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BROAD-SPECTRUM ANTIBACTERIAL AND ANTIOXIDANT POTENTIALS OF AQUEOUS *SYZYGIUM CUMINI* (L.) SKEELS LEAF EXTRACT

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ABSTRACT

The worldwide increase in antimicrobial resistance and illnesses associated with oxidative stress has heightened the quest for plant-derived alternatives possessing dual therapeutic benefits. In traditional medicine, Syzygium cumini (L.) Skeels is highly valued for its natural bioactive compounds. This research examined the antibacterial and antioxidant properties of its aqueous leaf extract employing standard in vitro techniques. The antibacterial efficacy was assessed against Escherichia coli, Klebsiella oxytoca, Pseudomonas aeruginosa, Staphylococcus aureus, and Proteus mirabilis using a turbidimetric assay, with the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) established through broth dilution and subculturing methods. The extract exhibited concentration-dependent inhibition in all bacterial strains, with MIC values between 60 and 80 mg/mL and MBC values from 80 to 100 mg/mL. MBC/MIC ratios of $\leqslant$1.67 indicate a bactericidal mechanism of action. Antioxidant activity, evaluated using DPPH and hydrogen peroxide (H_2O_2) scavenging assays, showed significant radical-neutralizing effects, achieving 80.8% (DPPH) and 85.6% (H_2O_2) inhibition at a concentration of 60 mg/mL. The extract demonstrated a strong, dose-dependent antioxidant profile, albeit with significantly reduced potency compared to ascorbic acid. These findings underscore the extensive bactericidal and oxidative stress-reducing capabilities of S. cumini aqueous leaf extract, affirming its ethnomedicinal significance. Its dual bioactivity facilitates its use in the creation of natural therapeutics and as a functional component in health-enhancing formulations. This research corresponds with SDG 3 (Good Health and Well-being) and bolsters circular bioeconomy initiatives by advocating for the utilization of safe, plant-derived resources for sustainable therapeutic advancement.

Keywords: Antibacterial activity, Antioxidant activity, Aqueous extract, MIC and MBC, *Syzygium cumini,* Turbidimetric assay

INTRODUCTION

The global growth in antimicrobial resistance (AMR) and oxidative stress-related illnesses has forced a shift towards alternative therapeutic agents, particularly those that come from nature. The excessive usage and improper use of synthetic antibiotics and antioxidants have resulted in concerning outcomes, such as resistant microbial strains and negative health impacts (Fatima *et al.,* 2023; Salam *et al.,* 2023). Consequently, medicinal plants recognized for their therapeutic significance in traditional medicine are being reassessed as possible sources of bioactive chemicals with dual antibacterial and antioxidant properties. *Syzygium cumini,* usually referred to as Jamun or black plum, has emerged as a promising choice among such plants due to its extensive range of biological activities.

Syzygium cumini, commonly known as jamun or black plum, has emerged as a compelling candidate in the search for natural therapeutic agents due to its rich phytochemical composition and broad-spectrum biological activities. Native to tropical regions of Asia, particularly the Indian subcontinent, *S. cumini* belongs to the Myrtaceae family and holds a long-standing place in traditional Ayurvedic and Unani medical systems, where it has been employed for the treatment of various ailments including diabetes, infections, inflammation, and gastrointestinal disorders

Syzygium cumini, referred to as jamun or black plum, has surfaced as a promising contender in this regard. This tropical tree, native to the Indian subcontinent, has been historically utilized in Ayurvedic medicine for numerous diseases, offering a diverse array of phytochemicals, including flavonoids, phenolic acids, and tannins (Vaishampayan and Grohmann, 2021; Alsadooni and Khudhair, 2024). These phytochemicals are shown to demonstrate extensive antibacterial properties, indicating that *Syzygium cumini* may function as a viable natural alternative against resistant bacterial strains (Khare *et al.,* 2021; Fernández-Fernández *et al.,* 2023). Moreover, its antioxidative qualities may enhance its therapeutic potential, underscoring the significance of this plant in both traditional medicine and contemporary biomedical applications (Rahman *et al.,* 2022; Kalapriya *et al.,* 2023).

