Journal Syifa Sciences and Clinical Research (JSSCR)
Volume 8 Number 1, 2026
Journal Homepage: http://ejurnal.ung.ac.id/index.php/jsscr, E-ISSN: 2656-9612 P-ISSN:2656-8187
DOI: https://doi.org/10.37311/jsscr.v8i1.35256

Acute Toxicity and Antioxidant Activity of Ethanol Extracts of Teak Leaves (*Tectona grandis* L.)

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ABSTRACT

Toxicity is the ability of a substance to cause damage that affects organisms, so toxicity tests can be used to determine the dosage for human safety. Natural antioxidants can protect the body against damage caused by reactive oxygen compounds, inhibit disease, and inhibit lipid peroxidation in food. Teak leaves (*Tectona grandis* L.) are thought to have antioxidant potential because they contain flavonoids. This study was conducted to determine the toxicity of teak leaf (*Tectona grandis* L.) ethanol extract using *the Brine Shrimp Lethality Test* (BSLT) method and to determine the antioxidant activity of teak leaf (*Tectona grandis* L.) ethanol extract using the *Ferric Reducing Antioxidant Power* (FRAP) method. The extract was obtained by maceration using 96% ethanol solvent for 3×24 hours and concentrated at 40°C. The results of The BSLT assay showed a dose-dependent mortality pattern, and probit analysis yielded an LC_{50} value of 1049.78 µg/mL (95% CI: 11.54–95,483.20 µg/mL), indicating low lethality under in-vitro screening conditions. In the FRAP assay, the extract exhibited ferric-reducing capacity, with increasing absorbance across concentrations of 10–300 µg/mL **(**mean ± SD: 0.2923–0.4727**)**. The conclusion of this study is The ethanol extract of teak leaves showed low lethality in the BSLT assay and exhibited ferric-reducing capacity in the FRAP assay under *in-vitro* conditions.

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Keywords:
Antioxidant; Toxicity; BSLT; FRAP; *Tectona grandis* L.

Received:	*Accepted:*	*Online:*
2025-11-11	2026-01-08	2026-01-25

1. Introduction

Tectona grandis, commonly known as teak, is a member of the Verbenaceae family and is widely found in Asian countries, including Indonesia, particularly in the Muna district of Southeast Sulawesi. *Tectona grandis* is a tropical plant that is considered a key component in traditional medicine and is used empirically to treat fever, lipid disorders, bronchial infections, diabetes, stomach ulcers, inflammation, cancer, and tuberculosis [1],[2],[3]. The use of medicinal plants as an alternative in community medicine continues to increase. Therefore, further research is needed to

ensure its use aligns with healthcare principles, meaning it must have a scientifically sound basis regarding efficacy, safety, and quality standards [4].

Acute toxicity testing is a fundamental method for assessing the safety of a chemical or substance by identifying toxic effects after administration of a dose within a 24-hour period [5] . Acute toxicity testing involves the administration of a single dose to assess toxic effects, particularly mortality, and to identify related symptoms in animal models. By systematically evaluating the safety profile of natural products, these toxicity studies bridge the gap between traditional use and scientific validation, ensuring their safe and effective application in healthcare[6] . *The Brine Shrimp Lethality Test* (BSLT) is a testing method that uses the test animal Artemia salina Leach. This species was chosen because it is the first natural bioassay capable of detecting the toxicity level of a compound from natural materials. Artemia salina Leach serves as the standard test animal in toxicity testing using the BSLT method[7] . The BSLT method is used to test the toxicity of a compound or extract obtained from plants and animals such as sea sponges [8]. This method is chosen because it is simple, fast, economical, easy to apply, has a good level of reliability, and is able to provide representative results. The BSLT test is used to assess the general toxicity level of a sample by utilising Artemia salina Leach shrimp larvae [9].

Antioxidants are compounds that play an important role in preventing oxidative stress, which is a condition that occurs when there is an imbalance between the amount of free radicals and antioxidants in the body. Free radicals themselves are highly reactive molecules because they have one or more unpaired electrons in their orbitals. Basically, the human body has endogenous antioxidants that can neutralise free radicals if their numbers are not excessive, but if endogenous antioxidants are insufficient, the body needs exogenous antioxidants [10].

