf of this plant is used as astringents and exists anti-inflammatory property [3]. The fresh leaves make a paste and apply on the affected areas on the skin and also helps in the healing of wounds faster. It is also used in the treatment of vomiting, hemorrhoids, asthma, bronchitis, mouth wash, mouth ulcerations, dysentery and anti-venom. The juice of Jamun leaf is orally given as an antidote to treat for opium poisoning and centipede bite. Fresh leaf paste are mixed with milk and taken orally to treat indigestion and diabetes. The tender leaf of Jamun is taken normally to treat Jaundice and can be used for strengthening gums and for controlling constipation [4].

In recent years, scientists have paid a lot of attention to phytochemicals and the ways they might improve human health. Particularly interesting is the quest for anti-inflammatory, hypoglycemic, and cancer-fighting compounds in various plant foods such vegetables, fruits, teas, spices, and medicinal herbs [5]. Nanotechnology has become a revolutionary field of research, offering exciting opportunities to design and engineer materials with unique properties at the nanoscale. Nanoparticles possess distinctive qualities due to their size, shape, morphology, and large surface area which contribute to their potential applications [6]. Nanotechnology has been a breakthrough tool used in various fields such as pharmaceuticals for antimicrobial, anticancer drug delivery, health for tumour detection as well as agriculture for plant protection and nutrition. Nanoparticles, especially the metal and metal oxide measuring less than 100 nm in diameter have effectively treated infectious diseases in response to antibiotic failure due to microbial resistance. Researchers have shown that metal-based nanomaterials such as copper,

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silver, gold, titanium, and zinc exhibit a broad spectrum of antimicrobial activity against various bacterial strains [7].

MATERIALS AND METHODS

Collection of Plant Material

Fresh and healthy jamun (Syzygium cumini) leaves were collected from a local area. Care was taken to select disease-free and mature leaves. The collected leaves were washed thoroughly with tap water to remove dust and soil particles, followed by washing with distilled water to remove any remaining impurities. The clean leaves were then shade-dried to remove surface moisture.

Preparation of Jamun Leaves Extract

The washed jamun leaves were finely chopped into small pieces. 10g of chopped leaves were taken and added to 100 ml distilled water in a beaker. The mixture was heated for 10 minutes to allow the phytochemicals present in the leaves to dissolve into the water. After heating, the solution was allowed to cool to room temperature. The cooled extract was filtered using filter paper to remove solid leaf residues. The clear filtrate obtained was used as jamun leaves extract for the synthesis of copper nanoparticles [6].

Green Synthesis of Copper Nanoparticles

0.1 M $CuSO_4$ was prepared in distilled water. Jamun leaf extract was added dropwise into $CuSO_4$ solution in the ratio (10:1) under continuous stirring to ensure proper mixing and interaction between the extract and copper ions. The pH of the reaction mixture was adjusted to 7 using 0.1 N NaOH while maintaining continuous stirring. The reaction mixture was incubated for 24 hours in dark conditions. The colour change from green to dark green, indicates the formation of copper nanoparticles [5].

Phytochemical Screening

Detection of Alkaloids (Wagner's Test) 2-3 drops of Wagner's reagent were added to 1 ml plant extract. Formation of brown or reddish precipitate indicates the presence of alkaloids.

Detection of Saponins (Froth Test)

1 ml Extract were diluted with 5 ml distilled water and was then shaken vigorously. Formation of foamy like liquid at the top of the mixture confirms the presence of saponin.

Detection of Phenols (Iodine Test)

To 1 ml of plant extract, few drops of 10% ferric chloride was added. Formation of blue or green colour confirms the presence of phenols.

Detection of Flavonoid (Alkaline Reagent Test)

1 ml extract was taken in a test tube and few drops of concentrated NaOH. Test tube was observed for development of deep yellow color, which becomes colorless on the addition of dilute acids. It shows the presence of flavonoids.

Detection of Steroids (Salkowskis Test)

0.5 ml extract was mixed with 2ml of Chloroform. 1 ml Concentrated sulfuric acid was added carefully
Along the wall. A reddish-brown color at the interface was an indicative of steroidal ring.

Detection of Tannins

5ml of the extract and a few drops of 0.1% ferric chloride solution were added. Greenish black precipitates indicated presence of tannins [3].

Characterization of Copper Nanoparticles

The formation of copper nanoparticles was confirmed using UV–Visible spectrophotometry. A sample of the nanoparticle solution was scanned over 200nm to 700nm wavelength range. The appearance of a characteristic absorption peak confirmed the successful synthesis of copper nanoparticles [5].

