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Original Research Article

In Vitro Antioxidation, *α*-Glucosidase, and *α*-Amylase Inhibitory Activities of Different Solvent Extracts of Thai Traditional Diabetic Medicine

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ABSTRACT

Globally, interest in the treatment of diabetes mellitus with medicinal plants is growing. Treatment of diabetes mellitus using traditional recipes in the Royal Textbook of King Rama V has been widely documented. The present study was aimed at investigating the effects of the recipe extracts of a Thai traditional diabetic medicine on antioxidation, α-glucosidase, and α-amylase inhibitory activities. Recipe-I comprised 6 Thai medicinal plants without any additional medicinal material, while Recipe-II was composed of 6 Thai medicinal plants with 2 additional medicinal components (ammonium alum and potassium nitrate). Constituent plants of the 2 recipes were extracted with aqueous (H_2O), 50% ethanol (HE), and 95% ethanol (E). Phytochemical screening was conducted on the various extracts to determine the total flavonoid content (TFC) and total phenolic content (TPC). Three different assays, including FRAP (ferric reducing antioxidant power), DPPH (2,2-diphenyl-1-picrylhydrazyl), and ABTS (2,2′-azino-bis-[3-ethylbenzothiazoline-6-sulfonic] acid) radical scavenging were used to evaluate the antioxidant potential of the various extracts. The inhibitory activities of the recipe extracts against α-glucosidase and α-amylase enzymes were also evaluated. The results showed that Recipe-II-E was significantly higher in both TPC and TFC. The antioxidant activity of Recipe-I-E and Recipe-II-E was more potent in DPPH and FRAP assays, while Recipe-II-E was significantly more potent in the ABTS assay. Concerning the α-glucosidase and α-amylase inhibitory activities, Recipe-I-H_2O was significantly ($p < 0.05$) more effective among the test extracts. The findings of this study revealed that the test recipe extracts possess noticeable *in vitro* antioxidant potential and α-glucosidase and α-amylase inhibitory activities.

Keywords: Antioxidation, Flavonoids, Phenolic compounds, α-glucosidase, α-amylase

Introduction

Thai traditional anti-diabetic herbal formula contains eight medicinal components, which have been documented in a textbook of King Rama V in Thai traditional medicines.[1] The anti-diabetic herbs in this recipe are *Momordica cochinchinensis*, *Tiliacora triandra*, *Imperata cylindrica*, *Schumannianthus dichotomus*, *Asparagus racemosus* Willd, and *Calamus rotang*. The remaining two components are alum and potassium nitrate.[1] The original anti-diabetic herbal formula included six plants and two elements that are not commonly found in herbal drugstores. According to the Codex General Standard for Food Additives (GSFA), alum and potassium nitrate adversely affect health.[2] The first plant, *M. cochinchinensis* (Cucurbitaceae), is a type of perennial melon grown throughout Southeast Asia.[3] The roots of the plant consist of various chemical components including saponins, momordins I and II.[3]

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Bangladesh folk medicine has been used in all parts of the planet for the treatment of various ailments, such as cancer, diabetes, liver diseases, skin infections, itches, rheumatoid arthritis, colic, and blood purification.[4] Some pharmacological activities were reported on antioxidation,[5] anticancer,[6] anti-inflammation,[7] and antiulcer.[8] The second plant, *T. triandra* Ya Nang (Minispermaceae), is a common indigenous plant found in Southeast Asia, including Thailand.[9] The roots of *T. triandra* have been widely used in folk medicine in Thailand as an antipyretic agent for all kinds of fever.[10] This plant's root extract contained two pure alkaloid compounds called tiliacorinine and tiliacorine with anti-malarial activity.[11-12] The root extracts also exhibit antipyretic activity[13] In addition, leaf extracts show hypoglycemic activity in normal and streptozotocin-induced diabetic rats.[14] *I. cylindrica* (Poaceae), which is the third plant is mainly grown in places such as Northern Africa, Turkey, Iraq, and Central Asia, as well as in Liaoning and Hebei, China. It is sweet in taste, cold in nature, and has the effects of cooling blood, arresting bleeding, clearing heat, and inducing diuresis.[15] To treat hematuresis, jaundice with damp-heat pathogen, emesis, hemorrhage and reducing fever and causing diuresis, anti-fever, and anti-inflammatory effects, the rhizome of *I. cylindrica* may be used alone or in conjunction with other herbal remedies.[16] The major phytochemical constituents identified in *I. cylindrica* are coumarins, flavonoids, chromones, phenols, glycoside and triterpenoid.[15,17] Modern pharmacology investigations on *I. cylindrica* have indicated that several substances from *I. cylindrica.* exhibit a wide range of biological activities such as immunomodulatory,[18] antibacterial,[19] antitumor[20], antioxidant,[21] and liver protection activities both in *vitro*.[22] The fourth plant, *S. dichotomus* (Marantaceae) is also found in India, Myanmar, Thailand, Vietnam,

