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ORIGINAL ARTICLE

Effect of a Thai Folk Recipe on Phytochemical Screening, Antioxidant Activities, and α-Glucosidase Inhibition by Different Solvent Extracts

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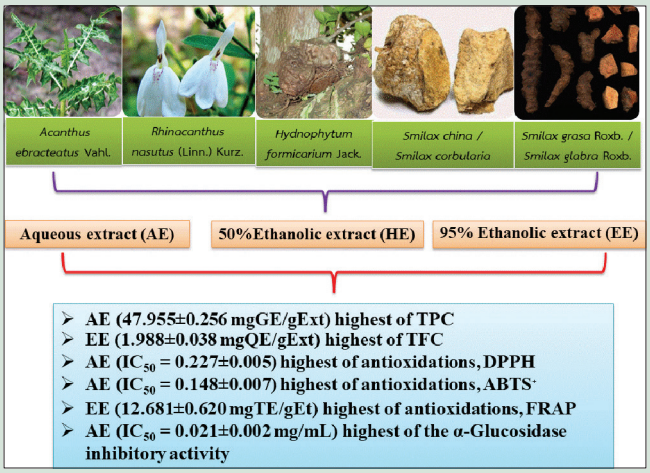
ABSTRACT

Context: Thai traditional medicine (TTM) has been widely use to treatment of various diseases. **Aims:** The aims of this study were determined on phytochemical screening, antioxidations, and α-glucosidase inhibition by different solvent extractions. **Materials and Methods:** The five medicinal plants from a TTM recipe were extracted using aqueous, 50% ethanol and 95% ethanol. The phytochemical screening were determined on total phenolic (TPC) and flavonoid (TFC) contents. Their antioxidant activities were tested using by 2,2-diphenyl-1-picrylhydrazy (DPPH) radical scavenging assay, 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS+) radical scavenging assay and ferric reducing antioxidant power (FRAP) assay. Glucose transferase mechanism was evaluated using by α-glucosidase inhibitory assay. **Results:** The results found that the recipe was ingredient with TPC and TFC. The aqueous extract (AE) (47.955 ± 0.256 mgGE/gExt) was significantly highest amount on TPC while, the ethanolic extract (EE) (1.988 ± 0.038 mgQE/gExt) was showed significantly highest amount on TFC. The antioxidations, DPPH, and the AE (IC_{50} = 0.227 ± 0.005) were significantly more potent on free radical scavenging. ABTS+, AE (IC_{50} = 0.148 ± 0.007), Hydro-ethanolic extract (HEE) (IC_{50} = 0.161 ± 0.008), and EE (IC_{50} = 0.151 ± 0.007) were not different on this method. FRAP, EE (12.681 ± 0.620 mgTE/gEt) were significantly more potent on cation radical reducing. The α-Glucosidase inhibitory activity, AE (IC_{50} = 0.021 ± 0.002 mg/mL) and HEE (IC_{50} = 0.076 ± 0.003 mg/mL) were significantly more potent on EE (IC_{50} = 0.292 ± 0.010 mg/mL) and Acarbose® (IC_{50} = 1.05 ± 0.110 mg/mL). **Conclusion:** The study confirms traditional use of a Thai folk herbal plants on antioxidation and α-glucosidase inhibition. The recipe was contained with also TPC and TFC might be more potential to antioxidant activities and anti-α-glucoxidase enzyme. Future study, we should be performed to clarify the mechanisms, major active compounds and *in vivo*.

Key words: Antioxidation, flavonoids, phenolic compounds, Thai folk recipe, α-glucosidase

SUMMARY

The recipe of Thai folk medicine in this study ingredient with phenolic compounds and flavonoid contents. The recipe were composed with phenolic compounds and flavonoids contents which chemical substance was more potent to antioxidation, and stronger to α-glucosidase inhibitory activity.



Abbreviations Used: TPC: Total phenolic content; TFC: Total flavonoid content; DPPH: 2,2-diphenyl-1-picrylhydrazy radical; ABTS+: 2,2 -azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical; FRAP: Ferric reducing antioxidant power; AE: Aquoes extract; HE: hydro-ethanolic extract; EE: Ethanolic extract; IC_{50}: Inhibitory concentration; mgGE/gExt: Gallic acid equivalent; mgQE/gExt: Quercetin equivalent; TE: Trolox equivalent.

