Phytochemical Screening, Antioxidant, and α-Glucosidase Inhibitory Activities of Different Solvent Extracts from *Leersia hexandra* and *Elephantopus scaber*

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Abstract:

The aim of this research was to determine on phytochemical screening, antioxidant and α-glucosidase inhibitory activities of *Leersia hexandra* and *Elephantopus scaber* by using different solvent extractions. Both plants (1:1;w:w) of recipe were extracted using by different solvents including aqueous (ALE) and 80% ethanol (ELE). Phytochemical screening was determined on total phenolic (TPC) and flavonoid (TFC) contents in plants. The antioxidant activities were determined by, 2,2-diphenyl-1-picrylhydrazy (DPPH) radical scavenging and 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonate) ($ABTS^+$) assay. The α-glucosidase inhibitory assay was evaluated on glucose transferase mechanism. This experimental study found that the recipe showed high among of total phenolic and flavonoid contents especially ELE (40.024±0.952 mgGE/gExt and 0.072±0.001 mgQE/gExt,). The ELE (IC_{50} = 0.082±0.0025 mg/mL) was significantly exerted on free radical scavenging activity higher than ALE (IC_{50} = 0.122±0.0033 mg/mL). $ABTS^+$ radical scavenging activity, the ELE (IC_{50} = 0.0048±0.00018 mg/mL) was significantly stronger than Trolox (IC_{50} = 0.0086±0.00063 mg/mL), known as standard substances. α-glucosidase inhibitory activity, ALE (IC_{50} = 0.022±0.001 mg/mL) and ELE (IC_{50} = 0.098±0.002 mg/mL) were significantly more effect in inhibited α-glucosidase enzyme than acarbose (IC_{50} =1.054±0.113 mg/mL), known anti-diabetic drug. The recipe has been having the phenolic and flavonoid contents which chemical substances were known as anti-oxidation and anti-diabetic property, Pharmaceutical activities were showed on antioxidant and α-glucosidase inhibitory activities. Further, also chemical compositions, major active compound(s) and *in vivo* were clarified in next study.

Keywords: Phytochemical screening, Phenol, Flavonoids, Antioxidant, α-glucosidase

Introduction

Herbal medicines have been developed not only to improve ancient traditional therapeutics, but also as an alternative solution for health problems [1]. Thai traditional medicine is folklore medicine inherited from Thai ancestor [2]. 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. This traditional medicine recipe is once recipe from Thai traditional medicine which composed with two kinds of medicinal plants. First plant, *Leersia hexandra*, Swamp Rice grass, name in Thai is Yaa-Sai [3] and second plant, *Elephantopus scaber*, Prickly-leaved elephant's foot, name in Thai is Do-Mai-Ru-Lom in weight ratio 1:1 (w:w) [4]. Thai ancestor still believed that this recipe has been claimed usage to treatment of diabetes.

The review literatures of each plant in the recipe was revealed in many scientific reports. *L. hexandra* (family: Poaceae) is grass species used in traditional medicine to treat many diseases including hypertension and diuretic. The phytochemical studies have demonstrated the presence of plants are

polyphenol, flavonoids and terpenoids. The pharmacological activities were reported such as hypertension, antioxidant and anticancer [5-6].

E. scaber (family: Asteraceae) has been used as traditional medicine in many countries. It has been popular as a medicinal herb in many countries of Southeast Asia [7]. In Thailand, it has been used as traditional medicine including diuretic, tonic, antihelminthic and aphrodisiac [7]. The most important of these biologically active constituents of plants are elephantopin, triterpenes, stigmasterol, epifriedelinol and lupeol. Other compounds are copaene, isopropyl, dimethyl, hexahydronaphthalene, cyclosativene and Zingiberene from the essential oils [8]. This plant has been extensively screened and proved for anticancer activity, which is mainly for its deoxyelephantopin containing. Many other biological activities such as antimicrobial [9-12], hepatoprotective [13], antioxidant [14-16], antidiabetic [17-19], anti-inflammatory [14], analgesic [17], anti-asthamatic[20] and anticancer [21] have been reported in various research articles[7-8].

