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The Potential of Active Compounds in Gelinggang Leaf Extract as a Natural Antimicrobial for Laying Hens

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ABSTRACT

Cassia alata L. (gelinggang) has been widely reported to contain bioactive secondary metabolites with antimicrobial properties. The advantages of utilizing *Cassia alata L.* encompass its antibacterial, antifungal, antioxidant, antiviral, antitumor, anti-inflammatory, antidiabetic, antihepatotoxic, and cytotoxic properties. This *in vitro* laboratory study aimed to evaluate the nutritional composition, phytochemical profile, and antibacterial activity of gelinggang leaf extract as a potential natural antimicrobial feed ingredient. Antibacterial activity was evaluated against *Escherichia coli* and *Salmonella* sp. using the agar well diffusion method. Proximate analysis revealed that *Cassia alata* leaf powder contained 20.35% crude protein, 10.05% crude fiber, 5.32% crude fat, 5.45% ash, and 51.44% nitrogen-free extract. Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, tannins, phenolics, glycosides, triterpenoids, and steroids. The ethanol extract demonstrated moderate inhibitory antimicrobial activity against *Escherichia coli and Salmonella sp.* at concentrations ranging from 50% to 100%; in contrast, lower concentrations exhibited weak activity inhibition. *Cassia alata* leaf extract demonstrated nutritional value with high protein content (20.35%) and moderate *in vitro* antibacterial potential, indicating its promise as a natural antimicrobial candidate.

Keywords: Antibacterial activity, Animal feed additive, Bioactive compound, *Cassia alata*

INTRODUCTION

Indonesia, a tropical country, is rich in floral biodiversity, much of which remains underexplored, particularly in poultry science and broiler chickens. Indonesia, a tropical country with high plant biodiversity, offers significant potential for developing natural feed additives for poultry production. The global poultry industry has faced increasing restrictions on the use of antibiotic growth promoters (AGPs) due to concerns regarding antimicrobial resistance and food safety. Since 2018, Indonesia has officially banned the use of AGPs in animal feed, prompting the exploration of safe and effective natural alternatives to support poultry health and productivity (Hashemi and Homa, 2011). Plant-derived additives are considered relatively safe due to their natural origin and minimal adverse effects. However, a scientific evaluation of their nutritional characteristics and biological activities is necessary before proceeding with further application.

Plant-derived compounds, known as phytoadditives, have gained increasing attention as potential substitutes for AGPs due to their bioactive secondary metabolites, such as flavonoids, phenolics, tannins, and saponins, which exhibit antimicrobial, antioxidant, and immunomodulatory activities (Oladeji et al., 2020). Plant-based phytoadditives can be administered via feed or drinking water. *Cassia alata L.*, commonly known as gelinggang, is a plant with significant potential but limited investigations in the poultry industry (Amao et al., 2014). *Cassia alata L.* has different local names in Indonesian, such as Chinese ketepeng and kupang leaf (Fajri et al., 2018). It is a shrub that can grow up to five meters tall, characterized by pinnate compound leaves, and belongs to the legume family (Bharathi et al., 2022). Scientifically, *Cassia alata* has been reported to exhibit several pharmacological properties, such as antibacterial, antifungal, and antioxidant. According to Bharathi et al. (2022), *Cassia*

