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Effects of *In Vitro* Simulated Gastrointestinal Digestion on the α-Glucosidase Inhibitory Activities of Thai folk Remedies: Synergistic Effects with Acarbose

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ABSTRACT

α-Glucosidase, an enzyme that plays a crucial role in glucose absorption in the intestine, is a key therapeutic intervention in the management of diabetes mellitus. The inhibition of this enzyme is critical for controlling postprandial hyperglycemia. The present study aims to investigate the stability of phenolic content, total flavonoid content, and α-glucosidase inhibition activities of six Thai folk anti-diabetes remedies by *in vitro* simulated gastrointestinal digestion methods, as well as to evaluate their synergistic effect with acarbose. The TFD-04 decoction and 90% EtOH extract showed higher total phenolic and flavonoid content (86.83 mg GAE/g extract, 10.00 mg QE/g extract) and (75.32 mg GAE/g extract, 12.45 mg QE/g extract), respectively. The α-glucosidase inhibitory activities (maltase and sucrase) of TFD-04 were found maximum in decoction and GD-decoction with IC_{50} values of 0.25±0.02 and 0.78±0.04 mg/mL, respectively, when compared with acarbose with statistical significance ($P< 0.05$). The IC_{50} values of 0.59±0.02 and 1.59±0.02 mg/mL, respectively. In addition, the decoction extract of TFD-04 showed the most potent synergistic inhibitory effect with acarbose. A kinetic analysis showed that TFD-04 showed uncompetitive inhibition against intestinal maltase. This study suggests that decoction extracts of TFD-04 may serve as effective anti-hyperglycemic remedies in the field of diabetic therapy.

***Keywords*:** α-Glucosidase inhibitory activity, Thai folk anti-diabetes remedies, *in vitro* simulated gastrointestinal digestion, Synergistic inhibition

Introduction

Type II diabetes is a long-term metabolic disorder that primarily affects the elderly and leads to high blood sugar levels (hyperglycemia). This condition is primarily caused by factors such as sedentary lifestyle, genetic predisposition, and other related dietary habits.[1,2] If a patient is unable to regulate their blood sugar levels under normal conditions, it can result in various complications affecting the eyes, kidneys, heart, and blood vessels.[3] Several methods are available to control and maintain sugar levels, one of which involves managing and reducing postprandial glucose levels. This can be achieved by targeting and inhibiting the activity of α-glucosidase in the small intestine, leading to the decreased and delayed absorption of glucose.[4] Medications that effectively inhibit these enzymes include acarbose, voglibose, and miglitol.[5] Patients administered this particular group of drugs may experience side effects related to the digestive and excretory systems, including symptoms such as bloating, diarrhea, and abdominal pain, particularly when exposed to high doses.[6]

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Many researchers are currently searching for α-glucosidase inhibitor compounds from natural sources for potential new drug development. The methanol extract of *Lagerstroemia speciosa* (L.) Pers. stems. [7], *Smilax glabra* Roxb rhizomes, *Smilax glabra* Roxb rhizomes and *Tinospora crispa* (L.) Hook.f. & Thomson vines.[8], as well as the ethanolic extracts of *Momordica charantia* L. fruits[9], *Cinnamomum zeylanicum* Blume leaves[10]and *Terminalia catappa* L. leaves[11], along with the water and ethanol extracts of *Zea mays* L. seeds[12], were demonstrated to have α-glucosidase inhibitors. In another investigation by Konsue *et al* (2020), it was discovered that aqueous and ethanol extracts derived from formula extract in traditional recipes demonstrated inhibitory abilities on α-glucosidase.[13]

Caring for a patient with diabetes can impose a substantial financial burden, highlighting the need to seek affordable treatment options. Folk medicine remains a viable alternative method that retains its intrinsic value in addressing health care concerns within diverse communities throughout various regions. By incorporating locally derived wisdom and traditional remedies, which involve the utilization of indigenous herbs, vegetables, and local foods, folk medicine plays a crucial role in the care and management of patients with diabetes. Herbal remedies have long been used in Thailand to manage diabetes using individual herbs or herbal medicine formulations. The use of single herbs in diabetes treatment predominantly relies on medicinal plants, often complementing the administration of drugs.[14] The decoction is the most prevalent method of preparation, although boluses, powders, and other forms are also utilized. Traditional medicine, however, commonly combines two or more herbs and is typically administered as decoctions, tablets, boluses, powders, and other formulations. In the present study, greater emphasis was placed on individual herbs than on formulation studies. The findings revealed

a substantial body of research has highlighted the effectiveness of various herbs in reducing blood sugar levels. However, there is a paucity of research focusing on herbal medicine formulations, and limited information is available regarding the underlying mechanisms and processes involved in effectively reducing blood sugar levels.[15]

The stability of phytochemicals presents in Thai folk antidiabetic remedies upon entering the gastrointestinal tract in humans is not well understood. The bioavailability of chemical compounds such as phenolics and flavonoids exposed to variations in temperature, pH, and digestive enzymes can be affected by these physiological changes.[16] Hence, it is crucial to investigate the stability and absorption of these compounds within the digestive tract to enhance their comprehension and assessment of their potential biological properties.[17] The efficiency of *in vitro* gastrointestinal digestion models for determining the stability of phytochemicals under gastrointestinal conditions has been well established and is widely accepted. Biochemical methods were employed to determine the *α*-glucosidase inhibitory activity before and after digestion, allowing for the evaluation of the stability of these activities during the digestion process.[18] However, the specific effects of different digestion phases on the physicochemical properties and bioactivity of Thai folk antidiabetic remedies have not yet been investigated.