Testing antioxidant activity using the *Ferric Reducing Antioxidant Power* (FRAP) method is a method used to determine antioxidant activity by reducing Fe(III) to Fe(II) through complexation with 2,4,6-Tripyridyl-s-Triazine (TPTZ). The FRAP test is based on electron transfer. FRAP cannot detect compounds that act through radical reduction (hydrogen transfer). The FRAP testing method is simple, fast, inexpensive, and does not require special equipment. Antioxidants play an important role in the prevention and treatment of metabolic disorders caused by oxidative stress[11] .

This study aims to evaluate the acute toxicity and antioxidant activity of ethanol extract of teak leaves (*Tectona grandis* L.) through the Brine Shrimp Lethality Test (BSLT) and measurement of reduction capacity using the Ferric Reducing Antioxidant Power (FRAP) method.

2. Method

This study was conducted at the Pharmacy Faculty Laboratory and the Medical Faculty Laboratory of Halu Oleo University. The type of research used was experimental, with acute toxicity testing using the BSLT (*Brine Shrimp Lethality Test*) method and antioxidant activity testing using the FRAP (*Ferric Reducing Antioxidant Power*) method on ethanol extracts of teak leaves **(***Tectona grandis* L.**).**

Materials

Aquades, ascorbic acid, oxalic acid 2.5%, aluminium foil (Bagus®), hydrochloric acid (HCl) 2 N, concentrated sulphuric acid, trichloroacetic acid 10%, anhydrous acetic acid, phosphate buffer (0.2 M pH 6.6), leaves of *Tectona grandis* L., $FeCl_3$ 0.1%, sterile gauze, cotton wool, potassium ferricyanide 1%, *potassium dichromate. universal* pH paper, 96% ethanol solvent. Mayer's reagent, Wagner's reagent, Dragendroff's reagent,

Liebermann-Burchard's reagent, dimethyl sulfoxide (DMSO), iron (III) chloride reagent, plastic wrap (*Total®*), tissue, *Artemia salina* Leach larvae eggs, magnesium powder.

Equipment

Vacuum Rotary Evaporator (Buchi®), oven (Stuart®), analytical balance (Explorer Ohaus®), dropper pipette, vial bottles, standard filter paper, knife, blender, *cutter*, spatula, chemical glassware (Pyrex®), measuring glassware (Pyrex®), water bath, probe, vacuum tube, centrifuge, cuvette, UV-Vis spectrophotometer (Genesys-20), one set of aquarium equipment, stirring rod, blender, funnel, Erlenmeyer flask, test tube clamp, pH meter, dropper pipette, measuring pipette, volumetric pipette, reaction tubes, 40-watt light bulb, glass jars, porcelain dishes, Petri dishes, water jug, *Buchner* funnel, macerator, plastic bottles, aerator, magnifying glass (*Joy Art MF-60*), glassware, UV light, chamber, *hair dryer*.

Sample collection and preparation

Samples of *Tectona grandis* L. leaves were collected in Danagoa Village, Tongkuno Subdistrict, Muna Regency, Southeast Sulawesi. The sample preparation process began with the collection and sorting of *Tectona grandis* L**.** leaves, followed by wet sorting, washing, shredding, drying, and dry sorting. After the drying stage was completed, the dried leaves were ground using an electric chopper to obtain fine-particle simplisia powder, which was then stored in a clean, dry container protected from light exposure.

Extraction

Tectona grandis L. simplisia was extracted using the maceration method with ethanol solvent until all parts of the simplisia were completely submerged. The maceration process lasted for 3 × 24 hours and was repeated until a clear filtrate was obtained. The maceration filtrate is then separated from the crude drug residue using filter paper, and subsequently evaporated using *a rotary vacuum evaporator* at a temperature of 40–45°C at a speed of 65–90 rpm until a thick extract is formed.

Toxicity testing using the *Brine Shrimp Lethality Test* (BSLT)

Preparation of *Artemia salina* egg hatching containers

The container for hatching Artemia salina eggs was prepared using a 22 × 32 cm vessel, which was then divided into two parts, namely the large side (dark) and the small side (light), using thick plastic sheets that had been perforated with a diameter of about 2 mm. The large part was covered with aluminium foil to create dark conditions, while the small part was illuminated using a lamp. Next, artificial seawater is poured into the container and aerated to support the hatching process.

