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Regular Article

Comparative study of alpha-glucosidase inhibition of four Vietnamese medicinal plants *Combretum quadrangulare*, *Dicranopteris linearis*, *Psychotria adenophylla*, and *Garcinia schomburgkiana*: *In vitro* and *in vivo* studies

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Four medicinal plants *C. quadrangulare*, *D. linearis*, *P. adenophylla*, and *G. schomburgkiana* growing in the South of Vietnam were investigated for their alpha-glucosidase inhibition. The crude methanol extract of *C. quadrangulare* was determined to be the most active extract, then was selected for further *in vivo* assays including antidiabetic study and toxicity. *In vitro* alpha-glucosidase inhibition of four medicinal plants *C. quadrangulare*, *D. linearis*, *P. adenophylla*, and *G. schomburgkiana* was screened using standard procedures. *In vivo* antidiabetic activity, acute toxicity and subchronical toxicity of *C. quadrangulare* leaves was assessed on Swiss albino mice. Swiss albino mice were induced with diabetes by intraperitoneal injection of alloxan at a dose of 150 mg/kg body weight. High-performance liquid chromatography with evaporative light scattering detector (HPLC-ELSD) were used to detect the bioactive components of *C. quadrangulare* leaves. All crude extracts from the studied plants showed promising alpha-glucosidase inhibition, with IC_{50} values ranging from 2.4 to 35.3 µg/mL. The methanol extract of *C. quadrangulare* leaves was determined to be the most active extract. This extract was then selected for antidiabetic assay using alloxan induced model of type 2 diabetes mellitus mice. The results indicated that the extract at a dose of 400 mg/kg can effectively decrease blood glucose levels that is comparable to that of glibenclamide 2 mg/kg. This compound showed moderate activity toward alpha-glucosidase. Therefore, our study indicated that C. *quadrangulare, D. linearis*, *P. adenophylla*, and *G. schomburgkiana* extract are potential materials for producing α-glucosidase inhibitor drugs.

Key words: Antidiabetic, alpha-glucosidase, cycloartane, acute toxicity, and chronic toxicity

Significance

Extracts of *C. quadrangulare*, *D. linearis*, *P. adenophylla*, and *G. schomburgkiana*, showed potent inhibitory activities toward alpha-glucosidase. Methanolic extract from *C. quadrangulare* exhibited the highest level of activity and was reported for the first time in an *in vivo* study on alpha-glucosidase. Low toxicity of *C. quadrangulare* extract was recorded at doses of 200 mg/kg and 600 mg/kg in mice.

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Introduction

Type 2 diabetes mellitus (T2DM) is a disease that induces problem in human regulation of blood sugar level [1]. In 2021, more than 536 million adult people (the age ranging 20–79) had diabetes mellitus, being over 10.5% of the global adult population [2]. Effective T2DM treatments based on blood glucose control and side-effects reduction [3]. For pharmacological therapy, there are three well-known alpha-glucosidase inhibitors: acarbose, miglitol, and voglibose [4]. The development of novel anti-T2DM agents has drawn the extensive attention of biochemists to reduce the limitations of currently commercial drugs [1]. The use of traditional medicine or natural products in T2DM treatment has developed due to their low toxicity and economic perspective [1,3–5].

Combretum quadrangulare Kurz (*C. quadrangulare*), namely Tram Bau in Vietnamese, is indigenous to Vietnam and has been applied for various traditional uses. The seeds, leaves, the stem bark are used in Vietnam and Thailand as traditional medicines as antihepatitis, antipyretic, and antidysenteric [6]. The most useful application is an anthelmintic agent [6]. In the South-West provinces of Vietnam, *C. quadrangulare* was used as a supporting agent to control blood sugar level. Ethnobotanical uses of some *Combretum* species growing in Africa were considered to be antidiabetic sources [7]. Diverse pharmacological properties of this plant were previously reported [8]. The alcoholic extract of leaves showed potent alpha-glucosidase inhibition [9]. Cycloartanes were believed to be the strong alpha-glucosidase inhibitors [10,11]. Previous studies indicated that there are nine cycloartanes isolated from the Vietnamese *C. quadrangulare* [8,10–12]. Among them, combretic acid D was determined to be a non-competitive inhibitor with an IC_{50} value of 13.9 μM [11].

Dicranopteris linearis (Burm. F.) Underw. is a common fern widely distributed in Vietnam. Comprehensive reviews indicated that ferns are well-known sources with many traditional uses: hepatoprotective activity, antihyperglycemic activity, leishmanicidal activity, and trypanocidal activity [13,14]. Chemical and biological data of *D. linearis* revealed various pharmacological properties: anticancer, antibacterial, antioxidant, analgesic, and anti-HIV activities [15–19]. This plant is used as a traditional medicine in East-Asia countries for treatments of several diseases: fever (Malaysia), intestinal worms (Indochina) [20], asthma, infertility in women (India), wounds (Papua New Guinea) [21]. Alpha-glucosidase inhibition of leaves of *D. linearis* was reported previously but the activities of other parts from this Vietnamese bio-source have not been investigated [22]. *Psychotria adenophylla* Wall is native to South of Vietnam. Both chemical and biological data of *P. adenophylla* are currently limited [22]. More recently, the alpha-glucosidase inhibition of leaves of the Vietnamese *P. adenophylla* has been reported but there is no data regarding the activity of other parts of this plant [22].

Garcinia schomburgakiana Pierre is an edible tree common in Vietnamese tropical forests. This plant was traditionally used for treatment of coughs, menstrual disorders, and diabetes in Vietnam [23]. In Thailand, the traditional ethnomedicinal uses of *G. schomburgakiana* leaves, root and fruit include the improvement of menstrual blood quality, treatment of diabetes, and as a laxative [24]. Pharmaceutical data of *G. schomburgkiana* revealed their strong cytotoxicity against various cancer cell lines and anti-inflammatory [23,25]. More recently, two benzoylphloroglucinols schomburgkianone I and guttiferone K isolated from the Thai fruits were reported for their potent alpha-glucosidase [26]. In the same year, Jaisamut and Vongsak reported the inhibitory effect against alpha-amylase and alpha-glucosidase of the bark, fruit, and leaf extracts of Thai *G. schomburgkiana*, revealing strong inhibition for both enzymes [27]. However, little is known about alpha-glucosidase inhibition of Vietnamese *G. schomburgkiana* [28].