The main focus of this study was to investigate the effects of *in vitro* gastrointestinal simulated digestion on the chemical properties and *α*-glucosidase inhibitory activities of Thai folk anti-diabetes remedies, including Krom Luang Chomphon or Mor Phon recipes and recipes of folk medicine. In addition, we explored the combined effects of these remedies and acarbose. The findings obtained from this study contribute to the understanding and potential development of Thai folk remedies as treatments or adjuvants for the prevention and treatment of diabetes mellitus.

Materials and Methods

Chemicals and reagents

Ethanol, sodium carbonate, methanol, aluminum chloride, sodium hydroxide, sodium chloride, hydrochloric acid, phosphate buffer, Folin-Ciocalteu reagent, maltose and sucrose were purchased from Merck (Darmstadt, Germany). Amylase enzyme, pepsin enzyme, porcine enzyme, lipase enzyme, rat intestinal acetone powder, quercetin and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Glucose-kit was purchased from Human (Magdeburg, Germany). Acarbose (Glucobay®, 100 mg) was purchased from Bayer Healthcare (Leverkusen, Germany).

Plant materials

Eighteen plants (Table 1), *Lagerstroemia speciosa* (L.) Pers., *Senna siamea (Lam.) H.S.Irwin & Barneby*, *Phyllanthus amarus* Schumach. and Thonn., *Tectona grandis* L.F., *Smilax corbularia* Kunth, *Tinospora crispa* (L.) Hook.f. & Thomson, *Drynaria quercifolia* (L.) J. Sm., *Senna alata* (L.) Roxb, *Acanthus ebracteatus* Vahl., *Momordica charantia* L., *Cinnamomum zeylanicum* Blume, *Zea mays* L., *Pluchea indica* (L.) Less, *Piper sarmentosum* Roxb., *Scoparia dulcis* L., *Terminalia catappa* L., *Pandanus amaryllifolius* Roxb. ex Lindl. and *Smilax glabra* Roxb. were selected from Mor Phon recipes and recipes of folk medicine, which have been indicated to treat diabetes. All plants were purchased from Kantang district Trang province Thailand, in May 2022 (7°24' 20.00"N 99° 30' 55.00"E) and Thai traditional drugs stores in Thung Song district Nakhon Sri Thammarat province Thailand, in June 2022 (8°7' 40.49"N 99° 40' 22.93"E).

Herb extract preparation process

All plants used in the preparation of the extract were either Thai folk anti-diabetes remedies, namely TFD-01, TFD-02, and TFD-03, or Krom Luang Chomphon, Mor Phon, and folk medicines, denoted as TFD-04, TD-05, and TFD-06, respectively (Table 1). A voucher specimen was deposited in the Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chaing Mai University. To ensure their botanical identification, the specimens were carefully examined and authenticated by Asst. Prof. Kanyatorn Yincharoen the Program of Traditional Thai Medicine at the Faculty of Science and Technology, Rajamangala University of Technology, Srivijaya. To prepare plant samples for analysis, they underwent a series of drying procedures. Initially, the plants were air-dried and chopped into smaller pieces. The chopped samples were dried in a hot-air oven at 50 °C for 48 h. Throughout this process, the moisture content of the samples was measured and analyzed using an infrared machine. The drying process was continued until the moisture content reached ~ 6%. To extract each Thai folk antidiabetic formula, a standardized procedure was followed. Initially, 300 g of the chosen formula was placed in a boiling pot along with a piece of calico cloth, which aided in the preparation of the decoction. The mixture was heated and simmered until water evaporated, achieving a 1:3 ratio. Subsequently, the decoction was allowed to cool to room temperature. After cooling, the extract was carefully filtered using Whatman® No.1 filter paper to remove solid particles. The filtered extract was then concentrated by evaporating the solvent using a pressure evaporator to concentrate the desired components. To eliminate any remaining moisture and to obtain a dry powder, the concentrated extract was freeze-dried. The resulting extract was finally stored at -20 °C until further analysis and testing. An additional step was involved in the extraction process of the Thai folk antidiabetic formula. Initially, 300 g of the formula was weighed precisely and macerated using 200 mL of 90% EtOH. The maceration process lasted for seven days. Subsequently, the extract was obtained by applying pressure using a rotary evaporator. The resulting extract from the Thai folk anti-diabetes formula was then stored at -20 °C until it was ready for subsequent testing and evaluation.

Total phenolic content

This study implemented a test methodology that had been previously outlined.[12] The total phenolic content of the extract obtained from the Thai traditional remedy was evaluated using the widely employed Folin-Ciocalteu method, which quantifies phenolic compounds in various extracts. To perform the analysis, 0.2 mL of each extract (at a concentration of 30 mg/mL) was combined with 15.8 mL of deionized water. Subsequently, 1 mL of Folin-Ciocalteu reagent and 1 mL of 20% sodium carbonate solution were added to the mixture. The resulting solution was then incubated at 27°C for 2 h to allow for a chemical reaction to occur. After incubation, the absorbance of the solutions was measured at 750 nm using a spectrophotometer. The total phenolic content was determined by comparing the absorbance values to a calibration curve established using gallic acid as the standard. The results are expressed as gallic acid equivalent (GAE), which serves as an indicator of the phenolic content in the extract derived from the Thai folk anti-diabetes remedy.

