PHYTOCHEMICAL SCREENING AND α-GLUCOSIDASE INHIBITORS ACTIVITY OF *MITRAGYNA SPECIOSA* KORTH.

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Abstract

The objective of this research was to study the basic phytochemical compounds of extracts from different leaves of the kratom using 5 solvents namely hexane, dichloromethane, ethyl acetate, ethanol and water. The bioactive phytochemicals in red vein kratom leaves included terpenoids, saponins, tannins, flavonoids, and alkaloids. And the green vein kratom leaves included terpenoids, saponins, flavonoids, and alkaloids. According to the α-glucosidase inhibitors activity (1U/mL) using *in vitro* kratom leaf extract, it was found that the red vein kratom leaves had a higher inhibitory effect on the α-glucosidase enzyme than the green vein kratom leaves. It was also observed that ethyl acetate extract of red vein kratom with α-glucosidase inhibitors activity (IC_{50} =17.28 mg/mL) was similar to that of acarbose positive control (IC_{50} =15.74 mg/mL) (there was a statistically significant difference ($p<0.5$).

Keywords: Crude extract, phytochemicals, kratom, α-glucosidase inhibitors activity

INTRODUCTION

Kratom (*Mitragynaspeciosa*) is a perennial plant in the family Rubiaceae, native to Southeast Asia (SEA). Thailand has a long history of kratom use.[1]Kratom is used to listed in the Narcotic Drugs Act, Category 5, 1979, in Thailand.[2]Which under Section 3 refers only to *M. speciosa*. Kratom leaves or extracts are typically eaten for pain and other medical treatments, including as an aid to agricultural labor [1].Kratom and kratom alkaloids are classified as central nervous system (CNS) disorders.[3]Kratom contains the main alkaloid, mitragynine, which accounts for approximately 66% of the total alkaloids in kratom leaves [4].Preliminary studies of mitragynine suggest that it may be developed into an analgesic drug.[4, 5]Kratom has thus become a natural product that can be commercially available over the Internet.[6, 7]The US Drug Enforcement Administration lists kratom as a regulated drug for use. In case studies, many Americans are using kratom to treat themselves with various conditions.[8-12]Although there is limited evidence supporting the benefits of kratom in the treatment of various ailments, kratom is believed to be curative.

According to Thai medicine textbooks, Kratom leaves have properties to suppress and treat diabetes.[13, 14]Diabetes is caused by abnormal secretion of the hormone insulin, resulting in hyperglycemia.[15]At present, the trend of diabetes is increasing rapidly, which is expected to increase to 592 million by 2035.[16]To treat diabetes, doctors prescribe medications that reduce or prevent the absorption of glucose such as acarbose, myglitol, and voglibose. These drugs act by inhibiting α-glucosidase, an enzyme that plays a key role in catalyzing the hydrolysis of starch into glucose. The absorption of glucose from the small intestine into the bloodstream is reduced. In the article titled "Does kratom have medicinal properties?", which studied the wisdom and popularity of using kratom by folk healers through interviews, it was found that they used kratom for medicinal purposes. The belief of indigenous healers arose from the experience of healing but not the study of scientific methods. The researcher was interested in studying phytochemical compounds in different leaves of kratom such as red vein kratom leaves andgreen vein kratom leaves.In addition, a comparative study was conducted on the inhibitory activity of α-glucosidase, an enzyme that plays an important role in catalyzing the hydrolysis of starch into glucose of red vein kratom leaves andgreen vein kratom leaves.

MATERIALS AND METHODS

Equipment and chemicals

The red vein kratom leaves andgreen vein kratom leaves obtained from Ratchaburiprovince, Thailand which were preliminarily examined by means of identifying kratom leaves in the control group by using kratom leaves from the same source(**Figure 1**). After that, the experiment was carried out when the initial identity was checked, starting from cleaning the kratom leaves with clean water, then drying them at 30 °C until completely dry (weighing until the weight was stable) and grinding them thoroughly.