Free radical stress is a common theme which underlines etiology of several degenerative disorders. The production of free radicals is linked to the inflammatory process. Free radicals prime the immune response, recruit inflammatory cells and are innately bactericidal [22-23]. The most common reactive oxygen species (ROS) in particular free radicals include superoxide ($O_2^{\cdot}$) anion, hydrogen peroxide (H_2O_2), peroxyl ($ROO^{\cdot}$) radicals, and reactive hydroxyl ($OH^{\cdot}$) radicals. The nitrogen derived free radicals are nitric oxide ($NO^{\cdot}$) and peroxynitrite anion ($ONOO^{\cdot}$). In treatment of these diseases, antioxidant therapy has gained an immense importance. Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used in processed foods have side effects and are carcinogenic [24-25].

Diabetes mellitusis a well-known metabolic disorder, which is characterized by an abnormal postprandial increase of blood glucose level. The control of postprandial hyperglycemia is believed to be important in the treatment of diabetes mellitus. α-Glucosidase secreted from intestinal chorionic epithelium is responsible for the degradation of carbohydrates. α-Glucosidase inhibitors slow down the process of digestion and absorption of carbohydrates by competitively blocking the activity of glucosidase. Consequently, the peak concentration of postprandial blood glucose is reduced and the blood sugar level comes under control. α-Glucosidase inhibitors can offer several advantages and has been recommend by the Third Asia-Pacific Region Diabetes Treatment Guidelines as the first-line of treatment for lowering postprandial hyperglycemia [26]. Several α-glucosidase inhibitors, acarbose was obtained from natural sources, can effectively control blood glucose levels after food intake and have been used clinically in the treatment of diabetes mellitus [27]. In clinically, they have been associated with serious gastrointestinal side effects. It is necessary to search for alternatives that can display α-glucosidase inhibitory activity but without side reactions [28].

However, this traditional medicine recipe was widely used to treat many diseases, but there is no any scientific report. Therefore, this study aimed to determine on phytochemical screening (TPC and TFC), anti-oxidant activity (DPPH and ABTS) and α-glucosidase inhibitory for confirmed pharmaceutical preliminary.

Materials and methods

Sample Collection

Leersia hexandra and *Elephantopus scaber* in recipe were collected from Maha Sarakham and Amnat Charoen province, northeastern of Thailand. The specimens were identified and deposited at the Faculty of Medicine, Mahasarakham University, Thailand (code; *L. hexandra*: MSU.MED-LH0001/SS and *E. scaber*: MED-ES00 01/SS). All of the fresh materials were cleaned and dried at 40°C for 18 hr in a hot air oven and then powdered.

Preparation of Extracts

The aqueous extract (ALE) was prepared by distilled water for 15 min at 100°C in Electric boiler (1:10 w/v). The boiling process was repeated twice. The ethanolic extract (ELE) was macerated with 80% ethanol for 3 hr at 60°C in sonication bath (1:10 w/v). The sonicating process was repeated three times. The residue powder was excluded by using filter papers. The filtrate was evaporated using by a rotary evaporator

(Heidolph Laborota 4000, Germany) and freeze-dried to obtain brown extract. The extracts were kept in the fridge at -20°C until be used.

Total phenolic content assay

Total phenolic content was determined according to a modified procedure [29]. 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 by UV-Vis Spectrophotometer after mixed 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 [30]. The extracts from recipe will be mixed with 100 µL of 5% aluminum chloride (w/v), 400 µL of 2.5% $NaNO_2$ After 5 min, 500 µL of 5% $AlCl_3$. The mixture was allowed to stand at room temperature for 10 min. The solution was mixed 2,000 µL distilled water. The samples were measured at 415 nm. The TFC was calculated from a standard quercetin equivalent (mgQE/gExt).

DPPH radical scavenging assay

2,2- diphenyl- 1- picrylhydrazy (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 [31]. DPPH was dissolved in ethanol to a 0.039 mg/mL. The plant extract at various concentrations was diluted with distilled water to get sample solution. The sample solution with 100 µL following which 900 µL DPPH (0.1 mM) working solution. After a 30 min reaction kept in the dark at ambient temperature then absorbance of the solution was measured at 515 nm. In this study, Trolox and ascorbic acid were used as standard substances. Blank was 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 (%) = $(A_0-A_1)/A_0$ x 100 where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

$ABTS^+$ Radical Scavenging assay

In ABTS assay, the plants extract was allowed to react with $ABTS^+$, a model stable free radical derived from 2,2-azinobis (3-ethylvenzothiazolin-6-sulphonic acid) ($ABTS^+$) [32]. 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 distilled water 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 (%) = $(A_0-A_1)/A_0$ x 100 where A_0 is the absorbance of the control and A_1 is the absorbance of the 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 Taepongsorat, *et al*. (2019) with slight modifications [33]. Briefly, a volume of 120 µL of the sample solution and 100 µL of 0.1 M phosphate buffer (pH 6.8) containing α-glucosidase solution (0.2 U/mL) was incubated at 37ºC for 20 min. After preincubation, 100 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 320 µL of 0.2-M Na_2CO_3 into each well, and absorbance were read (A) and recorded at 405 nm by UV-Vis Spectrophotometer and compared to a control which had 120 µL of buffer solution. 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 A1 is the absorbance of the sample. IC_{50} values were calculated by the graphic method.

