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α -glucosidase inhibition activities of crude extract and mitragynine from *Mitragyna Speciosa* Korth

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Abstract---The capability in holding the activities of α -glucosidase (1U/mL) of crude extract and mitragynine from *Mitragyna speciosa* Korth 10 mg/mL. Crude ethyl acetate extracts $61.52 \pm 0.47\%$ (IC₅₀ = 17.28 mg/mL) which closed to acarbose $78.94 \pm 2.97\%$ (IC₅₀ = 15.74 mg/mL). Minimum density acarbose 0.1562 mg/mL, $4.95 \pm 5.96\%$, crude hexane extracts 2.5 mg/mL, $0.78 \pm 11.52\%$, crude dichloromethane extracts 2.5 mg/mL, $4.40 \pm 7.73\%$, crude ethyl acetate extracts 0.3125 mg/mL, $0.51 \pm 5.20\%$, crude ethanol extracts 1.25 mg/mL, $0.29 \pm 2.54\%$, crude methanol extracts 0.625 mg/mL, $0.71 \pm 0.49\%$, fractions c (Mitragynine) 5 mg/mL, $1.54 \pm 4.50\%$, crude water extracts, fractions a and b, it was found that there was no activity holding of glucosidase enzyme which meant that korth had the ability to hold the activity of α -glucosidase enzyme in extracting by ethyl acetate. It was also another treatment option and to reduce adverse effects from medication in patients with type 2 diabetes. According to research, the effects of consuming korth leaves should be cautious. Studies on the neuroprotective effects of mitragynine showed that mitragynine could stimulate the nervous system just like cocaine, thereby causing nausea and vomiting. Additionally, korth leaf extract had been reported to be toxic to the liver and kidneys.

Keywords--- α -Glucosidase, *mitragyna speciosa* Korth, Mitragynine, crude, extracts.

Introduction

Diabetes mellitus is caused by the abnormality exposure of insulin that leads to Hyperglycemia¹. The number of diabetes patients is increasing rapidly. It is predicted that in 2035, the number will rise to 592 million patients². In the treatment of diabetes, doctors will order pills that suppress the absorption of glucose such as acarbose, which acts by inhibiting α -glucosidase (α -glucosidase), an enzyme that plays an important role in catalyzing the hydrolysis of starch into glucose. However, these drugs, if used for a long time, can cause side effects in patients such as liver toxicity and adverse gastrointestinal symptoms and makes longer treatment time with different medication³. In 2006, the American Embassy in Thailand and Office of the Narcotics Control Board Ministry of Justice has supported the preparation of books *Mitragyna speciosa* Korth in Thai society Part of the content of this book. It was shown in the percentage of diseases treated by southern folk healers with *Mitragyna speciosa* Korth. One of them is using *Mitragyna speciosa* Korth to treat diabetes for 63.3%⁴. *Mitragyna speciosa* Korth is in Rubiaceae found mostly in South East Asia, especially Thailand, and Malaysia. The main active ingredient is Mitragynine⁵ derived from the belief of science that comes from experience passed down from generation to generation of folk medicine. In the treatment of diabetes, it is very interesting to analyze and experiment with scientific experiments to determine the trend. Indeed, mitragynine can be used to treat diabetes according to the beliefs and experiences, and wisdom of the local folk medicine or not.

Research objectives

Capability in holding the activities of α -glucosidase (1U/mL) of crude extract and mitragynine from *Mitragyna speciosa* Korth.

Research Methodology

Tools and Chemical substance

Mitragyna speciosa Korth leaves were derived from Ratchaburi province. The leaves were tested by identification and the research is controlled by using the same origin of the leaves. The leaves were cleaned, dried at 30 degrees, and grinded. Chemical substance are *p*-NPG (*p*-nitrophenyl- α -D-glucopyranoside) 5.0 mM, α -glucosidase in sodium phosphate buffer (pH 6.8) 0.2 U/mL, sodium phosphate buffer (pH 6.8) 10.0 mM, sodium carbonate (Na₂CO₃) 1.0 mM, DMSO (Dimethyl sulfoxide), acarbose (10 mg/mL), hexane, dichloromethane, ethyl acetate, ethanol, methanol and water. Research tool, EZ Read 2000 Microplate Reader.