Figure 1.The red vein kratom leaves andgreen vein kratom leaves.

The reagents consisted of 0.02M *p*-nitrophenyl-α-D-glucopyranoside(*p*-NPG), 0.10 M sodium phosphate buffer (pH 6.8),0.10 M sodium phosphate buffer (pH 6.8) containingα-glucosidase (1 U/mL), 1.0 M sodium carbonate(Na_2CO_3), 80% methanol or dimethyl sulfoxide (DMSO), crude extract of kratom leaves, acarbose

(positive control), hexane, dichloromethane, ethyl acetate, ethanol, distilled water, standard laboratory glassware, EZ Read 2000 Microplate Reader.

Preparation of crude extract from kratom leaves

Preparation of crude extracts using solvents were as follows: hexane,dichloromethane,ethyl acetate, ethanol and distilled water.In the experiment, a volume of 200 mL of solvent was used. Initially, 20 g ofkratom leaf powder were weighed and then extracted with 200 mL of solvent per time. In each experiment, the extracted crude extract was filtered andevaporated by rotary evaporator until the solventwas completely evaporated, then the crude extract was weighed and calculated as a percentage.

Test of kratom phytochemical compounds [17]

Anthraquinones test

About 0.2 g of crude extract was weighed, then 10 mL of 10%sulfuric acid (H_2SO_4) solution was added and heated on a waterbath for 5 minutes, then filtered while hot. After that, let the solution cool to room temperature, add 2-3 drops of10% ammonia(NH_3) solution and notice a reddish-pink color indicating that anthraquinone was found.

Terpenoids test using Salkowski test

About 0.2g of the crude extract was weighed, then added 2 mL of dichloromethane and shaken and gradually added concentrated H_2SO_4.If a reddish-brown color was formed between the junctions of the solution, then terpenoids were found.

Flavonoids test

About 0.2g of the crude extract was weighed, then the extract was dissolved using 3 mL of 50% ethanol, and 2-3 small pieces of magnesium wire were added. Later, it was boiled and dripped with concentrated hydrochloric acid (HCl). If the solution was yellow, orange, or red, flavonoids were found.

Saponins test

A bubble test was performed by weighing about 0.2 g of crude extract, then adding 5 mL of distilled water, bringing to a boil and filtering. Subsequently, the filtrate was added to 2-3 mL of distilled water and shaken vigorously. If bubbles were observed, saponins were found.

Tannins test

About 0.2g of the crude extract was weighed, then added 5 mL of distilled water, heated on a water bath and filtered. After that, 2-3 drops of ferric chloride($FeCl_3$)were added to the filtered liquid. If the result was green-black or blue-black, then tannins were found.

Alkaloids test

About 0.2g of the crude extract was weighed, then dissolved in 15mL of2%H_2SO_4 solution, warmed for 2-3 minutes and filtered. The filtered liquid was then dripped with Dragendorff's reagent. If there was an orange-red precipitate, alkaloids were found.

Method for testing theα-glucosidase inhibitors activity of crude extract from kratom leaves[18]

The test kit could be divided into 4 sets and pipetted to a 96 well-plate to the required volume as shown in **Table 1**.

Table 1. Volume of α-glucosidase inhibitors.

Test kit	0.1 M Sodium phosphate buffer (pH 6.8) (µL)	Test sample (µL)	1 U/mL α-glucosidase (µL)	DMSO (µL)
A (E+S)	100	-	20	20
B (S)	120	-	-	20
C (E+T+S)	100	20	20	-
D (T+S)	120	20	-	-

Note: E=Enzyme, T=Test Sample, S=Substrate.

All substances were mixed together and incubated at 25 °C for 15 minutes. Next, 20µL of 0.02M*p*-NPG (Substrate) was added to each well and mixed well then incubated at 250°C for 5 minutes. After that, 40µL of 1.0 M Na_2CO_3 solution was added to inactivate the reaction in each well and absorbance was measured at a wavelength of 405nm using an EZ Read 2000 Microplate Reader.