C. quadrangulare, *D. linearis*, and *G. schomburgkiana* with their Vietnamese names as “Tram bau”, “Guot”, and “Bua dong”, respectively are believed to be valuable biosources to control blood sugar levels. *G. schomburgkiana* is considered to be an antidiabetic product in Thailand with its Thai name as Madan.

In this study, four plants *C. quadrangulare*, *D. linearis*, *P. adenophylla*, and *G. schomburgkiana* (Figure 1) growing in the South of Vietnam were collected and investigated. The extracts of these four plants were prepared and evaluated for their alpha-glucosidase inhibition. The crude methanol extract of *C. quadrangulare* was determined to be the most active extract, then was selected for further *in vivo* assays: antidiabetic study and toxicity.

Materials and Methods

Source of Plant Material

Various parts of *Combretum quandrangulare* (leaves, flowers, stem barks, roots, and seeds) were collected in Ca Mau Province (Vietnam) from June to July 2022. Its scientific name was defined by Prof. Tran Cong Luan (deposited as No UE-P002).

D. linearis leaves and aerial parts were collected in Ba Ria Province (Vietnam) from June to July 2023. The scientific name was identified as *Dicranopteris linearis* (Burm. F.) Underw. by Dr. Dang Van Son (deposited as No UE-P017).

Leaves and stems of *P. adenophylla* were collected in Binh Chau, Ba Ria-Vung Tau Province, Vietnam, from May to July 2023. The scientific name of the material was identified as *P. adenophylla* by Dr. Dang Van Son and stored as No UE-P018.

G. schomburgkiana (fruits, leaves, stem barks, and roots) were collected in Tay Ninh province (Vietnam) from June to

Figure 1 *Combretum quadrangulare*, *Dicranopteris linearis*, *Psychotria adenophylla*, and *Garcinia schomburgkiana*.

July 2023. Botanical identification was conducted by Dr. Dang Van Son with the voucher specimen coded No UE-P010.

Preparation of Extracts

100 g of *C. quadrangulare* leaves was exhaustedly extracted with methanol (5×500 mL) to provide the crude MeOH extract. The crude methanolic extract was subsequently applied to liquid-liquid partition using two different solvents *n*-hexane and EtOAc to obtained the corresponding *n*-hexane (extract H) and ethyl acetate (extract EA) extracts. The water-soluble layer was evaporated to provide the MeOH extract (namely extract M). Similarly, *C. quadrangulare* (stem barks, roots, and seeds), *D. linearis* (leaves and aerial parts), *P. adenophylla* (leaves and stems), and *G. schomburgkiana* (fruits, leaves, stem barks, and roots) were applied to the above-mentioned method. 100 g of each material was used to prepare extracts that were named as shown in Table S1. Large-scale extraction was applied to *C. quadrangulare* leaves (5 kg) to yield 789.5 g of the methanolic extract. This crude extract was then used for further *in vivo* tests.

Alpha-Glucosidase Inhibition Assay

Tested samples included: the crude MeOH extract, extracts H, EA, and M of each type of material (described in section "Preparation of extracts"). Each sample was dissolved in DMSO, then diluted in different concentrations. The alpha-glucosidase enzyme and *p*-nitrophenyl-*β*-D-glucopyranoside (pNPG) were dissolved in sodium phosphate buffer at pH 6.9. The conditions followed the previous reports [4]. The prepared extracts (each 50 µL) were preincubated with alpha-glucosidase at 37°C for 20 min. The mixture was then added by 40 µL of pNPG and continued for 20 min. Finally, sodium carbonate (130 µL, 0.2 M) was added to stop the reaction. The blank samples were constructed for each sample by substituting the enzyme with phosphate buffer. The control was set up by substituting the extracts with 5% DMSO. Additionally, the blank of the control was prepared by replacing both the extracts and enzyme with with 5% DMSO and phosphate buffer, respectively. The percentage inhibition of the samples was calculated using the given formula:

$$\text{Inhibition (\%)} = \left(1 - \frac{ODs - ODsb}{ODc - ODcb}\right) \times 100$$

Where: ODs: the OD_{405} value of the reaction mixture with sample; ODsb: the OD_{405} value of the reaction mixture of sample balnk; ODc: the OD_{405} value of the reaction mixture of control; ODc: the OD_{405} value of the reaction mixture of control blank.

HPLC Experiments Detected the Bioactive Compounds 1 and 2 from C. Quadrangulare

Compounds **1** and **2** were available from the previous report [11]. The ethyl acetate (coded EA roots, stems, fruits, flowers, and stems) and methanol (coded Me roots, stems, fruits, flowers, and stems) extracts of different parts of *C. quadrangulare* were prepared. High performance liquid chromatography analysis (HPLC Agilent 1260 Infinity II) with two detectors (Diode Array detector-DAD and Evaporative light scattering detector-ELSD) was conducted for prepared samples. 35 µL of each sample (at the concentration of 1 mg/mL) was injected separately. Solvents acetonitrile (ACN) and water were used with a gradient system changed during analysed 60 mins: 5% to 10% ACN in 5 min, 10% to 30%

ACN in 15 min, 30% to 80% ACN in 10 min, 80% to 100% ACN in 5 min, then 100% A in 5 min. A Luna C18 column (Phenomenex, 150 mm×4.6 mm, 5 μm) and a C18 guard column (Phenomenex, Torrance, CA, USA) were employed for this analysis.

Animals for in Vivo Assays

Swiss albino mice (20–30 g BW) of ten weeks old and both sexes were obtained from The Institute of Drug Testing of Ho Chi Minh City. All experimental animal protocols were performed in compliance with the Vietnamese animal ethics and approved by the Review Board of Faculty of Pharmacy (TDTUFP.202301). The mice underwent a period of acclimatization within the animal housing facilities for seven days prior to commencing the experiment. The mice were housed in appropriately sized cages, experienced natural light-dark cycles and were kept at room temperature. They were provided with unrestricted access to standard food and tap water on a regular basis.