Crude extract process

Preparation of crude extract using solvents as follows: hexane, dichloromethane, ethyl acetate, ethanol, methanol and water (200 mL) by weighing 20 grams of ground kratom leaves powder, extracting with 200 ml of solvent each time, evaporating the crude extract each time until the solution is completely evaporated. Then weigh the extracted crude extract.

Chemical composition separation of mitragynine and derivatives found by using the technique column chromatography

Dissolve the substance with ethyl acetate for 5-6 drops, thin-layer chromatography (TLC) to find the position of all the substances with mobile phase, ethyl acetate: hexane in the ratio of 7:3 and bring TLC sheet to see under UV light. The position of the substance will be defined in opaque circle and separated the mixture with column chromatography by silica gel as absorbent and ethyl acetate: hexane in the ratio of 2:8 in mobile phase, followed by collect fractions and test each fraction with the TLC technique, the lower one will fall first. Then, increasing eluting to ethyl acetate: hexane in the ratio of 7:3 and collect fractions until the substance is fully tested and combine fractions with the same substance in round bottom flasks, dry, and weigh. After that, then evaporate the eluting solvent with a rotary evaporator. Leave to dry and weigh the resulting substance. The extracted substance was analyzed by Nuclear Magnetic Resonance (NMR) technique using CDCl_3 as a solvent and identified by Fourier transform infrared spectroscopy (FTIR) technique.

Method for testing the inhibitory activity of α -glucosidase six of *Mitragyna speciosa* crude extract and pure mitragynine

Prepare concentrated crude extract of 0.1562, 0.3125, 0.625, 1.25, 2.5, 5 and 10 mg/mL dissolved with dimethyl sulfoxide. The solution used in concentration with the volume of 20 μL in 96 well-plate, and sodium phosphate buffer 0.1 M (pH 6.8) 50 μL , add α -glucosidase (1 U/mL) 20 μL . Mix all substances and heat at 25°C for 15 minutes. Measure the absorbance at wavelengths of 405 nm for one time and put solution *p*-NPG 0.02 M (Substrate) of 20 μL in each mixture and heat at 25°C for 5 minutes. Then put solution Na_2CO_3 of 10 μL to stop the reaction in each mixture and measure light absorbance at wavelength 405 nm by EZ Read 2000 Microplate Reader.

Statistical Analysis

Analyze the average for the other 3 times. (mean $\pm$ standard derivation), analysis of variance (ANOVA), Least significant difference (LSD), Duncan multiple range test (DMRT), and find the coefficient by using SPSS Program. Concentrations of extracts that inhibit α -glucosidase activity IC_{50} by using program IC_{50} Calculator to find the coefficient of the data with SPSS.

Result and Discussion

Crude extract weight

From the study of *Mitragyna speciosa* with solvent hexane, dichloromethane, ethyl acetate, ethanol, methanol and water, the results of crude extract from each solvent are hexane 2.00%, dichloromethane 5.00%, ethyl acetate 3.00%, ethanol 12.00%, methanol 15.00%, and water 4.00% (percent of extraction %yield (w/w) is calculated from (dried weight of crude extract/dried weight of leaves before extraction) $\times 100$).

Chemical composition separation of mitragynine and derivatives found in *Mitragyna speciosa* by technique column chromatography

When the dissolution of the chemical substance in *Mitragyna speciosa* with the technique of column chromatography found that substances are three fractions which are **a**, **b**, and **c** presented in Figure 1

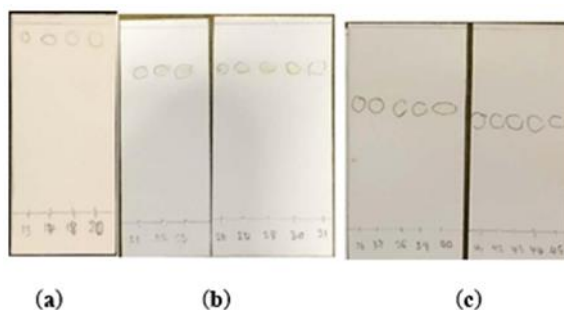


Figure 1. Spots of chemical substance fractions a, b and c on TLC Sheet

Proof of fractions a with FTIR technique, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$

