

# Bioinsecticidal Activity of Bintaro (*Cerbera odollam*) Leaf Extract against *Thrips parvispinus* Infesting Cayenne Pepper (*Capsicum frutescens*)

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## Abstract.

The increasing reliance on synthetic insecticides for pest management has raised concerns regarding environmental contamination, pest resistance, and adverse effects on non-target organisms. Therefore, botanical insecticides have received increasing attention as alternative pest control agents. *Cerbera odollam* or bintaro in Indonesia, is a tropical plant species containing bioactive secondary metabolites with potential insecticidal activity. This study aimed to evaluate the effectiveness of *C. odollam* leaf extract against *Thrips parvispinus* infesting cayenne pepper (*Capsicum frutescens*) and to determine the lethal concentration (LC<sub>50</sub>) within 96 h of exposure. A completely randomized design (CRD) was used, consisting of four extract concentrations, namely 1.0%, 1.5%, 2.0%, and 2.5%, a positive control using Regent 50 SC, a commercial fipronil-based insecticide, and a negative control (distilled water). The extract was applied by spraying onto cayenne pepper plants infested with *T. parvispinus*, followed by observations for seven consecutive days. Mortality percentage, mortality rate, and LC<sub>50</sub> values were evaluated. The results demonstrated that *C. odollam* leaf extract significantly increased thrips mortality. The highest extract concentration, 2.5%, caused 100% mortality and a mortality rate of 0.55 individuals per 24 h, showing no significant difference from Regent 50 SC. Probit analysis showed an LC<sub>50</sub> value of 2.15% after 96 h of exposure. These findings indicate that *C. odollam* leaf extract has potential as a botanical insecticide candidate for controlling *T. parvispinus* in cayenne pepper cultivation.

**Keywords:** Botanical insecticide, cayenne pepper, *Cerbera odollam*, LC<sub>50</sub>, mortality rate and *Thrips parvispinus*.

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## 1. INTRODUCTION

Cayenne pepper (*Capsicum frutescens* L.) is an important horticultural crop cultivated in tropical and subtropical regions and contributes to household income in many production areas. However, its productivity is often reduced by insect pests that damage vegetative and reproductive tissues. These damages are commonly caused by

thrips (*Thrips parvispinus* Karny), which attack young leaves and flowers, causing leaf curling, stunted growth, flower malformation, and substantial yield losses [1], [2] (Ferayanti, 2010; Najooan et al., 2016). Thrips infestations may cause yield losses of up to 23%, particularly under environmental conditions that support rapid population growth (Ferayanti, 2010). This pest damages plant tissues by piercing and sucking activity, which reduce photosynthetic capacity, weakens plant growth, and ultimately affecting crop productivity [2].

Synthetic insecticides remain the main control method used by farmers because they provide rapid pest suppression. However, repeated and intensive application may lead to pest resistance, pesticide residues, soil and water contamination, and harmful effects on non-target organisms (Pathak et al., 2022; Muñoz-Bautista et al., 2025). These concerns have intensified the search for alternative pest management approaches that maintain efficacy while minimizing environmental risks. Among the available alternatives, plant-based or botanical insecticides have attracted considerable attention because they are generally biodegradable, environmentally compatible, and derived from renewable plant resources, making them suitable components of sustainable pest management programs [3], [4].

Botanical insecticides contain a wide range of bioactive secondary metabolites capable of affecting insects through multiple mechanisms, including acute toxicity, feeding deterrence, repellency, oviposition inhibition, and disruption of growth and development [5], [6]. Their broad spectrum of biological activities, coupled with rapid biodegradability and reduced environmental persistence, has increased interest in their use as sustainable alternatives to conventional synthetic insecticides [5]. Numerous plant-derived compounds have demonstrated efficacy against economically important agricultural pests, including thrips, aphids, whiteflies, and lepidopteran larvae [7], [6].