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INTRODUCTION

Thailand, Land of Smiles, is a country in Southeast Asia's Indochina peninsula known as so many diversity of herbal plants. Thai ancestor bring them to folk medicine have been used to healing since the past until now. In each recipe will be consist with also equal of plant, dosage, herbal part, and indication use.[1] A recipe from Thai folk herbal medicine which ingredients with five medicinal plants. The recipe have been used to therapeutic many diseases including hypertension, cancer, cardiovascular disease, aging, and atherosclerosis especially diabetes.[2]

First plant, *Acanthus ebracteatus* Vahl. (*Acanthaceae*), Ngueak plaa mo, is a spiny plant which distributed throughout of Southeast Asia. Especially in Thai folk medicine, use all of parts of this plant have been treated of various diseases such as skin diseases, fever, cough, hypertension,

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hepatitis, asthma, arthritis, infectious diseases, and various types of cancers.[3-6] The plant consists of various chemical components including alkaloids, triterpenoids, steroids, glycosides, and polysaccharides. Some pharmacological activities were reported on antioxidantion, hepatoprotection, anti-inflammation, antitumor, antimutagenic, and anticarcinogen.[3,5,6] The active compounds of this plant were revealed that component with β-sitosterol, stigmasterol, lupeol, and benzoxazoline-2-one.[5]

Second plant, *Rhinacanthus nasutus* (L.) Kurz (*Acanthaceae*), Tong Pan Chang, is a medicinal shrub widely distributed in Southeast Asia. Thai folk medicine has been used this shrubs for the treatment of various diseases, such as cancer, fungal infections, skin diseases[7] rheumatism, eczema, pulmonary tuberculosis, influenza virus, liver diseases, peptic ulcers, helminthiasis, scurvy, inflammation, hypertension and obesity, leprosy, poison toxicity, herpes simplex virus, measles virus, and polio virus. The plant has been reported to pharmacological activities possess antiviral, antitumor, antiproliferative, anticancer, antimicrobial, anti-inflammatory, antidiabetic, and antioxidant activities.[8] Phytochemical studies revealed that the plant contains with secondary metabolites such as flavonoids, steroids, terpenoids, anthraquinones, lignin groups, and napthoquinones.[9,10]

Third plant, *Hydnophytum formicarum* (*Rubiaceae*), Hua Roi ROO, is a medicinal plant which is widely used in Southeast Asia, especially in Thailand. The plant lives as an epiphyte on big trees and develops mutualistic symbiosis with ants.[11] Pharmacological activity of this plant is antimicrobial and antioxidant activities,[11] anti-cancer, cure heart problems, treat chest pains, and as an anti-inflammatory.[12] The phytochemical analysis showed of alkaloids, saponins, flavonoids, essential oils, saponins, terpeniods, phenolic compounds, and resins.[13]

Fourth plant, *Smilax china* L. (*Smilacaceae*), Khoa Yen Nuea, is a climbing plant species which grows in the southern parts of Asia. Some literatures revealed that the plant was derived compound extract has various pharmacological properties including anti-inflammation, anti-cancer, antioxidantion[14], induced urination[15], rheumatic arthritis, injuries from falls, fractures, contusion, strain[16] anti-diabetic, anti-psoriatic, and digestive properties.[17] The main active components are stilbenes, flavonoids, polyphenols, and steroidal saponins.[14-17]

Final plant, *Smilax glabra* (Smilaceae), Khoa Yen Tai, is widely distributed in tropical and temperate regions throughout the world, especially in East Asia. It have been demonstrated that the rhizomes of the plant can be used to treatment many diseases including acute bacterial dysentery, colds, cancer, nephritis, mercury poisoning, rheumatoid arthritis, colitis and skin disorders, liver injury, hyperinsulinemia and cancer, antidiabetic, and jaundice.[18,19] The main chemical constituents of the plant rhizomes are includes isoflavone, taxifolin, astilbin, smitilbin, engeletin, dihydroquercetin, eurryphin, resveratrol, and 5-*O*-caffeoylshikimic acid.[19]