Statistical data analysis

Data analysis was performed using three iterations of mean ± standard derivation, analysis of variance (ANOVA), Least Significant Difference (LSD), and Duncan Multiple Range Test (DMRT). Data correlation was searched using SPSS program.

The concentration of the extract that inhibits the α-glucosidase enzyme at 50% (IC_{50}) was determined using the IC_{50} Calculator program.

RESULTS AND DISCUSSION

Amount of crude extract

According to the study on the extraction of red vein kratom leaves andgreen vein kratom leaves using solvents: hexane, dichloromethane,ethyl acetate, ethanol anddistilled water, the results shown in **Table 2**.The percentage of crude extract of red vein kratom leaves using various solvents showed the mass of the crude as follows: Ethanol had apercentage of mass at a maximum weight of 35% and ethyl acetate; 30%, dichloromethane; 25%, hexane; 24% and distilledwater had a percentage of mass at a minimum weight of 3%, respectively.The percentage of crude extract of green vein kratom leaves using various solvents showed the mass of the crudeas follows: Ethanol had a percentage of mass at a maximum weight of 22% and ethyl acetate; 8%, dichloromethane; 6%, hexane; 6% anddistilled water

had a percentage of mass at a minimum weight of 2%, respectively.All of the various solventsshowed the maximum presence in all ofred vein kratom leaves and green vein kratom leaves can soluble well in ethanol as a polar solvent.

Table 2. Percentage of crude extracts obtained from various solvents.

Solvents	Percentage of crude extracts obtained from various solvents (%w/w)	
	Red vein kratom leaves	Green vein kratom leaves
H	24	6
D	25	6
EA	30	8
E	35	22
W	3	2

Note: H = hexane, D = dichloromethane, EA = ethyl acetate, E = ethanol, W = distilled water. The percentage of extraction as a %yield (w/w) could be calculated as (dry weight of the extract/dry weight of the plant before extraction) x 100.

Primary phytochemical constituents of extracts from different parts of kratom using different solvents

Preliminary phytochemical testing of crude extracts from red vein kratom leaves using various solvents had the following results: hexane contained saponins and alkaloids; dichloromethane contained saponin and alkaloids; ethyl acetate contained alkaloids; ethanol contained terpenoids, tannins and alkaloids; and distilled water contained terpenoids, flavonoids and alkaloids. In addition, phytochemical testing of crude extracts from the green vein kratom leaves using various solvents showed the following results: hexane contained saponins and alkaloids; dichloromethane contained saponins and alkaloids; ethyl acetate contained flavonoids and alkaloids; ethanol contained terpenoids, saponins and alkaloids; and distilled water contained terpenoids, saponins and alkaloids as shown in **Table 3**.

Table 3. Phytochemicals found in red vein kratom leaves andgreen vein kratom leaves.

Phytochemical	*Mytragynaspeciosa*Korth									
	Red vein kratom leaves					Green vein kratom leaves				
	H	D	EA	E	W	H	D	EA	E	W
Anthraquinones	-	-	-	-	-	-	-	-	-	-
Terpenoids	-	-	-	+	+	-	-	-	+	+
Saponins	+	+	-	-	-	+	+	-	+	+
Tannins	-	-	-	+	-	-	-	-	-	-
Flavonoids	-	-	-	-	+	-	-	+	-	+
Alkaloids	+	+	+	+	+	+	+	+	+	+

Note: H = hexane, D = dichloromethane, EA = ethyl acetate, E = ethanol and W = distilled water, -means not detected, + means detected.

Test on the α-glucosidase inhibitors activity of crude extract in red vein kratom leaves and green vein kratom leaves

Mitragynine is an important and abundant substance in kratom, an alkaloid that may play a role in the α-glucosidase inhibitors activity.[19]According to the phytochemical composition test, it was found that kratom leaves contained alkaloids in crude extracts in all solvents. Therefore, the researcher chose the kratom leaves for extraction to test the α-glucosidase inhibitors activity.