Induction of Experimental Diabetes

All Swiss albino mice were fasted for overnight after which blood was collected from the tail vein to assess fasting blood glucose levels and body weight are recorded. The precise amount of alloxan for each individual animal was measured based on their respective body weights. Subsequently, the alloxan was dissolved in 0.9% (w/v) normal saline immediately before injection. The mice, having undergone an overnight fast, received intraperitoneal (IP) injections of alloxan at a dose of 150 mg/kg body weight (BW) to induce the experimental diabetic condition. Post alloxan administration, both food and water were reintroduced to the mice after a 30-minute interval. Exactly seven days following the alloxan injection, the plasma glucose levels of each mouse were meticulously assessed. Animals with fasting blood glucose levels surpassing 200 mg/dL were identified and included in the subsequent phases of the study. Blood samples for glucose level analysis were carefully collected from the tail of each mouse, ensuring minimal discomfort and distress.

Extracts Oral Administration to Diabetic Mice

The diabetic mice were randomly divided into six groups: Group I (Normal mice + distilled water), Group II (Normal mice+extract 200 mg/kg BW), Group III (Diabetic control+distilled water), Group IV (Diabetic mice+glibenclamide 5 mg/kg BW), Group V (Diabetic mice+extract 200 mg/kg BW), and Group VI (Diabetic mice+extract 400 mg/kg BW). All groups of mice received daily administration throughout a span of 21 consecutive days. Blood glucose levels (BGL) and body weight (BW) were assessed by extracting blood samples from the tails of each individual mouse. A weekly monitoring of BGL and BW was conducted over a course of three weeks. The fasting glucose levels and BW of the mice were determined on days 1, 7, 14, and 21 of the experimental periods.

Subchronical Oral Toxicity

Assess subchronical toxicity of the test sample by evaluating changes in hematological and biochemical parameters and alterations in organ histology, liver and kidney histopathology, between the control group and the test group after administering a pharmacologically active dose to mice for a specified period. Subacute toxicity assessment of the test sample was performed for 14 and 28 days, following the "Guidelines for Preclinical and Clinical Testing of Traditional Medicines and Herbal Medicines" issued by the Ministry of Health under Decision No. 141/QĐ-K2ĐT dated 27/10/2015. Mice in the test group were administered the expected therapeutic dose for humans and a dose three times higher than the therapeutic dose (the expected dose would allow for the observation of toxicity signs in the test animals) for 14 and 28 days. The test was conducted in parallel with a control group under the same conditions [29–31].

Acute Oral Toxicity

Mice were administered the test sample at the same dose under stable conditions. Observe any reactions for the first 72 hours and for 14 days. Then mice (fasted for 12 hours) were administered the maximum possible dose of the test sample via oral gavage, administered volume 50 ml/kg, according to "Guidelines for Preclinical and Clinical Testing of Traditional Medicines and Herbal Medicines" issued by the Ministry of Health under Decision No. 141/QĐ-K2ĐT dated 27/10/2015. Monitor and record general movements, behavioral changes, fur conditions, food intake, excretion, and mortality within the first 72 hours. If no abnormal signs or deaths occurred in the mice after the initial 72 hours, continue monitoring for 14 days (Figures S1–S5).

Statistical Analysis

Data are expressed as the mean (±) with the standard error of the mean (SEM). Statistical significance was set at $p<0.05$. Differences between groups in *in vivo* assays were analyzed using the Kruskal-Wallis and Mann-Whitney tests with SPSS 22.0 software.

Results

Alpha-Glucosidase Inhibition of Extracts of *C. Quadrangulare*, *D. Linearis*, *P. Adenophylla*, and *G. Schomburgkiana*

Extracts/fractions of different parts from *C. quadrangulare*, *D. linearis*, *P. adenophylla*, and *G. schomburgkiana* were evaluated for alpha-glucosidase inhibition (Table 1). As shown in Table 1, all crude MeOH extracts of all tested parts of *C. quadrangulare* showed potent activity with IC_{50} values ranging from 4.0 to 20.4 µg/mL. Among them, the extract of *C. quadrangulare* leaves is the most active extract. The ethyl acetate extracts of leaves, flowers, stem, seeds, and roots also showed good activity (IC_{50} range: 4.6-147.5 µg/mL) while all *n*-hexane and the water-soluble extracts of different *C. quadrangulare* parts are less or inactive.

Crude extracts of leaves of *D. linearis* and *P. adenophylla* were more active than those of stem and aerial part. Extract of fruits of *G. schomburgkiana* revealed significant inhibition with an IC_{50} value of 27.7 µg/mL while the activities of other extracts of this plant were weak.

In comparison to previously reported combretic acids D and E (1 and 2) [11], crude methanol of leaves from *C. quadrangulare* and extract M of leaves from *P. adenophylla* exhibited superior alpha-glucosidase inhibitory activity (IC_{50} values of 4.0 µg/mL and 3.4 µg/mL, respectively).

Among four studied medicinal sources, *C. quadrangulare* showed the best inhibition against alpha-glucosidase.

HPLC Analysis of Extracts of *C. Quadrangulare*

Cytoartanes were a group of compounds that showed potent alpha-glucosidase inhibition [11]. Combretic acids D (**1**) and E (**2**) (Figure 2) were determined to be the most active compounds with IC_{50} values of 13.9 and 30.7 µM, respectively. To detect the presence of these two compounds in extracts of *C. quadrangulare,* an HPLC analysis with two detectors DAD and ELSD analysis was conducted for different extracts of *C. quadrangulare* (Figures 3–5). As shown in Figure 3, compounds **1** and **2** were detected at retention times (RT) 37.3 and 26.3 mins, respectively. The crude methanolic extracts of different parts of *C. quadrangulare* (namely Me roots, Me stems, Me leaves, Me fruits, Me flowers) were prepared and analyzed, as shown in Figure 4. The derived ethyl acetate extracts of each sources (namely EA roots, EA stems, EA leaves, EA fruits, EA flowers) were also analyzed due to their significant activity (see Figure 5). The low content of both compounds are detected in extracts of roots and stems.