Syzygium cumini is a member of the Myrtaceae family and is indigenous to tropical areas of Asia, especially the Indian subcontinent. It possesses a profound historical application in Ayurvedic and Unani medical systems for the treatment of diabetes, infections, inflammation, and digestive ailments (Mishra *et al.,* 2021). Diverse plant components, such as leaves, bark, fruit, and seeds, are utilized to create decoctions, powders, and extracts. The phytochemical analysis of *S. cumini* has identified a diverse array of flavonoids, phenolic acids, tannins, glycosides, and terpenes, which enhance its biological activity (Asha and Radhamoni, 2023; Devi *et al.,* 2023). These chemicals have been independently linked to antibacterial, antioxidant, anti-inflammatory, and hypoglycemic properties, underscoring the plant's diverse medicinal potential (Rahman *et al.,* 2022; Kalapriya *et al.,* 2023).

The extraction procedure is crucial for optimizing the health benefits of medicinal plants. Aqueous extraction is beneficial as it preserves a broader range of bioactive chemicals while reducing the possible toxicity associated with chemical solvents. This technique is consistent with traditional

therapeutic practices, hence increasing its cultural significance and acceptance (Alqahtani *et al.,* 2022; Arya *et al.,* 2022; Alsadooni and Khudhair, 2024). Studies demonstrate that aqueous extracts from several plants exhibit significant antioxidant and antibacterial characteristics, validating the therapeutic potential of aqueous *Syzygium cumini* extracts (Alqahtani *et al.,* 2022; Kalapriya *et al.,* 2023).

Asha and Radhamoni, (2023) revealed that water-based extracts exhibited equivalent or even superior antioxidant properties relative to those obtained from organic solvents. Devi et al. (2023) highlighted that flavonoid and phenolics, prevalent in aqueous extracts, are effective free radical scavengers. These antioxidants mitigate oxidative stress, a harmful condition associated with aging, cancer, neurological disorders, and cardiovascular problems (Kalapriya *et al.,* 2023)). The antibacterial activities of *S. cumini* have been corroborated in numerous research. Methanolic and aqueous extracts have showed inhibitory effectiveness against therapeutically relevant pathogens such as *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Tambe *et al.,* 2021). Furthermore, prior research by Yusuf, et al. (2020) demonstrated that essential oil extracted from *S. cumini* leaves has antibacterial properties against foodborne microorganisms in cheese. The active constituents were discovered as sesquiterpenes, including trans-α-bergamotene, β-pinene, and α-santalene.

This study offers a deeper quantitative and mechanistic comprehension of *S. cumini's* bioactivity, building on existing findings. The turbidimetric method was utilized to evaluate antibacterial activity by detecting changes in optical density over time, facilitating accurate calculation of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC). Furthermore, antioxidant activity was evaluated by two established assays: DPPH radical scavenging and hydrogen peroxide (H_2O_2) scavenging. Collectively, these methodologies provide an extensive overview of the extract's antioxidant properties. Furthermore, this research contributes to the growing body of evidence advocating for plant-based alternatives to synthetic compounds. It aligns with global efforts toward sustainable healthcare as articulated in the United Nations Sustainable Development Goals (SDG 3: Good Health and Well-being; SDG 12: Responsible Consumption and Production). The use of aqueous extraction not only supports environmentally friendly practices but also enhances applicability in resource-limited settings where the use of organic solvents may pose practical or health-related challenges.

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh *Syzygium cumini* leaves were harvested from robust, mature trees situated in Irewolede Housing Estate, Ilorin, Kwara State, Nigeria (8.4629° N, 4.5524° E). The leaves were collected in the early morning, conveyed to the laboratory in sanitized, labeled paper bags, and promptly processed. The leaves were meticulously cleaned under running tap water and subsequently with distilled water to eliminate dust and debris. The samples were subsequently air-dried at ambient temperature (30 ± 2 °C). The desiccated leaves were ground in a sterile electric blender, and the resultant powder was preserved in an airtight container at 4 °C until extraction.

Aqueous Extraction of *S. Cumini* Leaves

Aqueous extraction involved immersing 6 g of powdered *S. cumini* leaves in 100 mLof distilled water. The mixture was intermittently agitated and permitted to rest for 24 hours at ambient temperature. Following extraction, the solution was filtered initially through muslin cloth and subsequently through Whatman No. 1 filter paper. The filtrate was concentrated using a water bath at 45 °C to yield a semi-solid extract, which was preserved at 4 °C in a sterile amber bottle for future use (Yusuf-Salihu *et al.,* 2024).

