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Research article

Antioxidant and Antibacterial Activities of Papaya (*Carica papaya* L.) Leaf Ethanolic Extract Against DPPH Radicals and *Staphylococcus aureus*

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Abstract

Papaya (*Carica papaya* L.) leaves contain bioactive compounds with potential antioxidant and antibacterial properties. This study aimed to investigate the effects of ethanol concentration on the total phenolic content (TPC), antioxidant activity, and antibacterial activity of papaya leaf extracts. The leaves were extracted by maceration using 70% and 96% ethanol for 24 h. TPC was determined using the Folin–Ciocalteu method, antioxidant activity was evaluated using the DPPH radical scavenging assay, and antibacterial activity against *Staphylococcus aureus* was assessed using the agar well diffusion method. The 70% ethanol extract exhibited significantly higher TPC than the 96% ethanol extract (65.27 ± 1.21% and 12.64 ± 0.27%, respectively; $p < 0.001$). The 70% ethanol extract also demonstrated greater DPPH radical scavenging activity, with an IC_{50} value of 1276.63 ± 23.61 µg/mL, compared with 1570.00 ± 37.09 µg/mL for the 96% ethanol extract. However, both extracts exhibited substantially lower antioxidant activity than the reference antioxidant butylated hydroxytoluene (BHT; IC_{50} = 52.73 ± 1.53 µg/mL). Antibacterial testing showed limited inhibition of *Staphylococcus aureus*, with no statistically significant differences between the two extracts at the evaluated concentrations. Overall, 70% ethanol extraction yielded higher TPC and greater DPPH radical scavenging activity than 96% ethanol extraction, although this advantage was not accompanied by a statistically significant improvement in antibacterial activity. Further phytochemical characterization, minimum inhibitory concentration determination, and safety evaluations are warranted to assess the potential pharmaceutical applications of papaya leaf extracts.

Keywords: antioxidant, antibacterial, papaya leaves, herbal candidate

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Introduction

Indonesia's rich botanical diversity offers substantial potential for identifying plant-derived compounds with therapeutic applications [1]. Among these botanical resources, papaya (*Carica papaya* L.) has attracted considerable scientific attention because of the traditional medicinal uses and reported biological activities of its leaves [2]. Phytochemical investigations have identified several flavonoid derivatives in papaya leaves. Phytochemical investigations have identified several flavonoid derivatives in papaya leaves, including quercetin 3-(2G-rhamnosylrutinoside), kaempferol 3-(2G-rhamnosylrutinoside), quercetin 3-rutinoside, myricetin 3-rhamnoside, kaempferol 3-rutinoside, quercetin, and kaempferol [3], [4], [5]. These compounds may contribute to the antioxidant and antibacterial properties of papaya leaves. Previous studies have demonstrated DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity in ethanolic extracts of papaya leaves and fruits [6], [7]. In addition, the antibacterial activity of papaya leaf ethanolic extracts has been reported against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, with inhibition zones of approximately 4 and 3 mm, respectively, at an extract concentration of 25 mg/mL. In contrast, the corresponding aqueous extract exhibited no detectable inhibitory activity against either bacterial species [8].

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The choice of extraction solvent is an important factor influencing the phytochemical composition and biological activity of plant extracts [9]. Ethanol is widely used for extracting bioactive compounds because its solvent polarity can be modified by adjusting the ethanol-to-water ratio [10]. In papaya leaves, extraction with 70% ethanol has been reported to recover various classes of secondary metabolites, including saponins, tannins, alkaloids, anthraquinones, flavonoids, steroids, terpenoids, and glycosides. Meanwhile, flavonoids, tannins, and glycosides have been identified in papaya leaf extracts prepared using 96% ethanol [11]. These findings suggest that ethanol concentration may influence the phytochemical profiles of papaya leaf extracts and, consequently, their biological activities. However, differences in the reported phytochemical profiles should be interpreted cautiously because they may also reflect variations in extraction procedures, plant materials, and analytical methods.