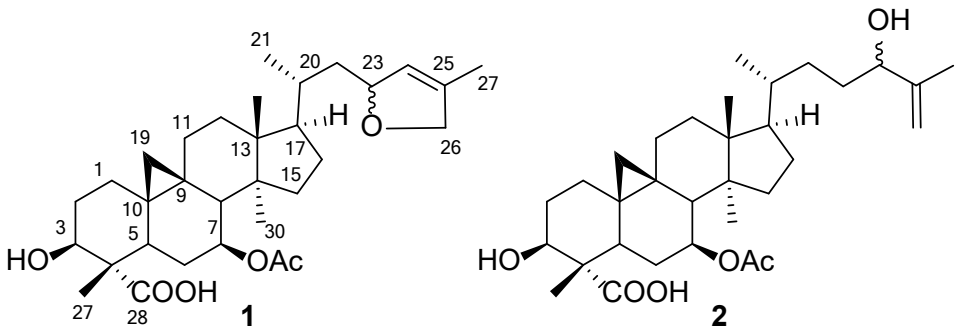

Figure 2 Chemical structures of combretic acids D (1) and E (2).

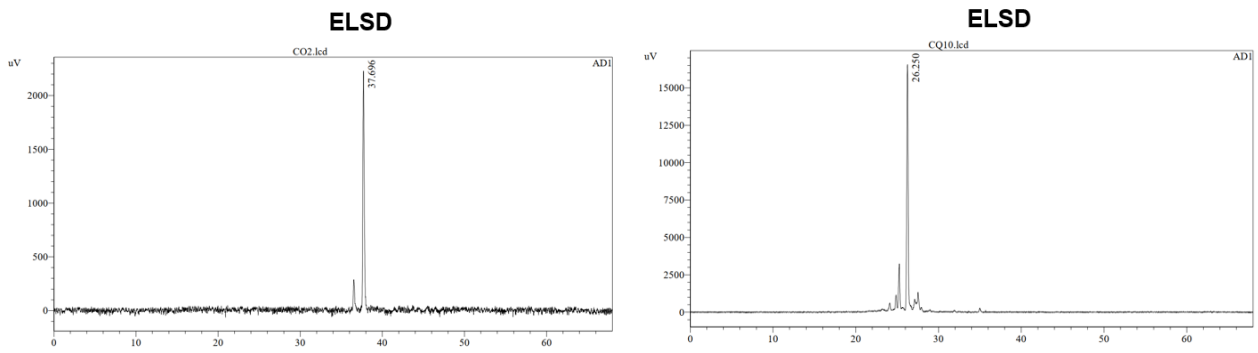

Figure 3 HPLC-ELSD chromatograms of compounds 1 (left) and 2 (right).

Table 1 Alpha-glucosidase inhibition (IC_{50}) by extracts of studied plants

Bio-source	Organ	Extracts	IC_{50} µg/mL
C. quadrangulare	Leaves	Crude MeOH	4.0 ± 0.1
		Extract **H**	>200
		Extract **EA**	4.6 ± 0.05
		Extract **M**	>200
	Flowers	Crude MeOH	61.3 ± 1.8
		Extract **H**	>200
		Extract **EA**	130.9 ± 1.7
		Extract **M**	>200
	Stem	Crude MeOH	8.3 ± 0.3
		Extract **H**	>200
		Extract **EA**	6.3 ± 0.1
		Extract **M**	>200
	Seeds	Crude MeOH	8.1 ± 0.2
		Extract **H**	>200
		Extract **EA**	145.7 ± 4.0
		Extract **M**	>200
	Roots	Crude MeOH	20.4 ± 0.8
		Extract **H**	>200
		Extract **EA**	100.0 ± 1.1
		Extract **M**	>200
D. linearis	Leaves	Crude MeOH	31.1 ± 0.03
		Extract **H**	>200
		Extract **EA**	154.1 ± 2.4
		Extract **M**	>200
	Aerial part	Crude MeOH	167.8 ± 2.1
		Extract **H**	>200
		Extract **EA**	>200
		Extract **M**	>200
P. adenophylla	Leaves	Crude MeOH	109.9 ± 1.2
		Extract **H**	56.6 ± 3.5
		Extract **EA**	6.7 ± 0.4
		Extract **M**	3.4 ± 0.1
	Stem	Crude MeOH	189.9 ± 4.6
		Extract **H**	>200
		Extract **EA**	66.8 ± 3.4
		Extract **M**	>200
G. schomburgkiana	Fruits	Crude MeOH	27.7 ± 0.5
		Extract **H**	>200
		Extract **EA**	66.0 ± 2.6
		Extract **M**	12.3 ± 0.5
	Leaves	Crude MeOH	>200
		Extract **H**	>200
		Extract **EA**	>200
		Extract **M**	>200
	Stem	Crude MeOH	>200
		Extract **H**	>200
		Extract **EA**	>200
		Extract **M**	>200
Acarbose (positive control)			201.9 ± 2.2