The α-glucosidase inhibitors activity of 1 U/mL in red kratom leaves had a positive control of acarbose.[19]In this regard, there was a percentage ofα-glucosidase inhibitors activity at a minimum concentration of 0.3125 mg/mL and a maximum concentration of 10 mg/mL, representing 4.39±0.79, 26.82±8.93, 33.78±0.84, 40.74±7.38, 56.51±11.38 and 78.94±2.97, respectively(**Figure 2**). The crude extract of red vein kratom leaves using various solvents showed the percentage of α-glucosidase inhibitors activity as follows (**Table 4**): Hexane extract had a percentage of α-glucosidase inhibitors activity at a minimum concentration of 2.5 mg/mL and a maximum concentration of 10 mg/mL, representing 0.78±11.52, 5.71±10.82 and 14.53±8.49, respectively.Dichloromethane extract had a percentage ofα-glucosidase inhibitors activity at a minimum concentration of 2.5 mg/mL and a maximum concentration of 10 mg/mL, representing 4.40±7.73, 22.55±7.19 and 48.00±7.95, respectively. Ethyl acetate extract had a percentage of α-glucosidase inhibitors activity at a minimum concentration of 0.3125 mg/mL and a maximum concentration of 10 mg/mL, representing 0.51±5.20, 3.27±2.24, 9.27±3.08,21.29±3.21,38.89±2.22 and 61.52±0.47, respectively. Ethanol extract had a percentage of α-glucosidase inhibitors activity at a minimum concentration of 1.25 mg/mL and a maximum concentration of 10 mg/mL, representing 0.29±2.54, 2.96±5.72, 12.47±0.56 and 32.39±0.67, respectively. Distilled waterextract, on the other hand, showed no the α-glucosidase inhibitors activity.The percentage of α-glucosidase inhibitors activity in various crude extracts of red vein kratom leaves at maximum concentration of 10 mg/mLcompared with acarbose (positive control) as shown in**Figure 3**.

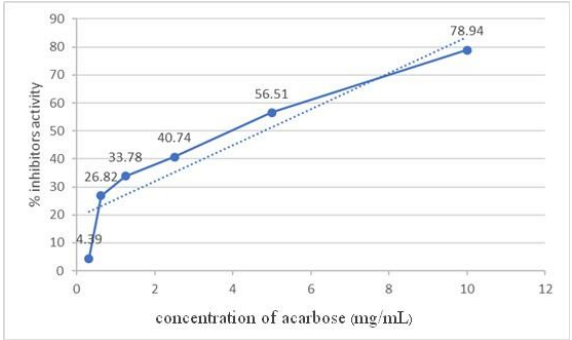

Figure 2. Percentage of α-glucosidase inhibitors activity in acarbose.

Table 4. Percentage of α-glucosidase inhibitors activity in crude extract of red vein kratom leaves.

Extract	Percentage of α -glucosidase inhibitors activity in crude extract of red vein kratom leave (mg/mL)					
	0.3125	**0.625**	**1.25**	**2.5**	**5**	**10**
H	ND	ND	ND	0.78 ±11.52^{aA}	5.71 ±10.82^{bA}	14.53 ±8.49^{cA}
D	ND	ND	ND	4.40 ±7.73^{aA}	22.55 ±7.19^{bB}	48.00 ±7.95^{cB}
EA	0.51 ±5.20^{aA}	3.27 ±2.24^{aA}	9.27 ±3.08^{bA}	21.29 ±3.21^{cB}	38.89 ±2.22^{dC}	61.52 ±0.47^{eC}
E	ND	ND	0.29 ±2.54^{aB}	2.96 ±5.72^{aA}	12.47 ±0.56^{bD}	32.39 ±0.67^{cD}
W	ND	ND	ND	ND	ND	ND
A	4.39 ±0.79^{aB}	26.82 ±8.93^{bB}	33.78 ±0.84^{bC}	40.74 ±7.38^{bC}	56.51 ±11.38^{cE}	78.94 ±2.97^{dE}