Total flavonoid content

This study was a new adjustment of the previous method.[19] The aluminum chloride colorimetric method was used to determine the overall flavonoid content of the extract derived from Thai folk remedies for diabetes. In summary, 20 µL of the extract (at a concentration of 5 mg/mL) was diluted with MeOH to obtain a 1 mL solution, which was then mixed with 5 mL of distilled water. Subsequently, 0.5 mL of a solution containing 5% sodium nitrite was added. After an incubation period of 10 min, a solution (0.5 mL) containing 5% aluminum chloride was added, and the mixture was left undisturbed for 6 min. 1 mol/L sodium hydroxide solution (3 mL) was added, and the final volume of the mixture was adjusted to 10 mL using double-distilled water. The mixture was left to stand for 20 min, and the absorbance was measured at 510 nm. Total flavonoid content was calculated using a calibration curve. The results are expressed as milligrams of quercetin equivalents per gram of dry weight.

In vitro digestion by gastric and pancreatic juices

The assay employed in this study was adapted from a previously described method.[19] To prepare gastric juice, 150 mg of porcine pepsin was mixed with 90 mg of NaCl in 50 mL of distilled water. The pH of the mixture was adjusted to 1.2 using HCl. Subsequently, the gastric juice was supplemented with the Thai folk anti-diabetes remedy (150 mg/mL) and the resulting mixture was incubated at 37°C for 1 h.

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Table 1: List of plants from Thai folk anti-diabetes recipe utilized in this study

No.	Name	Common name	Thai name	Family	Part	Voucher specimen	Abbreviations
1.	*Lagerstroemia speciosa* (L.) Pers.	Banaba	Inthaninnam	LYTHRACEAE	Leaves	PCMU0023352	(TFD-1)
	Senna siamea (Lam.) H.S.Irwin & Barneby	Cassod tree	Khilek	LEGUMINASEAE	Heartwood	PCMU0023350	
	Phyllanthus amarus Schumach. & Thonn.	Carry me seed	Luktaibai	EUPHORBIACEAE	Whole plant	PCMU0023355	
	Tectona grandis L.F.	Teak	Sak	VERBENACEAE	Leaves	PCMU0023351	
	Smilax corbularia Kunth	-	Hua Khao Yen Nea	SMILACACEAE	Rhizome	PCMU0023359	
2.	*Lagerstroemia speciosa* (L.) Pers.	Banaba	Inthaninnam	LYTHRACEAE	Leaves	PCMU0023352	(TFD-2)
	Tinospora crispa (L.) Hook.f. & Thomson	Patawali	Bora phet	MENISPERMACEAE	Stem	PCMU0023353	
	Drynaria quercifolia (L.) J. Sm.	Oak-leaf fern	Kra tae tai mai	POLYPODIACEAE	Whole plants	PCMU0023358	
	Senna alata (L.) Roxb	Ringworm bush	Chumhet thet	FABACEAE	Leaves	PCMU0023349	
	Senna siamea (Lam.) H.S.Irwin & Barneby	Cassod tree	Khilek	LEGUMINASEAE	Leaves	PCMU0023350	
	Phyllanthus amarus Schumach.	Carry me seed	Luktaibai	EUPHORBIACEAE	Whole plant	PCMU0023355	
	Acanthus ebracteatus Vahl.	Sea holly	Ngeak pla more	ACANTHACEAE	Whole plants	PCMU0023344	
	Smilax corbularia Kunth	-	Hua Khao Yen Nea	SMILACEAE	Rhizome	PCMU0023359	
	Momordica charantia L.	Bitter gourd	Mara	CUCURBITACEAE	Fruits	PCMU0023348	
	Cinnamomum zeylanicum Blume	Ceylon cinnamon	Op choei thet	LAURACEAE	Bark	PCMU0023346	
3.	*Zea mays* L.	Corn	Kao pod	POACEAE	Silk	PCMU0023357	(TFD-3)
	Lagerstroemia speciosa (L.) Pers.	Banaba	Inthaninnam	LYTHRACEAE	Leaves	PCMU0023352	
	Pluchea indica (L.) Less	Indian marsh fleabane	Khlu	ASTERACEAE	Leaves	PCMU0023341	
	Piper sarmentosum Roxb.	Wild betel	Chu plu	PIPERACEAE	Leaves	PCMU0023356	
	Scoparia dulcis L.	Macao tea	Krot nam	SCROPHULARIACEAE	Leaves	PCMU0023338	
	Drynaria quercifolia (L.) J. Sm.	Oak leaf fern	Kratae tai mai	POLYPODIACEAE	Whole plants	PCMU0023358	
4.	*Terminalia catappa* L.	Tropical almond	Hukwang	COMBRETACEAE	Leaves	PCMU0023347	(TFD-4)
5.	*Tectona grandis* L.F.	Teak	Sak	LAMIACEAE	Leaves	PCMU0023351	(TFD-5)
	Pandanus amaryllifolius Roxb. ex Lindl.	Pandanus palm	Toei hom	PANDANACEAE	Leave	PCMU0023354	
6.	*Phyllanthus amarus* Schumach. & Thonn.	Carry me seed	Luktaibai	EUPHORBIACEAE	Whole plants	PCMU0023355	(TFD-6)
	Smilax corbularia Kunth.	-	Hua Khao Yen Nea	SMILACACEAE	Rhizome	PCMU0023359	
	Smilax glabra Roxb.	-	Hua Khoa Yen Tai	SMILACACEAE	Rhizome	PCMU0023358	

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After the reaction was terminated by boiling in water, 5 mL samples of gastric juice containing Thai folk anti-diabetes remedy were reserved for pancreatic digestion. For pancreatic juice, 1000 mg of pancreatin was mixed with 50 mL of phosphate buffer (20 mM, pH 8.0). Next, 5 mL of the pancreatic juice was added to each of the extracts containing the Thai folk anti-diabetes remedy. At 37°C, the reaction mixture was incubated for a duration of 2 h. Liquid nitrogen was employed to halt the reaction, and subsequent centrifugation of the samples was carried out at 6,000 rpm and 4°C for 30 min. The resulting solution was filtered through Whatman No. 541 filter paper. To halt enzyme activity, the samples were subjected to boiling water at 100°C for 20 minutes, and subsequently stored at 4°C for further analysis.