Flavonoids are phenolic compounds that widely found in nature and distributed in plant leaves, seeds, barks, and flowers.[20] The phenolic compounds are secondary metabolites present in different parts of the plants provide protection against the pathogens and also protect the plants from ultraviolet radiations.[21] Epidemiological and human intervention studies reported the potentially beneficial effects of phenolic rich plant foods against several chronic conditions including cardiometabolic diseases, neurodegeneration, and certain kinds of cancer. Supplementation with phenolic compounds may represent an effective means of providing potential bioactive compounds to consumers, as a part of a strategy to enhance the health benefits attributed to plant-based food products.[22]

Antioxidation is a chemical process which cause to stop oxidation of cells within human body.[23] Oxidative stress, which results from an improper balance between reactive oxygen species and their metabolites and antioxidant defense, is a factor in the pathogenesis of various diseases.[24] Antioxidant compounds act through several chemical mechanisms such as hydrogen atom transfer, single electron transfer, and the ability to chelate transition metals. The importance of antioxidant mechanisms is to understand the biological meaning of antioxidants, their possible uses, their production by organic synthesis or biotechnological methods, or for the standardization of the determination of antioxidant activity.[25] The reaction between hydrogen peroxide and superoxide radical yields the hydroxyl radical (•OH) which is highly reactive and damaging to most biomolecules.[26]

Enzymes, pancreatic α-glucosidase affect to glucose degradation and absorption. Rapid degradation of dietary starch by α-glucosidase leads to elevated postprandial hyperglycemia. It has been shown that activity of human pancreatic α-glucosidase in the small intestine correlates to an increase in postprandial glucose levels, the control of which is therefore an important aspect in treatment of Type II diabetes. Inhibitors of pancreatic α-glucosidase delay carbohydrate digestion causing a reduction in the rate of glucose absorption and lowering the postprandial serum glucose levels.[27,28] Acarbose is a synthetical α-glucosidase inhibitors know as are widely applied in the treatment of patients with Type II diabetes. On the other hand, the control of postprandial blood glucose surges is critical for the treatment of diabetes Type II.[29]

The study confirms the efficacy of a Thai folk herbal plants to antioxidation and α glucosidase inhibition. The revealed antioxidant activities and anti-α-glucoxidase enzyme, beneficial effects to biochemical profiles of the entire formula extract provided valuable insight for the next-step research of this herbal formula. Further investigations on the *in vivo* hypoglycemic effect of the whole formula extract and chemical compounds responsible for its effect should be performed to clarify the mechanisms and active compounds.

MATERIALS AND METHODS

Collection of plants materials

The five plants of the recipe were collected from different area in Thailand. *A. ebracteatus* was harvested from Samut Prakarn province. *R. nasutus* were collected from Prachin Buri provice. *H. formicarum* were collected from Trat provice. *S. china* and *S. glabra* were collected from Lum Phun provice. The specimens were identified and deposited at the Faculty of Medicine, Mahasarakham University, Thailand (code-; *A. ebracteatus*: MSU. MED-AE0001/AN, *R. nasutus*: MSU. MED-RN0001/AN, *H.formicarum*-:MSUMED-HF0001/AN,*S.china*-:MSU.MED-SC0001/AN and *S. glabra*-: MSU. MED-SG0001/AN). All of the raw materials were cleaned and dried at 60°C for 48 h in a hot air oven then powdered.

Preparation of extracts

The aqueous extracts (AE) of the recipe were prepared by boiling with distilled water for 10 min (1:10 w/v). The boiling process was repeated twice. The hydroethanolic and ethanolic extracts (EE) extracts were macerated with 50% ethanol and 95% ethanol for 7 days (1:4 w/v). The residue powder was excluded using filter papers (Whatman, Germany). The filtrate was evaporated using by a rotary evaporator (Heidolph Laborota 4000, Germany) and freeze-dried to obtain dark brown extract. The extracts were kept in refrigerator at −4°C until be used.

