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Preliminary Phytochemical Analysis and the Anti-Diabetic Effect of Leaf Extracts of *Symplocos cochinchinensis* (Lour.) Moore ssp. *Laurina* (Retz.) Nooteb. Against α-Amylase and α-Glucosidase

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ABSTRACT

One of the ways of controlling postprandial glucose level is to inhibit carbohydrate-hydrolyzing enzymes like α-amylase and α-glucosidase. This study was undertaken to provide *in vitro* evidence for the potential inhibitory activity of crude extract and different fractions of *Symplocos cochinchinensis* leaves on α-amylase and α-glucosidase enzymes. Crude and fractionated extracts were obtained by percolation and liquid-liquid extraction. *In vitro* antidiabetic activity of all extracts was assessed based on α-amylase and α-glucosidase inhibition. Furthermore, phytochemical screening was done via chemical reactions, and the total polyphenol (TPC) and flavonoid (TFC) contents were measured via colorimetric methods using *S. cochinchinensis* leaves. Results showed that *S. cochinchinensis* leaves contain polyphenols, flavonoids, saponins, triterpenoids, alkaloids, anthraglycosides, anthocyanosides, proanthocyanidins, tannins, polyuronics, and reducing agents. The TPC was 517.71 mg gallic acid equivalent/g dry mass while the TFC was 1.12 mg quercetin equivalent/g dry mass. The results indicated that crude extract and its fractions demonstrated inhibitory activities on both α-amylase and α-glucosidase, of which, the crude extract and ethyl acetate fraction had the highest inhibitory potential for both α-amylase and α-glucosidase of all the fractions tested, with IC_{50} values of 38.85 and 35.74 µg/mL (crude extract), 30.18 and 30.91 µg/mL (ethyl acetate fraction), respectively. These fractions were especially better than acarbose (45.49 and 53.18 µg/mL). Simultaneously, there was a significant correlation between the TPC of the *S. cochinchinensis* leaf extracts and their α-amylase and α-glucosidase inhibitory

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activities. Further *in vivo* studies should be performed to clarify the regulating effect of *S. cochinchinensis* leaves on postprandial blood glucose.

Keywords: α-amylase inhibition; α-glucosidase inhibition; Correlation; *Symplocos cochinchinensis* leaves; Phytochemicals

1. Introduction

Diabetes mellitus is a metabolic disease, characterized by carbohydrate, lipid, and protein metabolism disturbances and chronic hyperglycemia. It causes decreased pancreatic insulin secretion by pancreatic Langerhans β cells, an impeded action of insulin, or both. Other metabolism-related disorders, including cardiovascular disease, can lead to the damage, malfunction, or failure of various internal organs such as the liver, brain, heart, and kidneys [1]. According to an estimation by the International Diabetes Federation, approximately 463 million people suffer from diabetes in 2019, accounting for 9.3% of the world population, a percentage that is expected to grow by more than 10% by 2030. Around 700 million people are anticipated to suffer from diabetes by 2045 if no interventions are taken [2]. Prediabetes is defined as a state in which blood sugar levels are higher than normal but do not surpass the threshold to be considered diabetes; this state of prediabetes puts those in it at a higher risk of contracting diabetes. The American Diabetes Association reported that around 70% of pre-diabetic patients will eventually develop diabetes [3]. To prevent these patients from developing diabetes and other complications associated with the disease, one viable intervention is inhibiting the conversion of starch molecules to glucose done by the enzymatic hydrolysis of carbohydrates.

Type 2 diabetes (T2D) is a common metabolic disorder; as a matter of fact, more than 90% of all diabetic patients suffer from this particular type of the disorder. The treatment is costly and causes many complications negatively affecting the quality of the patient's life [4]. An imbalance between the level of blood glucose absorption and insulin secretion is what triggers T2D. In addition, postprandial hyperglycemia plays a crucial role in the development of T2D [5]. The control of blood glucose is vital for delaying or preventing T2D. α-amylase and α-glucosidase are the two essential enzymes involved in the digestion of carbohydrates [6]. α-amylase is linked to the breakdown of long-chain carbohydrates, while α-glucosidase breaks down starch and disaccharides into glucose [7]. These serve as the major digestive enzymes, and can facilitate the intestinal absorption process. Hence, α-amylase and α-glucosidase inhibitors are regarded as potential targets for diabetes treatment. Some drugs and dietary options can help to regulate plasma glucose level through the inhibition of starch-digesting enzymes (α-amylase), and/or the absorption of glucose (α-glucosidase). However, the leading inhibitors of carbohydrate metabolizing enzymes such as α-amylase and α-glucosidase in the gastrointestinal tract, acarbose and miglitol, are often reported to cause some noticeable side effects involving flatulence, abdominal pain, diarrhea, and other intestinal disturbances [8]. Understanding the situation, pharmacists aim to explore more options centered on natural compounds from herbs, to contribute to the treatment and support of those with diabetes. According to the World Health Organization, over 60% of the global population, and a hefty majority of inhabitants of developing countries in particular (80%), have used traditional treatments as well as medicinal plants for the purpose of curing diseases [9]. Many previous studies have shown that herbs play an extremely important role in treating diabetes due to their conventional acceptability and lesser side effects [10].

