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ORIGINAL ARTICLE

Biological Screening of Tri-Jannarose as a Recipe from Thai Traditional Medicine

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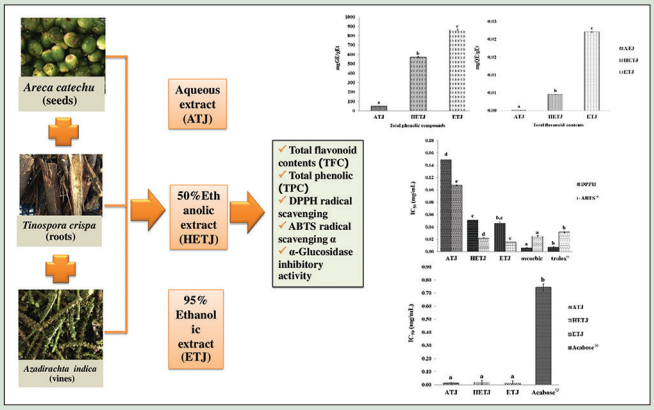
ABSTRACT

Context: Tri-Jannarose (TJ) is a Thai traditional medicine recipe, the ingredients of which are betel palm seed (*Areca catechu* L.), Siamese neem tree root (*Azadirachta indica* A. Juss.), and heart-leaved moonseed vines (*Tinospora cordifolia* [Thunb.] Miers). The equal mixture of three plants indicated to treatment of antipyretic, diuretic, expectorate, nourishment and appetizing. **Aims**: Phytochemical screening, antioxidant, and α-glucosidase inhibitory activities of TJ using different extractions were evaluated. **Materials and Methods:** The three plants of recipe were extracted using different solvents such as aqueous extract (ATJ), 50% ethanolic extract (HETJ), and 95% ethanolic extract (ETJ). The phytochemical screening was determined by total phenolic compounds and total flavonoid contents. The anti-oxidation were tested using by 2,2-diphenyl-1-picrylhydrazy (DPPH) radical scavenging and 2,2 -azinobis-(3-ethylbenzothiazoline-6-sulphonate) ($ABTS^+$) assay. The α-glucosidase inhibitory activity was determined for glucose transferase mechanism. **Results:** Phytochemical screening found that this recipe had both phenolic and flavonoid substances. The ETJ (IC_{50} = 0.0463 ± 0.002) was exerted on antioxidation higher than HETJ and ATJ (IC_{50} = 0.0511 ± 0.000 and 0.1485 ± 0.005 mg/mL). Surprisingly, $ABTS^+$ assay, ETJ (IC_{50} = 0.015 ± 0.000 mg/mL), and HETJ (IC_{50} = 0.022 ± 0.000 mg/mL) showed high effect on free radical scavenging activity than the standard controls, ascorbic acid (IC_{50} = 0.025 ± 0.001 mg/mL), and Trolox (IC_{50} = 0.032 ± 0.001 mg/mL). The α-glucosidase inhibitory activity found that all of the extract including ATJ (IC_{50} = 0.0127±0.02 mg/mL), ETJ (IC_{50} = 0.0154±0.01 mg/mL) and ETJ (IC_{50} = 0.0202±0.01 mg/mL) were more potent to inhibit α-glucosidase emzyme than acarbose (IC_{50} =0.745±0.026 mg/mL) as a positive control. **Conclusion:** The pharmaceutical preliminary scarring was confirmed to treatment on Thai traditional medicine. The recipe composed with phenolic compounds and flavonoids contents which chemical substance were more potent anti-oxidation, and bittersweet flavor was stronger to α-glucosidase inhibitory activity.

Key words: Antioxidant, phytochemical screening, Thai traditional medicine, Tri-Jannarose, α-glucosidase

SUMMARY

- Tri-Jannarose is a Thai traditional medicine ingredient with phenolic compounds and flavonoid contents. The recipe composed with phenolic compounds and flavonoids contents which chemical substance was more potent to anti-oxidation, and bittersweet flavor was stronger to α-glucosidase inhibitory activity.