*Cerbera odollam* Gaertn. is a tropical tree species belonging to the family Apocynaceae and is widely distributed throughout South and Southeast Asia. Phytochemical studies have revealed that the species contains various bioactive compounds, particularly cardenolides, alkaloids, flavonoids, phenolics, terpenoids, and saponins, which contribute to its biological activity [8], [9]. Previous studies have reported that extracts of *C. odollam* possess significant insecticidal activity against several insect pests, causing increased mortality, behavioral disruption, and developmental inhibition [10], [11]. For example, [10] reported that increasing concentrations of *C. odollam* leaf extract significantly increased mortality in *Spodoptera litura* larvae in a clear dose-dependent insecticidal response. Another recent investigations highlighted the broad biological activities of *C. odollam*-derived compounds, supporting their potential application in environmentally sustainable pest management programs [12]. These properties may be closely associated with its diverse and bioactive phytochemical composition, including cardenolides, alkaloids, flavonoids, phenolic compounds, terpenoids, and saponins [9].

Multiple mechanisms of botanical insecticides have been reported such as acute toxicity, feeding deterrence, repellency, growth disruption, and interference with

physiological processes that will lead to mortality [13], [14]. Although the insecticidal activity of *C. odollam* has been reported in several pest species, its effectiveness against *T. parvispinus* on cayenne pepper remains poorly documented. Therefore, this study aimed to evaluate the bioinsecticidal activity of *C. odollam* leaf extract against *T. parvispinus* and determined the concentration required to cause 50% mortality (LC<sub>50</sub>) based on *in planta* test.

## II. METHODS

### 2.1 Study Area and Experimental Period

The study was conducted from August to October 2021 at the Laboratory of Biology (Botanical Unit) for plant extraction and Greenhouse of the Faculty of Mathematics and Natural Sciences, Universitas Halu Oleo, Kendari, Southeast Sulawesi, Indonesia for field trial using cayenne pepper.

### 2.2 Experimental Design

The experiment was arranged in a completely randomized design (CRD) consisting of six treatments with four replicates per treatment. Each replicate consisted of one cayenne pepper plant infested with 10 2<sup>nd</sup>-instar nymphs of *T. parvispinus*, with a total of 40 insects per treatment. The treatments comprised four concentrations of *Cerbera odollam* leaf extract, one positive control, and one negative control. The treatment codes were defined as follows: DW = distilled water as the negative control; CI = commercial fipronil-based insecticide (Regent 50 SC) as the positive control; CoLE-1% = 1.0% *C. odollam* leaf extract; CoLE-1.5% = 1.5% *C. odollam* leaf extract; CoLE-2% = 2.0% *C. odollam* leaf extract; and CoLE-2.5% = 2.5% *C. odollam* leaf extract. The concentration range was selected based on Wahidah [8], who reported the insecticidal potential of *C. odollam* extract at concentrations between 1.0% and 2.5%.

### 2.3 Plant Materials and Insect Culture

Mature leaves of *C. odollam* were collected from Universitas Halu Oleo campus area. Dark green leaves at approximately the eighth position from the shoot apex were selected as the extraction material. This position was used to represent physiologically mature leaves, which have been reported to contain higher concentrations of secondary metabolites than younger leaves [16]. Populations of *T. parvispinus* were obtained from naturally infested cayenne pepper plants and maintained under greenhouse conditions before use in the bioassay. Infested plants were enclosed in a screened cage (1 m<sup>3</sup>) to prevent insect escape and to facilitate population multiplication following the method described by Idrus et al., [17]. Thrips individuals were collected using a fine brush and by tapping infested flowers and leaves onto a white tray, following the procedure described by Subagyo et al., [18].

### 2.4 Preparation of *Cerbera odollam* Leaf Extract

Fresh leaves of *C. odollam* were washed thoroughly and oven-dried at 70°C until a constant weight was achieved. The dried material was subsequently ground into

fine powder using a laboratory blender. Approximately 2 kg of fresh leaves yielded 300 g of dried powder. The powdered material was extracted by maceration using 2 L of 95% ethanol for 48 h to maximize the extraction of secondary metabolites. After maceration, the extract was filtered through filter paper and concentrated using a rotary evaporator at 50°C until a crude extract was obtained [19].

