RESEARCH ARTICLE

In-vitro antidiabetic activity of ethanolic extract from Khla (*Schumannianthus dichotomus* Roxb.) rhizome

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ABSTRACT

Schumannianthus dichotomus Roxb. in Thai, namely "Khla," is notably distributed in South and South-East Asia regions. In Thai traditional medicine, rhizome has a bland taste, and after decoction, it is used to reduce body heat and fever and treatment of skin diseases. Recently, the methanolic extract of rhizome had reported the hepatoprotective and hypoglycemic activities in mice. Aims of this study were conducted to determine the nutritional value of rhizome and to evaluate α-glucosidase, and α-amylase inhibitory activities of ethanol extract from the rhizome (KE).

The rhizome had a high content of dietary fiber and iron, while it was low in calories, fat, and carbohydrates. Thus, it can become an ingredient of a healthy diet. The total phenolic content of KE contained 229.8 ± 6.23 mg of gallic acid equivalent (GAE)/g. There was strongly inhibited α-glucosidase (IC_{50} = 0.11 ± 0.03 mg/mL) and comparable with the antidiabetic drug acarbose (IC_{50} = 0.10 ± 0.03 mg/mL). Therefore, KE was slightly inhibited by α-amylase (IC_{50} = 0.42 ± 0.05 mg/mL), when compared with acarbose (IC_{50} = 0.016 ± 0.001 mg/mL). Thus, the phytochemicals of different extracts are necessary to be identified. Therefore, the advance techniques were the limitation of our study.

Keywords: *Schumannianthus dichotomus Roxb.*, Antidiabetes, α-Glucosidase inhibition, α-Amylase inhibition.

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INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disease that is significantly concerning for the global population. The etiology of DM is either insulin deficiency or abnormal insulin function. The 463 million of individuals have DM in 2019, which will increase up to 578 million by 2030 and 700 million by 2045. Hyperglycemia leads to various complications, including diabetic retinopathy, nephropathy, atherosclerosis, hypercoagulability, cardiovascular diseases, abdominal adiposity, hypertension, dyslipidemia, cerebrovascular incidents, peripheral vascular afflictions, dermatological manifestations, cognitive impairment, auditory deficits, and psychological distress. The continuous utilization of certain synthetic substances is cause adverse reactions and non-harmful and cost-effective pharmaceuticals are still in demand. Thus, traditional treatments are commonly used of herbal remedies and the integration of botanical uses into contemporary and advanced medicinal practices has increased. A total of 21,000 plants around the world have been recorded for therapeutic uses by The World Health Organization (WHO), and over 400 botanical varieties are accessible for managing diabetes.[1] Recently, Thai traditional medicine has been supported by the government and integrated into the national health policy. The Thai government has expected that 10 and 5% of total traditional medicine use will facilitated by local health services at the subdistrict level and in community hospitals, respectively. In rural regions of Thailand, traditional healers are still important within the public health services due to there are ease to availability rather than modern medicine. In Thai communities, many people are still respected and assured of the healing outcome of medicinal uses.[2]

The plants belong to the *Marantaceae* family and are commonly rhizomatous perennial herbs and predominantly grow in clay or clay-loam soil within moist or swampy tropical forests. The genus *Schumannianthus* includes the three species can be identified, namely *S. dichotomus*, *S. monophyllus*, and *S. virgatus* (Roxb.) Rolfe. *S. dichotomus* (Roxb.) in Thai, namely "Khla", and stem of *S. dichotomus* is mostly utilized in handcraft in Thailand. There is a common height of 3-5 m with 2 to 5 cm of basal diameter, and is notably distributed in South and South-East Asia regions such as India, Bangladesh, Myanmar, Vietnam, Malaysia, Indonesia and Thailand.[2-4] Traditional uses of rhizome are relief of fever and skin diseases, and the stem is applied for earache treatment.[2,5]

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(a) (b)

Figure 1: (a) Characteristic of "Khla", *S. dichotomus* rhizome (b) The pieces of rhizome