alata exhibits a wide range of pharmacological potentials, including antibacterial, antifungal, antioxidant, antiviral, antitumor, anti-inflammatory, antidiabetic, antihepatotoxic, and cytotoxic effects. These biological activities are attributed to the secondary metabolites present in *Cassia alata* leaves, including phenolic compounds (flavonoids such as kaempferol and glycosides), anthraquinones (rhein, chrysophanol, aloe-emodin, and alatonal), fatty acids (oleic, palmitic, and linolenic acids), steroids, and terpenoids (Oladeji et al., 2020; Fajri et al., 2023). Broiler chickens fed commercial diets containing *Cassia alata* leaf extract at different inclusion levels demonstrated no significant changes in the hematological profile (erythrocytes, leukocytes, hemoglobin). *Cassia alata L.* is a tropical medicinal plant rich in bioactive compounds with reported antimicrobial activity. Anthraquinones are naturally occurring compounds found in different plants, such as gelinggang leaf (Ulfah et al., 2018). Anthraquinones function as antibacterial or antimicrobial agents that help prevent inflammation in animals (Kumar et al., 2015). Several studies have demonstrated the inhibitory effects of *Cassia alata* leaf extracts against pathogenic bacteria, including *Escherichia coli* (*E. coli)*, with inhibition levels varying with extract concentration and extraction method (Owayale et al., 2005; Happy et al., 2019; Ashari et al., 2022). Ethanol extracts have been reported to exhibit stronger antibacterial activity than extracts from other solvents, indicating the importance of extraction efficiency in isolating active compounds (Owayale et al. 2005). Similarly, Owayale et al. (2005) reported that ethanol extracts of *Cassia alata* leaves exhibited stronger inhibitory effects against *E. coli* than methanol and petroleum ether extracts. Despite these findings, information regarding the nutritional composition, phytochemical profile, and *in vitro* antibacterial activity of *Cassia alata* leaves remains limited. The present study aimed to determine the proximate composition, identify bioactive compounds, and evaluate the *in vitro* antibacterial activity of *Cassia alata* leaf extracts, using laboratory methods as an initial step to evaluate their potential as natural antimicrobial agents for animal feed.

MATERIALS AND METHODS

Ethical approval

The experiment had been approved by the faculty of animal science, Bogor Agricultural University, Indonesia, and according to the Bogor Agricultural University Animal Ethics Committee.

Production of *Cassia alata* leaf extract

The present study was conducted *in vitro* to evaluate the nutritional composition, phytochemical profile, and antibacterial activity of *Cassia alata* leaf extracts.

Figure 1. Production process of *Cassia alata* leaf extract

Fresh *Cassia alata* leaves were collected from Bogor, West Java, Indonesia. The plant material was authenticated at the Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Indonesia. Only mature leaves were used in the present study. The production of *Cassia alata* extract began with the collection of the plant material around the IPB University Dramaga campus. The leaves were separated from the stems, as only the leaves were during the present experiment. The leaves were washed with distilled water and dried using a dome dryer at approximately 60°C for 48 hours to prevent thermal degradation of bioactive compounds. The dried leaves were ground using a grinder and sieved to obtain fine leaf powder (Depkes, 2000). Two extraction methods were used, including ethanol maceration and traditional infusion extraction (Somchit et al., 2003). For ethanol maceration, *Cassia alata* leaf powder was soaked in 96% ethanol at a 1:5 (w/v) ratio for 72 hours at room temperature, following the method described by the Indonesian Ministry of Health (Depkes, 2000). The mixture was stirred twice daily. After maceration, the extract was filtered using Whatman No. 41 filter paper and concentrated using a rotary evaporator to remove the solvent. The concentrated extract was stored in dark glass bottles at 4°C until analysis. For infusion extraction, *Cassia alata* leaf powder was boiled in distilled water (1:10 w/v) for 15 minutes, allowed to cool, and filtered. The filtrate was used as the infusion extract. The filtrate was concentrated by rotary evaporation for about 3 hours to remove the solvent, yielding a concentrated extract of *Cassia alata* leaves (Figure 1). The resulting concentrated extract was collected in dark glass vials and stored for later use as a treatment in the present study.

Nutrition content

The chemical composition of the gelinggang leaves was analyzed by proximate analysis, which included the determination of moisture, ash, crude fat, crude protein, crude fiber, and nitrogen-free extract (NFE), using standard procedures of the Association of Official Analytical Chemists (AOAC, 1970). All analyses were performed in duplicate at the laboratory of feed technology science, Bogor Agricultural University, Indonesia.

Qualitative analysis of the active compounds in gelinggang leaves

Qualitative analysis of secondary metabolites in *Cassia alata* leaf powder was conducted using the Indonesian Materia Medika (MMI), Volume VI (Ministry of Health of the Republic of Indonesia, 1995). The screening identified the presence of alkaloids, saponins, tannins, phenolics, flavonoids, glycosides, triterpenoids, and steroids based on standard color and precipitation reactions.