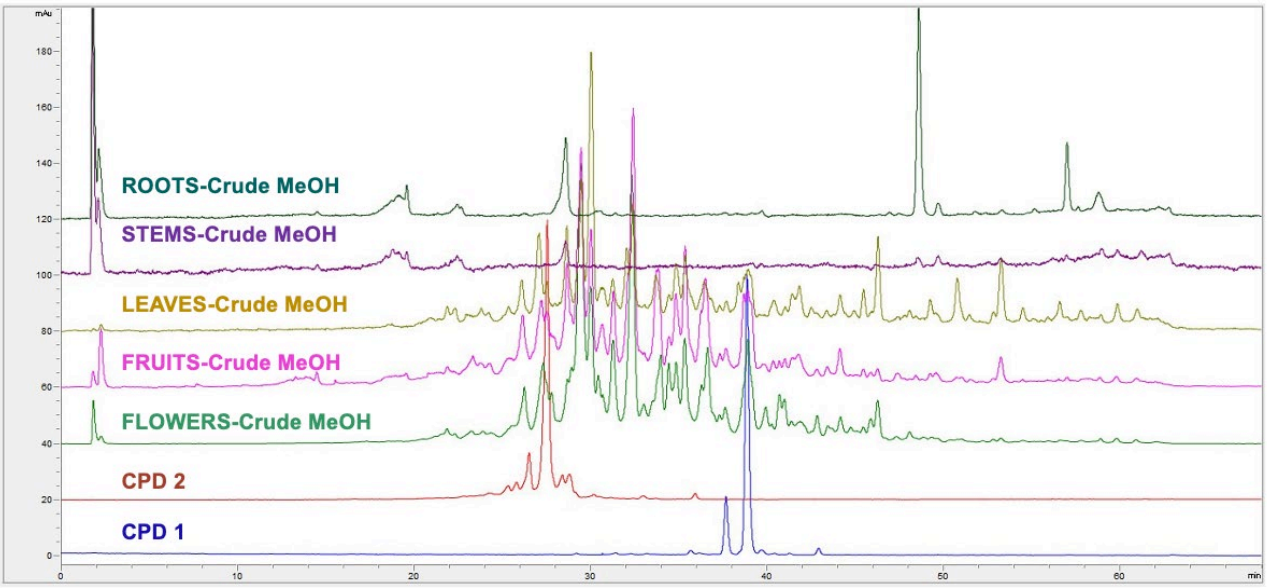

Figure 4 HPLC-ELSD chromatograms of methanolic extracts of different parts of *C. quadrangulare* in comparison with compounds **1** and **2**.

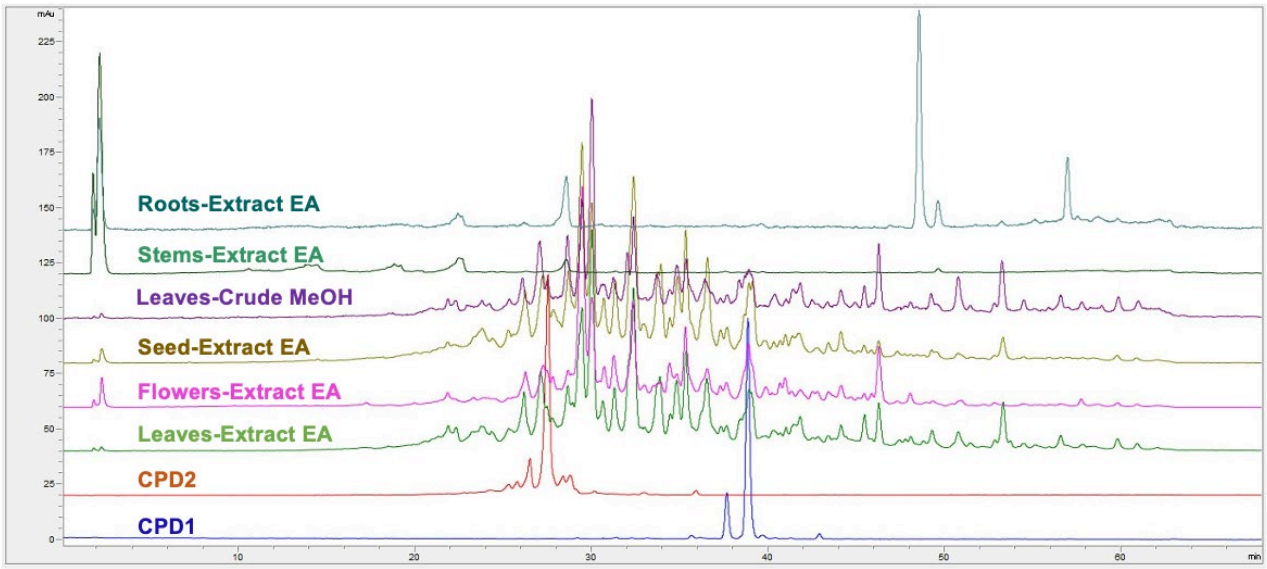

Figure 5 HPLC-ELSD chromatograms of extracts EA of different parts of *C. quadrangulare* in comparison with compounds **1** and **2**.

Antidiabetic Assays of Extract of *C. Quadrangulare* Leaves

As mentioned above, the extract has been evidenced to be effective in inhibition of alpha-glucosidase. To further confirm its potential antidiabetic activity, a study on the diabetic mouse model was conducted at the oral administration concentration of 200 and 400 mg extract per kilogram body weight (mg/ kg BW) (Figure 6). The result showed that the group of normal mice that consumed water (group I) and the dose of 200 mg/kg (group II) maintained blood glucose levels within the normal range throughout the 21-day observation period. However, group II exhibited a slight but non-significant reduction in blood glucose levels compared to the water-consuming group. Blood glucose levels in the group of mice injected with alloxan (group III) were significantly increased from 254.3 to 275.5 mg/dL after 21 days of testing. Notably, both the groups of mice administered with glibenclamide (at a dose of 2 mg/kg BW, namely group IV) and 400 mg/kg BW of extract (group VI) were able to reduce blood glucose levels from 248.3 to 152 mg/dL for group IV and 249 to 150.6 mg/dL for group VI after 21 days of oral administration. Indeed, group IV demonstrated a notable reduction in blood glucose levels after 7 days of oral administration, whereas group VI exhibited significant reduction after 14 days of oral administration. Conversely, the group consumed with 200 mg extract/kg BW (group V) did not show a significant

reduction in blood glucose levels during testing period (Figure 6). The results suggest that a dose of 400 mg extract/kg BW could effectively decrease blood glucose levels compared to that of glibenclamide at the dose of 2 mg/kg BW. Furthermore, the mice's body weights did not significantly change among the groups during the 21-day observation period (Figure 7).