Previous studies have demonstrated that solvent composition can influence the extraction of antioxidant compounds. Aqueous extracts of papaya seeds have been reported to exhibit higher total phenolic content (TPC) and stronger antioxidant activity than methanolic extracts [12]. Furthermore, extraction using 70% ethanol has been shown to facilitate the recovery of polar constituents, particularly hydrophilic antioxidants, resulting in greater antioxidant activity than extraction using 50% and 95% ethanol in the plant material investigated [13]. Other investigations have indicated that the optimal ethanol concentration for antioxidant extraction may vary depending on the phytochemical composition of the plant material [14]. Similarly, extracts prepared using 60% ethanol have demonstrated greater DPPH radical-scavenging activity, whereas 80% ethanol was more effective when antioxidant activity was assessed using the ABTS assay [15]. Collectively, these findings indicate that extraction efficiency and measured antioxidant activity depend on solvent composition, plant material, and the analytical assay employed. Nevertheless, the comparative effects of 70% and 96% ethanol on the antioxidant and antibacterial activities of papaya leaf extracts require further investigation.

Therefore, this study aimed to compare the antioxidant and antibacterial activities of papaya (*Carica papaya* L.) leaf extracts prepared using 70% and 96% ethanol. Antioxidant activity was evaluated using the DPPH radical-scavenging assay, while antibacterial activity was assessed against *Staphylococcus aureus*. By comparing these two extraction conditions, this study sought to provide preliminary evidence regarding the influence of ethanol concentration on the biological activities of papaya leaf extracts and their potential as sources of bioactive ingredients for further cosmetic research.

Materials and Methods

Materials

The chemicals and reagents used in this study included 70% and 96% ethanol, gallic acid, Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), and 2,2-diphenyl-1-picrylhydrazyl (DPPH). *Staphylococcus aureus* (S. aureus) was employed as the test microorganism for evaluating antibacterial activity, while Mueller–Hinton agar (MHA) was used as the culture medium. Distilled water was used for reagent preparation and dilution, as appropriate.

Methods

Sample preparation and extraction of Carica papaya L. leaves

Fresh leaves of *Carica papaya* L. were collected from a plantation in Jatinom, Klaten, Indonesia. The plant material was taxonomically identified by a biologist at Universitas Ahmad Dahlan, Indonesia, under identification certificate No. 036/lab.Bio/B/III/2019. The collected leaves were cleaned, dried, and subsequently ground into a fine powder. For extraction, 250 g of powdered leaves was separately macerated with either 70% or 96% ethanol at a solid-to-solvent ratio of 1:4 (w/v) for 24 h. The maceration process was repeated to improve the extraction efficiency and recovery of bioactive constituents. The resulting extracts were concentrated using a rotary evaporator at 60°C to remove the solvent. The concentrated extracts were then transferred into tightly sealed containers and stored at temperatures below 20°C until further analysis.

Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of *Carica papaya* L. leaf extracts was determined using the Folin–Ciocalteu colorimetric method, following a previously reported procedure with minor modifications [16]. A gallic acid stock solution (1 mg/mL) was serially diluted to prepare calibration standards at concentrations of 0.010, 0.0125, 0.015, 0.0175, 0.020, and 0.0225 mg/mL. Each standard solution was mixed with Folin–Ciocalteu reagent (diluted 1:10) at a volume ratio of 1:1 and vortexed for 1 min. After an 8-min reaction period, 0.8 mL of sodium carbonate solution (7.5%, w/v) was added, and the mixture was vortexed for an additional 40 s. Following color development, the absorbance was measured at 750 nm. The papaya leaf extracts were analyzed using the same procedure. TPC was calculated from the gallic acid calibration curve and expressed as a percentage of gallic acid equivalents (% GAE).

Determination of DPPH radical scavenging activity

The antioxidant activity of *Carica papaya* L. leaf extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, following a previously reported method with minor modifications [17]. The extracts
obtained using 70% and 96% ethanol were separately dissolved in ethanol to prepare test solutions at concentrations ranging from 500 to 2,000 µg/mL. For each concentration, 1 mL of the extract solution was mixed with 1 mL of freshly prepared 1 mM DPPH solution. The mixtures were allowed to react for a predetermined incubation period, after which the absorbance was measured at 517 nm using a UV–Vis spectrophotometer. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{control} represents the absorbance of the DPPH solution without extract, and A_{sample} represents the absorbance of the DPPH solution in the presence of the extract.