Antimicrobial inhibition test

The antibacterial activity of *Cassia alata* leaf extracts was evaluated *in vitro* using the agar well diffusion method, following the procedure described by Davis and Stout (1971). Ethanol (96%) maceration extract and infusion extract were tested and compared with a commercial supplement (Vitachick®, Indonesia) as the positive control, while sterile distilled water served as the negative control. The best-performing extract was subsequently used in the feeding trial. Antibacterial activity against pathogenic bacteria (*E. coli* and *Salmonella* sp.) was tested using the minimum inhibitory concentration (MIC) method with microplate assays (Eloff, 1998).

Pathogenic bacteria *E. coli* and *Salmonella* sp. were subcultured on nutrient agar slants and incubated at 37°C for 24 hours. The bacterial suspensions were then spread evenly onto nutrient agar plates. Wells with a diameter of 5 mm were made using a sterile cork borer, and 50 µL of extract solution at concentrations of 100%, 50%, 25%, and 12.5% (v/v) were added into each well. All treatments were performed in duplicate. The plates were incubated at 37°C for 24 hours, after which antibacterial activity was determined by measuring the diameter of the clear inhibition zones around the wells using a digital caliper. The inhibition zones were recorded in millimeters and presented as mean values. Antibacterial activity was classified according to the inhibition zone diameter. Less than 5 mm (weak), 5-10 mm (moderate), 10-20 mm (strong), and over 20 mm (very strong), following the method of Alouw et al. (2022). The findings were reported descriptively using average inhibition zone diameters.

RESULT AND DISCUSSION

Nutrition ingredients

The proximate composition of *Cassia alata* leaf powder was analyzed by determining dry matter, ash, crude protein, crude fat, crude fiber, and NFE. The results of the proximate analysis are presented in Table 1.

The ash content (5.45%) indicated the presence of inorganic minerals, such as calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P), sodium (Na), iron (Fe),

zinc (Zn), and manganese (Mn), which are commonly found in medicinal plant leaves.

Table 1. Proximate analysis of *Cassia alata* leaf powder

Parameters	Percentage
Dry matter	92.61
Ash	5.45
Crude protein	20.35
Crude fiber	10.05
Crude fat	5.32
Nitrogen-free extract	51.44

These minerals are essential for metabolic regulation, enzyme activation, osmotic balance, and antioxidant defense mechanisms in animals (Anyasor et al., 2014). The relatively high crude protein content (20.35%) suggested that *Cassia alata* leaves may serve as a plant-based protein source for animal feed ingredients. Nitrogen-free extract constituted the largest fraction, reflecting the presence of non-structural carbohydrates such as sugars and glycosides, which are often associated with bioactive secondary metabolites (Aborisade et al., 2017). The crude protein content (20.35%) indicated that *Cassia alata* leaves possessed moderate protein value compared to other medicinal plants and could contribute nitrogenous compounds when incorporated into poultry diets. However, in contrast to soybean meal, which contains approximately 40-48% crude protein (Ravindra et al., 2014) and functions as a primary source of protein in poultry feed, the protein content in *Cassia alata* was lower and should, therefore, be regarded as a supplementary rather than a primary protein ingredient. The biological activities observed in medicinal leaves are more strongly associated with secondary metabolites than with protein content alone (Oladeji et al., 2020). The NFE in *Cassia alata* leaf accounted for 51.44% of the total composition and was the largest fraction. The NFE primarily consists of non-structural carbohydrates such as soluble sugars, starch, and glycosidic compounds. Non-structural carbohydrates function as readily available energy sources in animal nutrition. In medicinal plants, this fraction may include carbohydrate-bound secondary metabolites, such as flavonoid glycosides and saponins, which are known to contribute to antimicrobial and antioxidant properties (Aborisade et al., 2017). The relatively high NFE value in the present study indicated that *Cassia alata* leaves contain substantial carbohydrate components and bioactive compounds that may support both nutritional and functional roles.