Fractions **a** from *Mitragyna speciosa* weighs 0.0029 g. to characterize with FTIR technique, ^1H and $^{13}\text{C-NMR}$ with the following results of FTIR spectrum fractions **a** found stretching peak and bending of C-H at 2924 cm^{-1} and 2855 cm^{-1} peak of C-C and C=C of aromatic, the peak is 1461 cm^{-1} stretching peak of the carbonyl group at 1700 cm^{-1} and peak of N-H is more than 3000 cm^{-1} , $^1\text{H-NMR}$ δ : 5.05-5.20 (m, 1H), 2.70-2.80 (m, 1H), 2.20-2.35 (m, 1H), 1.50-1.85 (m, 12H), 1.15-1.50 (m, 46H), 0.50-1.10 (m, 8H) and $^{13}\text{C-NMR}$ δ : 135.0, 131.2, 124.4, 39.4, 37.1, 34.6, 32.7, 29.6, 28.0. From the value of chemical shift, it was found that proton (^1H) and carbon (^{13}C) at the different positions are not conformed to the structure of mitragynine and paynantheine of previous research. It is predicted to be triglyceride because it has a chemical shift value with the numbers of the proton (^1H) and carbon (^{13}C) at positions which tallied with the structure of triglyceride as in the previous report

Proof of fractions **b** with FTIR technique, ^1H -NMR and ^{13}C -NMR

Fractions **b** from *Mitragyna speciosa* weighs 0.0159 g to characterize with FTIR technique, ^1H , and ^{13}C -NMR with the following results of FTIR spectrum of fractions **b** found stretching peak and bending of C-H at 2925 cm^{-1} and 2855 cm^{-1} peak of C-C and C=C of the aromatic found peak of 1460 cm^{-1} found peak stretching of the carbonyl group at 1700 cm^{-1} and peak of N-H is more than 3000 cm^{-1} , ^1H -NMR δ : 7.51-7.58 (m, 2H), 7.39-7.49 (m, 3H), 7.28-7.39 (m, 6H), 7.20-7.28 (m, 1H), 6.56 (d, 1H), 4.10 (q, 2H), 3.73-3.87 (m, 2H), 3.60 (s, 1H), 2.87-3.19 (m, 2H), 1.65 (bs, 1H), 1.25 (t, 3H) and ^{13}C -NMR δ 169.3, 165.6, 161.5, 158.7, 141.0, 137.6, 130.7, 128.9, 127.3, 126.3, 124.0, 119.3, 90.6, 62.1, 60.6, 58.9, 52.1, 44.9, 36.2, 35.0, 32.4, 29.7, 14.0. From the value of chemical shift, it was found that proton (^1H) and carbon (^{13}C) at different positions are not conformed to the structure of mitragynine and paynantheine of previous research.

Proof of fractions **c** with FTIR technique, ^1H -NMR and ^{13}C -NMR

Fractions **c**, likely to be mitragynine weighs 0.0073 g to Characterize with FTIR technique, ^1H -NMR, and ^{13}C -NMR with the following results of FTIR spectrum of fractions **c** found peak stretching and bending of C-H at 2950 cm^{-1} peak of C-C and C=C of aromatic, it was found that peak is about 1462 cm^{-1} and stretching peak of carbonyl group at 1698 cm^{-1} peak of C-O from ester found peak at 1284 cm^{-1} and peak of N-H found more than 3000 cm^{-1} , ^1H -NMR δ : (mitragynine)^{7,8} 7.65 (s, 1H), 7.41 (s, 3H), 6.97 (dd, 1H), 6.88 (dd, 3H), 6.43 (dd, 3H), 3.86 (s, 3H), 3.71 (s, 3H), 3.69 (s, 3H), 3.14 (d, 1H), 3.10 (m, 2H), 3.02 (ddd, 1H), 2.99 (dd, 2H), 2.51 (m, 2H), 1.78 (m, 2H), 1.66 (m, 1H), 1.18 (m, 2H), 0.85 (t, 3H) and ^1H -NMR δ : (fractions **c**) 7.79 (s, 1H), 7.43 (s, 1H), 6.97 (s, 1H), 6.89 (s, 1H), 6.45 (d, 1H), 3.88 (s, 3H), 3.71 (s, 3H), 3.70 (s, 3H), 3.16 (d, 1H), 3.05 (m, 2H), 2.98 (d, 1H), 2.99 (m, 2H), 2.51 (m, 2H), 1.78 (m, 2H), 1.61 (m, 1H), 1.25 (s, 2H), 0.87 (t, 3H, $J=7.5\text{ Hz}$), ^{13}C -NMR δ : (mitragynine)^{7,8} 169.6, 160.5, 154.5, 137.2, 133.7, 121.8, 117.7, 111.5, 107.9, 104.1, 99.8, 61.5, 61.2, 57.8, 55.3, 53.8, 51.3, 40.7, 39.9, 30.0, 23.9, 19.1, 12.9 and ^{13}C -NMR δ : (fractions **c**) 169.2, 160.5, 154.5, 137.2, 133.5, 121.8, 117.7, 111.3, 107.7, 104.3, 99.8, 61.5, 61.2, 57.5, 55.3, 53.8, 51.3, 40.7, 39.9, 29.9, 23.9, 19.1, 12.9. From the value of chemical shift, it was found that proton (^1H) and carbon (^{13}C) at different positions are conformed to the structure of mitragynine of previous research^{8,9}