Antibacterial Activity

The agar well diffusion method was used to assess the antibacterial activity of produced copper nanoparticles (CuNPs) against Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli. Petri dishes were filled with sterile nutrient agar medium, which was then left to harden. The bacterial cultures were standardized (around 0.5 McFarland standard) and newly prepared. To guarantee even lawn culture production, sterilized cotton swabs were used to evenly swab the agar plate surfaces. A sterile cork borer was used to create aseptic wells with a diameter of about 6 mm in solidified agar. Using a micropipette, the wells were carefully filled with varying quantities of CuNPs (50 mg/ml for E. coli and 85 mg/ml for S. aureus). Additionally, a control well was kept with solvent or distilled water. The plates were kept undisturbed for 30

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minutes at room temperature to allow pre-diffusion of the samples. After this, the plates were incubated at 37°C for 24 hours. After incubation, the antibacterial activity was determined by measuring the diameter of the zone of inhibition (in mm) around each well using a ruler or Vernier caliper. The results were recorded as an indicator of antibacterial efficacy of the copper nanoparticles [5].

Antioxidant Activity

The antioxidant activity of biosynthesized copper nanoparticles (CuNPs) was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, as described in standard reported methods with slight modifications [1,2].

A freshly prepared DPPH solution (0.1 mM) was prepared by dissolving DPPH in methanol. Different concentrations of CuNPs were prepared in methanol (e.g., 10, 20, 30, 40, and 50 µL or corresponding mg/mL concentrations).

In each test tube, 1 ml of CuNP solution was mixed with 3 ml of DPPH solution. The reaction mixture was shaken gently and incubated in the dark at room temperature for 30 minutes to prevent light-induced degradation of DPPH.

After incubation, the absorbance of each sample was measured at 517 nm using a UV–Visible spectrophotometer. A control was prepared using DPPH solution without CuNPs, while methanol served as blank.

The percentage of radical scavenging activity was calculated using the following formula:

DPPH Scavenging Activity (%) = Ac – As/Ac x 100

Where

Ac = Absorbance of control

As = Absorbance of test sample. The IC_{50} value (concentration required to inhibit 50% of DPPH radicals) zwas determined from the graph of % inhibition versus concentration.

Antidiabetic Activity:

The antidiabetic activity of biosynthesized copper nanoparticles (CuNPs) was evaluated using the α-amylase inhibition assay as per standard reported methods with slight modifications [1], [2].

A reaction mixture containing α-amylase enzyme and different concentrations of CuNPs was prepared and pre-incubated at 37°C for 10–15 minutes. After incubation, starch solution (substrate) was added to initiate the enzymatic reaction. The mixture was further incubated under controlled conditions to allow enzymatic hydrolysis of starch.

The reaction was terminated by adding iodine reagent, which forms a colored complex with unhydrolyzed starch. The absorbance was measured at 565 nm using a UV–Visible spectrophotometer. A control was prepared without CuNPs to represent maximum enzyme activity.The percentage inhibition of α-amylase activity was calculated using the standard formula:

Amylase inhibition % = Ac – As/Ac x 100

Where

Ac = Absorbance of control

As = Absorbance of test sample

The decrease in absorbance in test samples indicated inhibition of α-amylase activity by CuNPs. The biosynthesized nanoparticles showed approximately 40% inhibition, suggesting moderate antidiabetic potential. The observed activity may be attributed to the interaction of CuNPs with the enzyme active site and the synergistic effect of phytochemicals from *Syzygium cumini* leaf extract..

RESULT AND DISCUSSION

UV–Visible Spectroscopy Analysis

The formation of copper nanoparticles was confirmed by UV–Visible spectroscopic analysis. The appearance of a characteristic absorption peak in the visible region as shown in the graph (Fig 1) indicated successful nanoparticle synthesis. An initial increase followed by stabilization of absorbance confirmed the formation and stability of CuNPs. This behavior suggests reduction of Cu^{2+} ions and capping by phytochemicals present in *Syzygium cumini* leaf extract. Bioactive contributors for antimicrobial and antioxidant properties.