α-Glucosidase inhibitory activity assay

α-Glucosidase inhibitory activity was evaluated using the method described by Damsud.[19] Acarbose was used as the positive control in the experimental setup. The extent of inhibition was quantified using the following formula: % inhibition = [(A0–A1)/A0] × 100. Here, A_0 represents the absorbance without the sample, and A_1 represents the absorbance of the sample. To determine the type of inhibition, Lineweaver-Burk plots were used, enabling the extraction of V_{max} and K_m values from the substrate concentration plot graph.

Combined effect of acarbose with Thai folk anti-diabetes remedies

Various concentrations of acarbose were used in combination with or without the Thai folk anti-diabetes remedies at a low concentration. The reaction was conducted in accordance with the previously described *α*-glucosidase inhibitory activity assay. The obtained results were reported as percentage inhibition relative to the corresponding control values. [20]

Statistical analysis

The *α*-glucosidase inhibitory activity was determined, and the IC_{50} data for the combined effect of the extract and acarbose were analyzed using GraphPad Prism 6.05 (GraphPad Software, San Diego, California, USA). The results are presented as mean ± S.E.M for three independent experiments. Analysis of variance (ANOVA) was conducted using one-way ANOVA at a significant level of 95% confidence. Statistical analysis was performed using SPSS version 20.0 (IBM SPSS, IBM Corp., Armonk, New York).

Results and Discussion

Traditional medicines have been used since ancient times, and numerous reports have highlighted their successful application in the treatment of both acute and chronic diseases. In Thailand, traditional Thai doctors have acquired their knowledge through practical experience, developing effective recipes to address a wide range of ailments, and documenting them through various means. The present study aimed to extensively investigate six Thai medicinal formulas for the treatment of diabetes, which were extracted using diverse methods. Specifically, the decoction method and 90% EtOH were employed, and the resulting extracts were evaluated using an *in vitro* simulated gastrointestinal digestion system. The extraction method chosen in this study represents a widely adopted approach for extracting herbs within household settings, renowned for its simplicity, convenience, and expeditiousness.[21]

Extractability of Thai folk anti-diabetic remedies

Various solvents were employed to extract Thai folk anti-diabetic remedies, as indicated in Table 2. The decoction extract varied from 4.87 to 1.26 g/100 g sample, while 90% EtOH ranged from 6.23-1.74 g/100 g sample. The 90% EtOH extract exhibited a higher yield, whereas the decoction produced lower yields. Based on the yield, the 90% EtOH extraction method has a higher solvent extractive power for obtaining phenolics and flavonoids, which may influence the bioactivity. Decoction and 90% EtOH extraction differ in their extraction mechanisms, with heat facilitating the extraction process in the former and the use of organic solvents in the latter.[22] Nevertheless, both methods demonstrated the capacity to extract a wide range of polar compounds including phenolics, flavonoids, and glycosides.[23]

Table 2: Effect of TPC and TFC in anti-diabetes folk medicinal recipe

No.	Solvent	Extract yield (g/100 g)	TPC (mg GAE/g)	TFC (mg QE/g)
TFD-01	Decoction	1.26 ± 0.05^{a}	62.22 ± 0.25^{b}	9.23 ± 0.47^{a}
	GD-Decoction	-	65.65 ± 1.15^{a}	8.21 ± 0.92^{b}
	PD-Decoction	-	18.32 ± 3.18^{e}	8.01 ± 0.27^{b}
	90% EtOH	1.74 ± 0.30^{a}	51.34 ± 0.14^{d}	10.31 ± 0.35^{b}
	GD 90% EtOH	-	54.32 ± 0.43^{c}	8.34 ± 0.28^{b}
	PD-90% EtOH	-	8.21 ± 1.10^{f}	6.21 ± 0.78^{c}
TFD-02	Decoction	3.41 ± 0.72^{b}	51.91 ± 0.45^{b}	6.44 ± 0.79^{a}
	GD-Decoction	-	55.21 ± 1.59^{a}	4.32 ± 0.15^{b}
	PD-Decoction	-	15.31 ± 1.21^{c}	3.21 ± 0.14^{b}
	90% EtOH	5.14 ± 0.31^{a}	54.54 ± 0.31^{a}	5.31 ± 0.34^{a}
	GD-90% EtOH	-	55.41 ± 2.75^{a}	3.32 ± 0.12^{b}
	PD-90% EtOH	-	15.32 ± 0.25^{c}	2.98 ± 0.13^{c}
TFD-03	Decoction	2.54 ± 0.15^{b}	29.53 ± 0.37^{b}	3.63 ± 0.78^{a}
	GD-Decoction	-	25.32 ± 0.32^{c}	1.24 ± 0.14^{c}
	PD-Decoction	-	6.32 ± 2.14d	1.02 ± 0.17^{c}
	90% EtOH	4.65 ± 0.01^{a}	31.54 ± 0.20^{a}	2.14 ± 0.24^{b}
	GD-90% EtOH	-	31.24 ± 0.64^{a}	0.98 ± 0.17^{d}
	PD-90% EtOH	-	5.74 ± 0.78^{d}	0.25 ± 0.10^{d}
TFD-04	Decoction	4.87 ± 0.54^{b}	86.83 ± 0.24^{a}	10.00 ± 0.27^{b}
	GD-Decoction	-	68.32 ± 1.85^{c}	9.32 ± 0.29^{c}
	PD-Decoction	-	51.23 ± 0.78^{e}	5.74 ± 1.57^{d}