Therefore, investigation of α-amylase and α-glucosidase inhibitors in medicinal plants has drawn many scientists' attention.

Symplocos cochinchinensis, a member of the Symplocaceae family, is used by the Vietnamese in herbal medicines to support the treatment of various ailments. Pharmacological studies have illustrated that extracts and isolated compounds from *S. cochinchinensis* leaves have extensive biological activities, such as antidiabetic, antilipidemic, antioxidant, antitumor, anti-inflammatory, and antimicrobial activity amongst other medicinal properties [11-14]. *S. cochinchinensis* leaves have been shown to include phenolics, flavonoids, iridoid, tannins, and saponins [14-15] Among various phytochemicals, polyphenol compounds, particularly flavonoids, are widely considered to be some of the most bioactive compounds, having been proven to possess a variety of therapeutic effects [16]. Previous findings have indicated that *S. cochinchinensis* leaf extracts exhibit α-glucosidase inhibitory activity [12]. As a follow-up to these findings, the objective of this study was to systematically screen the *in vitro* inhibitory activities of crude extract and constituent fractions of *S. cochinchinensis* leaves on α- amylase and α- glucosidase. Furthermore, the phytochemical characteristics of *S. cochinchinensis* leaves were determined by qualitative chemical tests and readings of the total content of polyphenols and flavonoids.

2. Materials and Methods

2.1 Plant-based material and sample preparation

The plant *Symplocos cochinchinensis* was collected in the dry season of August 2019 from Cham Island, Quang Nam Province, Vietnam. The Greenviet Biodiversity Conservation Centre and Southern Institute of Ecology, Vietnam Academy of Science and Technology identified and verified the samples; a voucher specimen was also deposited for *Symplocos cochinchinensis* (Lour.) Moore ssp. *Laurina* (Retz.) Nooteb. Leaves were selected in randomized fashion from mature plants about 2 m high, without making any distinction between young or old leaves.

The leaves were rinsed with tap water before being cleaned with distilled water to remove the dirt on the surfaces. They were then air dried to the standard drying weight loss in accordance with the Vietnam Pharmacopoeia 5th Edition. Afterwards, these dried samples were ground to a fine powder and kept individually in airtight PVE bags at the Research Center Ginseng and Medicinal Materials in Ho Chi Minh City (Sample code: TTS-SC-0819).

2.2 Chemicals and reagents

Ethanol 96% (OPC Pharmaceutical Company, Vietnam), *n*-hexane, chloroform, ethyl acetate, *n*-butanol (VN-Chemsol, Co. Ltd, Vietnam); α-amylase (from malt, HIMEDIA), DNSA reagent (3,5-dinitrosalicylic acid, China), methanol, gallic acid (HPLC≥98%), Folin-Ciocalteu's phenol reagent, acarbose, α-glucosidase (from *Saccharomyces cerevisiae*), and *p*-Nitrophenyl-α-D-Glucopyranoside were purchased from Sigma-Aldrich® Co. Ltd (USA).

2.3 Extraction and fractionation

For crude extract, dried powder was extracted by 45% ethanol (material-solvent ratio of 1:15 (g/mL)) and left in a percolator apparatus at room temperature for 24 hours. This extract was then collected at a rate of 2 mL/min and concentrated using a rotary evaporator at 60 °C under reduced pressure to produce the crude ethanol extract. The total extract was solubilized in distilled water and extracted with solvents of increasing polarity (*n*-hexane, chloroform, ethyl acetate, and *n*-butanol) with the aim of obtaining fractionated extracts as the final step. Eventually, six dried extracts, including CE (crude extract), *n*-hexane (F1), chloroform (F2), ethyl acetate (F3), *n*-butanol (F4), and

water (F5) extracts, were obtained and preserved in sterilized vials and stored in a refrigerator at 2-8 °C. The extracts were dissolved in double distilled water to yield a stock solution in the tests.

2.4 Preliminary qualitative phyto-chemical analysis

Preliminary qualitative phytochemical analysis was conducted to identify the presence of secondary metabolites in the leaves. The screening was performed in accordance with Ciulei's method with minor adjustments [17]. The different extracts of *S. cochinchinensis* leaves, including diethyl ether, ethanol, and aqueous extracts were tested for lipids, volatile oils, carotenoids, triterpenoids, alkaloids, proanthocyanidins, anthocyanosides, flavonoids, anthraquinones, coumarins, tannins, saponins, reducing agents, polyuronics, and organic acids using characteristic chemical reactions. Generally, tests for the presence (+) or absence (-) of phytochemical compounds involved adding an appropriate chemical agent to the preparation in a test tube.