The pharmacological investigation on *S. dichotomus* sparsely reported that its stem and rhizome had antinociceptive and antipyretic effects in mice models. The effects depend on sex characteristics. Total phenolic content and flavonoids contained in the rhizome are higher than in the stem. Qualitative analysis of the rhizome revealed that the methanolic extract contains phenols, tannins, flavonoids, alkaloids, carbohydrates, terpenes, and phytosterols. In contrast, the rhizome extract is lack of glycosides, saponins, and gums.[6] Recently, the methanolic extract of *S. dichotomus* rhizome had reported hepatoprotective and hypoglycemic activities in CCl_4-induced hepatotoxic and dextrose-induced hyperglycemic mice.[7] In Thai traditional medicine, rhizome has a bland taste, and after decoction, it is used to reduce body heat and fever, and treatment of skin diseases.[2] The Thai traditional herbal formula for diabetes treatment consists of eight medicinal ingredients, including *S. dichotomus* rhizome, which has been recorded in the note of King Rama V on traditional Thai medicine.[8] In addition, Thai herbal formula is mostly decocted with water or macerated with ethanol. Thus, our study was conducted to determine the nutritional value of rhizome and to evaluate α-glucosidase and α-amylase inhibitory activities of ethanol extract from *S. dichotomus* rhizome. The finding may support the antidiabetic activity of *S. dichotomus* rhizome through key enzymatic inhibition on the hypoglycemic effect.

MATERIALS AND METHODS

Plant Collection

S. dichotomus plants were collected from the Ton Khla handicraft community enterprise, Thung Fon district, Udon Thani province (17°29'06.6"N 103°11'49.6"E). The stems were cut from rhizomes and the roots of the rhizome were also removed. The rhizomes were cleaned and sliced into thin-layer pieces (Figure 1).

Nutrition Analysis of rhizome

The pieces of rhizome (1 kg) were transferred to the laboratory service within three days. The evaluation of rhizome nutrients included caloric content, carbohydrate composition, dietary fiber, lipid content, sodium levels, calcium concentration, iron content, as well as the presence of vitamin A, vitamin B1, and vitamin B2, which was conducted by The Central Laboratory Co., Ltd., located in Bangkok, Thailand following the guidelines from AOAC International.[9] The nutritional content of the *S. dichotomus* rhizome was represented per 100 g of sample and compared with the Thai Recommended Daily Intakes (Thai RDIs).[10]

Rhizome Extraction and Total Phenolic Content (TPC)

The pieces of rhizome shade-dried at 50°C, and grinding into a fine powder by herbal grinders. The 200 g of rhizome powder was macerated in 1 L of 95% ethanol (RCI Labscan, Thailand) with sonication at room temperature for 72 hours, and this extraction was processed in triplicate. The pooled ethanol extract was concentrated in constant weight by rotary vacuum evaporator and water bath, and the yield of extraction was calculated. The TPC of the ethanol extract from *S. dichotomus* rhizome (KE) was analyzed by the Folin-Ciocalteu method. The 20 µL of KE (5 mg/mL) dissolved in dimethyl sulfoxide, DMSO (RCI Labscan, Thailand), the 100 µL of (1:10 v/v) Folin-Ciocalteu reagent in distilled water and 80 µL of 7.5% Na_2CO_3 (RCI Labscan, Thailand) in distilled water were sequentially mixed. The mixture was incubated in room temperature in a dark room for 30 minutes, and the absorbance was determined at 765 nm using a microplate reader. The TPC was calculated from a standard curve of gallic acid ($Y = 80.742X + 0.031$, $R^2 = 0.9994$) and reported as mg of gallic acid equivalent (GAE)/g of KE from three times measurements.[11]

α-glucosidase Inhibitory Assay

The monitoring of *p*-nitrophenol, the product cleaved from *p*-nitrophenyl-α-D-glucopyranose (Sigma, Switzerland), was catalyzed by α-glucosidase in the presented of sample. KE (0.001, 0.01, 0.1, 1.0, 10.0 mg/mL) dissolved in 10% DMSO was the test sample, and acarbose (Sigma-Aldrich, Germany) dissolved in 30 mM phosphate buffer, pH 6.5 (0.0005, 0.005, 0.05, 0.5, 5.0 mg/mL) was the positive control. The 1-mg of α-glucosidase from *Saccharomyces cerevisiae* (Sigma, Germany) was dissolved with 13.9 mL of 50 mM phosphate buffer, pH 6.5. The substrate, 3 mg of *p*-nitrophenyl-α-D-glucopyranose was dissolved with 10 mL of 50 mM phosphate buffer and stood in room temperature for 15 minutes. Each diluted sample (20 µl) was added with 15 µl of enzyme solution and 75 µL of substrate solution, respectively. The mixture was incubated at room temperature for 15 minutes and 15% w/v glycine solution was used for stopping of reaction. The determination of *p*-nitrophenol was monitored by a microplate reader at 415 nm. The calibration curve between the inhibition of enzyme and concentration was performed and the result was calculated from three-time measurements and represented as 50% inhibitory concentration of sample, IC_{50}.[12]