No.	Solvent	Extract yield (g/100 g)	TPC (mg GAE/g)	TFC (mg QE/g)
	90% EtOH	6.23 ± 0.21^{a}	75.32 ± 0.18^{b}	12.45 ± 0.21^{a}
	GD-90% EtOH	-	55.65 ± 0.45^{d}	11.65 ± 1.14^{a}
	PD-90% EtOH	-	47.32 ± 2.65^{f}	10.65 ± 1.10^{b}
TFD-05	Decoction	2.31 ± 0.03^{b}	42.57 ± 0.45^{a}	4.42 ± 0.78^{a}
	GD- Decoction	-	32.14 ± 0.75^{b}	3.21 ± 0.21^{b}
	PD- Decoction	-	14.32 ± 1.10^{c}	2.14 ± 0.14^{c}
	90% EtOH	3.37 ± 0.14^{a}	31.74 ± 0.35^{b}	3.78 ± 0.65^{b}
	GD- 90% EtOH	-	32.32 ± 0.61^{b}	4.32 ± 0.47^{a}
	PD-90% EtOH	-	5.65 ± 0.27^{d}	3.45 ± 0.74^{b}
TFD-06	Decoction	3.87 ± 0.08^{a}	40.43 ± 0.64^{b}	5.61 ± 0.34^{a}
	GD-Decoction	-	41.32 ± 1.18^{b}	4.32 ± 1.09^{a}
	PD-Decoction	-	8.32 ± 1.17^{d}	1.47 ± 0.28^{d}
	90% EtOH	4.17 ± 0.11^{a}	43.24 ± 0.36^{a}	5.14 ± 0.56^{a}
	GD-90% EtOH	-	43.32 ± 3.18^{a}	3.87 ± 1.21^{b}
	PD-90% EtOH	-	15.21 ± 1.34^{c}	2.41 ± 1.13^{c}

TPC, Total phenolic content; and TFC, Total flavonoid content
All Values are means of three independent analyses ± standard deviation (n=3)
GAE: gallic acid equivalents; QE: quercetin equivalents; GD-decoction: gastric digested decoction extract; PD-decoction: pancreatic digested decoction extract; GD-90% EtOH: gastric digested 90% EtOH extract; PD-90% EtOH - Pancreatic digested 90% EtOH extract.
[a.f] The data marked by different letters in each column indicate a statistically significant difference (SPSS version 20.0, $P \leq 0.05$)

These compounds possess a diverse array of biological activities, including anti-cancer, antioxidant, and anti-diabetic activities.[24,25] The results of the extraction study revealed that 90% EtOH resulted in extraction percentages (% yield) ranging from 4.87 to 1.26 g/100 g sample, surpassing the efficacy of the decoction method. Notably, prolonged exposure to heat during the extraction process can adversely affect both the quantity and intensity of the extract.[26,27] Additionally, prolonged heating has the potential to induce structural modifications, leading to decomposition of the target substance during the extraction procedure.[28] Hence, the decoction method is well suited for the extraction of water-soluble and heat-resistant substances, specifically from medicinal herbs that contain constituents such as bark, roots, seeds, and fruits.[29] The extraction of substances from Thai medicinal plants presents an avenue for obtaining compounds that exhibit specific effects and allow for differentiation among various herb types. As a result, a diverse array of extraction methods has been developed, each tailored to suit a specific herb and the desired target compounds. Consequently, researchers are encouraged to exercise discretion in the selection of appropriate extraction techniques to ensure optimal and efficient utilization of extracted substances.[30]

Quantification of total phenolic, flavonoid content and their stability

The extract obtained from the Thai folk anti-diabetic remedy was analyzed to determine the total phenolic content, which varied widely, as shown in Table 2. The results revealed that TFD-04 derived from the decoction (86.83 mg GAE/g extract) and 90% EtOH (75.32 mg GAE/g extract) exhibited higher levels of total phenolics. Additionally, both the decoction (10.00 mg QE/g extract) and 90% EtOH (12.45 mg QE/g extract) yielded higher concentrations of flavonoids. The presence of a higher concentration of phenolic and flavonoid compounds in TFD-04 contributed significantly to its effectiveness in inhibiting *α*-glucosidase inhibitory activity. Therefore, TFD-04 was selected to investigate its combined effect with acarbose and to conduct a kinetic study. Bioaccessibility findings indicated that the phenolic and flavonoid contents of the Thai folk anti-diabetic remedy became readily available after gastric and pancreatic digestion. Table 2 presents the total phenolic and flavonoid contents of the extracts from the Thai folk anti-diabetic remedy. Following the digestion process, there was a decrease in the total phenolic content and flavonoid level of both the decoction 86.83 – 29.53 mg GAE/g extract, 10.00 – 3.63 mg QE/g extract) and 90% EtOH (75.32 – 31.74 mg GAE/g extract, 12.45 – 2.14 mg QE/g extract). Bioaccessibility analysis conducted on a traditional Thai anti-diabetic remedy revealed that the phenolic and flavonoid components present in both the decoction and 90% EtOH extracts exhibited high accessibility after gastric and pancreatic digestion. Based on the obtained results, it was observed that the accessibility of the phenolic content was higher following gastric digestion of both the decoction and 90% EtOH extracts. This phenomenon could be attributed to the low pH encountered during gastric digestion (pH 2.0), as this alteration in pH may enhance the bioaccessibility of these compounds. Likewise, a higher polyphenolic content has been observed during the gastric phase than during the pancreatic phase.[19,31,32]