Determination of antibacterial activity against Staphylococcus aureus

The antibacterial activity of *Carica papaya* L. leaf extracts against *Staphylococcus aureus* was evaluated using the agar well diffusion method, following a previously reported procedure with minor modifications [18]. Mueller–Hinton agar (MHA) was used as the culture medium. Wells measuring 5 mm in diameter were prepared in the inoculated agar plates, and 20 µL of each papaya leaf extract was introduced into the respective wells. The plates were subsequently incubated at 37 °C for 24 h. Following incubation, antibacterial activity was assessed by measuring the diameter of the clear inhibition zones surrounding the wells. The inhibition zone diameters were recorded in millimeters (mm) as indicators of antibacterial activity.

Results and Discussion

Total Phenolic Content (TPC) of Carica papaya L. Leaf Extracts

The calibration curve of gallic acid obtained using the Folin–Ciocalteu method is presented in Figure 1.

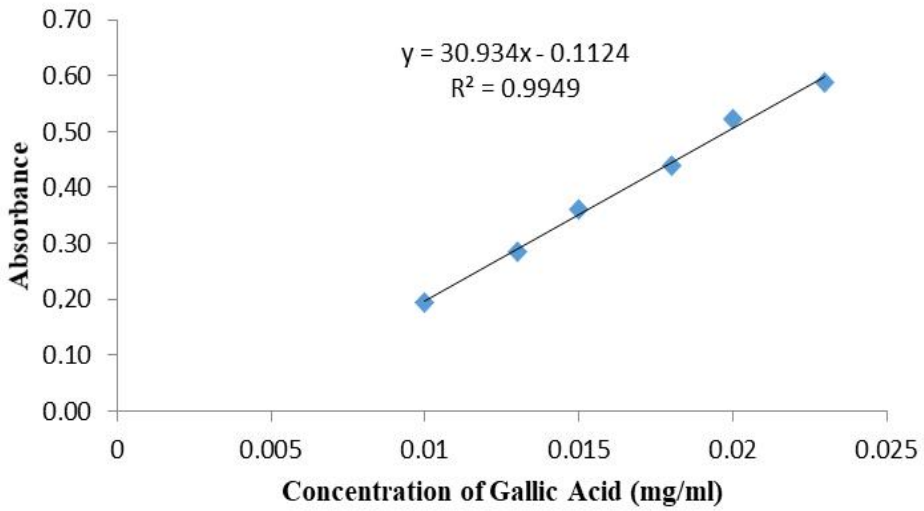


Figure 1. Gallic acid calibration curve for the determination of total phenolic content using the Folin–Ciocalteu method.

The total phenolic content (TPC) of *Carica papaya* L. leaf extracts was determined using the Folin–Ciocalteu colorimetric method, with gallic acid as the reference standard. Gallic acid solutions at concentrations ranging from 10 to 22.5 µg/mL were reacted with Folin–Ciocalteu reagent under alkaline conditions, and the absorbance was measured at 750 nm within an established operating time of 129–135 min. As illustrated in Figure 1, the calibration curve exhibited a strong linear relationship between gallic acid concentration and absorbance, with a regression equation of $y = 30.934x - 0.1124$ and a coefficient of determination (R^2) of 0.9949. This regression equation was subsequently used to quantify the TPC of the papaya leaf extracts. The resulting linear regression equation was used to determine

the TPC of *Carica papaya* L. leaf extracts obtained using 70% and 96% ethanol. The TPC values of both extracts are summarized in Table 1.

Table 1. Total phenolic content of *Carica papaya* L. leaf extracts obtained using 70% and 96% ethanol.

Sample	Total phenolic content (%)*	p-value
70% ethanol extract	65.27 ± 1.21	<0.001
96% ethanol extract	12.64 ± 0.27	—

*values are expressed as mean ± standard deviation (n = 6). The p-value represents the between-group comparison using a t-test. The percentage basis must be confirmed before final submission.