Statistical analysis

All assays were expressed as means ± standard deviation (SD) from five separate experiments (n = 5). Statistical analysis was carried out using 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

The ELE and ALE from the recipe has been have both TPC and TFC which ELE (40.024±0.952 mgGE/gExt; 0.072±0.0007 mgQE/gExt) showed significantly higher contents than ALE (11.424±0.158 mgGE/gExt; (0.017±0.0002 mgQE/gExt). (Table 1). Phenolic compounds have an aromatic ring with one or more hydroxyl groups and act as antioxidants [47]. In the study, extraction methods, 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 [34]. Some organic molecules are more polar and therefore more soluble in water. Thus, the chemical composition on aqueous extraction method were composed with polysaccharide, proteins and glycoside substances. The aqueous extraction may either contain non-phenolic or possess phenolic compounds that contain a smaller number of active groups than the other solvents [35]. Total phenolic compounds in methanol extract of *E. scaber* showed high TPC and significant antioxidant activity. The antioxidant activity increased with increasing concentration of extract [39]. *E. scaber* ingredients composed with phenolic, flavonoid, terpenoid and another compound might play a role in the antioxidant activity. These phytochemicals showed possess wound healing, anti-venom, anti-microbial, anti-inflammatory, anti-diabetic, cytotoxic and anti-tumour activities.

In this study DPPH radical scavenging activity, standard substances, ascorbic acid (IC_{50} = 0.004±0.0002 mg/mL) and trolox (0.016±0.0012 mg/mL) were show significantly more potent than all of the extracts from this recipe. While, The ELE (IC_{50} = 0.082±0.0025 mg/mL) was significantly exerted on free radical scavenging activity higher than ALE (IC_{50} = 0.122±0.0033 mg/mL). (Table 1). Surprisingly, $ABTS^{+}$ radical scavenging assay, the ELE (IC_{50} = 0.0048±0.00018 mg/mL) was significantly stronger on this method than trolox (IC_{50} = 0.0086±0.00063 mg/mL), known as standard substances but not ascorbic acid (IC_{50} = 0.0019±0.00004 mg/mL). (Table 1). The antioxidant activity in the experiment found that the extraction by using by ethanol provided high significantly free radical scavenging both DPPH and $ABTS^{+}$ methods which is related to the quantity of TPC and TFC. The ethanol extract gave the strong antioxidant capacity in the study which showed low values of IC_{50} [36]. The antioxidant activity of extracts varied depending on the polarity of solvent and the method used to extract bioactive compounds. Changing in solvent polarity alters its ability to dissolve a selected group of antioxidant compounds and influences the antioxidant activity estimation [37]. The antioxidant activity could be therapeutic importance in preventing oxidative stress involving in the development of several diseases [38]. There were some reports regarding the ethanol and aqueous extracts of *L. hexandra* showed that it has an antioxidant effect that protects the tissues from the deleterious effects of free radicals resulting in hypertension rat [5]. Methanol extract of *E. scaber* showed high TPC and the antioxidant activity increased with increasing concentration of extract [39].