α-Glucosidase inhibitory activity assay and their stability

The *α*-glucosidase inhibition of Thai folk anti-diabetic remedies was analyzed based on rat intestinal maltase and sucrase. The decoction formula TFD-04 and 90% EtOH were effective in inhibiting maltase and sucrase activity. The IC_{50} values for maltase inhibition were highest at 0.46 and 1.38 mg/mL for decoction and 90% EtOH extract, respectively. For sucrose inhibition, the IC_{50} values were 0.78 for the decoction extract and 1.47 mg/mL for 90% EtOH extracts. However, the observed inhibition was lower than that observed with acarbose. The *α*-glucosidase inhibitory activity of both the decoction and 90% EtOH decreased following digestion. The determination of the velocity (V_{max}) and Michaelis-Menton (K_m) of the enzyme involved plotting the reciprocal of enzyme velocity against the reciprocal of maltose concentration in the absence and presence of the inhibitor (Table 4). Based on the obtained data, the TFD-04 decoction extract exhibited uncompetitive inhibition of the *α*-glucosidase enzyme, as evidenced by a decrease in both K_m (from 121.4 to 91.3 mM) and V_{max} (from 3.6 to 4.3 mM/min) value at concentrations of 0.5 and 0.25 mg/mL, compared to the control group. This mode of uncompetitive inhibition of the *α*-glucosidase was also observed during the gastric phase (K_m :100.0 and 83.3 mM) and pancreatic phase (Km-169.6 and 104.3 mM). This study aimed to examine the *α*-glucosidase inhibitory activity of TFD-04, a preparation derived from a single herb (*Terminalia catappa* L.), against maltase and sucrase in the rat intestine. These findings demonstrated a significant and potent inhibitory effect that surpassed the efficacy of acarbose. Notably, the decoction method and extraction with 90% EtOH yielded extracts with superior inhibitory properties.

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Table 3: α-Glucosidase inhibitory activity of different anti-diabetes folk medicinal recipe

No.	Solvent	α-Glucosidase inhibitory IC_{50} (mg/mL)	
		Maltase	**Sucrase**
TFD-01	Decoction	>2.00[b]	1.44 ± 0.03[a]
	GD- Decoction	>2.00[b]	1.98 ± 0.08[b]
	PD- Decoction	>2.00[b]	>2.00[b]
	90% EtOH	>2.00[b]	>2.00[b]
	GD- 90% EtOH	>2.00[b]	>2.00[b]
	PD-90% EtOH	>2.00[b]	>2.00[b]
TFD-02	Decoction	1.65 ± 0.05 [b]	1.32 ± 0.07 [a]
	GD- Decoction	1.58 ± 0.02[b]	>2.00 [b]
	PD- Decoction	1.95 ± 0.01[b]	>2.00 [b]
	90% EtOH	>2.00 [b]	>2.00 [b]
	GD- 90% EtOH	1.82 ± 0.03[b]	>2.00 [b]
	PD-90% EtOH	>2.00 [b]	>2.00 [b]
TFD-03	Decoction	>2.00 [b]	>2.00 [b]
	GD- Decoction	>2.00 [b]	>2.00 [b]
	PD- Decoction	>2.00 [b]	>2.00 [b]
	90% EtOH	>2.00 [b]	>2.00 [b]
	GD- 90% EtOH	>2.00 [b]	>2.00 [b]
	PD-90% EtOH	>2.00 [b]	>2.00 [b]
TFD-04	Decoction	0.46 ± 0.02[a]	0.78 ± 0.04 [a]
	GD- Decoction	0.25 ± 0.02[a]	0.98 ± 0.04 [a]
	PD- Decoction	0.89 ± 0.01[b]	1.25 ± 0.01[a]
	90% EtOH	1.38 ± 0.03[b]	1.47 ± 0.03 [a]
	GD- 90% EtOH	>2.00[b]	1.89 ± 0.07 [b]
	PD-90% EtOH	>2.00[b]	>2.00 [b]
TFD-05	Decoction	>2.00 [b]	>2.00 [b]
	GD-Decoction	>2.00 [b]	>2.00 [b]
	PD-Decoction	1.87 ± 0.07 [b]	>2.00 [b]
	90% EtOH	>2.00 [b]	>2.00 [b]
	GD-90% EtOH	>2.00 [b]	>2.00 [b]
	PD-90% EtOH	>2.00 [b]	>2.00 [b]
TFD-06	Decoction	1.98 ± 0.0 [b]	1.87 ± 0.05[b]
	GD-Decoction	>2.00 [b]	>2.00 [b]
	PD-Decoction	>2.00 [b]	>2.00 [b]
	90% EtOH	>2.00 [b]	>2.00 [b]
	GD-90% EtOH	>2.00 [b]	>2.00 [b]
	PD-90% EtOH	>2.00 [b]	>2.00 [b]
Acarbose	-	0.59 ± 0.02	1.59 ± 0.02

There was no statistically significant difference between means with the same letter in the same column.
[a] Significantly difference of IC_{50} lower than acarbose control, $P < 0.05$
[b] Significantly difference of IC_{50} higher than acarbose control, $P < 0.0$

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Table 4: The V_{max} and K_m value of α-glucosidase enzyme inhibition assay in TFD-04.