### 2.5 Preparation of Treatment Solutions

The crude extract was diluted with distilled water to obtain concentrations of 1.0%, 1.5%, 2.0%, and 2.5%. The dilution procedure followed the standard dilution equation. Treatment solutions were prepared as follows (**Table 1**):

**Table 1.** Composition of treatment solutions used in the bioassay.

Treatment code	Treatment description	Composition
DW	Negative control	100 mL distilled water
CI	Positive control	2.5 mL commercial fipronil-based insecticide (Regent 50 SC) diluted in 1.5 L water
CoLE-1%	1.0% <i>C. odollam</i> leaf extract	1.0 mL crude extract + 99.0 mL distilled water
CoLE-1.5%	1.5% <i>C. odollam</i> leaf extract	1.5 mL crude extract + 98.5 mL distilled water
CoLE-2%	2.0% <i>C. odollam</i> leaf extract	2.0 mL crude extract + 98.0 mL distilled water
CoLE-2.5%	2.5% <i>C. odollam</i> leaf extract	2.5 mL crude extract + 97.5 mL distilled water

### 2.6 Cultivation of Cayenne Pepper Plants

Seeds of cayenne pepper (*C. frutescens* L.) cv. Cakra Hijau were obtained from a local agricultural supplier. Prior to sowing, seeds were immersed in warm water (50–55°C) for 15–30 min to enhance germination. The seeds were sown in nursery trays, and the seedlings were transplanted into polybags measuring 6 × 25 cm at 21 days after sowing, when two to four true leaves had developed. The planting medium consisted of soil and cattle manure mixed at a ratio of 2:1. An additional 80 g of NPK fertilizer was incorporated into the medium before transplanting. Plants were maintained under greenhouse conditions through regular irrigation and manual weed control. Experimental plants were used for bioassays at 30 days after transplanting following the procedure of Rahalalu et al., [20].

### 2.7 Bioassay Procedure

Second-instar nymphs of *T. parvispinus* were selected for the experiment because this developmental stage is highly active on leaf surfaces (Hutasoit et al., 2017). Ten nymphs were introduced onto each experimental plant and allowed to feed for 24 h before treatment application. Subsequently, 100 mL of the respective

treatment solution was sprayed uniformly onto the entire plant canopy, including stems and leaves, using a hand sprayer. Application procedures followed those described by Astuti [21]. Treated plants were maintained under greenhouse conditions throughout the observation period.

### 2.8 Data Analysis

Observations were conducted daily for seven consecutive days after application (DAA). The evaluated parameters were mortality percentage and lethal concentration at 50% mortality ( $LC_{50}$ ). Mortality percentage was calculated following Sinaga [22], by dividing the number of dead insects by the total number of insects tested and multiplying the value by 100. Cumulative mortality was calculated daily from 1 to 7 DAA, and final mortality was determined based on the cumulative number of dead insects recorded at 7 DAA. Mortality percentage data were analyzed using one-way analysis of variance (ANOVA) at a significance level of  $\alpha = 0.05$  using SPSS version 20.0. When significant differences among treatments were detected, mean separation was performed using Tukey's honest significant difference (HSD) test. The  $LC_{50}$  value was estimated based on cumulative mortality at 96 h after application, corresponding to mortality recorded from 1 to 4 DAA. The mortality data for each concentration were converted into percentage values and entered into the  $LC_{50}$  Calculator by AAT Bioquest (<https://www.aatbio.com/tools/lc50-calculator>). The analysis was performed using a four-parameter concentration–response model, with extract concentration as the independent variable and mortality percentage as the response variable. The  $LC_{50}$  value was defined as the concentration of *C. odollam* leaf extract required to cause 50% mortality in the tested *T. parvispinus* population within 96 h of exposure. Control mortality was not corrected because no mortality was recorded in the negative control treatment.