Malaysia, and the Philippines.[23-24] It is a shrub with dichotomously branched stems and grows up to 3-5 meters tall with oblong, lanceolate leaves.[25-26] *A. racemosus* Willd (Asparagaceae) is the fifth plant. The roots of this plant are commonly used in various medicinal preparations.[27] The plant is found all over India and is commonly known in Thailand as sam-sib or rak-sam-sib. In Ayurveda, the dried root of a plant is used as a tonic remedy to promote fertility and reduce menopausal symptoms.[27] The major active constituents of *A. racemosus* are steroidal saponins, which are found in the root.[28-29] It is also used for anti-oxytocic activities, antihepatotoxic, hepatopathy, dyspepsia, and dysentery.[30] Recent reports on the plant indicate that the root extracts show antioxidant and antidiarrheal activities in animal models.[31-32] The phytochemical analysis showed flavonoids, polyphenols, and vitamin C, which were found to exhibit the greatest antioxidant activity.[33] The main active constituents, are steroidal saponins (Shatavarins I–IV) Shatavarin IV is a glycoside of sarsasapogenin having two molecules of rhamnose and one molecule of glucose.[34] Root extracts have been shown to have phytoestrogen, antidiarrheal, anti-dyspepsia, adaptogenic, cardioprotective, antibacterial, immunological adjuvant, and antitussive properties.[33-34] The methanolic and aqueous extracts of roots have been produced in tablet form, root powder in tablet form, and root extract in syrup form.[33-34] The sixth plant, *C. rotang* (Palmaearecaceae) is a native plant of southwest Asia. The basal part of the plant grows vertically for 10 meters and horizontally for about 200 meters or more. The roots contain alkaloids and flavonoids that are used in convulsions and cramps.[35] C. *rotang* has shown several medicinal properties including anti-viral, anti-diabetic, and antiinflammatory effects.[35] In general, the roots of *C. rotang* are used in various ailments to cure piles, burning sensation, cough, leprosy, and bleeding disorders. Also, it was used in the treatment of inflammation and immunomodulatory activity in humans.[35] The seventh component is ammonium alum $(NH_4)_2SO_4.Al_2(SO_4)_3.24H_2O$, which is a compound that is used in Thai traditional medicine. Alum has astringent medicinal properties for topical and oral administration in Thai traditional medicine. However, the alum that is being used in Thai herbal preparation must be specially heated using a unique method called *"Satu"*.[36-37] The final component in the anti-diabetic recipe is potassium nitrate (KNO_3), which has found wide applications in Thai traditional medicine. It has been used to treat various diseases, such as astringents, detoxication, and blood purification.[38]

The present study was conducted to compare the *in vitro* antioxidant activity, α-glucosidase, and α-amylase inhibitory activities of extracts from a conventional formula (Recipe-I) containing 6 herbs and the substitution formula (Recipe-II) containing 8 herbs of a Thai traditional diabetic medicine.

Materials and Methods

Collection of plant materials

The six constituent medicinal plants of the recipe were collected from different areas in Thailand. *Momordica cochinchinensis* was harvested from Nakhon Pathom province (13°48'49.6"N 100°02'06.0"E). *Tiliacora triandra* and *Imperata cylindrica* were collected from Kalasin province (16°33'41.6"N 103°37'04.5"E). *Schumannianthus dichotomus* was collected from Phichit province (16°12'19.5"N 100°18'51.4"E). *Asparagus racemosus* was obtained from Prachinburi province (14°06'08.5"N 101°35'11.3"E). *Calamus rotang* was collected from Sakon Nakhon province Thailand, in December 2020 - January 2021 (17°22'44.8"N 103°40'50.4"E). The specimens were deposited at the Faculty of Medicine, Mahasarakham University, Thailand with the following codes: MSU.MED-MC0001/ST (*M. cochinchinensis*), MED-TT0001/ST (*T. triandra*), MED-IC0001/ST (*I. cylindrical*), MED-SD001/ST (*S. dichotomus*), MED-AR0001/ST (*A. racemosus*), and MED-CR0001/ST (*C. rotang*). Alum and potassium nitrate were purchased from Chakkrawat herbal drugstore in Bangkok, in January 2021 (13°45'18.0"N 100°29'34.8"E). All the raw materials were cleaned and dried at 60°C for 48 hr in a hot air oven (Binder, FED115, Germany) and kept in a cool, dry place in an airtight container until used.

Preparation of plant extracts

The Recipe-I consisted of *M. cochinchinensis*, *T. triandra*, *I. cylindrical*, *S. dichotomus*, *A. racemosus*, and *C. rotating* (1:1:1:1:1:1). *Momordica cochinchinensis*, *T. triandra*, *I. cylindrical*, *S. dichotomus*, *A. racemosus*, *C. rotang*, alum, and potassium nitrate (1:1:1:1:1:1:0.0000012:0.0030015) were used to formulate the Recipe-II. The aqueous extracts (AE) of the recipes were prepared by boiling the components with distilled water (1:10 w/v) for 10 min. The boiling process was repeated twice. The hydro-ethanolic (HE) and ethanolic extracts (EE) extracts were macerated with 50 and 95% ethanol (1:4 w/v), respectively, for 7 days. The residual powder was excluded by using filter paper (Whatman filter paper No.1, Germany). The filtrate was evaporated using a rotary evaporator (Heidolph Laborota 4000, Germany) and freezed-dried to obtain a dark brown extract. The extracts were kept in the refrigerator at -4°C until used.