Note:H = hexane, D = dichloromethane, EA = ethyl acetate, E = ethanol, W = distilled water, A = acarbose, andND means not detected,$^{a,\ b,\ and\ c}$The horizontal mean, labeled with different letters, had a statistically significant difference($p< 0.05$)., $^{A,\ B,\ and\ C}$ The vertical mean, labeled with different letters, had a statistically significant difference ($p < 0.05$).

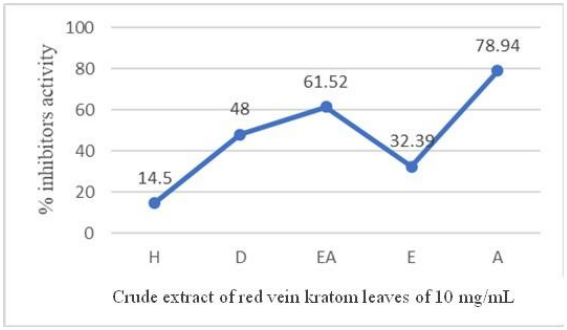

Figure 3. Percentage of α-glucosidase inhibitors activity in crude extract of red vein kratom leaves at maximum concentration of 10 mg/mL (H = hexane, D = dichloromethane, EA = ethyl acetate, E = ethanol and A = acarbose).

The α-glucosidase inhibitors activity of 1 U/mL in green kratom leaves had a positive control of acarbose.[19]In this regard, there was a percentage ofα-glucosidase inhibitors activity at a minimum concentration of 0.3125 mg/mL and a maximum concentration of 10 mg/mL, representing 4.39±0.79, 26.82±8.93, 33.78±0.84, 40.74±7.38, 56.51±11.38 and 78.94±2.97, respectively(**Figure 2**).The crude extracts of green vein kratom leaves from various solvents showed percentage of α-glucosidase inhibitors activity as follows(**Table 5**): Dichloromethane extract had a percentage of α-glucosidase inhibitors activity at a minimum concentration of 5 mg/mL and a maximum concentration of 10 mg/mL, representing 2.81±12.16 and 19.05±11.69, respectively. Ethyl acetate extract had a percentage of α-glucosidase inhibitors activity at a minimum concentration of 0.3125mg/mL and a maximum concentration of 10 mg/mL, representing 1.92±2.21, 6.76±1.79, 14.35 ±0.63, 23.95±1.29, 33.36 ±1.93 and 42.63±9.08, respectively. Theα-glucosidase inhibitors activity was not found inhexane,ethanol and distilled water extract. The percentage of α-glucosidase inhibitors activity in various crude extracts of green vein kratom leaves at maximum concentration of 10 mg/mLcompared with acarbose (positive control) as shown in**Figure 4**.

Table 5.Percentage of α-glucosidase inhibitorsactivity in crude extract of green vein kratom leaves.

Extract	Percentage of α -glucosidase inhibitors activity in crude extract of green vein kratom leave (mg/mL)					
	0.3125	**0.625**	**1.25**	**2.5**	**5**	**10**
H	ND	ND	ND	ND	ND	ND
D	ND	ND	ND	ND	2.81 $\pm12.16^{aA}$	19.05 $\pm11.69^{bA}$
EA	1.92 $\pm2.21^{aA}$	6.76 $\pm1.79^{abA}$	14.35 $\pm0.63^{bA}$	23.95 $\pm1.29^{cA}$	33.36 $\pm1.93^{dB}$	42.63 $\pm9.08^{eB}$
E	ND	ND	ND	ND	ND	ND
W	ND	ND	ND	ND	ND	ND
A	4.39 $\pm0.79^{aB}$	26.82 $\pm8.93^{bB}$	33.78 $\pm0.84^{bB}$	40.74 $\pm7.38^{bB}$	56.51 $\pm11.38^{cC}$	78.94 $\pm2.97^{dC}$

