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

Anirut Namwong, Pramote Thongkrajai¹, Ampa Konsue²

Ph.D. Candidate in Health Sciences Program, Faculty of Medicine, Mahasarakham University, ¹Health Sciences, Faculty of Medicine, Mahasarakham University, ²Thai Traditional Medicine Research Unit, Applied Thai Traditional Medicine, Faculty of Medicine, Mahasarakham University, Maha Sarakham, Thailand

**ABSTRACT**

Context: Thai traditional medicine (TTM) has been widely used to treatment of various disease. 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 2,2-diphenyl-1-picrylhydrazyl 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 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 (IC₅₀ = 0.227 ± 0.005) were significantly more potent on free radical scavenging. ABTS⁺, AE (IC₅₀ = 0.148 ± 0.007), Hydro-extract ethane (HEE) (IC₅₀ = 0.161 ± 0.008), and EE (IC₅₀ = 0.161 ± 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 (IC₅₀ = 0.021 ± 0.002 mg/mL) and HEE (IC₅₀ = 0.292 ± 0.010 mg/mL) and Acarbose® (IC₅₀ = 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 antioxidation activities and anti-α-glucosidase 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 flavonoid contents which chemical substance was more potent to antioxidation, and stronger to α-glucosidase inhibitory activity.

**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), Nguetak 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, diabetes.²

Abbreviations Used: TPC: Total phenolic content; TFC: Total flavonoid content; DPPH: 2,2-diphenyl-1-picrylhydrazyl radical; ABTS⁺: 2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonate) radical; FRAP: Ferric reducing antioxidant power; AE: Aqueous extract; HE: hydro-ethanolic extract; EE: Ethanolic extract; IC₅₀: Inhibitory concentration; mgGE/mL: Gallic acid equivalent; mgQE/gExt: Quercetin equivalent; mgTE/gEt: Trolox equivalent.

Correspondence: Dr. Ampa Konsue, Thai Traditional Medicine Research Unit, Applied Thai Traditional Medicine, Faculty of Medicine, Mahasarakham University, Maha Sarakham, 44000, Thailand. E-mail: ampa_ice@hotmail.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com
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 antioxidant, hepatoprotection, anti-inflammation, antitumor, antimutagenic, and anticarcinogenic.[5,3,4] The active compounds of this plant were revealed that component with β-sitosterol, stigmasterol, lupeol, and benzoxazoline-2-one.[2]

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, antiinflammatory, anticancer, antimicrobial, anti-inflammatory, anti diabetic, and antioxidant activities.[5] Phytochemical studies revealed that the plant contains with secondary metabolites such as flavonoids, steroids, terpenoids, anthraquinones, lignin groups, and napthoquinones.[2,5,6]

Third plant, Hydnophytum formicarum (Rubiacaeae), 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 in tropical and temperate regions throughout the world, especially in Thailand. It has been demonstrated that the rhizomes of the plant can be used to treat 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.[2,3,4] The phytochemical analysis of the plant revealed that component with phenolic compounds may represent an effective means of providing potential bioactive compounds to consumers, as a part of a strategy to protect against disease and to improve health. The active compounds of this plant were revealed that component with a-glucosidase delay carbohydrate digestion causing a reduction in the rate of glycemia and lowering the postprandial serum glycemia levels.[25,26] Acarbose is a synthetic a-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.[27]

The study confirms the efficacy of a Thai folk herbal plants to antioxidation and a-glucosidase inhibition. The revealed antioxidation activities and anti-a-glucosidase 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 Prakan province. R. nasutus were collected from Prachin Buri province. H. formicarum were collected from Trat province. S. chinensis and S. glabra were collected from Lum Phn province. The specimens were identified and deposited at the Faculty of Medicine, Mahasarakham University, Thailand (code; S. ebracteatus: MSU. MED-JF0001/AN, R. nasutus: MSU. MED-RN0001/AN, H. formicarum: MSUMED-HF0001/AN,S. chinensis: 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 Formica, Germany) and freeze-dried to obtain dark brown extract. The extracts were kept in refrigerator at –4°C until used.