Determination of total flavonoid content

The total flavonoid content (TFC) of the various plant extracts was estimated using the aluminum chloride colorimetric method developed by Yupparach *et al*.[39] The extracts from the recipe were mixed with 100 µL of 5% aluminum chloride (w/v), 400 µL of 2.5% $NaNO_3$ after 5 min, and 500 µL of 5% $AlCl_3$. Then, the mixture was allowed to stand at room temperature for 10 mins. The solution was mixed with 2,000 µL distilled water and the absorbance value was measured at 415 nm using a UV-visible spectrophotometer (Thermo Fisher Scientific, Genesys 150, USA). The TFC was calculated from a standard quercetin equivalent (mgQE/gExt).

Determination of total phenolic content

The total phenolic content (TPC) of the various plant extracts was determined according to a modified procedure by Konsue and Taepongsorat (2022).[40] An aliquot of 100 µL of each sample was oxidized with 500 µL of 0.2 N Folin-Ciocalteu's reagent and neutralized by adding 400 µL of 7.5% Na_2CO_3. The absorbance was measured at 765 nm after mixing at room temperature for 30 min. The results were expressed as gallic acid equivalents (mgQE/gExt).

Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacity of the various medicinal plant extracts was estimated spectrophotometrically following the procedure of Benzie and Strain (1996), which was modified by Rajurkar and Hande (2011).[42-43] In a reaction tube, ferric reducing antioxidant power (FRAP) reagent was prepared by mixing 300 mM acetate buffer, 10 mL TPTZ in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ in the proportion of 10:1:1 at 37°C. The FRAP reagent (900 µL) was mixed with 100 µL of the appropriately diluted plant extract and mixed thoroughly. After the solution was incubated at room temperature for 5 min, the absorbance was measured at 593 nm against a control. In the FRAP assay, the antioxidant potential of the sample was determined from a standard curve plotted using the Fe_3^+ and expressed as mg of Trolox equivalent per gram of the sample.

DPPH radical scavenging assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity of wheat extract was estimated by the reduction of the reaction color between the DPPH solution and sample extracts, as previously described.[41] DPPH was dissolved in ethanol to make a concentration of 0.039 mg/mL. The plant extract at various concentrations was diluted with distilled water to prepare the sample solution. The extract solution (100 µL) was mixed with 900 µL of 0.1 $mmolL^{-1}$ DPPH ethanolic solution. After 30 minutes, the reaction was kept in the dark at room temperature. Then, the absorbance of the solution was measured at 515 nm. This study used Trolox® and ascorbic acid as standard substances. Blanks were run in each assay. The scavenging ability of DPPH radical was expressed as IC_{50} (mg/mL) and the inhibition percentage was calculated using Equation 1.

% DPPH radical scavenging activity = $[(A_0-A_1)/ A_0] \times 100$ …… (Equation 1)

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

$ABTS^+$ radical scavenging assay

In the $ABTS^+$ assay, the plant extract was allowed to react with $ABTS^+$, a model stable free radical, derived from 2,2-azinobis (3-ethylvenzothiazolin-6-sulphonic acid). The $ABTS^+$ assay was performed as described by Namwong *et al*. (2020).[41] The $ABTS^+$ solution (900 µL) was added to the extract (100 µL) and thoroughly mixed. The mixture was held at room temperature for 6 min. and absorbance was immediately measured at 734 nm. Trolox® and ascorbic acid solution in 95% ethanol was prepared and assayed under the same conditions. The ABTS scavenging ability was expressed as IC_{50} (mg/mL) and the inhibition percentage was calculated using Equation (1).

α-glucosidase inhibitory assay

All extracts were tested for their ability to inhibit α-glucosidase using an *in vitro* assay. The assay was performed as described by Yupparach and Konsue's (2012),[39] with slight modifications. Briefly, an aliquot of 180 µL of sample solution and 150 µL of 0.1 M phosphate buffer (pH 6.8) containing α-glucosidase solution (0.2 U/mL) were incubated at 37ºC for 20 min. After pre-incubation, 150 µL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) were added to each well and incubated at 37ºC for another 20 mins. Then the reaction was stopped by adding 480 µL of 0.2 M Na_2CO_3 into each well and absorbance was read at 405 nm. The system without α-glucosidase was employed as blank and acarbose was used as a positive control. The α-glucosidase inhibitory activity was expressed as inhibition (%) and was calculated using Equation 1.

α-amylase inhibitory assay

The α-amylase inhibitory activity of the various extracts was determined according to Yupparach and Konsue (2022),[44] with some modifications. A volume of 150 µL of α-amylase dissolved in 50 mM phosphate buffer containing 20 mM NaCl and 0.2 mM $CaCl_2$ at pH 6.9 starch solution was mixed with 200 µL of the extracts. Thereafter 150 µL of the starch solution (2% in water [w/v]) was added to each tube and incubated for 10 mins at 37°C. The reaction was terminated by the addition of 500 µL of 1% DNSA reagent (0.05 g of sodium sulfate, 1 g of sodium hydroxide, 0.2 g phenol, and 1 g of 3,5-dinitrosalicylic acid solution) and was boiled for 10 mins in a water bath at 85-90°C. Subsequently, 40% tartrate solution (500 µL) was added to each tube after boiling and allowed to cool at room temperature. Absorbance was measured at 540 nm with a UV-visible spectrophotometer. The blank with 100% enzyme activity was prepared by substituting the extract with 150 µL of the buffer. A blank reaction was similarly prepared using the plant extract at each concentration in the absence of the enzyme solution. A positive control sample was prepared using acarbose and the reaction was performed similarly to the reaction with plant extract as mentioned above. The α-amylase inhibitory activity was expressed as inhibition (%) and was calculated using Equation 1.