Phytochemical screening

The phytochemical screening confirmed that *Syzygium cumini* leaf extract contains a rich profile of bioactive compounds such as alkaloids, flavonoids, phenols, tannins,

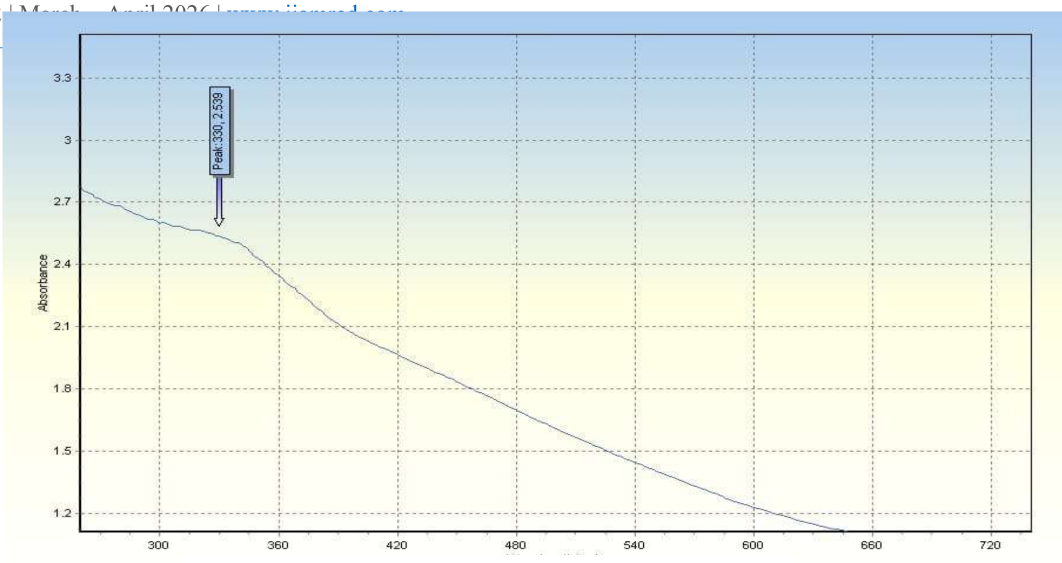

Fig 1: UV Characterization of CuNPs

saponins, and steroids. These phytochemicals play a crucial role in the **green synthesis of copper nanoparticles** by acting as: Reducing agents, Stabilizing/capping agents, Bioactive contributors for antimicrobial and antioxidant properties.

Antibacterial Activity

Copper nanoparticles were tested against *E. coli* and S. aures at different concentrations using the agar well diffusion method. The results showed a clear zone of inhibition, indicating antibacterial activity. The zone increased with increasing concentration of CuNPs.

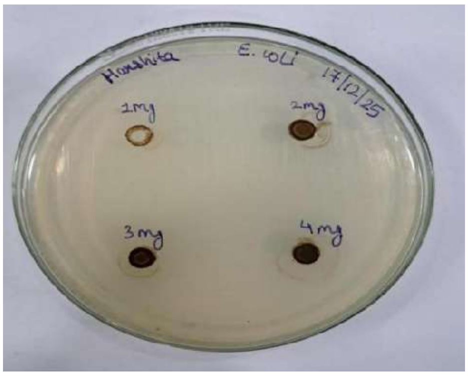

Fig 2: Antibacterial of nanoparticles against *E.coli*

Concentration of nanoparticles (mg/ml)	Zone of Inhibition in mm
1	-
2	12.26
3	12.83
4	14.05

Table 1: Antibacterial activity Against *E coli*

No inhibition was observed at the lowest concentration (20 µL), indicating that a minimum effective concentration is required for antibacterial activity. For *Staphylococcus aureus*, higher concentrations of CuNPs were used due to lower sensitivity at reduced doses.

Concentration of nanoparticles (mg/ml)	Zone of Inhibition in mm
1.7mg	11.33mm
3.4mg	17.97mm
5.1mg	18.22mm
6.8mg	21.24mm

Table 2: Antibacterial activity Against *S.aureus*

The results showed significant antibacterial activity with increasing zone of inhibition. The larger zone of inhibition confirms strong antibacterial activity against Gram-positive bacteria.

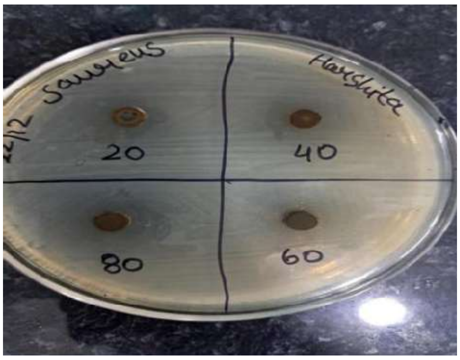

Fig 3: Antibacterial of nanoparticles against *S. aureus*

Antioxidant Activity

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The antioxidant potential of CuNPs was evaluated using the DPPH radical scavenging assay. A decrease in absorbance at 517 nm indicated free radical scavenging activity. The percentage inhibition increased with increasing concentration of CuNPs.