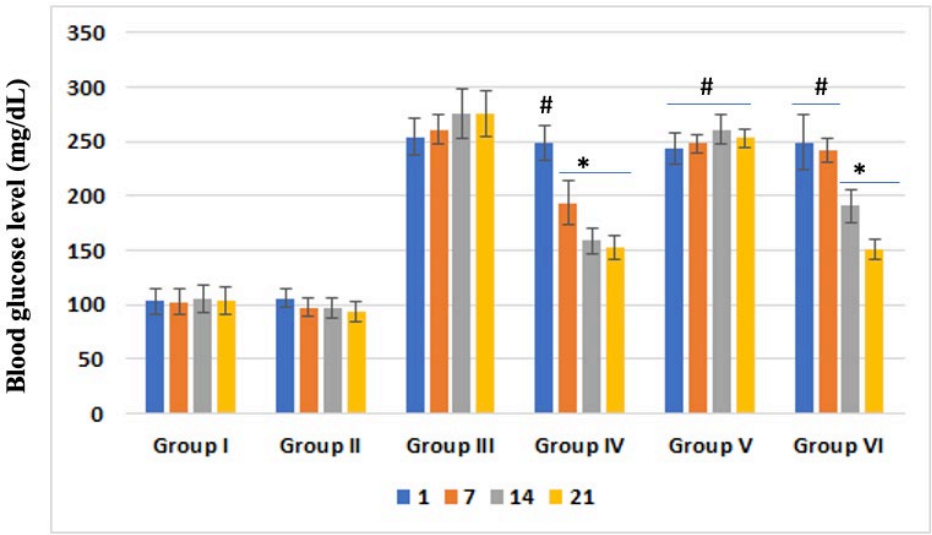

Figure 6 The effect of the extract on blood glucose level in diabetic mice during 1, 7, 14, and 21 days of oral administration. Group I (Normal mice+distilled water), Group II (Normal mice+200 mg extract/kg BW), Group III (Diabetic control+distilled water), Group IV (Diabetic mice+glibenclamide 5 mg/kg BW), Group V (Diabetic mice+200 mg extract/kg BW), and Group VI (Diabetic mice+400 mg extract/kg BW. Data are expressed as the mean (±) with the standard error of the mean (SEM). * Indicate not statistically significant difference between IV, V, and VI with I; # Indicate not statistically significant difference between IV, V, and VI with III.

Subchronical Toxicity Test of the *C. Quadrangulare* Extract

Before the experiment, mice were tested for hematological parameters and liver and kidney function, with results presented in Table S2. The mice used for the experiment were acclimatized to experimental conditions for five days (approximately seven weeks old). The laboratory parameters tested, including complete blood count (WBC, RBC, HgB, HCT, MCV, MCH, MCHC, RDW-CV, PLT), and biochemical tests (AST, ALT, albumin, total protein, total bilirubin, total cholesterol, glucose, creatinine, urea), fell within the reported ranges for normal mice [29–32]. Therefore, these mice were suitable for use in the toxicity study to assess the subchronical toxicity of the *C. quadrangulare* extract.

General condition and body weight: The results show that mice in the control group and the two groups that received *C. quadrangulare* extract at doses of 200 mg/kg and 600 mg/kg experienced an increase in weight ranging from 0.2 to 1.0 grams per day (see Table S3). During the first 21 days of the experiment, the body weight of the mice that received *C. quadrangulare* extract at both doses did not significantly differ from the control mice ($p>0.05$). In the final 7 days of the experiment, the two groups had slower weight gain compared to the control group, with the 600 mg/kg group showing a statistically significant lower average weight compared to the control group at the same time ($p<0.05$). When comparing within each group, male and female mice in the same group had no significant differences in body weight at most time points ($p>0.05$).

Hematological and blood glucose parameters: In terms of blood glucose levels, after 14 and 28 days, blood glucose levels in both groups at 200 mg/kg and 600 mg/kg were not significantly different from the control group at the same time points ($p>0.05$) (Table S4). However, when analyzing separately by gender, male mice in the 600 mg/kg group for 14 days had significantly higher blood glucose levels compared to female mice in the same group at the same time point ($p<0.01$), but these values still fell within the normal reference range.

Liver function: The results show that after 14 days, the AST levels in the groups of mice that received *C. quadrangulare* extract at doses of 200 mg/kg and 600 mg/kg were not significantly different from the control group ($p>0.05$) (Table S5). After 28 days, compared to the control group, the AST level in the 200 mg/kg group did not show statistically significant differences ($p>0.05$), while in the 600 mg/kg group, it was significantly lower ($p<0.05$) but still within the normal range. Regarding ALT levels, both groups of mice that received doses of 200 mg/kg and 600 mg/kg for 14 and 28 days did not exhibit significant changes compared to the control group ($p>0.05$). When comparing between male and female mice within the same group, the activities of AST and ALT were mostly not statistically different ($p>0.05$).

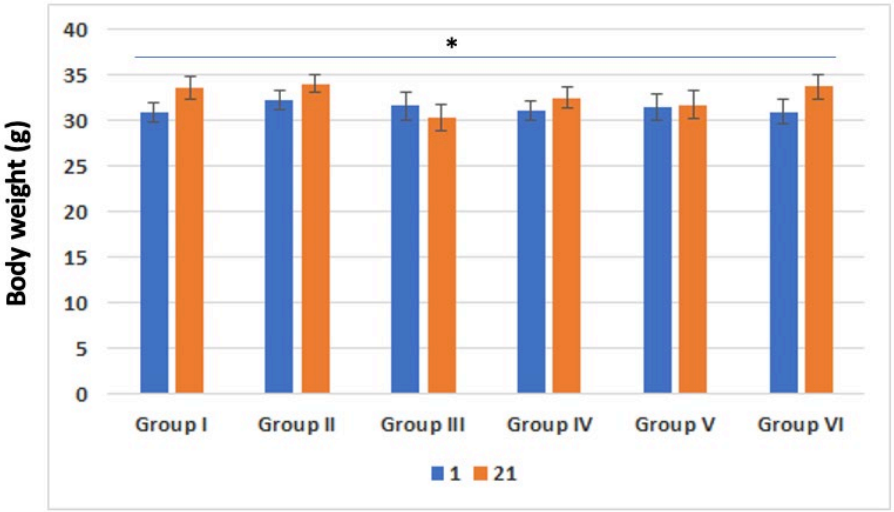

Figure 7 Body weight of diabetic mice during 1 and 21 days of the oral administration of the extract. Group I (Normal mice+distilled water), Group II (Normal mice+200 mg extract/kg BW), Group III (Diabetic control+distilled water), Group IV (Diabetic mice+glibenclamide 5 mg/kg BW), Group V (Diabetic mice+200 mg extract/kg BW), and Group VI (Diabetic mice+400 mg extract/kg BW. *Indicate not statistically significant difference among groups.

Kidney function: After 14 and 28 days, mice that received *C. quadrangulare* extract at doses of 200 mg/kg and 600 mg/kg did not exhibit significant changes in urea and creatinine levels compared to the control group ($p>0.05$) (Table S6). When comparing between male and female mice within the same group, the urea and creatinine levels were not statistically different ($p>0.05$) in the 200 mg/kg group. However, in the 600 mg/kg group, urea levels were higher in male mice compared to female mice within the same group ($p<0.01$) after 14 days, and creatinine levels were lower in male mice compared to female mice within the same group ($p<0.01$) after 28 days, but still within the normal range.