Test Organisms

The antibacterial activity of the extract was evaluated against five clinically relevant bacterial strains: *Escherichia coli*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Proteus mirabilis*. All test organisms were obtained from the Department of Microbiology, Kwara State University, Malete, Nigeria. The bacterial isolates were maintained on nutrient agar slants and sub-cultured freshly prior to use.

Antibacterial Activity Assay (Turbidimetric Method)

The antibacterial efficacy of the aqueous leaf extract of *Syzygium cumini* was assessed utilizing the turbidimetric method, as previously outlined by Yusuf-Salihu *et al.,* (2025). Test tubes containing 8 mLof nutritional broth were made and augmented with 1 mLof the extract at doses of 20, 40, 60, 80, and 100 mg/mL. Each tube was injected with 0.1 mLof a standardized bacterial suspension, calibrated to the 0.5 McFarland standard, roughly 1×10^8 CFU/mL. Positive control tubes comprised nutrient broth and inoculum devoid of extract, whereas negative controls included broth with extract but lacked inoculum. The tubes were incubated at 37 °C for 24 hours. Subsequent to incubation, the optical density (OD) of each tube was assessed at 600 nm utilizing a UV-Visible spectrophotometer. The antibacterial efficacy was quantified as the percentage inhibition of bacterial proliferation, determined using the formula:

$$\% \text{ growth inhibition} = \frac{\text{Dcontrol} - \text{Dtest}}{\text{Dontrol}} \times 100\%$$

This calculation measured the degree to which the nanoparticles inhibited bacterial growth.

$D_{control}$ represents the optical density of the control, while D_{test} denotes the optical density of the test.

Determination of MIC and MBC

The MIC was derived from the turbidimetric data as the lowest concentration of the extract that caused no visible turbidity and showed a substantial reduction in optical density (OD) at 600 nm relative to the control. To determine the MBC, 0.1 mL aliquots from tubes without apparent growth were aseptically plated onto nutrient agar and incubated at 37 °C for 24 hours. The MBC was defined as the lowest concentration at which no colony formation occurred, indicating complete bactericidal activity. All tests were conducted in triplicate, and the MBC/MIC ratio was calculated to classify the antibacterial action as either bactericidal (ratio ≤4) or bacteriostatic (ratio >4), following the standard interpretive criteria (Devi *et al.,* 2024).

Antioxidant Assays

DPPH Radical Scavenging Activity

The DPPH test was employed to assess the free radical scavenging capacity of the extract. In summary, 1 mL of different doses of the extract (6, 12, 24, 36, 48, and 60 mg/mL) was combined with 1 mL of 0.1 mM DPPH solution in methanol. The reaction mixture was incubated in darkness for 30 minutes, and absorbance was measured at 517 nm. Ascorbic acid served as the reference standard. The proportion of inhibition was computed accordingly (Yusuf-

Salihu *et al.,* 2024). The scavenging activity percentage was determined using the formula:

$$\%\text{DPPH scavenging effect} = \frac{AC - AS}{AB} x\ 100$$

Where: AC represents the absorbance of the control, and AS denotes the absorbance of the sample.

Hydrogen Peroxide (H_2O_2) Scavenging Activity

The scavenging activity of H_2O_2 was assessed by combining 0.6 mL of a 40 mM hydrogen peroxide solution in phosphate buffer with 1 mL of extract at different concentrations. Following a 10-minute incubation at ambient temperature, absorbance was assessed at 230 nm. Ascorbic acid served as the reference standard (Yusuf-Salihu *et al.,* 2024). The scavenging activity was quantified as a percentage in relation to the control using the formula:

$$\%\ H_2O_2\ \text{scavenging effect} = \frac{AC - AS}{AC} x\ 100$$

Where: AC represents the absorbance of the negative control, and AS denotes the absorbance of the sample.