Shrimp Larvae Hatching

Shrimp eggs are hatched two days before the toxicity test begins. The hatching container is divided into two parts, namely a dark side and a light side, then filled with artificial seawater. One part is illuminated using incandescent or neon lights to maintain the hatching temperature in the range **of 25–31°C** and stimulate the hatching process, while the other part remains in darkness with aluminium foil or black tape covering. A total of 300 mg of Artemia salina Leach eggs were placed in an aquarium containing seawater and left for 2 × 24 hours until they hatched into larvae (*nauplii*) ready for testing.

Preparation of Sample Extract Stock Solution, Positive Control and Negative Control

To prepare a negative control stock solution, prepare 2.5 mL of 5% DMSO in methanol and add it instead of the extract. From this stock solution, take 7.5 mL, 5 mL, 2.5 mL, 0.5 mL, 0.25 mL, and 0.05 mL of each concentration and place them in vials, then dilute with seawater to a volume of 10 mL and homogenize. The stock solution concentrations are 0 µg/mL, 10 µg/mL, 50 µg/mL, 100 µg/mL, 500 µg/mL, 1000 µg/mL, and 1500 µg/mL. The same treatment was applied to the positive control, the concentration of which was the same as the extract sample. The positive control was potassium dichromate or an anticancer compound such as vincristine sulfate [12].

Toxicity Test Procedure using the BSLT Method

Test solutions with concentrations of 0 µg/mL, 10 µg/mL, 50 µg/mL, 100 µg/mL, 500 µg/mL, 1000 µg/mL**,** and 1500 µg/mL were each taken in 5 mL, then placed in vials and added to 10 two-day-old shrimp larvae. The same procedure was applied for each concentration variation. Twenty-four hours after the addition of the extract, the number of dead larvae was observed. Each concentration was tested in three replicates, and the results were compared with the control. In total, 630 larvae were used in the entire test with three replicates.

The toxic effect was determined by observing the percentage of mortality of Artemia salina Leach larvae at each test concentration. The number of dead larvae in each vial was counted after 24 hours of observation. The percentage of mortality was calculated using the formula: number of dead larvae divided by the initial number of larvae, then multiplied by 100% for each replication. The results were then compared with the control and analysed using the probit method to obtain the **LC_{50}** value [13].

$$\% \, mortality = \frac{\text{Number of dead larvae}}{initial\ total\ number\ of\ larvae} \text{ x } 100\%$$

If the death of test animals in the control treatment is greater than 0% and less than 20%, it is calculated using the corrected mortality formula using the Abbott formula [14]:

$$P = \frac{\text{P1} - \text{C}}{100 - C} \text{ x } 100\%$$

Description:
P = corrected mortality (%)
P1 = mortality in treatment (%)
C = mortality in control (%)

Antioxidant Activity Testing Using the FRAP Method

Preparation of Stock Solutions of Samples and Comparators

A 1000 ppm stock solution was prepared by dissolving 25 mg of the extract sample in 96% ethanol up to the 25 ml mark on the measuring flask. Next, 3 ml, 2 ml, 1 ml, 0.5 ml, and 0.1 ml were taken from the 1000 ppm stock solution and placed in test tubes and diluted with ethanol to a volume of 10 ml and homogenised. The concentrations of the 1000 ppm standard solution of the extract sample were 10 ppm, 50 ppm, 100 ppm, 200 ppm, and 300 ppm. The same treatment was applied to the reference solution, namely weighing 25 mg of ascorbic acid dissolved in 2.5% oxalic acid to a 25 ml volumetric flask. Next, 3 ml, 2 ml, 1 ml, 0.5 ml, and 0.1 ml were taken from the 1000 ppm stock solution and placed in test tubes and diluted with oxalic acid solution to a volume of 10 ml and homogenised. The concentrations of the 1000 ppm

ascorbic acid standard solutions were 10 ppm, 50 ppm, 100 ppm, 200 ppm, and 300 ppm. The same treatment was performed on the ascorbic acid reference solutions.