2.5 Determination of total polyphenol content

The total polyphenol content was estimated by Folin-Ciocalteu's method using gallic acid as the standard [18]. To briefly illustrate, 200 µL test sample was mixed with 500 µL of Folin-Ciocalteu's reagent in 6 mL of double-distilled water. Then 1.5 mL of sodium carbonate solution (20% *w/v*) was poured into this mixture right after 5 min, and with distilled water, the volume reached 10 mL. The reaction was kept in dark conditions for 2 hours at room temperature. The absorbance was measured at 758 nm and all determinations were made in triplicate. The calibration curve used gallic acid as the standard. Using the calibration curve, the total polyphenol content was calculated and is expressed as milligram of gallic acid equivalent per gram of dry mass.

2.6 Determination of total flavonoid content

The flavonoid content was determined on the basis of the aluminum chloride colorimetric method, using quercetin as the standard [19]. First, 1.0 mg of quercetin was dissolved in 10 mL methanol by which the stock solution was made, then by serial dilutions, including methanol, the standard quercetin solutions were prepared. Briefly, 1 mL of aluminum chloride (2% *w/v*) was added to 1 mL diluted extract or standard quercetin solutions separately, and with methanol, the mixture volume reached 10 mL. Then, the solution was mixed and incubated for 15 min at room temperature. The absorbance of the reaction mixtures was calibrated at 416 nm using the UV–Vis spectrometer technique. The measurements were carried out in triplicate. The calibration curve was plotted using standard quercetin. The total flavonoid content was estimated using the calibration curve and is expressed as milligram of quercetin equivalent per gram of dry mass.

2.7 *α*-amylase inhibition assay

The previously described method-based *α*-amylase inhibitory activity of crude and fractionated extracts was carried out with some modification [20]. Firstly, 250 µL of each tested-extract at different concentrations was incubated with 250 µL of *α*-amylase (1 U/mL) in 0.02 M sodium phosphate buffer (pH 6.9) which contained 6 mM NaCl at 37 °C for 15 min. Then, 250 µL of 1% soluble starch was added to this mixture as a substrate and was then incubated at 37 °C for 20 min more; then, 500 µL of the DNSA color reagent was added and boiled for 10 min; after that, it was allowed cool to room temperature, at which point the absorbance was measured at 540 nm. Acarbose was used as a positive control. The measurements were done in triplicate, and the IC_{50} values, (the concentration of sample which leads to 50% inhibition of maximal activity) were determined.

2.8 α-glucosidase inhibition assay

The α-glucosidase inhibition assay was done as described by Dong *et al*. with slight modifications [21]. The reaction mixtures, including 60 µL of various concentrations of tested-extracts or acarbose and 50 µL of 0.1 M phosphate buffer (pH 6.8) containing α-glucosidase (0.2 U/mL), were incubated in 96-well plates at 37 °C for 10 min. Then, 50 µL of soluble *p*-nitrophenyl-α-D-glucopyranoside was added and incubated further at 37 °C for 20 min. After incubation, absorbance readings were recorded at 405 nm using a plate reader (Biotek, USA). The experiments were performed in triplicate, and acarbose was used as a positive control. The IC_{50} values were also determined. The percentage of enzyme (α-amylase and α-glucosidase) inhibition was estimated using the following formula: %Inhibition = $[(A_c - A_{0c}) - (A_t - A_{0t}) / (A_c - A_{0c})] \times 100$. In which, A_c is the absorbance of the samples with enzyme without test extract, A_{0c} is the absorbance of the samples without enzyme and test extract, A_t is the absorbance of the samples with enzyme and test extract, and A_{0t} is the absorbance of the samples with test extract without enzyme.

2.9 Statistical analysis

The results are expressed as mean ± SEM/SD (Standard error of the mean/Standard deviation), data were analyzed by Graphpad Prism software (version 8, Inc., La Jolla, CA, USA) using t-test and One-way ANOVA. Correlation coefficient (r) and coefficient of determination (R^2) were determined by the Pearson test, using Graphpad Prism software (version 8, Inc., La Jolla, CA, USA). *P* values < 0.05 are considered statistically significant.

3. Results and Discussion

3.1 Phytochemical analysis

The extracts were subjected to qualitative chemical tests for identifying various secondary metabolites present in the leaves of *S. cochinchinensis*. The results indicated the presence of triterpenoids, alkaloids, anthraglycosides, flavonoids, anthocyanosides, proanthocyanidins, tannins, saponins, polyuronics, and reducing agents (Table 1).

Table 1. Preliminary phytochemical analysis results of *S. cochinchinensis* leaves.

Metabolites	Diethyl ether extract	Ethanol extract		Aqueous extract		Presence
		Non-hydrolysis	Hydro-lysis	Non-hydrolysis	Hydro-lysis	
Lipids	-					-
Carotenoids	-					-
Volatile oils	-					-
Free triterpenoids	+				+	+
Triterpenoid hydrolysis			+		+	+
Alkaloids	-	+		-		+
Coumarins	-	-		-		-
Anthraglycosides				+		+
Flavonoids	+	+	+	+	+	+
Anthocyanosides		+		+		+
Proanthocyanidins		+		+		+
Tannins		+		+		+
Saponins		+		+		+
Organic acids		-		-		-
Reducing agents		+		+		+
Polyuronics				+		+

Note: (+):compound is present; (−): compound is absent; Empty cells: presence is possible but not conclusive.