Phytochemical screening

Determination of total flavonoid content

Flavonoid content was estimated using the aluminum chloride colorimetric method Chang *et al*.(2002).[30] The extracts from recipe will be mixed with 100 μL of 5% aluminum chloride (w/v), 400 μL of

2.5% Na_2NO_3. After 5 min, 500 µL of 5% $AlCl_3$ (w/v). The mixture will be allowed to stand at room temperature for 10 min. The solution was mixed 2000 µL distilled water. The results were measured at 415 nm. The total flavonoid content (TFC) was calculated from a standard quercetin equivalent (mgQE/gExt).

Determination of total phenolic content

Total phenolic content was determined according to a modified procedure Singleton *et al.* (1999).[31] The sample (100 µL) will be 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 measured at 765 nm after mixed and incubated in room temperature for 30 min. The results were expressed as gallic acid equivalents (mgGE/gExt).

Antioxidations

2,2-Diphenyl-2-picrylhydrazyl radical scavenging assay

2,2-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacities of wheat extracts were estimated by the reduction of the reaction color between DPPH solution and sample extracts as previously described by prior method Ursini *et al*. (1994).[32] DPPH was dissolved in ethanol to a 0.039 mg/mL. DPPH was dissolved in ethanol to a 0.039 mg/mL. The plant extract at various concentrations was diluted with distilled water to get a sample solution. Then, 100 µL of the sample solution following which 900 µL DPPH (0.1 mM) working solution. After a 30 min, reaction kept in the dark at ambient temperature then the absorbance of the solution was measured at 515 nm. In this study, will be used Trolox® and ascorbic acid as standard substances. Blanks were run in each assay. DPPH radical ability was expressed as IC_{50} (mg/mL) and the inhibition percentage calculated using the following formula: DPPH scavenging activity (%) = $(A_0-A_1)/A_0 \times 100$ where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical scavenging assay

In 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS) assay, the plants extract will be allowed to react with ABTS+, a model stable-free radical derived from ABTS + assay was performed Long and Halliwell (2001).[33] B The $ABTS^+$ (900 µL) was added to the extracts (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 80% ethanol was prepared and assayed under the same conditions. ABTS scavenging ability was expressed as IC_{50} (mg/mL) and the inhibition percentage calculated using the following formula: ABTS scavenging activity (%) = $(A_0-A_1)/A_0 \times 100$ where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Ferric reducing antioxidant power assay

The antioxidant capacity of the medicinal plants was estimated spectrophotometrically following the procedure of Benzie and Strain (1996) applied by Rajurkar and Hande (2011).[34,35] This reaction is monitored by measuring the change in absorbance at 593 nm. The ferric reducing antioxidant power (FRAP) reagent was prepared by mixing 300 mM acetate buffer, 10 ml tripyridyl triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in the proportion of 10:1:1 at 37°C. Freshly prepared working FRAP reagent was pipetted using 1–5 ml variable micropipette and mixed with 5 µl of the appropriately diluted plant sample and mixed thoroughly. An intense blue color complex was formed when ferric TPTZ (Fe_{3+} TPTZ) complex was reduced to ferrous form and the absorbance at 593 nm was recorded against a reagent blank (3.995 ml FRAP reagent + 5 µl distilled water) after 30 min incubation at 37°C. The calibration curve was prepared by plotting the absorbance at 593 nm versus different concentrations of $FeSO_4$. The concentrations of $FeSO_4$ were in turn plotted against concentration of standard antioxidant Trolox®. The FRA*P* values were obtained by comparing the absorbance change in the test mixture with those obtained from increasing concentrations of Fe^{3+} and expressed as mg of Trolox equivalent per gram of sample.

α-Glucosidase inhibitory assay

All extracts were tested for their ability in inhibiting α-glucosidase using *in vitro* assay. The assay method was assessed using Dong *et al.* (2012)[36] assay with slight modifications. Briefly, a volume of 60 µL of sample solution and 50 µL of 0.1 M phosphate buffer (pH 6.8) containing α-glucosidase solution (0.2 U/mL) was incubated at 37°C for 20 min. After preincubation, 50 µL of 5 mM *p*-Nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.8) was added to each well and incubated at 37°C for another 20 min. Then, the reaction was stopped by adding 160 µL of 0.2 M Na_2CO_3 into each well and absorbance was readings (A) were recorded at 405 nm and compared to a control which had 60 µL of buffer solution in place of the extract. The system without α-glucosidase was used as blank and acarbose was used as a positive control. The α-glucosidase inhibitory activity was expressed as inhibition (%) and was calculated as follows: % inhibition = $(A_0-A_1)/A_0 \times 100$ where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. IC_{50} values were calculated by the graphic method.