Abbreviations Used: TPC: Total phenolic compound, TFC: Total flavonoid content, DPPH: 2,2-diphenyl-1-picrylhydrazyl assay, $ABTS^+$: 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) assay, TJ: Tri-Jannarose, ATJ: Tri-Jannarose extracted with aqueous, HETJ: Tri-Jannarose extracted with 50% ethanol, ETJ: Tri-Jannarose extracted with 95% ethanol, *A. catechu: Areca catechu* L., *A. indica: Azadirachta indica* A. Juss, *T. cordifolia*: *Tinospora cordifolia* (Thunb.) Miers.

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INTRODUCTION

Thai traditional medicine is folklore medicine inherited from Thai ancestor. The drugs have been used for healing since the past until now. In each recipe might be consist with also approximately of some plant, some dosage, some herbal part and some indication to disease treatment. TJ is once recipe from Thai traditional medicine which ingredient composed with Sai-mark/betel palm seed (*Areca catechu* HYPERLINK "https://th.wikipedia.org/wiki/Carolus_Linnaeus"L.), Rak-Sa-Doa/ Siamese neem tree root (*Azadirachta indica* A. Juss.) and Toa-Bore-Ra-Pet/heart leaved moonseed vines (*Tinospora cordifolia* (HYPERLINK "https://th.wikipedia.org/w/index.php?title=Thunb.&action=edit&redlink=1"Thunb.) Miers) (w:w:w =1:1:1). Thai ancestor still believed that the bittersweet flavor of this recipe has been claimed usage to treatment of many diseases such as antipyretic, diuretic, expectorate, nourishment and appetizing.[1]

The review literature of each plant in the recipe was revealed in many publications. Betel palm (*A. catechu* L., family: Palmaceae) was a slender single trunk, 30-meter tall, and about 20-cm wide.[2] The most important seeds of these biologically active constituents of plants are alkaloids, flavonoids, tannin, triterpenes, fatty acids, and mineral and phenolic compounds.[3,4] The plant invites to attention of many researchers

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worldwide for its wide range of pharmacological activities included anti-oxidantion,[5-9] Antihyperglycemia,[10,11] anti-inflammation,[12] hypolipideamia,[13-15] α-glucosidase inhibitory, wound healing[16] and anticonvulsant.[17]

Siamese neem tree (*A. indica* A. Juss.) is a member of the Meliaceae family and it can grow into a big tree to a height of about 20–35 m. The tree has a complex of various constituents including nimbin, nimbidin, nimbolide, and limonoids, and such types of ingredients play a role in disease management through modulation of various genetic pathways and other activities. Quercetin and β-sitosterol were purified from fresh leaves and were known for antifungal and antibacterial activities.[18] Diterpenoids from root have reported antibacterial, antifungal, and anti-inflammation properties.[19,20] Earlier investigators have confirmed their role as anti-inflammatory, anti-arthritic, antipyretic, hypoglycemic, antigastric, antifungal, antibacterial, and antitumor activities.[21-23]

Heart-leaved moonseed vines (*T. cordifolia* [Thunb.] Miers) is in the family Menispermaceae and has a long medical history of being used as an antidiabetic remedy. This plant component contained the natural substances such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds, and polysaccharides. The plant were reported to possess hypoglycemic, anti-inflammatory immunomodulatory[24-26] anticholinesterase[27] antibacterial, antifilarial,[28-34] antioxidant,[35,36] and anti α-glucosidase[37] activity.

Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage. Although the antioxidant defenses are different from species to species, the presence of the antioxidant defense is universal. Antioxidants exist both in enzymatic and in nonenzymatic forms in the intracellular and extracellular environments.[38] Oxidative stress is characterized as an imbalance between the production of reactive species and antioxidant defense activity, and its enhanced state has been associated with many of the chronic diseases such as cancer, diabetes, and neurodegenerative and cardiovascular diseases. Based on that, many research groups have driven efforts to assess the antioxidant properties of natural products. These properties have been investigated through either chemical *(in vitro)* or biological *(in vivo)* methods, or both. The results of these researches have led some to suggest that the long-term consumption of food rich in antioxidants can retard or avoid the occurrence of many diseases.[39]

α-Glucosidase is well-renowned as a therapeutic target for the modulation of postprandial hyperglycemia. The α-glucosidase inhibitors were used in treatment to delay or interrupt gastrointestinal digestion of oligosaccharides and subsequent release of glucose. This consequently delays or reduces glucose absorption into the bloodstream following food uptake.[40] Acarbose, miglitol, and voglibose were used antiglucosidase drugs that are orally administered for the management of postprandial hyperglycemia. Pending to their undesirable side effects, there is great interest in the middle of researchers worldwide to explore for alternative glucosidase inhibitors.