As presented in Table 1, the extract obtained using 70% ethanol exhibited a substantially higher TPC (65.27 ± 1.21%) than that obtained using 96% ethanol (12.64 ± 0.27%). Statistical analysis using an independent-samples t-test indicated a significant difference between the two extracts ($p < 0.001$). The TPC of the 70% ethanol extract was approximately 5.16 times that of the 96% ethanol extract, suggesting that aqueous ethanol was more effective in recovering Folin–Ciocalteu-reactive constituents from papaya leaves under the extraction conditions employed.

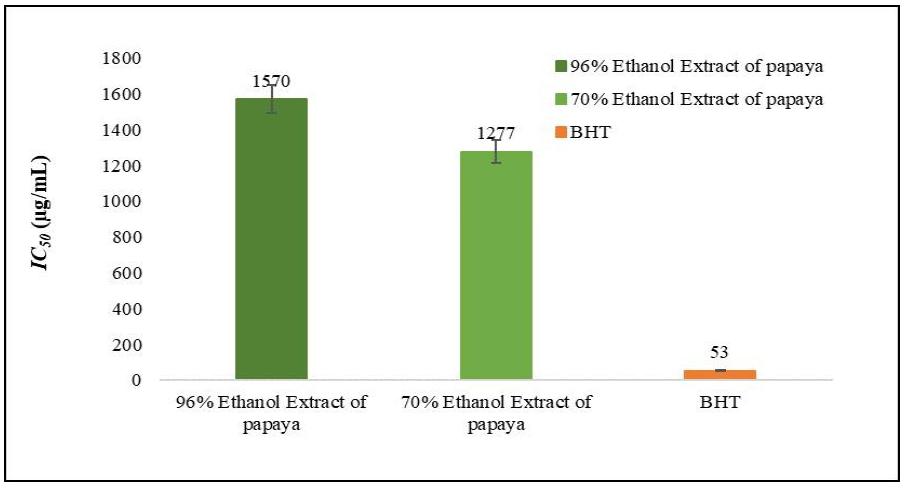
The higher TPC observed in the 70% ethanol extract may be associated with differences in solvent polarity and the presence of water in the extraction medium [19]. Water can facilitate the hydration and swelling of plant tissues, potentially improving solvent penetration and the release of phenolic constituents [20]. Furthermore, the intermediate polarity of aqueous ethanol may favor the extraction of a broader range of phenolic compounds compared with nearly absolute ethanol. Nevertheless, extraction efficiency depends on the chemical characteristics of individual phenolic constituents, the plant matrix, and the extraction conditions [21].

For comparison, a previous study reported a total phenolic content of 296.85 ± 14.25 g/kg (equivalent to 29.685 ± 1.425% w/w) in a papaya fruit extract prepared using 96% ethanol [7]. The TPC reported for the 70% ethanol papaya leaf extract in the present study was numerically higher than that reported for papaya fruit, whereas the 96% ethanol leaf extract exhibited a lower value. However, these cross-study comparisons should be interpreted cautiously because differences in plant tissues, extraction procedures, analytical conditions, and reporting bases may influence the measured TPC.

Antioxidant Activity of Carica papaya L. Leaf Extracts

The antioxidant activity of *Carica papaya* L. leaf extracts was evaluated using the DPPH radical scavenging assay, with butylated hydroxytoluene (BHT) as the positive control. The operating time ranges for the 70% and 96% ethanol extracts were 76–80 min and 62–71 min, respectively, whereas BHT exhibited an operating time range of 50–81 min. The absorbance measurements were performed at 517 nm. The antioxidant activity was expressed as the half-maximal inhibitory concentration (IC_{50}), representing the concentration required to scavenge 50% of DPPH radicals.

As illustrated in Figure 2, BHT exhibited the strongest DPPH radical scavenging activity, with an IC_{50} value of 52.73 ± 1.53 µg/mL. In comparison, the 70% and 96% ethanol extracts of papaya leaves showed IC_{50} values of 1276.63 ± 23.61 µg/mL and 1570.00 ± 37.09 µg/mL, respectively. These results indicate that both extracts exhibited substantially lower antioxidant activity than BHT. Nevertheless, the 70% ethanol extract demonstrated greater DPPH radical scavenging activity than the 96% ethanol extract, as indicated by its lower IC_{50} value.