RESULTS AND DISCUSSION

Antibacterial Activity of *Syzygium cumini* Leaf Extract

The aqueous extract of *Syzygium cumini* leaves demonstrated significant antibacterial activity against all tested bacterial strains, including *Escherichia coli*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Proteus mirabilis* as shown in Figure 1. The inhibition was concentration-dependent, with percentage activity values increasing consistently from 20 mg/mL to 100 mg/mL across all organisms. Notably, *P. mirabilis* showed the highest susceptibility, with 92.50% inhibition at the highest concentration tested, followed closely by *S. aureus* (91.07%) and *E. coli* (88.37%). These findings are consistent with previous studies, including Tambe et al. (2021), who reported that both methanol and aqueous extracts of *S. cumini* leaves exhibited inhibitory effects against pathogens such as *E. coli*, *S. typhi*, and *P. aeruginosa*, with inhibition zones ranging from 6-22 mm. Although methanol extracts showed greater efficacy in their study, the aqueous extract was still effective, highlighting the potential of water-soluble bioactives. Our use of a turbidimetric assay provides a more quantitative assessment of antibacterial activity, reinforcing the potency of aqueous extracts in inhibiting bacterial growth.

Additionally, the present findings expand on our previous research (Yusuf, et al., 2020), in which the essential oil of *S. cumini* leaves demonstrated antimicrobial activity against pathogenic bacteria and spoiling agents isolated from spoilt local cheese. In that study, inhibition was attributed to sesquiterpenes such as trans-α-bergamotene, β-pinene, and α-santalene. In contrast, the present study focuses on hydrophilic phytochemicals such as flavonoids, tannins, and polyphenols present in the aqueous extract, suggesting that *S. cumini* possesses a broad spectrum of antimicrobial compounds across different phytochemical classes. Together, these findings reinforce the therapeutic and preservative potential of *S. cumini*, particularly in developing natural antimicrobial agents for use in food safety, public health, and potentially nanoformulations. The consistency of activity across essential oil,

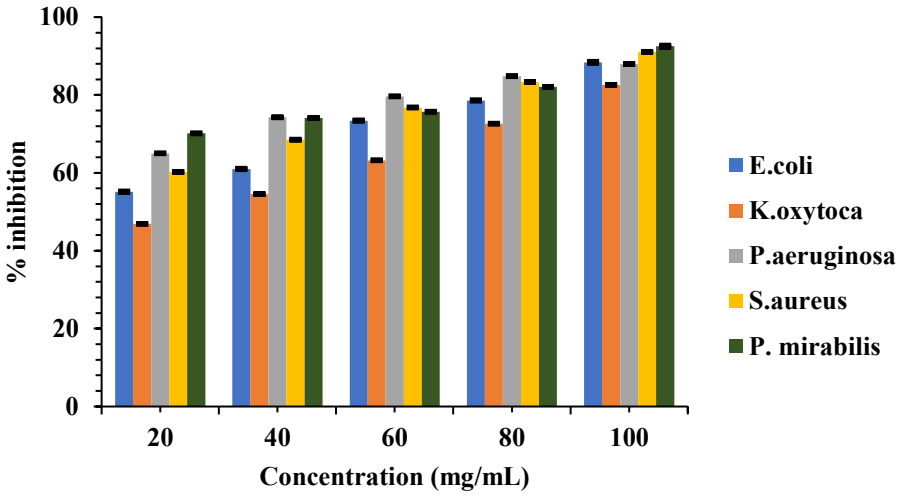

Figure 1: Percentage inhibition of bacterial growth by aqueous *Syzygium cumini* leaf extract at different concentrations. Error bars represent standard error of triplicate measurements

methanol, and aqueous fractions underscores the versatility of the plant and supports its continued exploration within sustainable biopharmaceutical and functional food research.

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

The MIC was defined as the minimum concentration that inhibits apparent bacterial growth, whereas the MBC was recognized as the minimum concentration that prevents bacterial colony formation during subculturing. In our study, MIC values ranged from 60 to 80 mg/mL, and MBC values ranged from 80 to 100 mg/mL, depending on the bacterial strain. Specifically, *Staphylococcus aureus* and *Proteus mirabilis* showed MICs at 60 mg/mL and MBCs at 80 mg/mL, indicating higher susceptibility. Conversely, *Klebsiella oxytoca* exhibited an MIC of 80 mg/mL and an MBC of 100 mg/mL, suggesting greater resistance (Table 1).

The MBC/MIC ratios for all tested organisms were ≤1.67, which, according to established criteria, indicates a bactericidal effect (Devi *et al.,* 2024). While these values are higher than those reported by Devi *et al.,* (2024), who found MIC and MBC values for *S. cumini* leaf extracts ranging from 625 to 2,500 µg/mL against various bacterial strains. The MBC/MIC ratios in both studies similarly support bactericidal action. The difference in absolute MIC/MBC values may be attributed to variations in extraction methods, bacterial strains, or plant source. Furthermore, a study by Jassim et al. (2024) demonstrated the antimicrobial activity of *S. cumini*

leaf extracts against both Gram-positive and Gram-negative bacteria, with MIC values of 104 µg/mL for Gram-positive and 208 µg/mL for Gram-negative bacteria. Although these values are lower than those observed in our study, differences in extraction methods, plant parts used, and bacterial strains tested could account for the variations.