3.2 Extraction yield of crude and fractionated extracts

The loss on drying (LOD) of the dried powdered material and crude ethanol extract was carried out according to regulations in Vietnam Pharmacopoeia 5th Edition. The LOD of dried powder and the crude extract was 9.73±0.41% and 15.50±0.34%, respectively, which met the standard for raw materials (≤13%) and condensed extracts (≤20%). The yields of crude and fractionated extracts of *S. cochinchinensis* leaves are presented in Fig. 1.

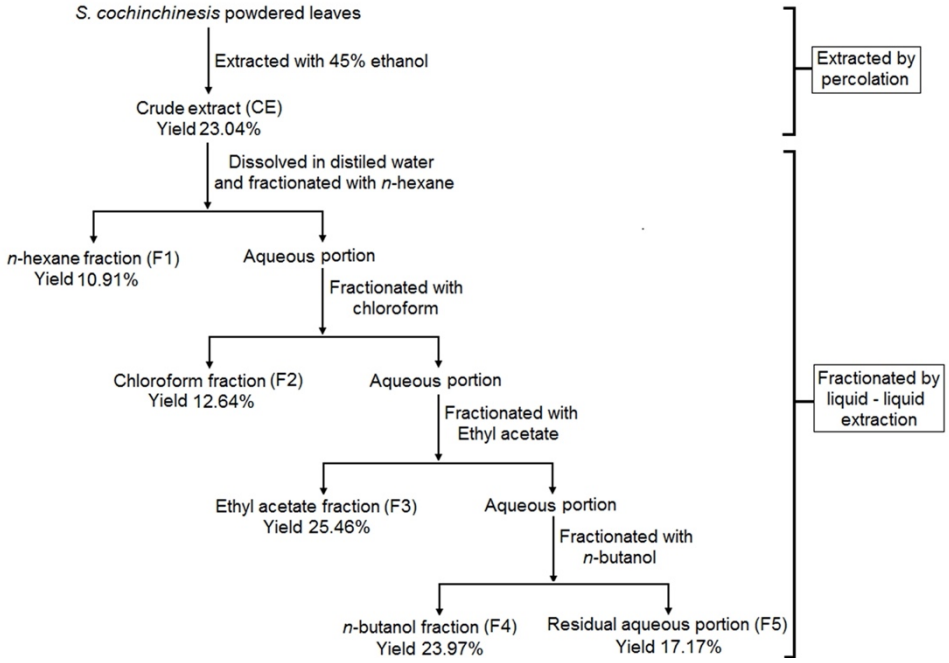

Fig. 1. The scheme for extraction and fractionation of *S. cochinchinensis* leaves.

3.3 Total polyphenol and flavonoid contents

Polyphenol compounds, ubiquitous secondary metabolites in vegetation, are known to have biological activities; perhaps the activities of the plant extracts in this study are attributable to these compounds [22]. The total polyphenol content for *S. cochinchinensis* leaf extract was estimated by Folin Ciocalteu's method using gallic acid as the standard. The reagent is a mixture of two acids: phosphotungstic acid and phosphomolybdic acid. This reagent, after the oxidation of phenols, is reduced to a mixture of molybdenum and tungsten (two blue oxides). The blue coloration produced has a maximum absorption in the region of 758 nm and is proportional to the total volume of polyphenol compounds originally present. The concentration of the gallic acid solution (150-400 µg/mL) conformed to Beer's Law at 758 nm with a regression coefficient (R^2) = 0.9907. The plot has a slope (m) = 0.0024 and intercept = -0.2135. The equation of the standard curve is $Y = 0.0024x - 0.2135$, $R^2 = 0.9907$ (Fig. 2A). The results obtained in this study showed a significant level of polyphenol compounds in the *S. cochinchinensis* leaf extract (Table 2).

Flavonoids-secondary metabolites, are commonly distributed in flora. More than 8000 flavonoids have been identified in plants [16, 23]. These metabolites are of great importance for flower coloration, producing yellow and other pigments. In addition, flavonoids are readily ingested by