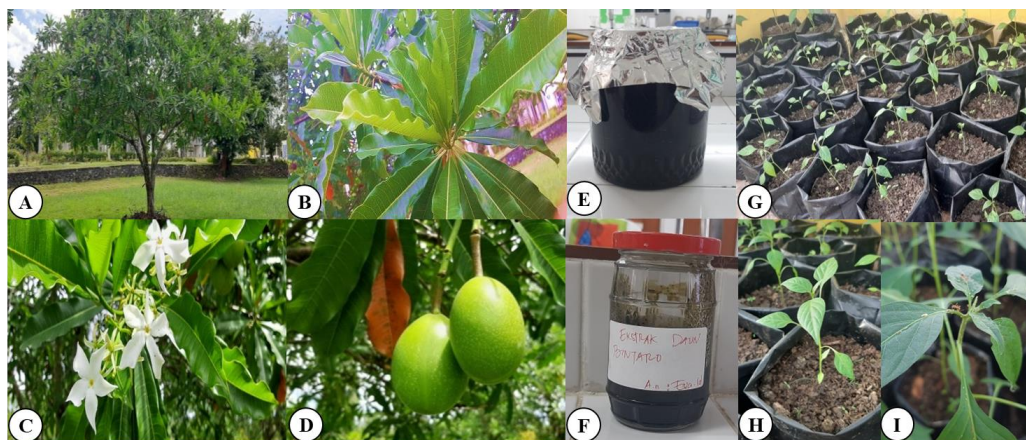
## III. RESULT AND DISCUSSION

### 3.1 Effect of *Cerbera odollam* Leaf Extract on Thrips Mortality

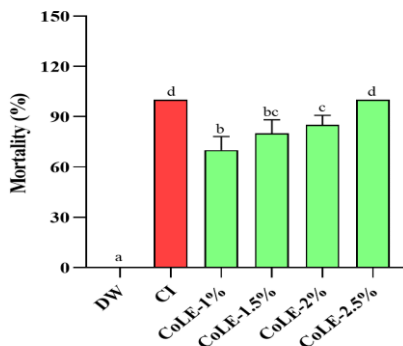
The preparation and application stages of *C. odollam* leaf extract are shown in **Figure 1**. Mature leaves were collected from *C. odollam* trees, followed by extraction and preparation of crude leaf extract for bioassay application. Cayenne pepper plants were maintained in polybags under greenhouse conditions and infested with second-instar nymphs of *T. parvispinus* before treatment application. Visible feeding symptoms were observed on infested leaves, including leaf curling, deformation, and surface injury, indicating active thrips infestation before extract application.

The application of *C. odollam* leaf extract significantly affected the mortality of *Thrips parvispinus* after seven days of observation (**Figure 2**). One-way ANOVA showed a highly significant treatment effect on mortality percentage ( $F_{5,18} = 201.24$ ,  $p < 0.001$ ). No mortality was found in the distilled water (DW) treatment, whereas

complete mortality was observed in the commercial insecticide (CI) and CoLE-2.5% treatments (non-significant). A concentration-dependent response was observed among the *C. odollam* leaf extract treatments. Mean mortality increased from 70.00% at CoLE-1% to 80.00% at CoLE-1.5%, 85.00% at CoLE-2%, and 100.00% at CoLE-2.5%. Tukey’s HSD test separated the treatments into distinct significance groups, with CoLE-1% showing lower mortality than the higher extract concentrations. CoLE-1.5% produced an intermediate response, whereas CoLE-2% resulted in significantly higher mortality than CoLE-1%. These results indicate that increasing extract concentration improved the insecticidal activity of *C. odollam* leaf extract against *T. parvispinus*.



**Fig. 1.** Preparation and application of *C. odollam* leaf extract for bioinsecticidal assay against *T. parvispinus* on cayenne pepper. (A) Mature *C. odollam* tree used as the source of leaf material; (B) leaf, (C) flower, and (D) fruit parts of *C. odollam*; (E) maceration of leaf material using ethanol; (F) crude leaf extract after concentration; (G) cayenne pepper plants prepared for bioassay; (H) experimental cayenne pepper plant after treatment application; and (H) cayenne pepper plant infested by *T. parvispinus*.



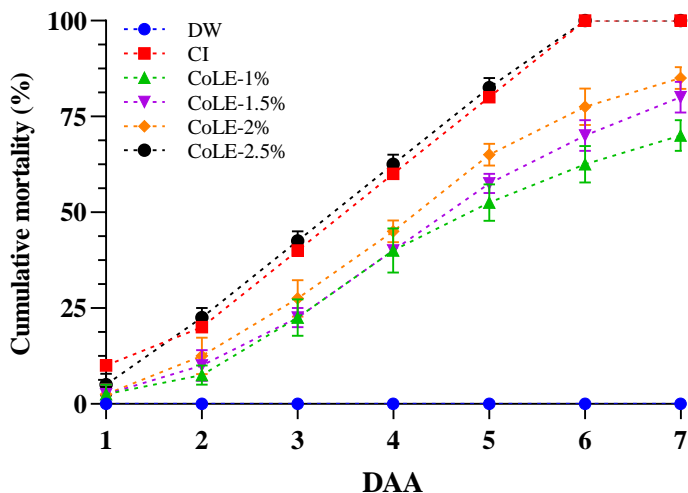
**Fig. 2.** Final mortality of *T. parvispinus* after seven days of exposure to *C. odollam* leaf extract. DW = distilled water; CI = commercial insecticide; CoLE = *C. odollam* leaf extract at different concentrations. Bars represent mean mortality ± SD from four replicates. Different letters above the bars indicate significant differences among treatments based on Tukey’s HSD test at  $p \leq 0.05$ .