Statistical analysis

The values from all assays were expressed as mean±standard deviation of the mean (SD) from five separate experiments (n = 5). Statistical analysis was carried out using the Statistical Package for Social Sciences (version 23; IBM). The data were analyzed by a one-way analysis of variance (ANOVA), followed by Duncan's multiple range tests. Differences at $p < 0.05$ were considered to be significant.

Results and Discussion

In each region of the world, there are different therapeutic methods for the treatment of various diseases according to geography, weather, living style, and natural resources. Thai traditional medicine is one of the most valuable heritages handed down from Thai ancestors. For the past decade, the government and private organizations have worked in collaboration to restore the values and popularity of Thai traditional medicine. Recently, the Ministry of Public Health has promoted the use of herbal medicine, and the Center for Herbal Information has collected data and performed scientific studies on this matter.[45]

Table 1: Phytochemical screening of different solvent extracts from the recipes

Sample	TPC	TFC
	mgGE/gExt	mgQE/gExt
Recipe-I-H_2O	13.830 ± 0.771^f	4.415 ± 0.137^d
Recipe-II-H_2O	14.855 ± 0.371^e	4.352 ± 0.142^d
Recipe-I-HE	37.025 ± 0.754^c	8.251 ± 0.162^c
Recipe-II-HE	35.134 ± 0.819^d	7.638 ± 0.143^c
Recipe-I-E	77.680 ± 1.283^b	20.900 ± 1.080^b
Recipe-II-E	99.123 ± 0.685^a	26.197 ± 1.164^a

TPC: Total phenol content; TFC: Total flavonoid content; Recipe-I-H_2O : Recipe-I- aqueous extract; Recipe-II-H_2O : Recipe-II- aqueous extract; Recipe-I-HE : Recipe-I- 50% ethanolic extract; Recipe-II-HE: Recipe-II- 50% ethanolic extract; Recipe-I-E Recipe-I- 95% ethanolic extract; Recipe-II-E: Recipe-II- 95% ethanolic extract; n = 5; Different letters indicated significant differences at $p < 0.05$.

Phytochemical screening of the various plant extracts

The total phenolic content in the test antidiabetic recipes showed that Recipe-II-E (99.123±0.685 mgGE/gExt) was significantly highest in amount than Recipe-I-E (77.680±1.283 mgGE/gExt), Recipe-I-HE (37.025±0.754 mgGE/gExt), Recipe-II-HE (35.134±0.819 mgGE/gExt), Recipe-II-H_2O (14.855±0.371 mgGE/gExt), and Recipe-I-H_2O (13.830±0.771 mgGE/gExt), as presented in Table 1. Concerning the total flavonoid content, it was observed that the Recipe-II-E (26.197±1.164 mgQE/gExt) was significantly highest in amount than Recipe-I-E (20.900±1.080 mgQE/gExt), Recipe-I-HE (8.251±0.162 mgQE/gExt), Recipe-II-HE (7.638±0.143 mgQE/gExt), Recipe-I-H_2O (4.415±0.137 mgQE/gExt) and Recipe-II-H_2O (4.352±0.142 mgQE/gExt), as shown in Table 1.

Traditional Thai medicine is a type of healing method derived from ancient Thai wisdom that has been used since antiquity.[45] The anti-diabetic recipe is in the Mor-ra-na-yan-nu-sut scripture that lists the components of medicinal plants, as well as their uses and dosages for treating diabetes.[1] Secondary metabolites from common plants have been proven to protect the body from UV radiation, diseases, and pests. These molecules are known as flavonoids or phenolic compounds.[46] The findings of the phytochemical screening revealed that the recipe also contains TPC and TFC, especially TFC originating from a combination of plants used to formulate the recipe that possesses flavonoid-like phytochemical components. A review of the literature revealed markers in the herbal ingredients of the recipe. Saponins, momordins I and II,[3] alkaloid,[11-12] coumarins, flavonoids, chromones, phenols glycoside and triterpenoid.[15,17] polyphenols, vitamin C,[33-34] and flavonoid,[35] are the principal bioactive substances in each herb used for the formulation.