Phytochemical screening

Determination of total flavonoid content

Phenolic content was estimated using the aluminum chloride colorimetric method Chang et al.[20] The extracts from recipe will be mixed with 100 µL of 5% aluminum chloride (w/v), 400 µL of
2.5% Na₂NO₃. After 5 min, 500 µL of 5% AlCl₃ (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% Na₂CO₃. 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, we will be used Trolox® and ascorbic acid as standard substances. Blanks were run in each assay. DPPH radical ability was expressed as IC₅₀ (mg/mL) and the inhibition percentage calculated using the following formula: DPPH scavenging activity (%) = (A₀−A₁)/A₀ × 100 where A₀ is the absorbance of the control and A₁ 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 IC₅₀ (mg/mL) and the inhibition percentage calculated using the following formula: ABTS scavenging activity (%) = (A₀−A₁)/A₀ × 100 where A₀ is the absorbance of the control and A₁ 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).[24,25] 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 tripriyld triazine (TPTZ) in 40 mM HCl and 20 mM FeCl₃·6H₂O 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 (Fe³⁺·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 FeSO₄. The concentrations of FeSO₄ were in turn plotted against concentration of standard antioxidant Trolox®. The FRAP values were obtained by comparing the absorbance change in the test mixture with those obtained from increasing concentrations of Fe³⁺ 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 Na₂CO₃ 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 = (A₀−A₁)/A₀ × 100 where A₀ is the absorbance of the control and A₁ is the absorbance of the sample. IC₅₀ 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 the EE (1.188 ± 0.047 mgQE/gExt) was significantly highest amount than AE (1.988 ± 0.038 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 (IC₅₀ = 0.016 ± 0.0003), and ‘Trolox’ (IC₅₀ = 0.044 ± 0.0008).

<table>
<thead>
<tr>
<th>Different solvent extracts</th>
<th>TPC (mgGE/gExt)</th>
<th>TFC (mgQE/gExt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous extract</td>
<td>47.955±0.256</td>
<td>1.188±0.047</td>
</tr>
<tr>
<td>Hydroethanolic extract</td>
<td>34.609±0.192</td>
<td>0.772±0.013</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>38.667±0.797</td>
<td>1.988±0.038</td>
</tr>
</tbody>
</table>

TPC was measured with gallic acid equivalents (mgGE/gExt). TFC was measured with quercetin equivalent (mgQE/gExt). Different letters indicated significantly different at P<0.05. TPC: Total phenolic content; TFC: Total flavonoid content.
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 antioxidant capacity assay in this method [Table 2].

2,2-azobisis-(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 higher 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 mg/TE/gEt) was significantly more potent to reducing electron than AE (9.543 ± 0.440 mg/TE/gEt) and HEE (6.416 ± 0.255 mg/TE/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.[17,18] Review literatures were reported to isolating of bioactive flavonoid and phenolic compounds: Isoliquiritigenin, protocatechuicdehyde, 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.[19] Their phenolics chemical constituents of some plant included flavonoids and tannins and other phenolic contents showed high DPPH-free radical scavenging activity.[20] 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 antioxidation 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.[21] 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.[22]

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.[23] The overall results indicated that R. nasutus has equivalent antidiabetic potential that might be suitable Candidate for antiabetic drug.[24] S. chinensis, 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.[25,26]

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.

Acknowledgements

The authors thank Faculty of Medicine, Mahasarakham University, Maha Sarakham, Thailand for financial supported.

Financial support and sponsorship

The study was partially financially supported by the Faculty of Medicine, Mahasarakham University, Maha Sarakham, Thailand.

Conflicts of interest

There are no conflicts of interest.

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

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

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


2. Nantasenee, C. Diabetes Mellitus in Thai Traditional Medicine Perspective. Thesis submitted to M.Sc., Faculty of Eastern Medicine, Rang Sit University; 2013.