The insecticidal activity observed in the present study is consistent with previous reports indicating that *Cerbera odollam* possesses bioactive compounds capable of suppressing insect survival. Extracts derived from *C. odollam* have been shown to increase mortality in several agricultural pests, including *S. litura* and *S. frugiperda*, with mortality levels generally increasing as extract concentration increased [11], [23]. Those effects are likely associated with the presence of secondary metabolites, including cardenolides, alkaloids, saponins, terpenoids, and phenolic compounds, which have been reported to interfere with feeding activity, physiological processes, and insect development [9]. The present study demonstrated that increasing concentrations of *C. odollam* leaf extract resulted in higher mortality of *T. parvispinus*. Similar findings were reported by Wahidah [23], who demonstrated that concentrations between 1% and 2.5% of *C. odollam* extract produced significant insecticidal activity against agricultural pests. The increasing mortality observed with increasing extract concentration supports the concept that higher concentrations contain greater quantities of bioactive compounds capable of disrupting insect physiological processes. According to Risyadi [24], increasing concentration enhances the availability of toxic compounds, thereby improving insecticidal efficacy. Fadlilah [25], further explained that toxic phytochemicals accumulate within insect tissues, disrupt metabolic processes, and eventually cause mortality.

In addition to direct toxicity, botanical insecticides may interfere with feeding behavior, reproduction, molting, and developmental processes. Saenong [26], reported that plant-derived insecticides can suppress pest populations by inhibiting feeding activity, reducing reproductive capacity, disrupting egg development, and interfering with metamorphosis. These mechanisms collectively contribute to the observed mortality of *T. parvispinus*.

### 3.2 Cumulative Mortality Response of *T. parvispinus*

The cumulative mortality of *T. parvispinus* differed among treatments during the seven-day observation period (**Figure 3**). No mortality was recorded in the distilled water treatment, indicating that mortality in the treated groups was associated with insecticide or extract exposure. The commercial fipronil-based insecticide produced a rapid mortality response, reaching 60.0% at 4 days after application (DAA) and complete mortality at 6 DAA. A similar response was observed in CoLE-2.5%, which resulted in 62.5% mortality at 4 DAA and 100.0% mortality at 6 DAA. A progressive increase in mortality was recorded in all *C. odollam* leaf extract treatments. At 4 DAA, cumulative mortality reached 40.0% in CoLE-1%, 40.0% in CoLE-1.5%, 45.0% in CoLE-2%, and 62.5% in CoLE-2.5%. By 7 DAA, mortality increased to 70.0%, 80.0%, 85.0%, and 100.0%, respectively. This pattern indicates that the insecticidal effect of the extract was both concentration- and exposure-dependent, with the highest concentration producing mortality achieving the commercial insecticide under the bioassay conditions.

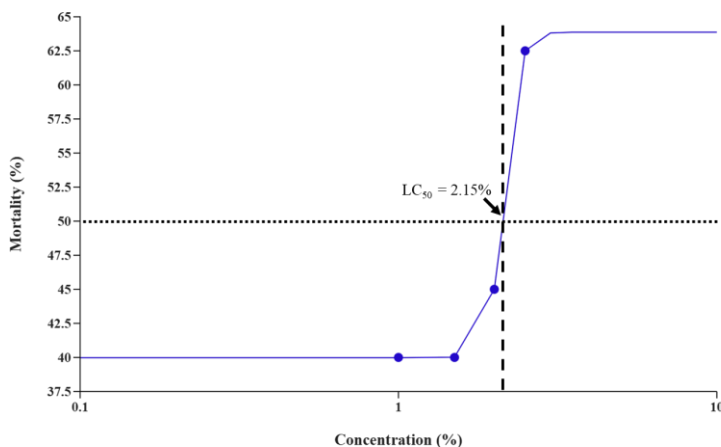


**Fig. 2.** Cumulative mortality of *T. parvispinus* following application of *C. odollam* leaf extract on cayenne pepper plants. Values represent mean from four replicates, with 10 second-instar nymphs per replicate. Error bars indicate SEM. DW = distilled water; CI = commercial insecticide; CoLE = *C. odollam* leaf extract; DAA = days after application.