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Figure 2. IC_{50} values of 70% and 96% ethanol extracts of *Carica papaya* L. leaves and butylated hydroxytoluene (BHT) against DPPH free radicals. Data are expressed as mean ± standard deviation (n = 5).

The difference in antioxidant activity between the two extracts may be associated with variations in their phytochemical composition resulting from the different extraction solvent concentrations [22]. Interestingly, the 70% ethanol extract also exhibited a higher total phenolic content (65.27 ± 1.21%) than the 96% ethanol extract (12.64 ± 0.27%), suggesting a possible contribution of phenolic constituents to the observed antioxidant activity. However, this relationship should be interpreted cautiously because antioxidant activity is influenced not only by the total concentration of phenolic compounds but also by their chemical structures, redox properties, and interactions with other extract constituents [23].

Both *Carica papaya* L. leaf extracts exhibited very weak DPPH radical-scavenging activity according to a previously reported IC_{50}-based classification, in which values exceeding 200 µg/mL are categorized as indicating very weak antioxidant activity [24]. The 70% ethanol extract demonstrated comparatively stronger antioxidant activity (IC_{50} = 1276.63 ± 23.61 µg/mL) than the 96% ethanol extract (IC_{50} = 1570.00 ± 37.09 µg/mL), although both extracts showed substantially lower activity than the reference antioxidant, butylated hydroxytoluene (BHT; IC_{50} = 52.73 ± 1.53 µg/mL). Nevertheless, these findings demonstrate measurable antioxidant potential under the experimental conditions employed. Comparisons with previous studies should be interpreted cautiously because differences in reported IC_{50} units, experimental conditions, and DPPH assay protocols may affect the comparability of antioxidant activity results [6], [7].

The antibacterial activities of *Carica papaya* L. leaf extracts obtained using 70% and 96% ethanol against *Staphylococcus aureus* were evaluated using the agar well diffusion method, as presented in Table 2.

Table 2. Inhibition zone diameters of *Carica papaya* L. leaf extracts at different extract concentrations.

Samples	8 mg/mL	4 mg/mL	1 mg/mL
70% ethanol extract	1.47 ± 0.05[a]	1.17 ± 0.05[a]	1.00 ± 0.00[a]
96% ethanol extract	1.37 ± 0.05[a]	1.07 ± 0.05[a]	1.00 ± 0.00[a]
Negative control	—	—	—

Note: Values are expressed as mean ± standard deviation (n = 3). The original table reports inhibition zone diameters in mm; the measurement unit requires verification. Identical superscript letters indicate no statistically significant difference within the comparison defined by the statistical test ($p \geq 0.05$). Negative-control results were not provided.

Both extracts exhibited relatively limited antibacterial activity within the investigated concentration range of 1–8 mg/mL. At the highest concentration (8 mg/mL), the 70% ethanol extract produced a numerically larger inhibition zone (1.47 ± 0.05) than the 96% ethanol extract (1.37 ± 0.05). A similar trend was observed at 4 mg/mL, with inhibition zone values of 1.17 ± 0.05 and 1.07 ± 0.05, respectively. At the lowest concentration (1 mg/mL), both extracts produced identical inhibition zone values of 1.00 ± 0.00. However, the reported statistical analysis did not indicate significant differences between the extracts at the tested concentrations.

The observed numerical increase in inhibition zone diameter with increasing extract concentration suggests a possible concentration-dependent antibacterial response [25]. Nevertheless, the absence of statistically significant differences indicates that this trend should be interpreted cautiously. The limited antibacterial activity may be associated with the relatively low extract concentrations, the concentrations of active antibacterial constituents, and differences in the ability of phytochemical compounds to diffuse through the agar medium.