Several studies have investigated *Cassia alata* materials in broiler chickens. Leaf extract supplementation was tested for its impact on the hematological profiles of broilers, showing no significant changes in erythrocyte, leukocyte, or hemoglobin levels at specific doses (Ashari et al., 2024). However, the inclusion of 0.8% *Cassia alata L.* leaf extract in broiler feed yielded the most favorable results regarding the surface area of the villi and the villus count (Arroziq et al., 2024). Another feeding trial reported positive effects on growth performance and carcass traits in broiler chickens, with the 1.0 g/kg diet inclusion showing the highest daily weight gain and improved carcass component weights (Yahaya et al., 2024). Additionally, a clinical study on the inclusion of aqueous *Cassia alata* leaf extract in broiler chickens' water revealed significant effects on hematological and biochemical parameters (Amao et al. 2014). Values at 0.8% extract were erythrocytes 2.81×10^{6}/mm³, hemoglobin 7.64 g/dL, leukocytes 15.95×10^{3}/mL, all within normal physiological ranges, suggesting no overt blood toxicity at these inclusion levels (Ashari et al., 2024). The feeding of *Cassia* sp. flour at different percentages, namely 0%, 3%, 6%, 9%, and 12%, in the study by Yakubu et al. (2017) showed significant differences in weight gain and FCR, with the best treatment being 9%.

Table 2. Qualitative analysis of bioactive compounds in *Cassia alata* leaf powder

Compound	Result	Methode
Alkaloid	+	MMI Jilid VI, Thn 1995 Lampiran 16
Saponin	+	
Tanin	+	
Phenolic	+	
Flavonoid	+	
Glycoside	+	
Tripenoid	+	
Steroid	+	

The active compound in gelinggang leaf flour

Qualitative phytochemical screening demonstrated that *Cassia alata* leaves contained alkaloids, flavonoids, saponins, tannins, phenolics, glycosides, triterpenoids, and steroids (Table 2). The presence of these compounds was consistent with previous studies of Oladeji et al. (2020) and Fajri et al. (2023) regarding the phytochemical compounds of *Cassia alata* leaves. Flavonoids inhibit bacterial growth by interfering with DNA replication enzymes and disrupting cytoplasmic membrane permeability, while phenolic compounds induce protein denaturation and damage bacterial cell wall integrity. These mechanisms collectively contribute to the antibacterial activity observed in the present study (Alouw et al., 2022). Saponins are able to reduce bacterial cell

stability by diffusing through the outer membrane and cell wall, subsequently interacting with the cytoplasmic membrane and causing membrane leakage that leads to cell death. In addition, saponins can alter the surface tension of the cell wall, allowing antibacterial compounds to penetrate the cell and interfere with metabolic processes, ultimately resulting in bacterial death (Ngazizah et al., 2017). Meanwhile, tannins can inhibit microbial cell adhesion, enzymatic activity, and protein transport within the inner cell layer. Disruption in protein transport impairs the cell's ability to carry out essential life functions, which in turn restricts microbial growth (Scalbert, 1991; Chung et al., 1998; Cowan, 1999).

Antimicrobial inhibition

Antibacterial activity was evaluated using the agar well diffusion assay, as evidenced by clear inhibition zones around the extract-filled wells. Figures 2 and 3 illustrate the results of the inhibition zone test obtained from gelinggang leaf extract, vitachick, and distilled water. The resulting diameter was subsequently measured, and the findings were presented in Tables 3 and 4.

Antibacterial activity was classified according to inhibition zone diameters. Based on the classification by Alouw et al. (2022), less than 5 mm was considered weak, 5-10 mm moderate, 10-20 mm strong, and over 20 mm very strong. At concentrations of 50% and 100%, the ethanol extract exhibited moderate antibacterial activity, while lower concentrations (25% and 12.5%) demonstrated weak or no inhibition. Conversely, the infusion extract exhibited consistently weak antibacterial effects. The current findings suggested that ethanol was more effective than water in extracting bioactive antibacterial compounds from *Cassia alata* leaves.

The increased inhibition zone diameter at higher extract concentrations indicated a dose-dependent antibacterial effect, consistent with the findings of Mahmudah et al. (2018) on medicinal plant extracts. This effect may be due to the increased levels of flavonoids, saponins, and tannins found in the ethanol extract.