S. No	Samples mg/mL	Decoction		GD-Decoction		PD-Decoction	
		Vmax (mM/min)	Km (mM)	Vmax (mM/min)	Km (mM)	Vmax (mM/min)	Km (mM)
1	0.5	3.6	121.4	3.3	100.0	4.2	169.6
2	0.25	4.3	91.3	4.2	83.3	4.3	104.3
3	Control	4.5	72.7	4.8	81.0	4.9	90.5

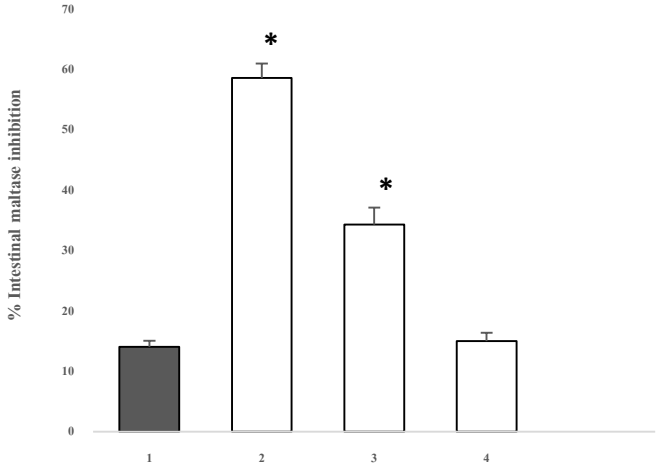

Figure 1: The combined effect of acarbose and TFD-04 on intestinal maltase inhibition. The experimental groups were as follows: (1) 0.5 mg/mL acarbose, (2) 0.5 mg/mL acarbose + 1 mg/mL decoction, (3) 0.5 mg/mL acarbose, (4) 0.5 mg/mL acarbose + 1 mg/mL GD-Decoction, and (5) 0.5 mg/mL acarbose + 1 mg/mL PD-Decoction. The results are expressed as means ± S.E.M, with a sample size of n = 3. Significance was determined using **P* < 0.001 compared to acarbose (0.5 mg/mL) alone.

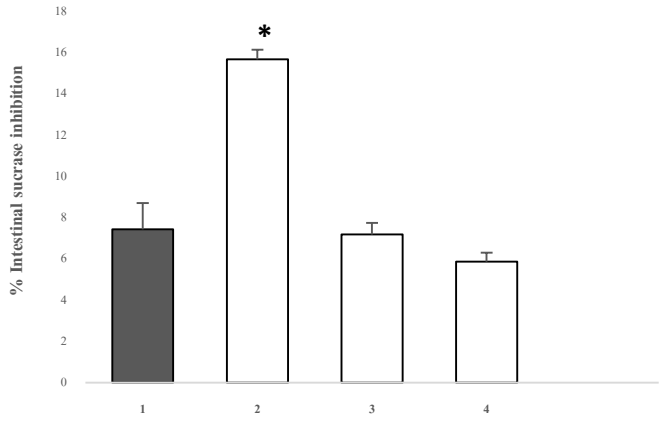

Figure 2: The combined effect of acarbose and TFD-04 on intestinal sucrase inhibition. The experimental groups were as follows: (1) 0.5 mg/mL acarbose, (2) 0.5 mg/mL acarbose + 1 mg/mL decoction, (3) 0.5 mg/mL acarbose, (4) 0.5 mg/mL acarbose + 1 mg/mL GD-Decoction, and (5) 0.5 mg/mL acarbose + 1 mg/mL PD-Decoction. The results are expressed as means ± S.E.M, with a sample size of n = 3. Significance was determined using **P* < 0.001 compared to acarbose (0.5 mg/mL) alone.

Moreover, the preparation exhibited a remarkable abundance of phenolic compounds and possessed the highest total flavonoid content compared to the other herbal formulations. The phenolic compounds and flavonoids present in *T. catappa*, namely tectochrysin, luteoin, kaempferol, 3,7,4′-trimethyl ether kaempferol, and gallic acid, have been reported to reduce blood glucose levels in animal models.[33-36] However, the inhibitory effects of these compounds on α-glucosidase in the rat intestine have not been investigated. The present study utilized enzymes isolated from the small intestine of rats, which possess functional similarities to enzymes found in the human digestive system. Notably, the inhibition of enzymatic activity was significantly influenced by the presence of hydroxy groups in the molecular structure of the inhibitor, specifically in phenolic and flavonoid compounds as well as glycosides. These structures facilitate strong binding interactions with the enzyme, leading to inactivation or conformational changes that impede hydrolysis with the substrate. As a result, enzymatic activity is inhibited, thereby preventing substrate hydrolysis.[4,37,38] Kinetic study of the glucosidase enzyme extracted from TFD-04 using the decoction extract revealed a distinctive pattern of kinetic inhibition, known as uncompetitive inhibition. In this mechanism, the inhibitor binds to the ES complex, causing structural modifications of the enzyme, thereby hindering the hydrolysis reaction, and preventing product formation. It was observed that the uncompetitive inhibitor possessed a large molecular size, rendering it incapable of accessing the active site of the enzyme.[39,40,41] Consequently, this inhibition pattern led to a reduction in both V_{max} and K_m values (Figure 3).