Statistical analysis

All assays were expressed as mean ± standard deviation from five separate experiments (n = 5). Statistical analysis was carried out using one-way analysis of variance followed by Duncan's multiple range tests. Differences at $P < 0.05$ were considered to be statistically significant.

RESULTS

Phytochemical screening

Total phenolic contents

Total phenolic contents in this recipe were showed that the AE (47.955 ± 0.256 mgGE/gExt) was significantly highest amount than HEE (34.609 ± 0.192 mgGE/gExt) and EE (38.667 ± 0.797 mgGE/gExt) [Table 1].

Total flavonoid contents

Total flavonoid contents in this recipe were showed that the EE (1.988 ± 0.038 mgQE/gExt) was significantly highest amount than AE (1.188 ± 0.047 mgQE/gExt) and HEE (0.772 ± 0.013 mgQE/gExt), respectively [Table 1].

Antioxidant activities

2,2-Diphenyl-2-picrylhydrazyl radical scavenging activity

DPPH-free radical scavenging activity, standard substances, ascorbic acid (IC_{50} = 0.016 ± 0.0003), and Trolox® (IC_{50} = 0.044 ± 0.0008)

Table 1: Total phenolic and flavonoid contents of different solvent extracts from the recipe

Different solvent extracts	TPC (mgGE/gExt)	TFC (mgQE/gExt)
Aqueous extract	47.955±0.256[b]	1.188±0.047[b]
Hydroethanolic extract	34.609±0.192[a]	0.772±0.013[a]
Ethanolic extract	38.667±0.797[a]	1.988±0.038[c]

TPC was measured with gallic acid equivalents (mgGE/gExt). TFC was measured with quercetin equivalent (mgQE/gExt). Different letters indicated significantly difference at P<0.05. TPC: Total phenolic content; TFC: Total flavonoid content

Table 2: Antioxidant and α-glucosidase inhibitory activities showed inhibitory concentration$_{50}$ of different solvent extracts from the recipe

Different solvent extracts and standard substances	DPPH (IC_{50}=mg/mL)	ABTS (IC_{50}=mg/mL)	FRAP (mg=TE/gExt)	α-Glucosidase (IC_{50}=mg/mL)
Aqueous extract	0.227±0.005^{c}	0.148±0.007^{c}	9.543±0.440^{b}	0.021±0.002^{a}
Hydro-ethanolic extract	0.334±0.005e	0.161±0.008^{c}	6.416±0.255^{a}	0.076±0.003^{a}
Ethanolic extract	0.271±0.001d	0.151±0.007^{c}	12.681±0.620^{c}	0.292±0.010^{b}
Ascorbic acid	0.016±0.0003^{a}	0.010±0.0002^{a}	-	-
Trolox®	0.044±0.0008^{b}	0.023±0.0004^{b}	-	-
Acarbose®	-	-	-	1.05±0.110^{c}

DPPH radical scavenging, ABTS^{+} and FRAP assay were used trolox® and ascorbic acid as standard substances. The α-glucosidase inhibitory system was used Acarbose® as positive control. Different letters indicated significantly difference at P<0.05. DPPH: 2,2-diphenyl-1-picrylhydrazy; FRAP: Ferric reducing antioxidant power; IC_{50}: Inhibitory concentration$_{50}$; ABTS: 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate)

were showed more potent than all of different solvent extracts from the recipe. The AE (IC_{50} = 0.227 ± 0.005) was significantly more potent on free radical scavenging than EE (IC_{50} = 0.271 ± 0.001) and HEE (IC_{50} = 0.334 ± 0.005), respectively. In this experiment, ascorbic like Vitamin C is a good standard substance on antioxidation capacity assay in this method [Table 2].