Antioxidant Activity using the FRAP Method

Was conducted to evaluate the extract's capacity to provide electrons for the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). 10 mM TPTZ solution (24,6-Tripyridyl-s-Triazine) in 40 mM HCl, 20 mM $FeCl_3.6H_2O$ (BDH), and 300 mM acetate buffer (pH 3.6) were combined in a 1:1:10 ratio to create a new FRAP reagent. 900 µL of FRAP reagent (pre-incubated for 10 min), 90 µL of distilled water, and 30 µL of the sample (0.5 mg/mL concentration) were combined to perform the antioxidant activity test. The combination was then left to react for ten minutes at 37 °C. The mixture's absorbance was then measured at 593 nm using spectrophotometry [15].

Antioxidant Activity Test Data Analysis

Antioxidant activity can be expressed as a percentage of inhibition using the following formula:

$$Inhibition\ Percentage\ (\%) = \frac{\text{blank absorbance} - \text{sample absorbance}}{blank\ absorbance} x\ 100\%$$

After obtaining the inhibition percentage for each concentration, the sample concentration and inhibition percentage obtained were plotted on the x and y axes, respectively, in the linear regression equation y = ax + b. This equation was used to determine the IC_{50} value for each sample[16].

The antioxidant activity is classified as very strong when the IC_{50} value is less than 50 µg/mL, strong when it ranges from 50 to 100 µg/mL, moderate when it lies between 101 and 250 µg/mL, weak when it is within 250 to 500 µg/mL, and inactive when the IC_{50} value exceeds 500 µg/mL [17].

3. Results and Discussion

Sample processing

Teak leaf samples (*Tectona grandis* L) were obtained in Danagoa Village, Tongkuno District, Muna Regency, Southeast Sulawesi Province. A total of 20 kg of samples obtained were washed with running water and then wet sorted to separate dirt or other foreign materials from the plants by removing unnecessary parts before drying so that good/suitable simplisia could be obtained for use. Next, a chopping process was carried out to facilitate the drying process[18] . The drying process was carried out under the sun with black cloth covering the top of the sample. After obtaining the dry sample, weighing was carried out, followed by dry sorting and simplisia refinement.

Extract Yield

This study utilised extraction through maceration, which was performed by placing plant powder and an appropriate solvent into a tightly sealed inert container at room temperature [19] . The advantage of this method is that it is easy and does not require heating, so there is little chance of the natural material being damaged or decomposed. The long maceration process and the fact that it is carried out in a static state during maceration allow many compounds to be extracted[20] . The solvent used was 96% ethanol. Ethanol is used as a solvent because it is a universal solvent due to its semi-polar nature, enabling it to dissolve both polar and non-polar compounds.

The results of the extraction of teak leaf samples (*Tectona grandis* L) were then processed using a rotary vacuum evaporator to separate the extract and solvent, yielding a concentrated extract. The concentrated extract obtained was then placed in a glass container and placed in *a water bath* to remove any remaining solvent from the extract. The concentrated extract was then weighed and its yield calculated (**Table 1**).

Table 1. Yield values of *Tectona grandis* L. extract

Dried crude drug (g)	Thick extract (g)	Yield (%)
729	45	6.17

The drying method of crude drugs and the extraction method affect the percentage (%) yield of crude drug extracts from medicinal plants. In addition to the percentage (%) yield of extracts, the drying method of crude drugs and the extraction method also affect the average total phenol content in medicinal plants [21] .

Toxicity Test using the *Brine Shrimp Lethality Test* (BSLT)

The acute toxicity test is designed to determine the toxic effects of a compound that will occur during a short exposure period. This test is conducted by administering a single concentration of the test compound to test animals. BSLT is a simple preliminary or pre-screening test of biological activity to determine the acute toxicity of a compound or extract using *Artemia salina* L larvae as test animals[22] . This method is indicated by the mortality rate of *Artemia salina* Leach shrimp larvae caused by the test extract. The results obtained are calculated as the LC_{50} (*Lethal Concentration*) value of the test extract, which is the concentration of the extract that can cause the death of 50% of the shrimp larvae during a 24-hour incubation period. Compounds with an LC_{50} <1000 µg/mL can be considered active compounds [23] .

Table 2. Calculation results of LC_{50}values for *Tectona grandis* L. and positive control (potassium dichromate).