Combined effect of acarbose with Thai folk anti-diabetes remedies

Studies have been conducted to investigate the synergistic action of TFD-04 and acarbose. Based on the IC_{50} values provided in Table 3 for the Decoction, GD-Decoction, and PD-Decoction extracts, the percentage of α-glucosidase inhibitory activity was examined in the rat intestine (maltase and sucrase). The results indicated that the decoction extract demonstrated the most effective synergistic effect when combined with acarbose, resulting in inhibition percentages of 60.25 and 15.45%, respectively (Figures 1 and 2), surpassing the efficacy of acarbose alone. The TFD-04 extract was obtained using the decoction method, employing both GD-decoction and PD-decoction *in vitro* gastrointestinal digestion simulation systems. Notably, decoction extract exhibited remarkable efficacy, particularly when administered in combination with acarbose. A significant enhancement in efficiency was observed compared with the use of acarbose alone, with a confidence level of **P* < 0.05. Furthermore, the kinetics of α-glucosidase enzyme inhibition was investigated by focusing on maltase intestinal inhibition. The primary objective of this study was to explore the potential synergistic effect between TFD-04 extract and the antidiabetic medication acarbose, providing valuable insights for optimizing the dosage of diabetes treatment. Acarbose is classified as a medication used to reduce postprandial blood glucose levels despite some adverse effects. It belongs to the α-glucosidase inhibitor class and has been investigated for the treatment of type 2 diabetes mellitus. Recent research has indicated that acarbose treatment is associated with a 25% decrease in the incidence of diabetes. When administered, acarbose reduced postprandial blood sugar levels by 20%, with an effect lasting up to 5 h. This delays glucose absorption, thereby preventing glucotoxicity and excessive insulin release. The minimum effective dose of acarbose is 150 mg per day, whereas doses exceeding 300 mg per day do not enhance the drug's inhibitory effect, as they surpass the saturated binding capacity of α-glucosidase. The most

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commonly observed adverse effect of acarbose is gastrointestinal discomfort, including flatulence, bloating, and abdominal distention, which occurs in a manner dependent on the dosage.[42]

Conclusion

This study evaluated six Thai folk anti-diabetes remedies using decoction and 90% EtOH extraction techniques and further evaluated bioaccessibility using *in vitro* gastrointestinal simulation of the human digestive system. TFD-04 exhibited remarkable efficacy and stability in the inhibition of *α*-glucosidase, maltase, and sucrase. Additionally, potentiating effects were observed when combined with acarbose. It is crucial to acknowledge that this study represents the first *in vitro* investigation of these compounds. Further efficacy studies at the clinical level are imperative to establish the therapeutic effectiveness in human subjects. Obtaining empirical data on the efficacy of these formulations would significantly contribute to the Thai traditional medicine community, alternative medicine practitioners, and individuals with diabetes seeking viable therapeutic alternatives based on Thai medicinal formulas in the future good.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.\

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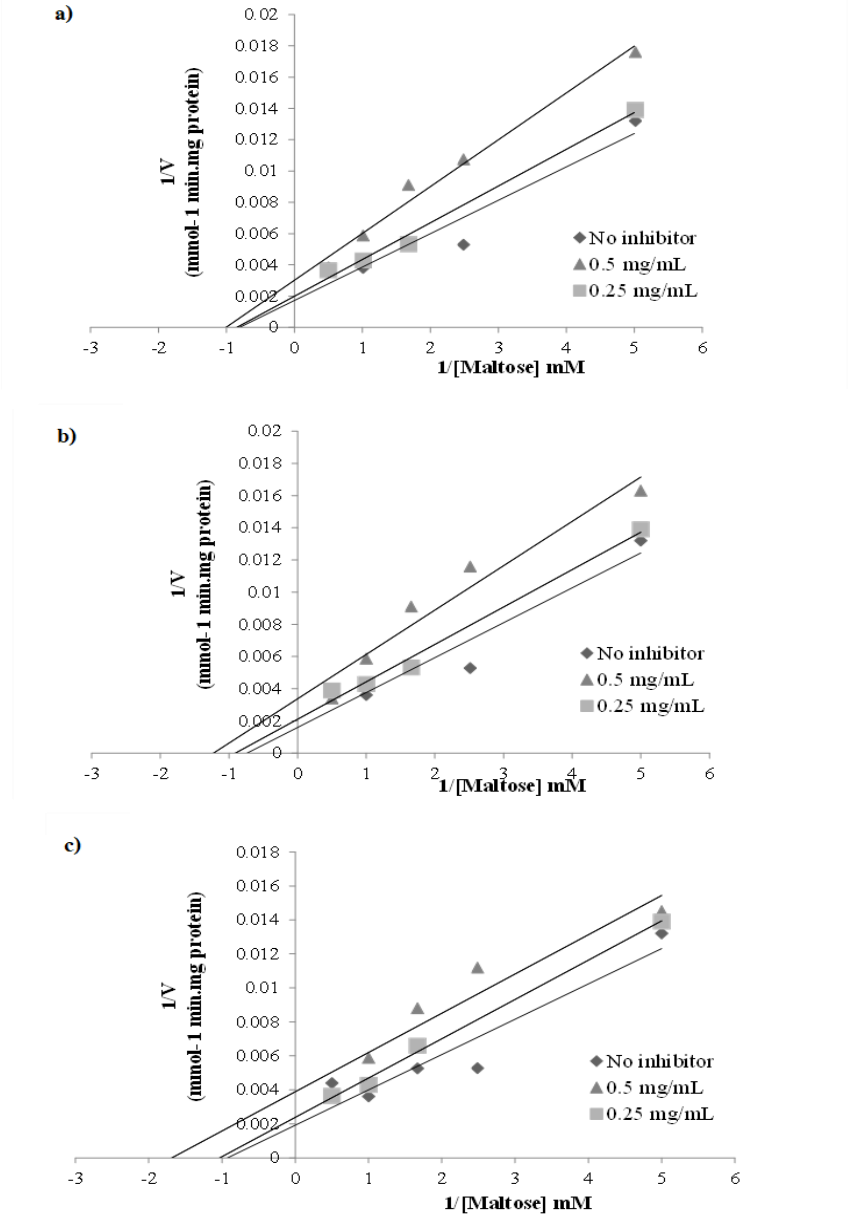

Figure 3: Lineweaver-Burk plot of TFD-04 on intestinal maltase inhibition a) Decoction; b) GD-Decoction c) PD-Decoction.

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