Antioxidant activity of the various plant extracts

In FRAP assay, the results showed that Recipe-II-E (293.518±5.631 mgTE/gExt) was significantly more potent in reducing electrons than Recipe-I-E (273.100±4.589 mgTE/gExt), Recipe-II-HE (162.294±3.084 mgTE/gExt), Recipe-I-HE (124.506±7.184 mgTE/gExt), Recipe-I-H_2O (53.130±0.766 mgTE/gExt), and Recipe-II-H_2O (35.033±0.483 mgTE/gExt) as highlighted in Table 2. Concerning the DPPH free radical scavenging activity, standard substance, ascorbic acid (IC_{50} = 0.0162±0.0003), and Trolox® (IC_{50} = 0.0444±0.0008) showed more potent activity than all the different solvent extracts from the recipe. The Recipe-I-E (IC_{50} 0.116±0.002 mg/mL) was significantly more potent on free radical scavenging than Recipe-II-E, Recipe-I-HE, Recipe-II-HE, Recipe-I-H_2O and Recipe-II-H_2O (IC_{50} 0.121±0.008, 0.166±0.004, 0.116±0.002, 0.241±0.008, 0.421±0.006 and 0.432±0.021, mg/mL, respectively). From the results, ascorbic-like vitamin C is a good standard substance on anti-oxidation capacity assay in this method. (Table 2). In the $ABTS^+$ assay, the effect of free radical scavenging activity from all of the different solvent extracts was not different. On the other hand, the standard substances, ascorbic acid (IC_{50} = 0.0099±0.0002 mg/mL) and Trolox® (IC_{50} = 0.0230±0.0004 mg/mL)

showed significantly higher activity than the crude extracts from plant Recipe-II-E (IC_{50} = 0.013±0.013 mg/mL), Recipe-I-E (IC_{50} = 0.017±0.005 mg/mL), Recipe-I-HE (IC_{50} = 0.027±0.003 mg/mL), Recipe-II-HE (IC_{50} = 0.031±0.007 mg/mL), Recipe-I-H_2O (IC_{50} = 0.081±0.004 mg/mL), and Recipe-II-H_2O (IC_{50} = 0.082±0.005 mg/mL). In this way, ascorbic, vitamin C was even more effective than those on anti-oxidation (Table 2). Some phenolic chemical components in plants, such as alkaloids, flavonoids, tannins, saponins, and other phenolic contents, have strong DPPH free radical scavenging ability.[3, 33] The current study demonstrates that the phenolic-enriched extract obtained from the recipe displayed a notable ability to scavenge DPPH and ABTS radicals. Additionally, the extract exhibited a substantial capacity to reduce ferric ions, indicating its potent antioxidant properties.

α-glucosidase and α-amylase *inhibitory effects of the various plant extracts*

Surprisingly, Recipe-I-H_2O was significantly higher in α-glucosidase and α-amylase inhibitory activity (IC_{50} = 1.293±0.331 and 0.124 ± 0.007 mg/mL) compared to Recipe-I-HE (IC_{50} = 0.226±0.015 and 0.226 ± 0.015 mg/mL), Recipe-II-HE (IC_{50} = 0.528±0.059 and 0.528 ± 0.059 mg/mL), and Acarbose®, a positive control drug known for its an anti-diabetic activity (IC_{50} =1.070±0.075 and 0.173 ± 0.026 mg/mL) respectively. H**owever**, Recipe-II-H_2O, Recipe-I-E, and Recipe-II-E extracts were observed not to be α-glucosidase and α-amylase inhibitory (Table 3).

Table 2: Antioxidant activity of different solvent extracts from the recipe using different assays

Sample	FRAP mgTE/gExt	DPPH IC_{50} (mg/mL)	$ABTS^+$ IC_{50} (mg/mL)
Recipe-I-H_2O	53.130 ± 0.766^{e}	$0.421 \pm 0.006^{e,f}$	0.081 ± 0.004^{d}
Recipe-II-H_2O	35.033 ± 0.483^{f}	0.432 ± 0.021^{f}	0.082 ± 0.005^{d}
Recipe-I-HE	162.294 ± 3.084^{d}	0.166 ± 0.004^{d}	$0.027 \pm 0.003^{b,c}$
Recipe-II-HE	124.506 ± 7.184^{c}	0.241 ± 0.008^{e}	0.031 ± 0.007^{c}
Recipe-I-E	273.100 ± 4.589^{b}	0.116 ± 0.002^{c}	$0.017 \pm 0.005^{a,b,c}$
Recipe-II-E	293.518 ± 5.631^{a}	0.121 ± 0.008^{c}	$0.013 \pm 0.013^{a,b}$
Ascorbic	-	0.0162 ± 0.0003^{a}	0.0099 ± 0.0002^{a}
Trolox	-	0.0444 ± 0.0008^{b}	$0.0230 \pm 0.0004^{a,b,c}$

FRAP: Ferric reducing antioxidant power assay; DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay, $ABTS^+$: 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid assay, Recipe-I-H_2O : Recipe-I- aqueous extract; Recipe-II-H_2O : Recipe-II- aqueous extract; Recipe-I-HE : Recipe-I- 50% ethanolic extract; Recipe-II-HE: Recipe-II- 50% ethanolic extract; Recipe-I-E Recipe-I- 95% ethanolic extract; Recipe-II-E: Recipe-II- 95% ethanolic extract; which used Trolox® and ascorbic acid as standard substances; n = 5; Different letters indicated significant differences at $p < 0.05$.

Table 3: α-glucosidase and α-amylase inhibitory activities of different solvent extracts from the recipe expressed as IC_{50}.