Cassia alata leaves contain several bioactive compounds, including flavonoids, saponins, and tannins, that contribute to their antibacterial effects (Oladeji et al., 2020). Flavonoids suppress nucleic acid synthesis by interfering with DNA and RNA synthesis, resulting in damage to the bacterial cell wall and intracellular organelles (Cushnie and Lamb, 2005). Saponins compromise cell membrane integrity by interacting with the cytoplasmic membrane, leading to membrane leakage and subsequent cell death (Ngazizah et al., 2017). Furthermore, saponins lower surface tension, facilitating the entry of antibacterial compounds into bacterial cells. Tannins, on the other hand, deactivate microbial adhesion, enzymatic activity, and protein transport systems, thereby restricting cellular functions and inhibiting bacterial growth (Scalbert, 1991; Cowan, 1999). According to the present results (data from Tables 2 and 3), the control variable did not influence the suppression of *E. coli* and *Salmonella* sp., therefore, the best results were obtained using traditional water-based extracts of ethanol.

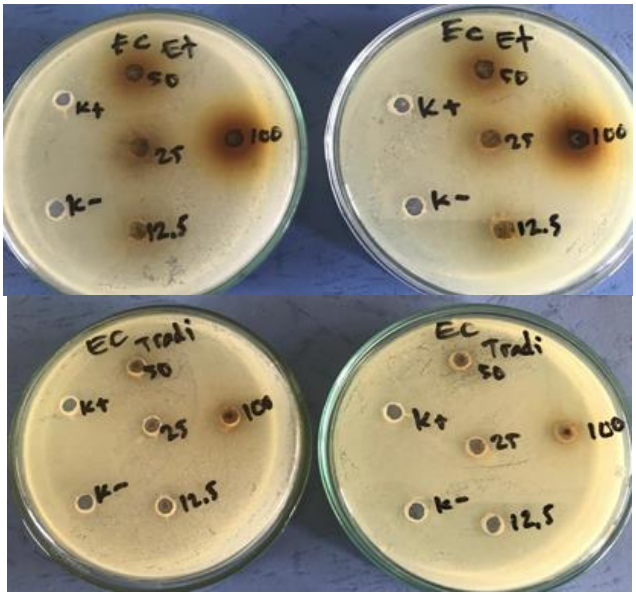

Figure 2. Antibacterial activity and inhibition zones of *Cassia alata* leaf extract (right) and Vitachick (left) against *Escherichia coli*

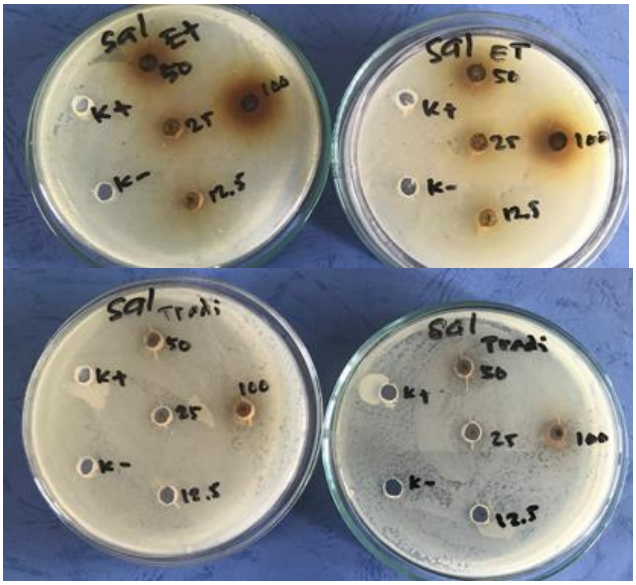

Figure 3. Antibacterial activity and inhibition zones of *Cassia alata* leaf extract (right) and Vitachick (left) against *Salmonella* sp.

The results of antibacterial activity tests of *Cassia alata* leaf extract against *E. coli* and *Salmonella* sp. bacteria showed inhibition zones at certain concentrations, measured based on the diameter of the inhibition zone (mm). The inhibition zone measurement method is a common approach in evaluating antibacterial activity, where the larger the diameter of the clear zone formed, the stronger the antibacterial activity (Bauer et al., 1966). In *E. coli* bacteria, the ethanol extract showed higher activity than the traditional extract, with the largest average inhibition zone at a concentration of 100% (13 mm), followed by 50% (10.5 mm) and 25% (8 mm), while at a concentration of 12.5% no inhibition zone was formed. Meanwhile, the traditional extract only showed inhibition at concentrations of 100% (6 mm) and 50% (5.5 mm).