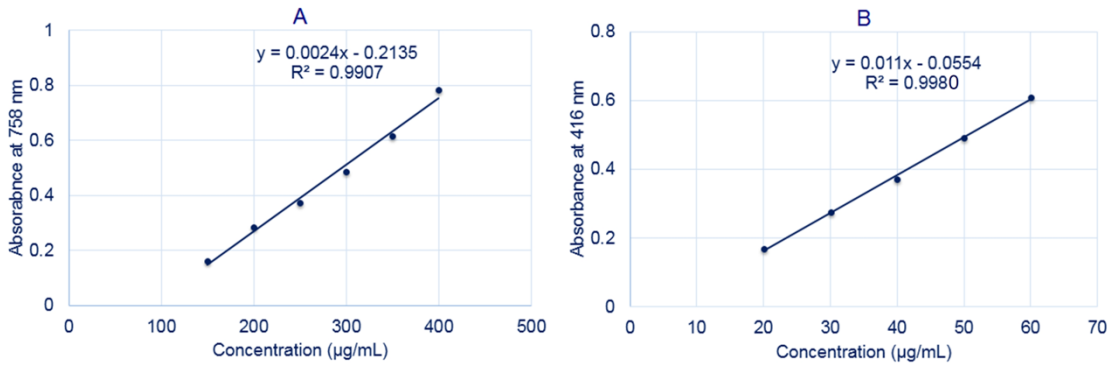

Fig. 2. Calibration line of gallic acid standard for total polyphenol content (A) and quercetin standard for total flavonoid content. All values are expressed as mean ± SEM (*n* = *3*).

Table 2. Total polyphenol content and total flavonoid content of *S. cochinchinensis* leaves.

Parameters	*S. cochinchinensis* leaves
Polyphenols (mg gallic acid equivalent/g dry mass)	517.71 ± 19.50
Flavonoids (mg quercetin equivalent/g dry mass)	1.12 ± 0.01

Note*:* All values are expressed as mean ± SEM *(n* = 3*)*.

humans, and seem to possess helpful attributes: antioxidant, anti-inflammatory, antidiabetic, anti-allergic, anticancer, and various other activities [16]. The total flavonoid content for *S. cochinchinensis* leaf extract was evaluated using the aluminum chloride colorimetric assay using quercetin as the standard. Aluminum chloride forms acid-stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxide groups of flavones and flavonols as well. Likewise, liable complexes with ortho-dihydroxide groups in the A- or B-ring of flavonoids are developed by this chemical compound. The quercetin solution (20-60 µg/mL) conformed to Beer's Law at 416 nm with a regression coefficient (R^2) = 0.9980. The plot has a slope (m) = 0.011 and intercept = 0.0554. The equation of standard curve is $Y = 0.011x - 0.0554$, $R^2 = 0.9980$ (Fig. 2B). The total flavonoid content of *S. cochinchinensis* leaf extract is shown in Table 2.

Furthermore, in this study the total polyphenol content of crude extract and its fractions was quantified, with results showing that the ethyl acetate fraction had the highest concentration of TPC (852.74 mg GAE/g d. w.) while the TPC of crude extract, *n*-hexane fraction, chloroform fraction, *n*-butanol fraction, and aqueous fraction was 738.47, 320.90, 397.08, 757.51, 511.35 mg GAE/g d. w., respectively.

3.4 *α*-amylase and *α*-glucosidase inhibitory activities

In this study, an *in vitro* *α*-amylase inhibition test was performed to figure out the inhibitory effect of the crude extract and different fractions of *S. cochinchinensis* leaves and then compare these to acarbose as a positive control. The results shown in Fig. 3A and Fig. 4 indicate that the crude and fractionated extracts of *S. cochinchinensis* leaves exhibited inhibitory activity against *α*-amylase in a dose-dependent manner. The level of inhibition for crude extract and its fractions at 80 µg/mL, listed in descending order is as follows,: ethyl acetate fraction (73.37%) > crude extract (70.92%) > *n*-butanol fraction (60.74%) > chloroform fraction (54.62%) > aqueous fraction (41.75%) > *n*-hexane fraction (31.29%) (Fig. 4A).

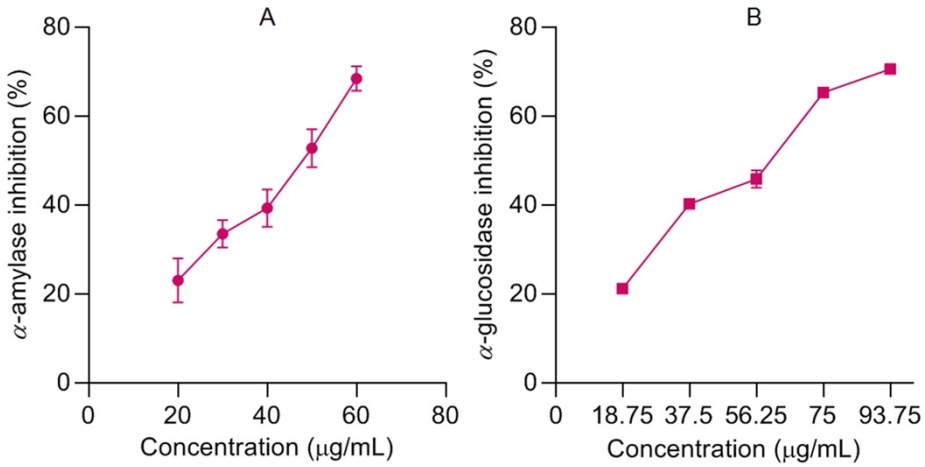

Fig. 3. Effect of acarbose for inhibition of α-amylase (**A**) and α-glucosidase (**B**). All values are expressed as mean ± SD ($n = 3$).