Our findings reinforce the potential of *S. cumini* aqueous leaf extract as a natural antimicrobial agent, particularly against both Gram-positive and Gram-negative bacteria. The bactericidal nature of the extract, as evidenced by the MBC/MIC ratios, underscores its therapeutic potential in combating bacterial infections.

Table 1: Minimum Inhibitory and Bactericidal Concentrations of *Syzygium cumini* Aqueous Leaf Extract

Organism	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC Ratio	Interpretation
Escherichia coli	60	100	1.67	Bactericidal
Klebsiella oxytoca	80	100	1.25	Bactericidal
Pseudomonas aeruginosa	60	100	1.67	Bactericidal
Staphylococcus aureus	60	80	1.33	Bactericidal
Proteus mirabilis	60	80	1.33	Bactericidal

Antioxidant Assays

DPPH Radical Scavenging Activity of Syzygium Cumini Leaf Extract

The aqueous extract of *Syzygium cumini* leaves exhibited remarkable DPPH radical scavenging activity, characterized by a clear dose-dependent increase from 62.51% at 6 mg/mL to 80.80% at 60 mg/mL (Figure 2). While ascorbic acid, used as the standard, achieved slightly higher inhibition (88.82% at 60 mg/mL), the antioxidant capacity of *S. cumini* remained consistently high across all tested concentrations. This performance underscores the presence of potent hydrogen-donating phytochemicals in the extract, likely flavonoids, phenolic acids, and tannins, which contribute to its free radical neutralizing ability.

When compared with our previous study on the antioxidant activity of shea butter waste (Yusuf-Salihu *et al.,* 2024), the superiority of *S. cumini* is evident. In that study, the most active shea extract (SNC1) recorded 68.45% DPPH inhibition at 60 mg/mL substantially lower than the 80.80% recorded for *S. cumini* at just 60 mg/mL. This suggests that *S. cumini* offers significantly higher antioxidant efficacy at lower dosages, making it a more efficient candidate for therapeutic or nutraceutical formulations. Further supporting these observations, Halim *et al.,* (2022) reported variations in antioxidant activity among different parts of blackberry using both DPPH and FRAP assays, which they attributed to differences in phenolic profiles. While blackberry seeds exhibited notable DPPH activity, the values were comparable to or slightly lower than those reported here for *S. cumini*, further reinforcing its potent radical scavenging ability.

Hydrogen Peroxide (H_2O_2) Scavenging Activity

Hydrogen peroxide scavenging activity is a vital indicator of antioxidant efficiency, reflecting the ability of bioactive compounds to neutralize reactive oxygen species before they induce oxidative damage. In this study, *Syzygium cumini* aqueous leaf extract demonstrated a robust and concentration-dependent H_2O_2 scavenging response, starting at 57.56% inhibition at 6 mg/mL and reaching an impressive 85.60% at 60 mg/mL (Figure 3). This near parity with ascorbic acid (84.43% at the same concentration) highlights the extract's high radical-scavenging potential, especially in aqueous environments where peroxide reactivity is of particular concern.

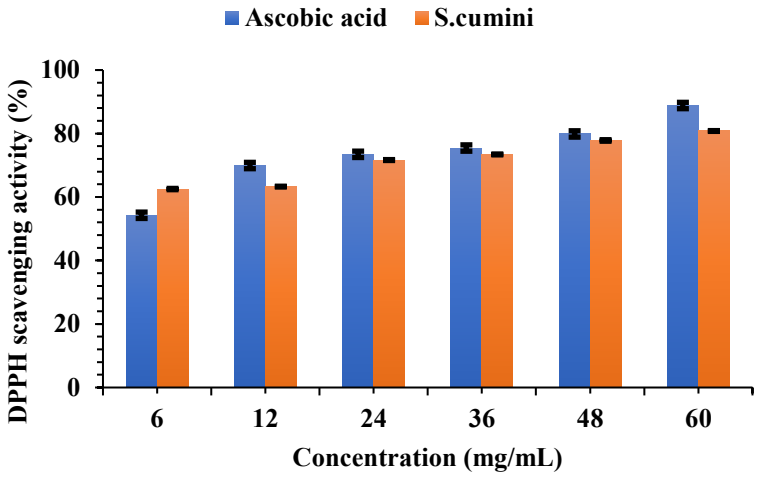

Figure 2: DPPH radical scavenging activity of aqueous *Syzygium cumini* leaf extract compared with ascorbic acid. Error bar represent standard error mean