Treatment group	Concentration (µg/mL)	% Mortality	LC_{50} µg/mL	95% CI (µg/mL)
Teak leaf extract (*Tectona grandis* L.)	10	0	1,049.78 ± 678.178	11.54 – 95,483.20
	50	0		
	100	13.33		
	500	30.00		
	1000	43.33		
	1500	63.33		
Positive control	10	0	103.50 ± 152.844	79.70 – 134.40
	50	13.33		
	100	60		
	500	93.33		
	1000	100.00		
	1,500	100.00		

This study used ethanol extract of teak leaves (*Tectona grandis* L.) and prepared a 2000 ppm stock solution of 50 ml, which was then diluted to 6 different concentrations, namely 10 µg/mL, 50 µg/mL, 100 µg/mL, 500 µg/mL, 1000 µg/mL, and 1500 µg/mL. with a positive control of potassium dichromate at the same concentration as the test sample and a negative control of 5% DMSO solvent and

seawater. The toxicity test was conducted with three repetitions/replicates to obtain accurate and reliable data. The percentage of mortality was then calculated for each treatment concentration and control. Probit analysis was used to examine the relationship between increasing concentrations and the mortality rate of the test larvae, assuming that higher concentrations would result in higher toxic effects. The data obtained were analysed using Probit analysis with Minitab 17 software, with the results shown in the following **Table 2**.

The **Table 2** and **Figure 1** shows that differences in extract concentration affect the mortality rate of test larvae. Ethanol extract of teak leaves (*Tectona grandis* L.) has a lower mortality rate compared to the positive control. The results of the BSLT test on ethanol extract yielded an LC_{50}value of 1149.40 with a larval mortality rate at a concentration of 1500 µg/mL = 63.33%; and a larval mortality rate at a concentration of 1000 µg/mL = 43.33%; larval mortality percentage at a concentration of 500 µg/mL = 30.00%; larval mortality percentage at a concentration of 100 µg/mL = 13.33%; larval mortality percentage at a concentration of 50 µg/mL = 0.00%; and larval mortality percentage at a concentration of 10 µg/mL = 0.00%. Meanwhile, in the positive control, the LC_{50}value obtained was 197.30 with % larval mortality at a concentration of 1500 µg/mL = 100.00%; and % larval mortality at a concentration of 1000 µg/mL = 100.00%; and % larval mortality at a concentration of 500 µg/mL = 93.33%; larval mortality at a concentration of 100 µg/mL = 60.00%; larval mortality at a concentration of 50 µg/mL = 13.33%; and larval mortality at a concentration of 10 µg/mL = 0.00%. The test results indicate that ethanol extract of teak leaves (*Tectona grandis* L.) has no toxic effect on Artemia salina Leach larvae.

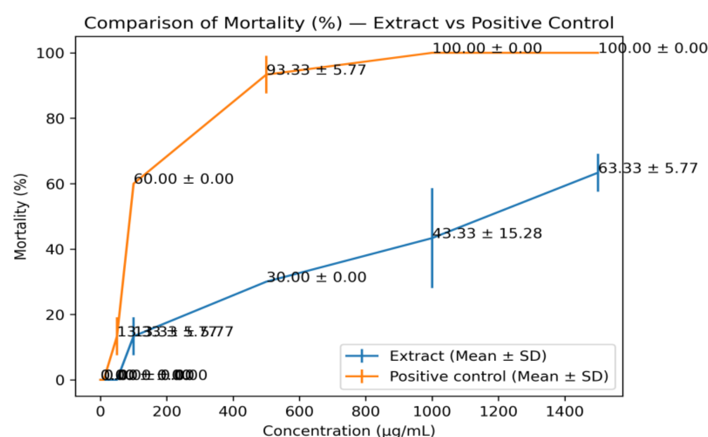

Figure 1. Curve of Percentage Mortality *of* Artemia *salina* Leach Larvae

Probit analysis using Minitab 17 showed that the LC_{50}value of teak leaf extract (*Tectona grandis* L.) was 1149.40 µg/mL. According to Meyer et al. (1982), an extract shows toxic activity in a toxicity test if it can cause the death of 50% of the test animals at a concentration of < 1000 ppm. Based on this statement, teak leaf extract (*Tectona grandis* L.) is not toxic. This is indicated by the LC50 value obtained at a concentration of 1149.40 ppm. Therefore, teak leaf ethanol extract (*Tectona grandis* L.) is safe to use as an alternative medicine [24].