Note:H = hexane, D = dichloromethane, EA = ethyl acetate, E = ethanol, W = distilled water, A = acarbose, andND means not detected, $^{a, b, and\ c}$The horizontal mean, labeled with different letters, had a statistically significant difference($p< 0.05$), $^{A, B, and\ C}$ The vertical mean, labeled with different letters, had a statistically significant difference ($p < 0.05$).

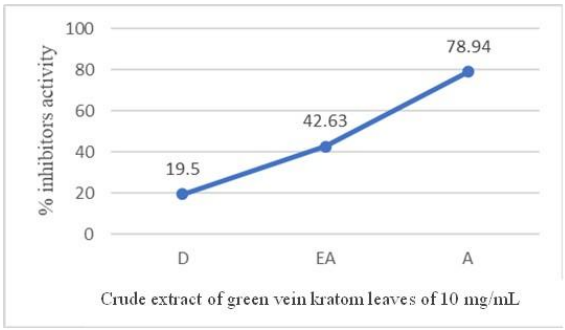

Figure 4.Percentage of α-glucosidase inhibitors activity in crude extract of green vein kratom leavesat maximum concentration of 10 mg/mL (D = dichloromethane, EA = ethyl acetate and A = acarbose).

To compare the concentrations of the extracts from the red vein kratom leaves and the green vein kratom leaves for the determination of IC_{50}, it was found that the inhibitory concentrations of the α-glucosidase activity of 50% of the positive controls were as follows: Acarbose was 15.74 mg/mL. Crude extract of red vein kratom leaves with ethyl acetate was 17.28 mg/mL.[19]Crude extract of ethanol was more 50 mg/mL. Green vein kratom leaves with 50 percent of α-glucosidase inhibitor activity were as follows: Crude extracts of ethyl acetate wasmore50 mg/mL as shown in **Table 6**.

Table 6. Concentrations of α-glucosidase inhibitors extracts at 50% (IC_{50}).

Extracts	**Concentrations of α-glucosidase inhibitors extracts at 50% (IC_{50}) (mg/mL)**	
	Red vein kratom leaves	**Green vein kratom leaves**
H	ND	ND
D	ND	ND
EA	17.28^{B}	$>50^{C}$
E	$>50^{C}$	ND
W	ND	ND
A	15.74^{A}	

Note: H = hexane, D = dichloromethane, EA = ethyl acetate, E = ethanol, W = distilled water, A = acarbose, andND means not detected, $^{A, B, \text{ and } C}$The vertical mean, labeled with different letters, had a statistically significant difference($p < 0.05$).

CONCLUSIONS

According to preliminary phytochemical testing of extracts from different leaf of the kratom using five solvents: hexane, dichloromethane, ethyl acetate, ethanol and distilled water. Details could be classified as follows. The red vein kratom leaves contained bioactive phytochemicals including terpenoids, saponins, tannins, flavonoids,

and alkaloids, but no anthraquinones. The green vein kratom leaves contained bioactive phytochemicals such as terpenoids, saponins, flavonoids, and alkaloids, but no anthraquinones and tannins.

According to the α-glucosidase inhibitors activity (1 U/mL) using *in vitro* kratom leaf extract, it was found that the red vein kratom leaves had a higher inhibitory effect on the α-glucosidase enzyme than the green vein kratom leaves. It was also observed that ethyl acetate extract of red vein kratom with α-glucosidase inhibitors activity (IC_{50}=17.28 mg/mL) was similar to that of acarbose positive control (IC_{50}=15.74 mg/mL) (there was a statistically significant difference ($p<0.05$).

However, a detailed study of the important phytochemicals of crude extracts and their bioactivity is interesting for further experiments.

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