Further, at the investigated concentrations, crude extract and all of its fractions displayed an inhibition of α-amylase via the IC_{50} values (Fig. 4B). The α-amylase inhibitory potential (IC_{50} value) of sample tests was ordered as follows: ethyl acetate fraction > crude extract > acarbose > *n*-butanol fraction > chloroform fraction > aqueous fraction > *n*-hexane fraction. It was clearly seen that the ethyl acetate fraction and crude extract inhibited α-amylase enzyme remarkably well, and their efficacy could be compared to the standard acarbose.

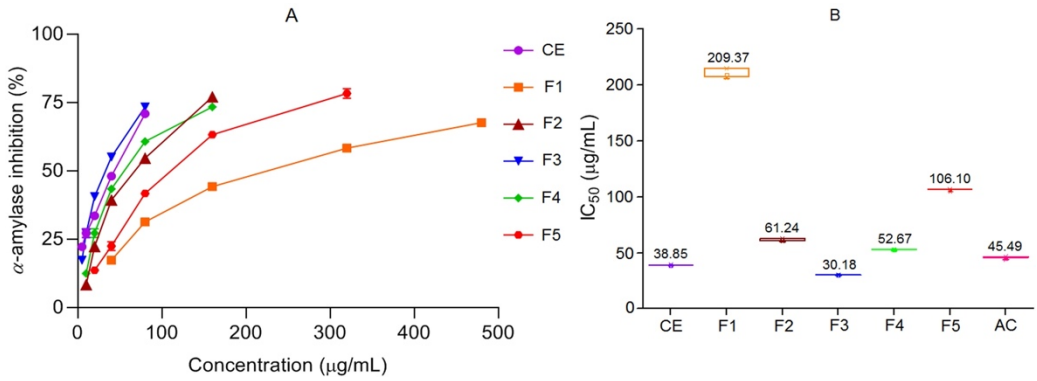

Fig. 4. α-amylase inhibitory activity of crude and fractionated extracts of *S. cochinchinensis* leaves. The percentage of inhibition (**A**) and IC_{50} values (**B**) of crude extract and its fractions. All values are expressed as mean ± SD ($n = 3$). CE, F1-5, AC*:* crude extract, *n*-hexane, *n*-butanol, ethyl acetate, chloroform, aqueous fractions, and acarbose, respectively. $^{**}p < 0.01$*:* F3, compared to CE extract, $^{****}p < 0.0001$*:* F3, compared to F1, F2, F4, and F5 fractions and AC standard, $^{**}p < 0.01$*:* F4, compared to F2 fraction and AC standard, $^{****}p < 0.0001$*:* F4, compared to F1, F3, and F5 fractions, $^{*}p < 0.05$*:* CE, compared to AC standard, $^{****}p < 0.0001$*:* other extracts, significantly different from one pair to another.

In terms of the α-glucosidase inhibitory potential, all extracts of *S. cochinchinensis* leaves inhibited α-glucosidase in a concentration-dependent manner, which was similar to the results of acarbose (Fig. 5A and Fig. 3B). In the present study, the level of α-glucosidase inhibition, in decreasing order, was crude extract (68.24%) > ethyl acetate fraction (67.89%) > acarbose (65.30%) > *n*-butanol fraction (58.99%) > chloroform fraction (50.27%) > aqueous fraction (43.35%) > *n*-hexane fraction (36.42%) at a concentration of 75 µg/mL (Fig. 5A). Also, α-glucosidase inhibition of sample tests based on IC_{50} values were in the same order as α-amylase inhibition. Interestingly, the ethyl acetate fraction and crude extract of *S. cochinchinensis* leaves also showed significantly ($p < 0.0001$) higher α-glucosidase inhibition, with an IC_{50} value of 30.91 µg/mL and 35.74 µg/mL, respectively, compared to acarbose at 53.18 µg/mL (Fig. 5B). The results suggest that the crude extract and ethyl acetate fraction from *S. cochinchinensis* leaves are able to inhibit α-amylase and α-glucosidase activity better than acarbose.