Antioxidant Activity Using the FRAP (*Ferric Reducing Antioxidant Power*) Method

The antioxidant activity of ethanol extract of teak leaves (*Tectona grandis* L.) in this study was evaluated using the Ferric Reducing Antioxidant Power (FRAP) method, which measures the ability of compounds in a sample to reduce Fe^{3+} ions to

Fe^{2+} under in vitro conditions. Increased Fe^{2+} complex formation is indicated by an increase in the absorbance value, thus the FRAP value reflects the ferric reduction capacity or the ability of compounds in the extract to act as electron donors.

The FRAP absorbance value of teak leaf extract increased with increasing test solution concentration (10–300 µg/mL), with mean ± SD results of 0.2923 ± 0.0055; 0.3243 ± 0.0050; 0.3880 ± 0.0010; 0.4313 ± 0.0023; and 0.4727 ± 0.0006, respectively. The concentration-response relationship pattern is shown in **Figure 2**, where increasing extract concentration is followed by an increase in FRAP absorption values. This indicates that the phytochemical components in the extract contribute to the ferric reduction activity in the FRAP reaction system.

Phytochemically, teak leaves are known to contain phenolic compounds, flavonoids, tannins, and other polyphenol derivatives that possess aromatic hydroxyl groups and have the potential to act as electron donors. These groups can play a role in the reduction mechanism through the donation of electrons or hydrogen atoms, thereby helping stabilize the oxidant compounds in the FRAP reaction system. The differences in standard deviation values between concentrations indicate natural variations in the reduction response of the extract, which may be influenced by the chemical composition of the secondary metabolite mixture within it.

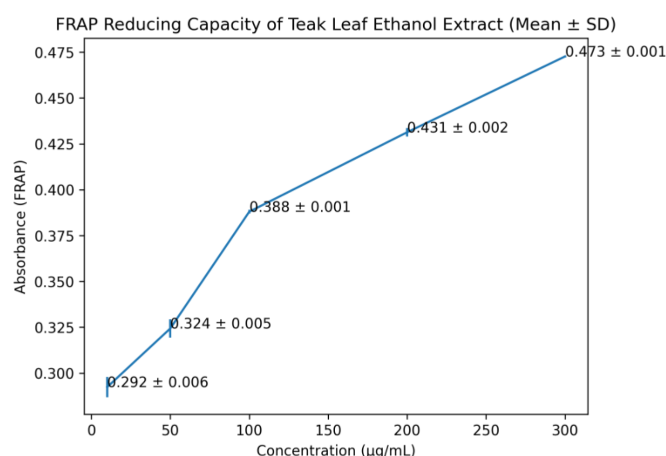

Figure 2. FRAP reducing capacity of teak leaf ethanol extract (*Tectona grandis* L.) expressed as mean ± SD at concentrations of 10–300 µg/mL.

Limitations of the study

This study has several limitations. The toxicity assessment was restricted to acute toxicity testing with a single-dose administration and a short observation period, so it does not yet reflect the potential for sub-chronic, chronic, or cumulative long-term toxicity. The antioxidant activity was evaluated only using the FRAP assay in vitro, which does not fully represent biological responses in vivo. In addition, the extract used was a crude ethanolic extract without fractionation or isolation of active compounds, so the specific bioactive constituents could not be identified. Nevertheless, the findings provide important preliminary evidence that may serve as a basis for further studies with a broader and more comprehensive evaluation design.

4. Conclusion

The ethanol extract of teak (*Tectona grandis* L.) leaves demonstrated relatively low lethality in the BSLT assay, with an LC_{50} value of 1149.40 µg/mL, which falls

within the low-toxicity category. In addition, the extract demonstrated ferric-reducing activity in the FRAP assay under in-vitro conditions, as indicated by an increase in absorbance with increasing concentrations (10–300 µg/mL), with mean ± SD values of 0.2923 ± 0.0055; 0.3243 ± 0.0050; 0.3880 ± 0.0010; 0.4313 ± 0.0023; and 0.4727 ± 0.0006, respectively.

Acknowledgment:
The authors would like to express their sincere gratitude to the Faculty of Pharmacy and the Faculty of Medicine, Halu Oleo University, for providing laboratory facilities and technical support throughout this study. Appreciation is also extended to all laboratory staff who assisted during sample preparation, extraction, and experimental analysis.

Conflicts of Interest:
The authors declare that there is no conflict of interest regarding the publication of this article.

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