Sample	Alpha-glucosidase IC_{50} (mg/mL)	Alpha-amylase IC_{50} (mg/mL)
Recipe-I-H_2O	1.293 ± 0.331^{b}	0.124 ± 0.007^{a}
Recipe-II-H_2O	N/A	N/A
Recipe-I-HE	1.527 ± 0.288^{b}	0.226 ± 0.015^{c}
Recipe-II-HE	5.324 ± 0.696^{C}	0.528 ± 0.059^{d}
Recipe-I-E	N/A	N/A
Recipe-II-E	N/A	N/A
Acarbose	1.070 ± 0.075^{a}	0.173 ± 0.026^{b}

Recipe-I-H_2O : Recipe-I- aqueous extract; Recipe-II-H_2O : Recipe-II- aqueous extract; Recipe-I-HE : Recipe-I- 50% ethanolic extract; Recipe-II-HE: Recipe-II- 50% ethanolic extract; Recipe-I-E Recipe-I- 95% ethanolic extract; Recipe-II-E: Recipe-II- 95% ethanolic extract; n = 5; N/A: Not available; Acarbose® was used as a positive control; Different letters indicated significant differences at $p < 0.05$.

As depicted in Table 3, the findings elucidate that Recipe-I-H_2O exhibited markedly elevated levels of inhibition against α-glucosidase and α-amylase enzymes compared to acarbose, a pharmaceutical drug used in diabetes management. These findings strongly suggest the potential of the recipe to possess natural antioxidants, α-glucosidase, and α-amylase activities. Phenolic compounds are a major class of bioactive components, which have been demonstrated to be better antioxidants *in vitro*.[46-47] Poly phenols possess the ideal chemistry for antioxidant activity because they have high reactivity as hydrogen or electron donors and also they are capable of chelating metal ions. Flavonoids, one of the major polyphenolic constituents of plants, are known for their efficient radical scavenging activity owing to their hydroxyl group at various positions.[48-51] Furthermore, the chemical composition of various plants in the recipe suggested that the study intended to separate carotenoids from *M. cochinchinensis*, which are well-known as widely used standard reagents for antioxidation.[5] The findings of the present study have revealed that the extracts derived from the recipe exert a more substantial effect on inhibiting the α-glucosidase enzyme compared to acarbose®, a well-known anti-diabetic medication. Several studies in the literature have documented that the ethanolic extract of *T. triandra* leaves effectively reduces blood glucose levels in diabetic rats over eight weeks. These findings indicate that the ethanolic extract of *T. triandra* employs distinct inhibitory mechanisms, which contribute to the inhibition of α-glucosidase activity. This, in turn, leads to a reduction in postprandial blood glucose levels in individuals with type-2 diabetes mellitus.[14] The overall findings of the present study showed that *C. rotatang* had comparable antidiabetic capabilities and would be an excellent candidate for the development of antidiabetic medications.[35] *T. triandra*, known for their constituents with reported hypoglycemic properties, have been recognized as containing alkaloid and flavonoid compounds. This composition could potentially contribute to their positive influence on carbohydrate metabolism in individuals with diabetes.[11-14]

Conclusion

The extracts from the recipe of the Royal Textbook of King Rama V in this study contained a high level of phenolic compounds, predominantly phenolic acids, and flavonoids, and displayed high antioxidant capacities in reagent-based assays. The extracts exhibited an array of potential health-enhancing properties, such as inhibition of α-glucosidase and α-amylase enzymes. The findings are in support of the traditional belief of the Thai ethnic population about the health-supporting functions of this recipe to treat diabetes mellitus. The other mechanisms of activities of this anti-diabetic recipe will be further investigated.

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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References