α-amylase Inhibitory Assay

This assay monitored the reducing sugar, the product cleaved from starch, catalyzed by α-amylase in the presented of sample. The 600 µL of KE (0.001, 0.01, 0.1, 1.0, 10.0 mg/mL) dissolved in 10%DMSO and 1.2 mL of starch in phosphate buffer (pH 6.9) containing 6.7mM NaCl was mixed. The reaction was started by filling 600 µL of pancreatic α-amylase (Fluka, Germany) and incubated at 37°C. The 600 μL of the reaction mixture was transferred to 300 μL of dinitrosalicylic acid (DNSA) solution (1% w/v DNSA, 30% w/v sodium potassium tartrate and 20% v/v 2N NaOH in distilled water), and kept in boiling water for stopping of reaction. The mixture was diluted with 2.7 mL of distilled water and absorbance was measured at 540 nm by spectrophotometer (T80, Oasis Scientific, USA). Acarbose (Sigma-Aldrich, Germany) dissolved in 30 mM phosphate buffer, pH 6.5 (0.00025, 0.0025, 0.0025, 0.025, 0.25, 2.5 mg/mL) was positive control. Negative control was a similar reaction mixture; therefore, it was without a sample contained. The calibration curve between the inhibition of enzyme and concentration was performed and the result was calculated from three-time measurements and represented as IC_{50}.[13]

RESULTS AND DISCUSSION

S. dichotomus rhizome had a high content of dietary fiber and iron, while it was low in calories, fat, and carbohydrates (Table 1). Thus, it can become an ingredient of a healthy diet, especially for overweight and weight control people. The extract was a dark brown solid and yielded approximately about 7%. *S. dichotomus* or "Khla" rhizome extract (KE) contained 229.8 ± 6.23 mg of GAE/g. There was strongly inhibited α-glucosidase (IC_{50} = 0.11 ± 0.03 mg/mL) and comparable with the antidiabetic drug acarbose (IC_{50} = 0.10 ± 0.03 mg/mL). Therefore, KE was slightly inhibited by α-amylase (IC_{50} = 0.42 ± 0.05 mg/mL), when compared with acarbose (IC_{50} = 0.016 ± 0.001 mg/mL) (Table 2). The calibration curve between the enzyme inhibition and concentration of α-glucosidase and α-amylase inhibitory assays were represented in Figures 2 and 3, respectively.

The utilization of medicinal plants is targeting diabetes management as a novel therapeutic agent, which is one of the most researched areas worldwide. Among the abundance of plants documented in folk medicine while, the majority of species are unexplored in their chemical constituents and pharmacological properties. Nevertheless, several studies have documented the hypoglycemic properties, which are exhibited by different plant components.[14-17] Recently, the methanolic extract of *S. dichotomus* rhizome had reported hypoglycemic activities in dextrose-induced hyperglycemic mice. There had contained catechin hydrate, (-) epicatechin and caffeic acid at relatively high concentrations when compared with other polyphenolic compounds. They had reported compounds are commonly known to exhibit hypoglycemic properties as antidiabetic agents.[7] We found α-glucosidase and α-amylase inhibitory activities of *S. dichotomus rhizome* extract, which corresponded to hypoglycemic properties as previous study.[7] Therefore, in our study, we were extracted *S. dichotomus* rhizome by ethanol, which was simulated the common preparation of Thai herbal plants and had less toxicity rather than methanol extract. Thus, the phytochemicals of different extracts from *S. dichotomus* rhizome is necessary to identify. Therefore, the advanced techniques were the limitation of our study.