Gross Anatomy of Organs: Observations of gross anatomy showed that after 28 days, organs such as the heart, lungs, liver, kidneys, and the digestive system in mice from all groups did not show any abnormalities (Figure S1).

Liver Histopathology: The results of liver histopathological analysis are presented in Figures S2-4 and Table S7. Continuous administration of *C. quadrangulare* extract at doses of 200 mg/kg and 600 mg/kg for 28 days may affect the histopathological structure of liver in test mice at a minimal level but does not alter the function of the liver (no impact on ALT and AST enzyme activity).

Renal Histopathology: The results of renal histopathological analysis in test mice after 28 days are presented in Table S8 and Figures S5-7. Administering *C. quadrangulare* extract to mice at doses of 200 mg/kg and 600 mg/kg continuously for 28 days did not affect the histopathological

In conclusion, administering *C. quadrangulare* extract to mice at doses of 200 mg/kg and 600 mg/kg continuously for 14 and 28 days did not have significant effects on the body weight of the mice, did not affect hematopoietic function, and did not impact liver and kidney function.

Acute Oral Toxicity Test of the *C. Quadrangulare* Extract

Tested mice (5 male and 5 female) were administrated a single dose at 37 g/kg BW. Toxic effects and lethality were observed during each 12 hours, lengthened to 14 days (see Table S9). Lethality, behaviors, macroscopic tissue injury, and total weight loss were observed. The tested results indicated that all tested mice are normal and healthy without lethality.

Discussion

Alpha-glucosidase inhibition of crude extracts of both leaves of *D. linearis* and *P. adenophylla* have been reported in the previous work [22]. However, alpha-glucosidase inhibition of other parts of both plants evidence is limited. There are a few studies regarding the alpha-glucosidase inhibition of the plants belonging to *Dicranopteris* and *Psychotria* genera. *Dicranopteris caudata*, *Psychotria malayana*, and *Psychotria viridiflora* revealed the potent inhibition effects on alpha-glucosidase [33–35]. Fruits, leaves, and barks of Thailand *Garcinia schomburgkiana* were reported to inhibit alpha-glucosidase enzyme [27]. Nevertheless, there is only one study regarding alpha-glucosidase inhibition of Vietnamese *Garcinia schomburgkiana* source were found.

In this study, we evaluated α-glucosidase of *C. quadrangulare*, *D. linearis* and *P. adenophylla* using different parts of these plants. Interestingly, all crude MeOH extracts of all tested parts of *C. quadrangulare* showed significant potential in α-glucosidase inhibitory characteristic. In addition, we found that crude MeOH extracts from *D. linearis* leaves and aerial parts, *P. adenophylla* leaves and stem, *G. schomburgkiana* fruits, leaves and stem also exert the inhibition of α-glucosidase effect. These results suggested that *C. quadrangulare*, *D. linearis* and *P. adenophylla* contain the compounds which exert alpha-glucosidase effects in many types of their tissue and that we could analyze chemicals from these different tissues of them to select promising subject for further studies.

According to Chabang and colleagues, cycloartane derivatives were able to inhibit sodium/glucose co-transporter-mediated glucose uptake in human renal proximal tubular cells [36]. Moreover, the previous study has evidenced the potential inhibition of cycloartane on starch hydrolysis enzymes [37]. The leaves of Vietnamese *C. quadrangulare* have been found to contain high content of cycloartanes and combretic acids D (1) and E (2) were determined to be the most active compounds [11]. Previous studies conducted on *C. quadrangulare* leaves have indicated that cycloartanes have been predominantly detected in the EtOAc extract [8–11]. In this study, *C. quadrangulare* crude MeOH and EtOAc extracts of all tested parts exhibited the inhibitory effects on alpha-glucosidase. High-performance liquid chromatography (HPLC) analysis was employed to elucidate the presence of combretic acids D (1) and E (2) in all tested samples of *C. quadrangulare* crude MeOH and EtOAc extracts. A literature review indicated that there are over 60 cycloartanes found in EtOAc extracts of *C. quadrangulare* seeds, flowers, and leaves (Figure S9). The presence of 1 and 2 in *C. quadrangulare* seeds, flowers, and leaves was demonstrated in Figures 4 and 5, suggesting that cycloartanes may be responsible for the substantial activity of these biosources.

Our results suggest that root and stem extracts have low content of compounds 1 and 2. HPLC chromatograms of crude MeOH and EtOAc extracts from *C. quadrangulare* root and stem showed complicated chemical data with distinct peaks. Little is known about chemical data and alpha-glucosidase inhibition of *C. quadrangulare* root and stem. Although lacking compounds 1 and 2 in their content, extracts from root and stem surprisingly showed significant alpha-glucosidase inhibition effects (Table 1). The inhibition of these extracts is even more significant than extracts from the flower which have high content of the compound 1 and 2. Consequently, it is plausible that the alpha-glucosidase inhibitory activity of *C. quadrangulare* is not solely regulated by compounds 1 and 2, suggesting the potential presence of bioactive chemicals in the root and stem of this plant that warrant further isolation and evaluation.

Extract M of leaves from *P. adenophylla* exhibited substantial alpha-glucosidase inhibitory activity, with an IC_{50} value of 3.4 μg/mL. As previously reported [22], the high activity of this extract may be attributed to the mixture of undefined lignins. The composition of this extract was determined through HPLC analysis [22].

Our results indicated that *C. quadrangulare* extracts have the highest inhibitory effects on alpha-glucosidase activity. Therefore, we evaluated the toxicity of this extract for further investigation of its pharmacological effects using mouse model. Evaluating toxicity of crude plant extracts is often performed after alpha-glucosidase inhibitory characteristic being identified *in vitro* [38–40]. *C. quadrangulare* extracts did not induce significant acute or subchronical toxicity.