A similar pattern was also observed in tests on *Salmonella sp*., where ethanol extracts produced average inhibition zones of 13 mm (100%), 10.5 mm (50%), and 7 mm (25%), while the 12.5% concentration showed no activity. Traditional extracts produced smaller inhibition zones, namely 6.75 mm (100%), 5.5 mm (50%), and 5.25 mm (25%). The absence of inhibition zones in the negative control (sterile distilled water) indicated that the solvent did not affect the test results, while the positive control confirmed the validity of the test method.

Based on the classification of antibacterial strength according to Davis and Stout (1971), inhibition zone diameters of 5-10 mm are categorized as moderate activity, while 10-20 mm are categorized as strong. Thus, ethanol extracts of *Cassia alata* leaves at concentrations of 100% and 50% are categorized as strong against both test bacteria, while traditional extracts tend to be in the moderate category. This antibacterial activity is thought to be related to the content of bioactive compounds such as flavonoids, tannins, and saponins, which are known to be capable of damaging bacterial cell membranes, inhibiting nucleic acid synthesis, and disrupting cell membrane permeability (Cowan, 1999). In general, these results indicate that the ethanol extraction method is more effective in extracting antibacterial active compounds than the traditional method, resulting in higher inhibitory activity against Gram-negative bacteria such as *E. coli* and *Salmonella sp*.

Table 3. Inhibition zone diameter of *Cassia alata* extract against *Escherichia*

No	Extract type	Rep.	Results of the extract inhibition test against *E. Coli* bacteria (mm)				Control	
			100%	50%	25%	12.5%	Vitachick (+)	Sterile distilled water (-)
1.	Traditional	U-1	6	5.5	0	0	0	0
		U-2	6	5.5	0	0	0	0
		Mean	6	5.5	0	0	0	0
2.	Ethanol	U-1	12	10	9	0	0	0
		U-2	14	11	7	0	0	0
		Mean	13	10.5	8	0	0	0

Traditional: Infusion extraction method, Rep: Replication, U1: Replication 1, U2: Replication 2, Vitachick: Commercial feed additives (positive control), Sterile distilled water: Negative control

Table 4. Inhibition zone diameter of *Cassia alata* extract against *Salmonella sp.*

No	Extract type	Rep.	Results of the extract inhibition test against *Salmonella sp.* bacteria (mm)				Control	
			100%	50%	25%	12.5%	Vitachick (+)	Sterile distilled water (-)
1.	Traditional	U-1	6,5	5,5	5	0	0	0
		U-2	7	6	5,5	0	0	0
		Mean	6.75	5.5	5.25	0	0	0
2.	Ethanol	U-1	14	10	7	0	0	0
		U-2	11	11	7	0	0	0
		Mean	13	10,5	7	0	0	0

Traditional: Infusion extraction method, Rep: Replication, U1: Replication 1, U2: Replication 2, Vitachick: Commercial feed additives (positive control), Sterile distilled water: Negative control

CONCLUSION

The current study demonstrated that *Cassia alata* leaf powder is rich in nutrients and bioactive compounds, including flavonoids, saponins, tannins, phenolics, glycosides, triterpenoids, and steroids. The ethanol extract exhibited higher *in vitro* antibacterial activity against *E. coli* and *Salmonella* sp. compared to the infusion extract, particularly at concentrations of 50-100%. The current results suggested that *Cassia alata* leaves could serve as a natural antimicrobial option for animal feed applications. However, further *in vivo* studies are required to confirm the efficacy of *Cassia alata* leaves bioactive compounds, assess safety, and determine the optimal dosage.

DECLARATIONS

Fundings

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Availability of data and materials

The datasets generated during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

Hasna Ariqoh conducted the laboratory analysis and drafted the manuscript. Yuli Retnani and Widya Hermana designed and supervised the study. Indah Wijayanti, Mahirah Firdaus, and Taryati assisted in data analysis and manuscript revision. All authors read and approved the final edition of the manuscript.

Competing interests

The authors declare no conflict of interest.

Ethical considerations

This study was conducted in accordance with the ethical principles of scientific study, including honesty, transparency, and responsibility. All data presented in this study are original, have not been fabricated or manipulated, and have not been published or submitted elsewhere. The manuscript was carefully checked to avoid plagiarism and duplicate publication. The authors confirmed that no AI tools were used to prepare this study.

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