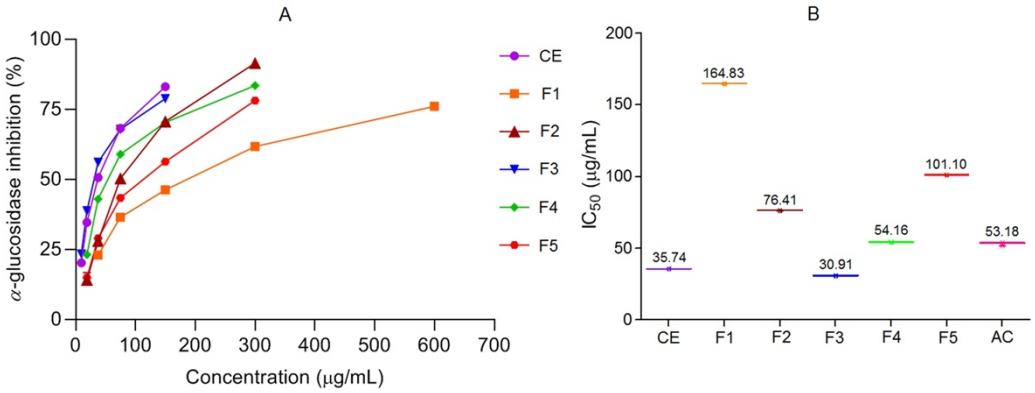

Fig. 5. α-glucosidase inhibitory activity of crude and fractionated extracts of *S. cochinchinensis* leaves. The percentage of inhibition (**A**) and IC_{50} values (**B**) of crude extract and its fractions. All values are expressed as mean ± SD ($n = 3$). CE, F1-5, AC*:* crude extract, *n*-hexane, chloroform, ethyl acetate, *n*-butanol, aqueous fractions, and acarbose, respectively. $^{ns}p > 0.05$*:* F4 fraction and AC standard*:* There is no significant difference. $^{****}p < 0.0001$*:* other extracts, significantly different from one pair to another.

3.5 Correlation between total polyphenol content and enzymes (α-amylase and α-glucosidase) inhibitory activities

The results of this study also showed that there was a significant correlation between the α-amylase and α-glucosidase inhibitory properties (IC_{50} values) and their estimated total polyphenol content of *S. cochinchinensis* leaves. The α-amylase and α-glucosidase inhibitory activities and TPC were significantly correlated with the R^2 value of 0.6122 and 0.7574, respectively (Fig. 6). These correlations indicate that the evaluated α-amylase and α-glucosidase inhibitory properties may be related to the presence of antioxidant bioactive compounds such as polyphenols.

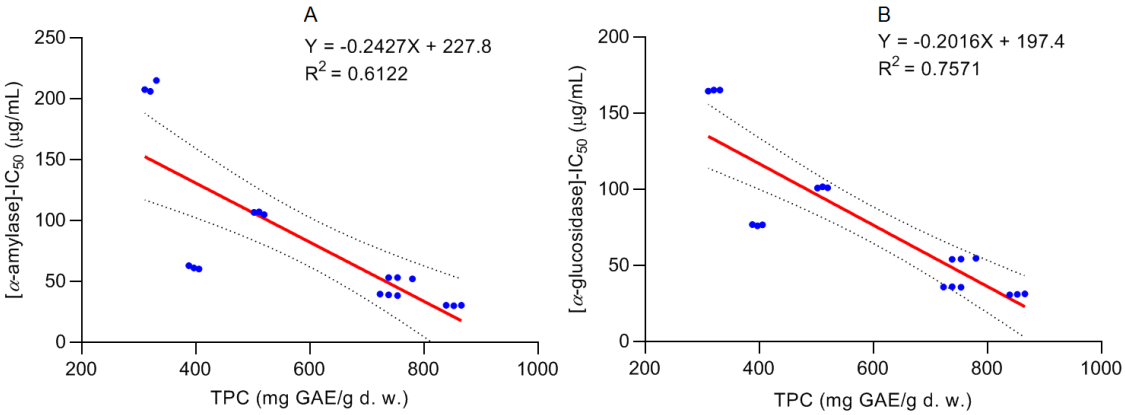

Fig. 6. Linear regression between total polyphenol content (mg GAE/g d. w.) and α-amylase inhibitory activity (A), α-glucosidase inhibitory activity (B).

Furthermore, the lowest IC_{50} for α-amylase and α-glucosidase enzymes reveal the highest α-amylase and α-glucosidase inhibitory activities. Coefficient of Pearson correlation was significantly negative if $-0.61 \leqslant r \leqslant -0.97$ and significantly positive if $0.61 \leqslant r \leqslant 0.97$ [24]. The present study showed that TPC in *S. cochinchinensis* leaf extracts had a significant and negative correlation with the IC_{50} values for α-amylase and α-glucosidase inhibitory activities ($r = -0.7824$ and $r = -0.8701$, respectively, $p < 0.05$) (Table 3). It can be predicted that phenolic compounds are the main contributor to the α-amylase and α-glucosidase inhibitory activities of *S. cochinchinensis* leaves.

Table 3. Pearson's correlation coefficient (r) and p (two-tailed) for total polyphenol content versus IC_{50} values for α-amylase and α-glucosidase inhibitory activities of *S. cochinchinensis* leaf extracts.

Correlation	Total polyphenol content	
	r	***p***
IC_{50} of α-amylase inhibitory ability	-0.7824	0.0001^{***}
IC_{50} of α-glucosidase inhibitory ability	-0.8701	$<0.0001^{****}$

$^{***}p < 0.001$ and $^{****}p < 0.0001$ are statistically significance.