2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical scavenging activity

ABTS + assay, the effect of free radical scavenging activity from all of different solvent extracts were not different. On the other hand, the standard substances, ascorbic acid (IC_{50} = 0.010 ± 0.0002) and Trolox® (IC_{50} = 0.023 ± 0.0004) were showed significantly higher the crude extract from plants AE (IC_{50} = 0.148 ± 0.007), HEE (IC_{50} = 0.161 ± 0.008) and EE (IC_{50} = 0.151 ± 0.007). The ascorbic, Vitamin C was yet continuous more effect highest than those on antioxidation in this method [Table 2].

Ferric reducing antioxidant power activity

In FRAP assay, the experiment showed that EE (12.681 ± 0.620 mgTE/gEt) was significantly more potent to reducing electron than AE (9.543 ± 0.440 mgTE/gEt) and HEE (6.416 ± 0.255 mgTE/gEt), respectively [Table 2].

α-Glucosidase inhibitory activity

In this experiment, surprisingly, AE (IC_{50} = 0.021 ± 0.002 mg/mL) and HAE (IC_{50} = 0.076 ± 0.003 mg/mL) were significantly more possess on α-glucosidase inhibition than EE (IC_{50} = 0.292 ± 0.010 mg/mL) and Acarbose (IC_{50} = 1.05 ± 0.110 mg/mL), positive substance known as an antidiabetic drug, respectively [Table 2].

DISCUSSION

In each region in the world, there are different therapeutic method to treatment of various diseases according to the geographic, weather, living style, and natural resources. Thai folk traditional medicine (TTM) is a kind of alternative medicine that it has been from Thai ancient inherited until now. Some recipes in TTM not yet any scientific reported. In the present study, we selected a Thai folk medicinal recipe from TTM for examined to pharmaceutical activities.

In our study, we found amount of both TPC and TFC in the mixture plant extract.[37,38] Review literatures were reported to isolating of bioactive flavonoid and phenolic compounds: Isoliquiritigenin, protocatechualdehyde, butin, and butein from a plant of this recipe, *H. formicarum* can serve as a new source enriched with potent antioxidative agents on the DPPH assay, the crude received from ethyl acetate extraction exhibits highest radical scavenging activity.[39] Their phenolics chemical constituents of some plant included flavonoids and tannins and other phenolic contents showed high DPPH-free radical scavenging activity.[40] In the present study, phenolic-enriched extract of the recipe exhibited obviously scavenging capacity for DPPH and ABTS radicals as well as significant reducing power for ferric ion. These findings strongly suggest the potential of recipe as a natural antioxidant and α-glucosidase activity. Phenolic compounds are a major class of bioactive components, which have been demonstrated to be better antioxidants *in vitro*. 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, were known for their efficient radical scavenging activity owing to their hydroxyl group at various positions.[41] In addition, chemical composition of some plant from the recipe was revealed that the study intends to isolate carotenoids from *R. nasutus* that carotenoids know widely used as an antioxidation standard reagent.[42]

Our study showed that the extract from the recipe has more effect on α-glucosidase enzyme inhibition than Acarbose® know as anti-diabetic drug. Some literature were reported to the phenolic compounds from *R. nasutus* leaf extract has been have rhinacanthus-rich extracts which a semipurified that contains 60% w/w of rhinacanthin-C reduced the fasting blood glucose levels. These finding suggest that the combination of rhinacanthus -rich extracts having different inhibitory mechanisms could be inhibit α-glucosidase activity, resulting in a reduction of postprandial blood glucose in type-2 DM.[43] The overall results indicated that *R. nasutus* has equivalent antidiabetic potential that might be suitable Candidate for antidiabetic drug.[44] *S. china*, the plant constituents reportedly possessing hypoglycemic activity have been identified as flavonoids and miscellaneous compounds could be due to a beneficial effect on carbohydrate metabolism in diabetes.[45,46]

CONCLUSION

In the present study, free radical scavenging capability assays (DPPH and ABTS) and FRAP determination were carried out to evaluate the antioxidant ability of the recipe. The results were demonstrated that the recipe possesses valuable antioxidant and α-glucosidase inhibitory activities. The results in the present study support the pharmacological basis of the recipe to type II diabetes treatment. The biological activities were confirmed to indication use of this recipe from Thai folk medicine. Next study, chemical compositions, major active compound(s) and *in vivo* will be clarified.

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Conflicts of interest

There are no conflicts of interest.

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