However, TJ recipe was widely used to treat many diseases, but there is no any scientific report. Therefore, this study were propose in investigate phytochemical screening, anti-oxidant activity and α-glucosidase inhibitory to for confirmed pharmaceutical preliminary.

MATERIALS AND METHODS

Collection of plant materials

The three plants in TJ were collected from Kalasin Province, northeastern Thailand. The specimens were identified and deposited at the Faculty of Medicine, Mahasarakham University, Thailand (code; *A. catechu*: MSU.MED-AC0001/SS, *Tinospora crispa*: MSU.MED-TC0001/SS, and *A. indica*: MED-AI0001/SS). All of the fresh materials were cleaned and dried at 60°C for 48 h in a hot air oven and then powdered.

Preparation of extracts

The ATJ extracts were prepared by boiling with distilled water for 15 min (1:10 w/v). The boiling process was repeated twice. The HETJ and ETJ extracts were macerated with 50% ethanol and 95% ethanol for 7 days (1:4 w/v). The residue powder was excluded using filter papers. The filtrate was evaporated using a rotary evaporator (Heidolph Laborota 4000, Germany) and freeze-dried to obtain a dark brown extract. The extracts were kept in the fridge at -20°C until used.

Total phenolic content assay

Total phenolic content was determined according to a modified procedure.[41] Sample (100 μL) will be oxidized with 500 μL of 0.2-N Folin–Ciocalteu reagent and neutralized by adding 400 μL of 7.5% Na_2CO_3. The absorbance was measured at 765 nm after mixing and incubated in room temperature for 30 min. The results were expressed as gallic acid equivalents (mgGE/gExt).

Total flavonoid content assay

Flavonoid content was estimated using the aluminum chloride colorimetric method.[42] The extracts from recipe (100 μL) will be mixed with 500 μL of 2.5% $NaNO_2$. After 5 min, 500 μL of 5% $AlCl_3$ (w/v) was added. The mixture will be allowed to stand at room temperature for 10 min. The solution was mixed with 2,000-μL distilled water. The results were measured at 415 nm. The total flavonoid content (TFC) was calculated from a standard quercetin equivalent (mgQE/gExt).

DPPH free radical scavenging activity

2,2-diphenyl-1-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.[43] 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, the absorbance of the solution was measured at 515 nm. In this study, we will use 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 was 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-ethylbenzothiazoline-6-sulfonate radical scavenging activity

The extract will be allowed to react with $ABTS^+$, a model stable-free radical derived from 2,2-azinobis (3-ethylvenzothiazolin-6-sulphonic acid) ($ABTS^+$) assay was performed.[44] 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 was 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.

α-Glucosidase inhibitory activity

All extracts were tested for their ability in inhibiting α-glucosidase using *in vitro* assay. The assay method was assessed using Dong *et al.* assay[45] with slight modifications. Briefly, a volume of 60 μL of the sample solution and 50 μL of 0.1-M phosphate buffer (pH 6.8) containing