1. Fine Arts Department. Textbook of Medicine, Royal Issue of Rama V, Volume 2. Bangkok: Amarin Printing and Publishing Public Company; 1999.238-239. (in Thai)
2. Codex Alimentarius FAO-WHO, 2018. Codex General Standard for Food Additives (GSFA, Codex STAN 192-1995). Available online: http://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/gsfa/en (accessed on 16 April 2019).
3. Chainumnim S, Suksamrarn S, Jarintanan F, Jongrungruangchok S, Wannaiampikul S, Tanechpongtamb W. Sonicated extract from the Aril of *Momordica cochinchinensis* inhibits cell proliferation and migration in aggressive prostate cancer cells. J Toxicol. 2022;27:1-12.
4. Rahmatullah M, Biswas A, Haq WM, Seraj S, Jahan R. An ethnomedicinal survey of cucurbitaceae family plants used in the folk medicinal practices of Bangladesh 1. Chron Young Sci 2012; 3:212-222.
5. Chuyen HV, Roach PD, Golding JB, Parks SE, Nguyen MH. Optimization of extraction conditions for recovering carotenoids and antioxidant capacity from Gac peel using response surface methodology. Int J Food Sci Technol 2017; 52:972-980.
6. Mazzio E, Badisa R, Eyunni S, Ablordeppey S, George B, Soliman KFA. Bioactivity-guided isolation of neuritogenic factor from the seeds of the Gac plant (*Momordica cochinchinensis*). Evid Based Complement. Altern. Med. 2018; 8953958–8953958. doi: 10.1155/2018/8953958
7. Jung K, Chin YW, Yoon Kd, Chae HS, Kim CY, Yoo H, Kim J. Anti-inflammatory properties of a triterpenoidal glycoside from *Momordica cochinchinensis* in LPS-stimulated macrophages. Immunopharmacol Immunotoxicol 2013; 35:8-14.
8. Kang JM, Kim N, Kim B, Kim JH, Lee BY, Park JH, Lee MK, Lee HS, Kim JS, Jung HC, Song IS. Enhancement of gastric ulcer healing and angiogenesis by *Cochinchina momordica* seed extract in rats. J Korean Med Sci 2010; 25:875-881.
9. Das G, Gouda S, Kerry RG, Cortes H, Prado-Audelo ML, Leyva-Gomez G, Tsouh Fokou PV, Gutiérrez-Grijalva EP, Heredia JB, Shin HS, Patra JK. Study of traditional uses, extraction procedures, phytochemical constituents, and pharmacological properties of *Tiliacora triandra*. J Chem. 2022; 7:8754528. doi.org/10.1155/2022/8754528
10. Rattana S, Padungkit M, Cushnie B. Phytochemical screening, flavonoid content, and antioxidant activity of *Tiliacora triandra* leaf extracts. In Proceedings of the 2nd Annual International Conference of Northeast Pharmacy Research, 2010; 60-63.
11. Nutmakul T, Pattanapanyasat K, Soonthornchareonnon N, Shiomi K, Mori M, Prathanturarug S. Antiplasmodial stage specific activity of tiliacorinine and yanangcorinine, and their interaction effects with chloroquine. Planta Med Lett 2016; 81(S01):P921. http://doi.org/10.1055/s-0036-1596922.
12. Jongchanapong A, Singharachai C, Palanuvej C, Ruangrungsi N, Towiwat P. Antipyretic and antinociceptive effects of Ben-cha-Lo-Ka-Wi-Chian remedy. J Health Res 2010; 24(1):15-22.
13. Singharachai C, Palanuvej C, Kiyohara H, Yamada H, Ruangrungsi N. Pharmacognostic specification of five root species in Thai traditional medicine remedy: Ben- Cha-Lo-Ka-Wi-Chian. Phcog J. 2011; 3(21):1-11. http://doi.org/10.5530/pj.2011.21.1.
14. Katisart T, Rattana S. Hypoglycemic activity of leaf extracts from *Tiliacora triandra* in normal and streptozotocin-induced diabetic rats. Pharmacogn J. 2017; 9(5):621-625.
15. Liu RH, Chen SS, Ren G, Shao F, Huang HL. Phenolic compounds from roots of *Imperata cylindrica* var. major. Chinese Herbal Medicines. 2013; 5(3):240-243.
16. Jung YK, Shin D. *Imperata cylindrica*: A review of phytochemistry, pharmacology, and industrial applications. Molecules. 2021; 26(5):1454. https://doi.org/10.3390/molecules26051454.
17. Padma R, Parvathy NG, Renjith V, Kalpana PR, Rahate P. Quantitative estimation of tannins, phenols and antioxidant activity of methanolic extract of *Imperata cylindrica*. Int J Res Pharm Sci. 2013; 4(1):73-77.
18. Fu L, Chen L, Liu R, Chen D. Chemical constituents of *Rhizoma Imperatae* and their anti-complementary activity. J. Chin. Med. Mater. 2010; 33:1871-1874.
19. Koh KH, Tham FY. Screening of traditional Chinese medicinal plants for quorum-sensing inhibitors activity. J. Microbiol. Immunol. Infect. 2011; 44:144-148.
20. Zeng J, Liu X, Li X, Zheng Y, Liu B, Xiao Y. Daucosterol inhibits the proliferation, migration, and invasion of hepatocellular carcinoma cells via Wnt/beta-catenin signaling. Molecules. 2017; 22:862. https://doi.org/10.3390/molecules22060862.
21. Zhou XR, Wang JH, Jiang B, Shang J, Zhao CQ. A study of extraction process and in vitro antioxidant activity of total phenols from *Rhizoma Imperatae*. Afr. J. Tradit. Complement. Altern. Med. 2013; 10:175-178.
22. Yin YS, Ou J, Wei JZ, Li YQ, Li XL, Li QC, Li KH. Effects of *Rhizoma Imperatae* and its compound decoction on the model of IgA nephropathy in rats. Lishizhen Med. Mater. Med. Res. 2011; 11:2659-2662.
23. Chowdhury MS, Uddin MS, Haque F, Muhammed N, Koike M. Indigenous management of patipata (*Schumannianthus dichotoma*) plantation in the rural homesteads of Bangladesh. Subtrop Agric Res Dev 2007; 5(1):202-207.
24. Mohiuddin M, Rashid MH. Survival and growth of vegetatively grown pati-pata (*Schumannianthus dichotomas*): An exploratory study. J For Sci. 1988; 17:20-25.
25. Maneenoon K, Khuniad C, Teanuan Y, Saedan N, Prom-in S, Rukleng N, Kongpool W, Pinsook P, Wongwiwat W. Ethnomedicinal plants used by traditional healers in Phatthalung Province, Peninsular Thailand. J Ethnobiology Ethnomedicine.2015; 11:43. https://doi.org/10.1186/s13002-015-0031-5.
26. Chowdhury D, Konwar BKr. Morphophenology and karyotype study of Patidoi (*Schumannianthus dichotomus* (Roxb.) Gagnep. Synonym *Clinogyne dichotoma* Salisb.) – a traditional plant of Assam. Current science. 2006; 91(5):648-651.
27. Kongkaneramit L, Witoonsardsilp W, Peeungvicha P, Ingkaninan K, Waranuch N, Sarisuta N. Antioxidant activity and antiapoptotic effect of *Asparagus racemosus* root