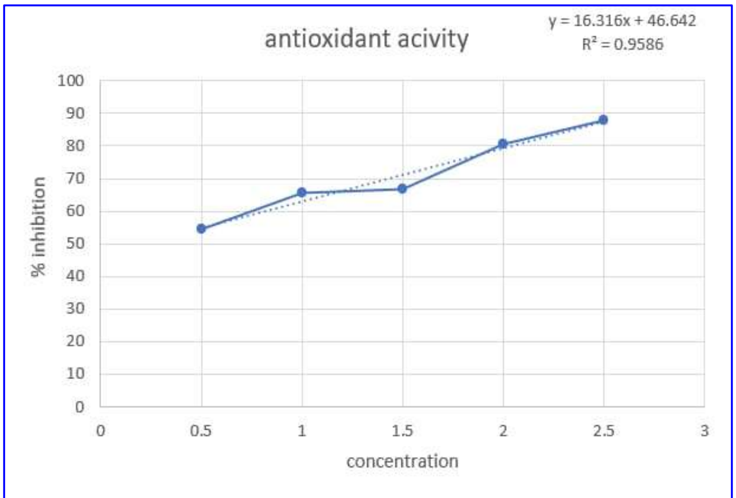
Fig 4: DPPH antioxidant assay reaction

Concentration of nanoparticles (mg/ml)	%scavenging activity
0.5	54.55%
1	65.71%
1.5	66.77%
2	80.71%
2.5	87.84%

Table 3: Antioxidant Activity of Copper Nanoparticles

The low IC_{50} value indicates strong antioxidant potential of copper nanoparticles.

Fig 5. Graph of concentration vs percentage inhibition



Alpha Amylase Inhibition Activity

The antidiabetic potential of copper nanoparticles was assessed using the alpha-amylase inhibition assay. The control sample showed high absorbance, indicating maximum enzyme activity. Test samples containing copper nanoparticles showed reduced absorbance. A maximum of 25.45 % inhibition of α-amylase activity was observed at 50 µg/ml. These observations indicated effective inhibition of alpha-amylase enzyme activity.

CONCLUSION

Copper nanoparticles synthesized using *Syzygium cumini* leaf extract were successfully prepared through a green synthesis approach. The phytochemicals present in the extract acted as reducing and stabilizing agents, enabling eco-friendly nanoparticle formation. The biosynthesized CuNPs exhibited significant antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, strong antioxidant potential with a low IC_{50} value (0.259 mg/ml), and moderate antidiabetic activity through α-amylase inhibition (~40%). Overall, the study confirms that green-synthesized CuNPs possess promising biomedical properties. However, further in vivo studies and toxicity evaluations are required to establish their safety and clinical applications

REFERENCE

[1] G. Eswarappa and R. K. Somashekar, "Jamun (*Syzygium cumini* L.), an underutilized fruit crop of India: An overview," Department of Environmental Science, University of Bangalore, Jnanabharathi Campus, Bangalore, Karnataka, India, 2020.

[2] Jonika, "Traditional medicinal plant: *Syzygium cumini*," *International Journal of Innovative Research in Technology*, vol. 12, no. 3, p. 526, 2025.

[3] H. Porika and M. Suchithra, "Jamun: An underutilized fruit loaded with nutraceuticals: A review," *Journal of Pharmacognosy and Phytochemistry*, vol. 11, no. 4, pp. 326–330, 2022.

[4] A. Semwal and N. Gupta, "Characterization of antioxidant properties in *Syzygium cumini* (Linn)," OPJS University, Churu, Rajasthan, India, n.d.

ISSN: **3107-6513**

[5] G. C. Jagetia, "Bioactive phytoconstituents and medicinal properties of Jamun (*Syzygium cumini*)," 2024, vol. 15, no. 2, pp. 101–115.

[6] A. Relhan, S. Guleria, A. Bhasin, A. Mirza, and J. L. Zhou, "Biosynthesized copper oxide nanoparticles by *Psidium guajava* plants with antibacterial, antidiabetic, antioxidant, and photocatalytic capacity," 2024.

[7] A. G. Kaningini, T. Motlhalamme, G. K. More, K. C. Mohale, and M. Maaza, "Antimicrobial, antioxidant, and cytotoxic properties of biosynthesized copper oxide nanoparticles (*CuO-NPs*) using *Athrixia phylicoides* DC," n.d.

[8] H. P. Moon, S. P. Moon, and N. A. Raut, "Seeds, leaves and stems of *Eugenia jambolana* (Jamun) as a tool for water purification," *Journal of Pharmacognosy and Phytochemistry*, vol. 13, no. 5, pp. 479–485, 2024.