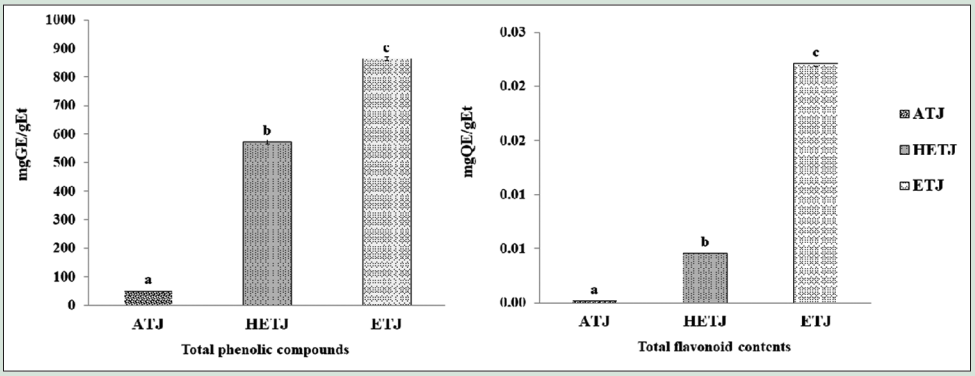

Figure 1: Phytochemical screening showed total phenolic compounds and total flavonoid contents of different extracts from Tri-Jannarose recipe. Total phenolic compounds were measured with gallic acid equivalents (mgGE/gExt). Total flavonoid contents were measured with quercetin equivalent (mgQE/gExt). Different letters indicated significantly different at $P < 0.05$

α-glucosidase solution (0.2 U/mL) was incubated in 96-well plates 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 were readings (A) and recorded at 405 nm by micro-plate reader 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 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 error of mean from three separate experiments ($n = 3$). 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 significant.

RESULTS

Total phenolic compounds and total flavonoid contents

Phytochemical screening showed that ETJ had both total phenolic compound (TPC) and TFC higher than different extracts. The TPC found that 95% ethanolic extract (865.15 ± 5.570 mgGE/gExt) was higher than hydroethanolic and aqueous extracts (573.44 ± 7.590 and 51.15 ± 0.421 mgGE/gExt, respectively). Moreover, TFC from 95% ethanol extract had still more content (0.0221±2.623 mgQE/gExt) than hydro ethanolic and aqueous extracts (0.0046±3.299 and 0.0002±2.857 mgQE/gExt, respectively) [Figure 1].

Antioxidant activities

DPPH-free radical scavenging activity

In this study, standard substances, ascorbic acid, and Trolox were shown to be more potent than all the extracts of TJ. The ETJ (IC_{50} = 0.0463 ± 0.002) was exerted on free radical scavenging activity higher than HETJ and ATJ (IC_{50} =0.0511 ± 0.000, 0.1485 ± 0.005 mg/mL, respectively) [Figure 2].

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

Surprisingly, using $ABTS^+$ assay, ETJ (IC_{50} = 0.015 ± 0.000 mg/mL) and HETJ (IC_{50} = 0.022 ± 0.000 mg/mL) showed the highest effect on free radical scavenging activity than the standard controls, ascorbic acid (IC_{50} = 0.025 ± 0.001 mg/mL), and Trolox (IC_{50} = 0.032 ± 0.001 mg/mL) [Figure 2].

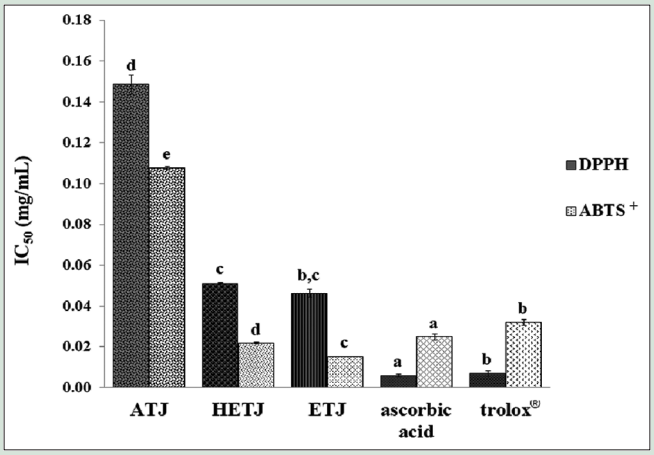

Figure 2: Antioxidant activities showed IC_{50} of different extracts from Tri-Jannarose recipe. DPPH radical scavenging and 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) assay were used Trolox® and ascorbic acid as standard substances. Different letters indicated significantly different at $P < 0.05$

α-Glucosidase inhibitory activity

In this experiment, α-glucosidase inhibitory activity found that the extract of TJ including ATJ (IC_{50} = 0.0127 ± 0.02 mg/mL), ETJ (IC_{50} = 0.0154 ± 0.01 mg/mL) and ETJ (IC_{50} = 0.0202 ± 0.01 mg/mL) were more effect to inhibit α-glucosidase emzyme than acarbose (IC_{50} =0.745 ± 0.026 mg/mL) as a positive control [Figure 3].