The gradual increase in mortality suggests that *C. odollam* leaf extract may act through both direct toxicity and delayed physiological disruption. Bintaro extracts have been reported to function as antifeedants that suppress insect feeding activity, reduce nutrient intake, inhibit growth, and eventually cause mortality [27, [28], [29]. This mode of action may explain why mortality in the extract treatments increased progressively rather than occurring immediately after application. The concentration-dependent response observed in this study is also consistent with Lina [30] and Purba [31], who reported that higher concentrations of botanical insecticides generally produce greater pest mortality because of the increased availability of toxic compounds. The insecticidal activity of *C. odollam* may be associated with bioactive secondary metabolites capable of interfering with physiological functions in insects. Compounds such as alkaloids and phenolics have been suggested to disrupt essential enzymatic processes, including those involved in nerve impulse transmission, thereby impairing normal insect function and increasing mortality [32]. In addition, botanical insecticides may affect insects through multiple pathways, including repellency, feeding inhibition, digestive disturbance, respiratory impairment, and nervous system disruption. These combined effects may contribute to the sustained mortality response observed in *T. parvispinus* following exposure to *C. odollam* leaf extract.

#### 3.4 $LC_{50}$ of *C. odollam* Leaf Extract against *T. parvispinus*

The  $LC_{50}$  value of *C. odollam* leaf extract against *T. parvispinus* was estimated using mortality data recorded at 96 h after application. The concentration–response curve was generated with a four-parameter model (**Figure 4**).



**Fig. 4.** Concentration–response curve of *C. odollam* leaf extract against *T. parvispinus* at 96 h after exposure. The  $LC_{50}$  value was estimated based on mortality responses at 1.0%, 1.5%, 2.0%, and 2.5% extract concentrations.

The analysis estimated an  $LC_{50}$  value of 2.15% at 96 h after application. This value represents the concentration of *C. odollam* leaf extract required to cause 50% mortality in the tested *T. parvispinus* population under the bioassay conditions. The increasing mortality response along the concentration gradient indicates that the insecticidal effect of *C. odollam* leaf extract was, again, concentration dependent. Although complete mortality was not reached within 96 h at the tested extract concentrations, mortality exceeded 50% at CoLE-2.5%, indicating toxicity during the early exposure period. In insect toxicology, a lower  $LC_{50}$  value generally indicates stronger biological activity because mortality can be achieved at a lower concentration [33]. Therefore, the  $LC_{50}$  estimate obtained in this study indicates that *C. odollam* leaf extract has potential as a botanical insecticide candidate for the management of *T. parvispinus*.

#### IV. CONCLUSION

The leaf extract of *Cerbera odollam* Gaertn. showed bioinsecticidal activity against *Thrips parvispinus* Karny on cayenne pepper (*Capsicum frutescens* L.). Mortality increased with extract concentration, with CoLE-2.5% producing 100% mortality at 7 days after application and an effect comparable to the commercial fipronil-based insecticide. The  $LC_{50}$  value was estimated at 2.15% after 96 h of exposure, indicating that moderate extract concentrations were sufficient to induce 50% mortality under the bioassay conditions. These results indicate that *C. odollam* leaf extract has potential as a botanical insecticide candidate for managing *T. parvispinus* in cayenne pepper cultivation. Further studies are needed to evaluate its effects on different developmental stages of *T. parvispinus*, identify the active

compounds responsible for toxicity, assess possible phytotoxic effects, and confirm its field-scale performance under different agroecological conditions.

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### Author Contributions

Conceptualization, methodology, investigation, data collection, data analysis, interpretation of results, and manuscript preparation were carried out by the authors. All authors reviewed and approved the final version of the manuscript.

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### Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

### Data Availability Statement

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

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