Potential antidiabetic activity of *C. quadrangulare* extracts is confirmed in our study using diabetic mouse model. Oral administration of the extracts significantly attenuated alloxan-induced abnormal blood glucose level (Figure 6). Until now, acarbose, miglitol and voglibose are the most often used alpha-glucosidase inhibitors in clinical practice. Therefore, screening for natural products *in vivo* to vary the options for clinical practice has been raising interest in recent years [41]. Oral administration of *Chrysophyllum caimito* (75 mg/kg) significantly decreases sugar blood level in diabetic rats [42]. *Heteromorpha arborescens* leaves' hydro-methanolic crude extract significanly attenuated the increase of blood glucose level in streptozotocin-induced diabetic mice [43]. We showed in this study that the extract of *C. quadrangulare* leaves emeliorated alloxan-induced diabetes in mouse model and can be considered as a potential natural product for treatment of diabetes.

The alloxan-induced diabetes mouse model is frequently employed to simulate type 1 diabetes. Nevertheless, the elevation of blood glucose levels in alloxan-treated mice can also be influenced by AGI medications. Alloxan-induced diabetes models have also been utilized as an in vivo model to assess the effects of the herbal extract on blood glucose levels in numerous previous studies [44–49]. Although the alloxan model is not directly representative of type 2 diabetes, it enables the assessment of glucose-lowering effects in a model where glucose regulation has been compromised. This is pertinent for comparing treatments that influence blood glucose levels, such as the extract's alpha-glucosidase inhibition mechanism, which reduces postprandial glucose absorption in the intestine, thereby indirectly lowering blood glucose. The inclusion of glibenclamide in this model highlights the potential of the extract to regulate glucose levels independently of insulin secretion, a valuable attribute for the management of type 2 diabetes. Although a type 2 diabetes model may provide a more pertinent comparison, employing the alloxan model facilitates an effective evaluation of the extract's glucose-lowering efficacy in relation to a well-established hypoglycemic agent.

The effects of *C. quadrangulare* extract only significantly exert positive effects on blood glucose level after 14-day observation while glibenclamide exert its effects after 7-day observation. Glibenclamide, a sulfonylurea drug, primarily induces insulin secretion through its interaction with ATP-sensitive potassium channels on pancreatic b-cells. This

interaction results in depolarization and calcium influx, subsequently triggering insulin release. This mechanism results in a rapid decrease in blood glucose levels, as seen in Group IV of our study (Figure 6), where glibenclamide administration led to a significant reduction in blood glucose as early as day 7. In contrast, the plant extract primarily inhibits α-glucosidase, thereby delaying carbohydrate digestion and reducing glucose absorption in the intestines. This indirect mechanism does not directly stimulate insulin secretion but rather retards glucose entry into the bloodstream. Consequently, the extract's hypoglycemic effect manifested more gradually, with substantial outcomes observed after 14 days (Group VI). Furthermore, the extract's slower metabolic processing may further delay its hypoglycemic effect. These distinctions emphasize that while glibenclamide provides a rapid onset of action, the plant extract may offer a more sustained and long-term impact on glycemic control without directly altering insulin levels. This potential reduction in the risk of hypoglycemia associated with insulin-stimulating agents is noteworthy.The delay in the action of *C. quadrangulare* extract could also be explained by the low metabolism of plant extract which delays the induction of hypoglycemic effects [50]. However, after 21-day observation, effects on blood glucose level of glibenclamide and the extracts turned out to be similar (Figure 6). The alike phenomenon is observed in the research about hypoglycemic activity of celery herb extract *Apium graveolens* in blood glucose level of laboratory rats [51]. This phenomenon indicated that hypoglycemic effects of *C. quadrangulare* extract is related to the inhibition of glucose absorption in the intestine and is not due to the stimulation of insulin excretion of the pancreas [51,52]. Alpha-glucosidase inhibitor and glibenclamide are usually used as options to combine with first-line therapy such as metformin when it is not enough to achieve the desired glycemic target. The combination of metformin with alpha-glucosidase inhibitors is less effective in HbA_{1c} in comparison with glibenclamide. However, alpha-glucosidase inhibitors are reported to have more beneficial effects than glibenclamide in long-term efficacy and long-term safety [52]. This information is in fact consistent with our results. These results indicate the potential of the *C. quadrangulare* extract in the positive regulation of blood glucose levels in diabetic patients.

A previous study indicated benzoylphloroglucinol-type compounds from *G. schomburgkiana* are bioactive compounds [28]. Compared to alpha-glucosidase inhibition of co-occurred compounds G1-G11 in the same source (Figure S8), compound 4 was less active than other benzoylphloroglucinol derivatives *epi*-guttiferone Q (G1) or guttiferones I-K (G2-G3) [28]. This indicated that the larger number of isoprenyl units in G1-G3 might affect the activity.

Conclusion

Four Vietnamese medicinal plants *Combretum quadrangulare*, *Dicranopteris linearis*, *Psychotria adenophylla*, and *Garcinia schomburgkiana* growing in the South of Vietnam were investigated potent alpha-glucosidase inhibition. All prepared extracts of the titled plants showed good activity. Among tested extracts, the crude methanolic extract of *C. quadrangulare* leaves was the most active extract and then selected for antidiabetic assay. A dose of 400 mg extract/kg showed good antidiabetic activity. Toxicity of *C. quadrangulare* extract at doses of 200 mg/kg and 600 mg/kg to mice did not have significantly harmful effects.

Conflict of Interest

The authors have no conflicts of interest to declare.

Author Contributions

Conceptualization, T.-H.-T. D., T.-H.D., H.-T.N.; methodology, T.-H.D., N.-H.N., H.-H.N.; software, N.-H.N, H.-H.N.; formal analysis, T.-H.D., D.-T.M, H.-T.N.; investigation, T.-H.-T. D., T.-H.D., H.-T.N.; resources, N.-H.N, H.-H.N.; data curation, T.-S. V., H.-T.N.; writing—original draft preparation, T.-H.-T. D., T.-H.D., H.-T.N.; writing-review and editing, T.-H.-T. D., T.-H.D., H.-T.N.; supervision, T.-H.-T. D., T.-H.D., H.-T.N. All authors have read and agreed to the published version of the manuscript.

Ethical Approval

Animal experiments were reviewed and approved by the Review Board of Faculty of Pharmacy of Ton Duc Thang University (TDTUFP.202301).

Data Availability

The evidence data generated and/or analyzed during the current study are available from the corresponding author on reasonable request and partly shown in Supporting information file.

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