DISCUSSION

In the study, extraction method, solvent polarity is frequently used for recovering phenolic compounds from plant. Ethanol is an organic solvent which has been known as a good solvent for phenolic substance extraction and lowly hazard to human consumption.[46] The aqueous an organic solution with show high polarity. Thus, the chemical composition on aqueous extraction method were composed with polysaccharide, proteins and glycoside substances. The aqueous extraction may either contain nonphenolic or possess phenolic compounds that contain a smaller number of active groups than the other solvents.[47]

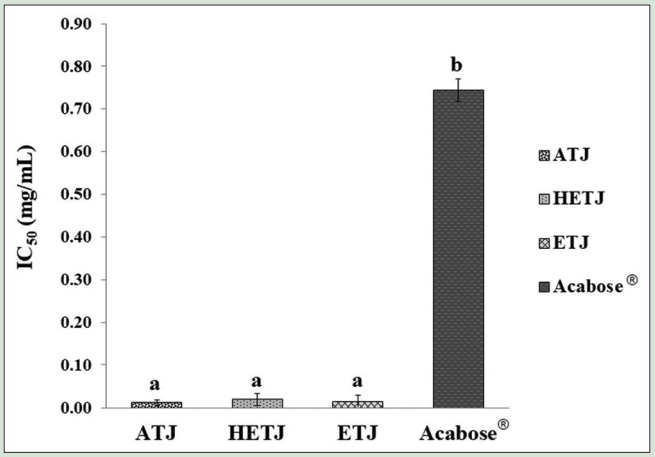

Figure 3: α-Glucosidase inhibitory activity showed IC_{50} of different extracts from Tri-Jannarose recipe. The system was used Acarbose® as a positive control. Different letters indicated significantly different at $P < 0.05$

The antioxidant activity in the experiment found that the extraction by using by 95% ethanol provided high significantly free radical scavenging both DPPH and $ABTS^{+}$ methods cause the solvent extraction showed the high amounts of TPC and TFC. It is clear that 95% ethanol extract gave the strong antioxidant capacity in the study which showed low values of IC_{50}.[48-51] The antioxidant activity of extracts varied depending on the polarity of solvent and the method used to extract bioactive compounds. Change in solvent polarity alters its ability to dissolve a selected group of antioxidant compounds and influences the antioxidant activity estimation.[52] The antioxidant activity could be of therapeutic importance in preventing oxidative stress involved in the development of several diseases.[53] There has been some report regarding antioxidant activity components in root bark of *A. indica* that the plant ingredients compost with flavonoid, quercetin might play a role in the antioxidant activity.[54]

In this study, the α-glucosidase inhibitory activity was obtained stronger than the positive control, acarbose. Any scientific report review that *T. crispa* as a plant in the recipe were composed with some alkaloids such as borapetoside C, lysicamine and liriodenine.[53] They showed strong inhibitory activity against α-glucosidase. A number of alkaloids from natural sources have been proven efficacious in curing various ailments. The alkaloids are several examples which are useful in beneficial treatment of diabetes.[55] However, could be linked to more than one mechanism including insulin sensitizing, insulin releasing, gluconeogenesis inhibition and α-glucosidase inhibition.[56] The effect of phytomedicines can be better evaluated by studying synergistic effects through multitarget effects or effects on pharmacokinetic or physicochemical properties. Thus, it is worthwhile to evaluate further the effective components of isolated compounds *in vivo* rather than make a conclusion based on enzyme inhibition assay only.[57]

CONCLUSION

TJ is a Thai traditional medicine ingredient with phenolic compound and flavonoid contents. The recipes were potent antioxidant and α-glucosidase inhibitory activities. Furthermore, isolation and active compound(s) were evaluated. The pharmaceutical preliminary was confirmed usage indication in Thai traditional medicine. However, any sign or symptoms were clarified in next study.

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Conflicts of interest

There are no conflicts of interest.

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