The use of herbs in the development of supplements and alternative drugs for diabetes treatment has rapidly expanded worldwide recently. To treat diabetes mellitus, one of the applied strategies involves the control of postprandial blood glucose excursion through the inhibition of α-amylase and α-glucosidase in the gastrointestinal glucose absorption, thereby decreasing the postprandial glucose level and preventing diabetes symptoms [5]. An *in vitro* inhibitory effect of crude and fractionated extracts of *S. cochinchinensis* leaves on α-amylase and α-glucosidase was evaluated in this study. The rise of post-prandial glucose level has been reported to correlate closely with the activity of human pancreatic α-amylase in the small intestine; the control of this activity, therefore, is imperative in treating type 2 diabetes mellitus. α-amylase is an enzyme that takes the first step in the hydrolysis of starches; if this enzyme is inhibited, the subsequent steps in carbohydrate metabolism will stagnate, resulting in limiting the formation of glucose after a meal, therefore helping patients stabilize blood sugar [25]. The final step in the so-called carbohydrate digestion produces absorbable monosaccharides, and is mediated by α-glucosidases in epithelial tissue of the small-intestine. The breakdown of dietary polysaccharides into more simple

saccharides in the gastro-intestinal tract is impeded by the inhibition of these enzymes, thus reducing postprandial hyperglycemia [26]. Data presented here suggests that all crude and fractionated extracts of *S. cochinchinensis* leaves are promising *α*-amylase and *α*-glucosidase inhibitors; of these, the ethyl acetate fraction exhibited the highest *α*-amylase and *α*-glucosidase inhibitory activities. Interestingly, crude 45% ethanol extract being in mixed form and unpurified, presented a higher *α*-glucosidase inhibitory effect than acarbose did, the standard drug which has been purified and commercialized.

Through qualitative phytochemical analysis of multiple extraction solvents, several phytochemicals including polyphenols, flavonoids, triterpenoids, alkaloids, saponins, anthraglycosides, anthocyanosides, proanthocyanidins, tannins, polyuronics, and reducing agents were detected. Therefore, the inhibitory effect of crude and fractionated extracts of the *S. cochinchinensis* leaves might be attributable to the presence of several phytochemicals. Flavonoids, a heterogeneous group of plant polyphenols, have been widely reported to inhibit activity of *α*-amylase and *α*-glucosidase, *in vitro*, *in vivo,* and *in silico* [27-30]. More importantly, some investigations have inferred that total flavonoid and polyphenol content are directly proportional to the capacity to inhibit *α*-amylase and *α*-glucosidase [31]. The ethyl acetate fraction, being rich in flavonoid compounds, was demonstrated to regulate glucose homeostasis effects by inhibiting *α*-glucosidase, which slows the absorption of glucose into the blood leading to a risk reduction of hyperglycemia. Polyphenol compounds also inhibit the activities of carbohydrate hydrolyzing enzymes due to their ability to bind with the proteins [32]. Additionally, the presence of a variety of other secondary metabolites in *S. cochinchinensis* leaves may contribute to the *α*-amylase and *α*-glucosidase inhibitory effects. A previous study demonstrated that *S. cochinchinensis* leaves and its compounds had antidiabetic activity [15]. The hexane extract of *S. cochinchinensis* leaves had a significant effect on high fat diet-low streptozotocin rats, inducing type 2 diabetes in the rats [11]. A previous study revealed that the ethanol extract of *S. cochinchinensis* bark presented antidiabetic activity by its inhibition of *α*-glucosidase (IC_{50} = 82.07 μg/mL) and enhanced insulin sensitivity [12], comparing this to results from this study show that the potential for glucosidase inhibition of ethanol extract of *S. cochinchinensis* leaves (IC_{50} = 35.74 μg/mL) is better. Another study also illustrated that the methanolic extract of *S. cochinchinensis* bark caused a significant decrease in blood glucose and a notable increase in plasma insulin as well as liver glycogen levels in treated diabetic rats [13].

According to the present findings, it is suggested that one of the mechanisms by which *S. cochinchinensis* leaves exhibit the hypoglycemic potential is the prevention of carbohydrate hydrolyzing enzymes (*α*-amylase and *α*-glucosidase). The results prove that *S. cochinchinensis* leaves can be applied for the treatment of diabetes mellitus by regulating postprandial blood glucose.

4. Conclusion

In conclusion, some phytochemicals found in *Symplocos cochinchinensis* leaves such as triterpenoids, alkaloids, flavonoids, tannins, saponins, and flavonoids are proposed as the main compound group. *S. cochinchinensis* leaves' crude and fractionated extracts indicated considerable inhibitory effects of *α*-amylase and *α*-glucosidase when compared with acarbose. The crude 45% ethanol extract and ethyl acetate fraction exhibited higher *α*-amylase and *α*-glucosidase inhibitory activities than the other extracts. Furthermore, the crude 45% ethanol extract and ethyl acetate fractions could be used for isolating pure compounds and subsequent pharmacological tests.

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