Table 1: Nutritive value of *S. dichotomus* rhizome

Nutrition value	*Unit*	*Per 100 g*	*Per serving size*	*RDI (%)*
Energy	kcal	100.32	100	-
Energy from fat	kcal	1.08	0	-
Total fat	g	0.12	0	0
Saturated fat	g	ND	0	0
Cholesterol	mg	ND	0	0
Protein (%N × 6.25)	g	0.96	<1	-
Total carbohydrate	g	23.85	24	8
Dietary fiber	g	21.16	21	84
Sugar	g	2.54	3	-
Sodium	mg	14.23	15	1
Vitamin A	μg	ND	(0.0)	0
Vitamin B1	mg	ND	(0.0)	0
Vitamin B2	mg	0.047	(0.05)	2
Calcium	mg	34.08	(34.08)	4
Iron	mg	12.99	(12.99)	9

ND = not determined

Table 2: Total phenolic content (TPC) and *in-vitro* antidiabetic activities of rhizome extract (KE)

Sample/Assay	*TPC*	*α-glucosidase inhibition**	*α-amylase inhibition**
(Units)	(mg GAE/g)	(mg/mL)	(mg/mL)
KE	229.8 ± 6.23	0.11 ± 0.03	0.42 ± 0.05
Acarbose	-	0.10 ± 0.03	0.016 ± 0.001

*Results were represented as IC_{50}; GAE = gallic acid equivalent

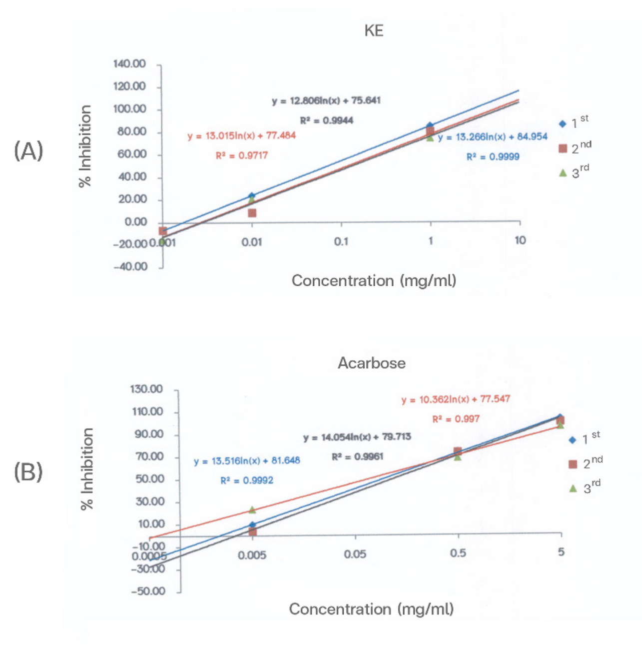

Figure 2: The calibration curves of α-glucosidase inhibitory assay for (A) KE and (B) acarbose

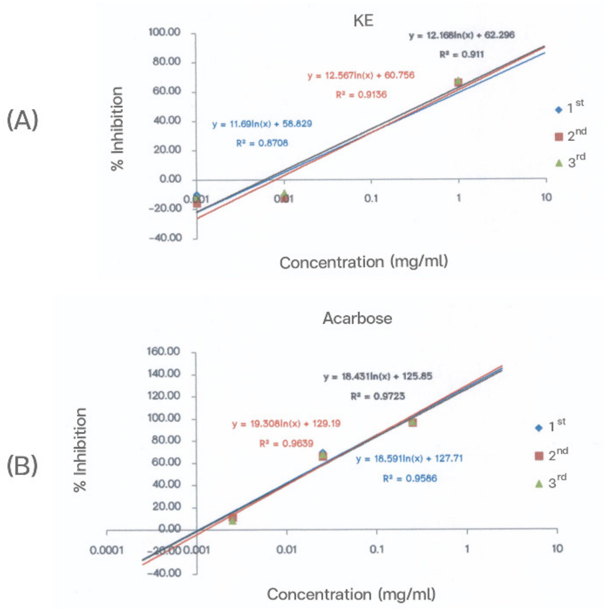

Figure 3: The calibration curves of α-amylase inhibitory assay for (A) KE and (B) acarbose

CONCLUSION

The *S. dichotomus* rhizome had a high content of dietary fiber and iron, while it was low in calories, fat, and carbohydrates. Ethanol rhizome extract (KE) contained high phenolic content (. There was strongly inhibited α-glucosidase and comparable with acarbose. Therefore, KE was slightly inhibited by α-amylase.

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