[9] S. Ahmed, M. Saifullah, A. Ahmad, B. L. Swami, and S. Ikram, "Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract," *Journal of Radiation Research and Applied Sciences*, vol. 9, no. 1, pp. 1–7, 2016.

[10] P. Mukherjee, A. Roy, B. P. Mandal, G. K. Dey, P. Mukherjee, and M. Ghatak, "Green synthesis of highly stabilized nanoparticles using plant extracts," *Nano Research Letters*, vol. 3, pp. 1–10, 2008.

[11] S. Iravani, "Green synthesis of metal nanoparticles using plants," *Green Chemistry*, vol. 13, no. 10, pp. 2638–2650, 2011.

[12] J. Singh, T. Dutta, K. H. Kim, M. Rawat, P. Samddar, and P. Kumar, "Green synthesis of metals and their oxide nanoparticles: Applications for environmental remediation," *Journal of Nanobiotechnology*, vol. 16, no. 1, pp. 1–24, 2018.

[13] M. Rai, A. Yadav, and A. Gade, "Silver nanoparticles as a new generation of antimicrobials," *Biotechnology Advances*, vol. 27, no. 1, pp. 76–83, 2009.

[14] S. M. Ahmed, "Antioxidant and antimicrobial activities of plant-mediated nanoparticles," *Applied Nanoscience*, vol. 10, pp. 1–15, 2020.

[15] V. K. Sharma, R. A. Yngard, and Y. Lin, "Silver nanoparticles: Green synthesis and antimicrobial studies," *Advances in Colloid and Interface Science*, vol. 145, pp. 83–96, 2009.

[16] A. K. Mittal, Y. Chisti, and U. C. Banerjee, "Synthesis of metallic nanoparticles using plant extracts," *Biotechnology Advances*, vol. 31, no. 2, pp. 346–356, 2013.

[17] S. K. Ghosh, S. Kundu, M. Mandal, S. Nath, and T. Pal, "Studies on the evolution of silver nanoparticles in aqueous solution," *Journal of Nanoparticle Research*, vol. 4, pp. 337–341, 2002.

[18] K. B. Narayanan and N. Sakthivel, "Biological synthesis of metal nanoparticles by microbes," *Advances in Colloid and Interface Science*, vol. 156, pp. 1–13, 2010.

[19] S. K. Prasad and S. Elumalai, "Biofabrication of copper nanoparticles using plant extracts and their antibacterial activity," *Journal of Nanomedicine & Nanotechnology*, vol. 5, no. 6, pp. 1–5, 2014.

[20] R. A. Singh, A. S. Yadav, and V. K. Singh, "Green synthesis of metal nanoparticles using medicinal plants and their applications," *International Journal of Green Nanotechnology*, vol. 3, no. 2, pp. 45–58, 2011.

[21] N. N. Mariselvam, S. Elumalai, and P. Hemalatha, "Plant mediated synthesis of copper oxide nanoparticles and their antimicrobial activity," *Journal of Applied Pharmaceutical Science*, vol. 6, no. 5, pp. 123–129, 2016.

[22] A. Singh and S. M. Vishwakarma, "Phytochemical and pharmacological importance of *Syzygium cumini*," *International Journal of Pharmaceutical Sciences Review and Research*, vol. 21, no. 1, pp. 96–101, 2013.

[23] P. N. Aiyelaagbe and A. O. Osamudiamen, "Phytochemical screening of plants used in Nigerian herbal

ISSN: 3107-6513

medicine," *Journal of Medicinal Plants Research*, vol. 3, no. 5, pp. 286–290, 2009.

[24] S. Ahmed, M. Ikram, and A. B. Y. Khan, "Green synthesis of metal nanoparticles: A review," *Colloids and Surfaces B: Biointerfaces*, vol. 75, pp. 1–10, 2010.

[25] S. C. Sharma, "Bioactive compounds in *Syzygium cumini* and their medicinal applications," *Pharmacognosy Reviews*, vol. 8, no. 15, pp. 108–115, 2014.

[26] R. S. Verma and S. K. Gupta, "Antioxidant and antidiabetic activity of plant mediated nanoparticles," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 10, no. 3, pp. 100–106, 2017.

[27] M. Thiruvengadam, J. Chung, and I. M. Jang, "Biogenic synthesis of nanoparticles using plant extracts and their biomedical applications," *Frontiers in Microbiology*, vol. 9, pp. 1–15, 2018.

[28] A. K. Das, S. Debnath, and P. B. Ghosh, "Medicinal importance of *Syzygium cumini* and its phytoconstituents," *Journal of Ethnopharmacology*, vol. 210, pp. 233–241, 2018.