extracts in human lung epithelial H460 cells. Exp Ther Med. 2011; 2(1):143-148.
28. Naquvi KJ, Dohare S, Shuaib M. *In vitro* antioxidant activity *Asparagus racemosus* root. Int J Biomed Res. 2011; 2(4):228-35.
29. Potduang B, Meeploy M, Giwanon R, Benmart Y, Kaewduang M, Supatanakul W. Biological activities of *Asparagus racemosus*. Afr J Tradit Complement Altern Med. 2008; 5(3):230-7.
30. Hayes PY, Jahidin AH, Lehmann R, Penman K, Kitching W, De Voss JJ. Structure revision of shatavarins I and IV, the major component from the roots of *Asparagus racemosus*. Terahedron Lett. 2006; 47:6965-6969.
31. Visavadiya N, Narasimhacharya RL. *Asparagus* root regulates cholesterol metabolism and improves antioxidant status in hypercholesteremic rats. 2009; 6(2):219-226.
32. Chopra RN, Chopra IC, Handa KL, Kapur LD. Indigenous drugs of India, Calcutta, Academic Publishers, 1994; 607.
33. Bopana N, Saxena S. *Asparagus racemosus*-Ethnopharmacological evaluation and conservation needs. J Ethnopharmacol 2007; 110(1):1-15.
34. Khare CP. Indian medicinal plants: an illustrated dictionary. Heidelberg: Springer, 2008; 72-73.
35. Gupta A, Chaphalka SR. Assessment of immunomodulatory activity of aqueous extract of *Calamus rotang*. Avicenna J Phytomed. 2017; 7(3):199-205.
36. Phrutivorapongkul A, Rungsimakan S. Quality evaluation of the exsiccated alum. J Thai Trad Alt Med. 2020; 18(3):573-583.
37. Picheansoonthon C. Medicinal elements. 2nded. Bangkok: Amarin Printing and Publishing Public Co., Ltd. 2013; 58-60. (in Thai)
38. Picheansoonthon C, Maenmas C, Jirawong V. Description of King Narai's medicines treatise. Bangkok: Amarin Printing and Publishing Public Co., Ltd, 2001.640-641. (in Thai)
39. Yupparach P, Sumalee A, Konsue A. Phytochemical screening and biological activities of a remedy from A-thi-sa-ra-wak scripture as a folkloric diabetic medicine. Trop J Nat Prod Res. 2022; 6(6):863-867.
40. Konsue A, Taepongsorat L. Phytochemical screening and antioxidant activity of longevity remedy from national Thai traditional medicine scripture (formulary special edition). Trop J Nat Prod Res. 2022; 6(6):868-871.
41. Namwong A, Thongkrajai P, Konsue A. Effect of a Thai folk recipe on phytochemical screening, antioxidant activities, and α-Glucosidase inhibition by different solvent extracts. Phcog Res. 2020; 12:225-229.
42. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal Biochem. 1996; 239:70-76.
43. Rajurkar NS, Hande SM. Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. Ind J Pharm Sci. 2011; 73(2):146-151.
44. Yupparach P, Konsue A. Phytochemical screening and antioxidant activities of *Cornukaempferia chayanii* extracts and *in vitro* inhibitory effects on digestive enzymes. Trop J Nat Prod Res. 2022; 6(5):705-708.
45. Chotchoungchatchai S, Saralamp P, Jenjittikul T, Pornsiripongse S, Prathanturarug S. Medicinal plants used with Thai traditional medicine in modern healthcare services: a case study in Kabchoeng Hospital, Surin province, Thailand. J Ethnopharmacol 2012; 141(1):193-205.
46. De la Rosa LA, Moreno-Escamilla JO, Rodrigo-Garcia J, Alvarez-Parrilla E. Phenolic compounds. In E. M. Yahia & A. Carrillo-Lopez (Eds.), Postharvest physiology and biochemistry of fruits and vegetables. 2019; 253-271.
47. Assefa ST, Yang EY, Chae SY, Song M, Lee J, Cho MC, Jang S. Alpha glucosidase inhibitory activities of plants with a focus on common vegetables. Plants. 2019; 9(1):2.
48. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002; 10(3):178-82.
49. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Method Enzymol. 1999; 299:152-178.
50. Ursini F, Mairorino M, Morazzoni P, Roveri A, Pifferi G. Antioxidant flavonoid (IdB 1031) affecting molecular mechanisms of cellular activation. Free Rad Biol Med. 1994; 16:547-553.
51. Long LH and Halliwell B. Oxidation and generation of hydrogen peroxide by thiol compounds in commonly used cell culture media. Biochem Biophys Res Commun. 2001; 286:991-994.