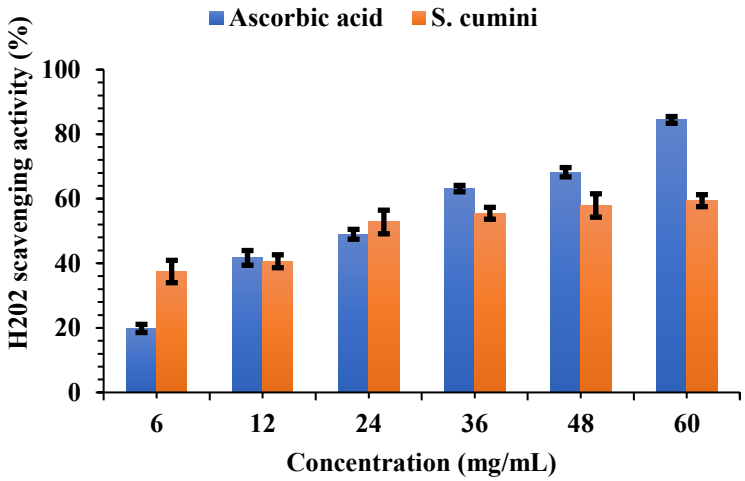

Figure 3: Hydrogen peroxide (H_2O_2) scavenging activity of aqueous Syzygium cumini leaf extract compared with ascorbic acid across concentrations. Error bar represent standard error mean

The performance of *S. cumini* notably surpasses that of previously investigated natural sources, including various shea butter waste extracts. In our earlier study (Yusuf-Salihu *et al.,* 2024), the best-performing waste sample, SNS, recorded only 38.46% inhibition at 60 mg/mL, while SNC1, SNWW, and SNC2 showed even lower activity. In contrast, *S. cumini* achieved over twice the scavenging activity at 1,000 times lower concentration, signifying a far more potent antioxidant composition, likely attributed to its enriched profile of flavonoids, phenolic acids, and tannins. These findings also align with the trend observed in other plant-based studies. For instance, Revathi et al. (2023) reported that *Anisomeles malabarica* achieved 88.67% inhibition at 90 mg/mL, while *Coldenia procumbens* reached 73% at the same dose, both considerably less efficient than *S. cumini* on a per-concentration basis. Similarly, orange peel extract showed 72.75% inhibition at 100 mg/mL(Olaitan *et al.,* 2024), and *Bersama abyssinica* extracts demonstrated only moderate, unspecified activity at higher concentrations (Alemu *et al.,* 2024). These comparisons collectively highlight *S. cumini* as a highly effective hydrogen peroxide scavenger, delivering superior outcomes with minimal dosage.

The minimal standard errors observed among replicates further substantiate the reliability and reproducibility of this result. Collectively, these data confirm that *Syzygium cumini* aqueous extract is an effective, natural source of antioxidants that can alleviate peroxide-induced oxidative stress. Its robust performance, attained without organic solvents, bolsters its suitability for medicinal applications and clean-label antioxidant compositions in the nutraceutical, pharmaceutical, and food preservation industries.

CONCLUSION

This study established that *Syzygium cumini* (L.) Skeels' aqueous leaf extract exhibits antibacterial and antioxidant properties. The extract demonstrated concentration-dependent inhibition against Gram-positive and Gram-negative bacteria, with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values ranging from 60 to 100 mg/mL. The MBC/MIC ratios indicated a bactericidal mechanism. Furthermore, the extract displayed significant free radical scavenging activity in both DPPH and hydrogen peroxide assays, affirming its antioxidative potential. These results corroborate the traditional applications of *S. cumini* (L.) Skeels and underscore its promise as a natural therapeutic agent for addressing microbial infections and oxidative stress. Additional research on compound isolation, mechanisms of action, and safety assessments is advised to facilitate its integration into drug and functional product development.

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