In this experiment, α-glucosidase inhibitory activity, the result found that ALE (IC_{50} = 0.022±0.001 mg/mL) and ELE (IC_{50} = 0.098±0.002 mg/mL) which can inhibit activity of α-glucosidase enzyme more than acarbose (IC_{50} = 1.054±0.113 mg/mL). (Table 1). In this study, the α-glucosidase inhibitory activity was obtained stronger than the positive control, acarbose. Phytochemicals have also been isolated from plants includes elephantopin, triterpenes, stigmasterol epifriedelinol and lupeol [8]. They showed strong inhibitory activity against α-glucosidase. These studies showed α-glucosidase inhibitors slow down the process of digestion and absorption of carbohydrates by competitively blocking the activity of glucosidase [26]. On the basis of literatures published worldwide, summarized in a list of 411 natural products isolated from medicinal plants that showed α-glucosidase inhibitory activity [38]. Structurally these natural product inhibitors compose of terpene, alkaloid, quinine, flavonoid, phenol, phenylpropanoid, and steroid frameworks. These inhibitors are rich in organic acid, ester, alcohol, and allyl functional groups. A majority of the compounds reported contain flavonoid, terpene, and phenylpropanoid ring structures [38]. Recently, several studies have determined that flavonoid compounds can be very effective in inhibiting α-glucosidase activity [45]. A total of 103 flavonoids showed glycosidase inhibitory activity including xanthones,

flavanones, flavans, anthocyamins, chalcones, and other structural motifs [38]. Thirty-seven polyphenols from plants have shown promising α-glucosidase inhibitory activity. Gallic acid, an important constituent of many plants species showed strong inhibitory activity against glucosidase both *in vitro* and *in vivo* [46]. The effect of phytomedicines can be evaluated by studying synergistic effects through multitarget effects or effects on pharmacokinetic or physicochemical properties. There has been some reports regarding 28Nor-22(R) with a 2, 6, 23-trienolide, a major steroid isolated from the acetone extract of the *E. scaber* decreased blood level glucose in STZ diabetic rats [41]. This may be due to a stimulating effect on insulin release from regenerated β-cells of the pancreas or increased cellularity of the islet tissues [41]. Bioactive compounds of *E. scaber* had also successfully identified in some compounds such as deoxyelephantopin, isodeoxyelephantopin, scabertopin, isoscabertopin, elescaberin, 17, 19-dihydrodeoxyelephantopin and a terpenoid which showed a broad range of biological functions [8]. The ethyl acetate root extract and methanol leaf extract of *E. scaber* showed antihyperglycemic effect by reducing the blood glucose level, glycosylated hemoglobin, a change in the lipid profile and kidney functions, liver and muscle glycogen, serum insulin levels and histopathological studies [42]. Aqueous extract of leaves and roots into alloxan induced diabetic rats significantly reduced serum glucose, glycosylated hemoglobin and the activity of gluconeogenic enzyme glucose-6-phosphatase [43]. However, could be linked to more than one mechanism including insulin sensitizing, insulin releasing, gluconeogenesis inhibition and α-glucosidase inhibition [44]. 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 [45].

Conclusions

The recipe are combines from *L. hexandra* and *E. scaber* as a Thai traditional remedy, present experiments provides a preliminary data that the recipe ingredients with phenolic compounds and flavonoid contents. The pharmaceutical activities were show more potent on antioxidant and α-glucosidase inhibitory activities. In this study, the pharmaceutical preliminary was confirmed to use of this recipe from Thai traditional medicine. However, chemical compositions, major active compound(s) and *in vivo* need to be clarified in next study.

Table 1 Total phenolic (TPC), and flavonoid contents (TFC), DPPH, and ABTS radical scavenging activities, and α-glucosidase inhibitory activities of different solvent extracts from combination of *Leersia hexandra* and *Elephantopus scaber*.

Samples	TPC mgGE/gExt	TFC mgQE/gExt	DPPH IC_{50}(mg/mL)	$ABTS^{+}$ IC_{50}(mg/mL)	α-Glucosidase IC_{50} (mg/mL)
ALE	11.424±0.158b	0.017±0.0002b	0.122±0.0033d	0.0095±0.00054d	0.022±0.001a
ELE	40.024±0.952a	0.072±0.0007a	0.082±0.0025c	0.0048±0.00018b	0.098±0.002a
ascorbic acid	-	-	0.004±0.0002a	0.0019±0.00004a	-
trolox	-	-	0.016±0.0012b	0.0086±0.00063c	-
acarbose	-	-	-	-	1.054±0.113b

ALE was extracted with aqueous. ELE was extracted with 80% ethanol. TPC was measured with gallic acid equivalents (mgGE/gExt). TFC was measured with quercetin equivalent (mgQE/gExt). Antioxidant activities and α-glucosidase inhibitory activities showed IC_{50} of different extracts from recipe. DPPH and $ABTS^{+}$ radical scavenging activity were used trolox and ascorbic acid as standard substances. α-glucosidase inhibitory activities was used Acarbose as a positive control. Different letters indicated significantly different at $p < 0.05$.

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