These findings are consistent with previous evidence demonstrating the antibacterial activity of papaya leaf ethanolic extracts against *Staphylococcus aureus*, with reported inhibition zone diameters of 3 and 16 mm at extract concentrations of 25 and 100 mg/mL, respectively [8]. Although these findings suggest that higher extract concentrations may produce greater bacterial growth inhibition, direct comparisons should be made cautiously because differences in extraction procedures, bacterial strains, experimental conditions, and inhibition zone measurement methods may affect the results.

Interestingly, the 70% ethanol extract exhibited a substantially higher total phenolic content and greater DPPH radical scavenging activity than the 96% ethanol extract. However, these differences were not accompanied by a statistically significant improvement in antibacterial activity against S. aureus. This finding suggests that the compounds contributing to antioxidant activity may not necessarily exert comparable antibacterial effects under the experimental conditions. Furthermore, antibacterial activity may depend on the specific chemical structures, concentrations, and mechanisms of action of individual phytochemical constituents rather than total phenolic content alone [26].

Overall, ethanol concentration influenced the phenolic content and DPPH radical scavenging activity of papaya leaf extracts, whereas its effect on antibacterial activity against S. aureus was not statistically significant in the

reported comparisons. These findings highlight the importance of considering biological activity independently when evaluating plant extracts for potential pharmaceutical applications. Further investigations involving higher extract concentrations, minimum inhibitory concentration (MIC) determination, phytochemical characterization, and additional bacterial strains are warranted to establish the antibacterial potential of C. papaya leaf extracts.

Conclusion

The 70% ethanol extract of *Carica papaya* L. leaves exhibited a higher total phenolic content (65.27 ± 1.21%) than the 96% ethanol extract (12.64 ± 0.27%). The 70% ethanol extract also demonstrated greater DPPH radical scavenging activity, as indicated by its lower IC_{50} value (1276.63 ± 23.61 µg/mL) compared with the 96% ethanol extract (1570.00 ± 37.09 µg/mL). Nevertheless, both extracts exhibited substantially lower antioxidant activity than the reference antioxidant BHT. Antibacterial testing against *Staphylococcus aureus* revealed limited inhibitory effects, with no statistically significant differences between the extracts under the investigated conditions. Overall, 70% ethanol was more effective than 96% ethanol in recovering Folin–Ciocalteu-reactive constituents and producing an extract with greater DPPH radical scavenging activity, but this advantage did not translate into a statistically significant improvement in antibacterial activity. Further studies involving minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination, phytochemical characterization, and comprehensive safety assessments are required to establish the potential pharmaceutical applications of these extracts.

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Declarations

Author contribution	:	MA contributed to the conceptualization, research design, methodology, supervision, data interpretation, and manuscript review and editing. AEW contributed to sample preparation, extraction procedures, laboratory experiments, and data collection. RDM contributed to the determination of total phenolic content, antioxidant activity assessment, data analysis, and preparation of the initial manuscript draft. IN contributed to antibacterial activity testing, interpretation of experimental findings, and manuscript review and editing. NAN contributed to the literature review, interpretation of results, and critical revision of the manuscript. All authors reviewed and approved the final version of the manuscript.
Funding statement	:	This research was financially supported by the Institute for Research and Community Service (LPPM), Universitas Ahmad Dahlan, Yogyakarta, Indonesia, through the Fundamental Research Grant scheme.
Conflict of interest	:	The authors declare that they have no conflicts of interest.
Ethics Declaration	:	This study involved laboratory-based investigations of plant extracts, including chemical assays and in vitro antibacterial testing. No human participants or animals were involved. Ethical approval requirements should be confirmed in accordance with the relevant institutional regulations.
Additional information	:	No additional information is available for this article.

Informed Consent Statement

Not applicable. This study involved laboratory-based experiments using *Carica papaya* L. leaf extracts and did not involve human participants or identifiable personal data.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this manuscript, the authors used artificial intelligence (AI)-assisted technology solely to improve the language, grammar, and readability of the text. The authors subsequently reviewed and edited the AI-assisted content and take full responsibility for the accuracy, integrity, and scientific content of the manuscript. AI-assisted technology was not used for experimental data generation, statistical analysis, interpretation of results, or formulation of scientific conclusions.

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