Test the inhibitory activity of α -glucosidase of *Mitragyna speciosa* crude extract, and pure mitragynine

Test the inhibitory activity of α -glucosidase of 1 U/mL of crude extract of *Mitragyna speciosa* and pure mitragynine with column chromatography technique with positive control factor acarbose that can inhibit the activity of α -glucosidase by Table 1.

Table 1
Percent of the inhibitory activity of α -glucosidase of acarbose from the crude extract from *Mitragyna speciosa* and pure mitragynine

Solution	Percent of the inhibitory activity of α -glucosidase of acarbose from crude extract from <i>Mitragyna speciosa</i> and pure mitragynine						
	0.1562	0.3125	0.625	1.25	2.5	5	10
Hexane	ND	ND	ND	ND	0.78	5.71	14.53
					$\pm 11.32^{1A}$	$\pm 10.82^{1A}$	$\pm 8.49^{1A}$
Dichloro-methane	ND	ND	ND	ND	4.40	22.55	48.00
					$\pm 7.73^{1A}$	$\pm 7.19^{1A}$	$\pm 7.95^{1B}$
Ethyl acetate	ND	0.51	3.27	9.27	21.29	38.89	61.52
		$\pm 5.20^{1A}$	$\pm 2.24^{1A}$	$\pm 3.08^{1A}$	$\pm 3.21^{1A}$	$\pm 2.22^{1C}$	$\pm 0.47^{1C}$
Ethanol	ND	ND	ND	0.29	2.96	12.47	32.39
				$\pm 2.54^{1A}$	$\pm 5.72^{1A}$	$\pm 0.56^{1B}$	$\pm 0.67^{1B}$
Methanol	ND	ND	0.71	2.77	3.40	18.00	32.02
			$\pm 0.49^{1A}$	$\pm 1.97^{1A}$	$\pm 1.17^{1A}$	$\pm 1.33^{1A}$	$\pm 1.20^{1B}$
Fractions c (Mitragynine)	ND	ND	ND	ND	ND	1.54	8.24
						$\pm 4.50^{1A}$	$\pm 3.29^{1A}$
Acarbose	4.95	4.39	26.82	33.78	40.74	56.51	78.94
	$\pm 5.96^{1A}$	$\pm 0.79^{1A}$	$\pm 8.93^{1A}$	$\pm 0.84^{1A}$	$\pm 7.38^{1A}$	$\pm 11.38^{1A}$	$\pm 2.97^{1B}$

abc Horizontal average with different alphabets has different statistic value ($p \leq 0.05$). ABC Vertical average with different alphabets has different statistic values ($p \leq 0.05$). ND Not Detected, Water fractions a and fractions b Not Detected every concentration. The concentration of extract substance can suppress the activities of suppressing enzyme α -glucosides for 50% (IC_{50}). In the comparison of the concentration of extract substance sample to find the value of IC_{50} found that the concentration of suppressing the activities of enzyme α -glucosides 50% of positive control factors such as acarbose (15.74 mg/ml) and ethyl acetates (17.28 mg/ml) as in Table 2

Table 2
The concentration of extract substance sample to find the value of IC_{50}

Extract	The value of IC ₅₀
Hexane	ND
Dichloromethane	>50
Ethyl acetate	17.28
Ethanol	>50
Methanol	>50
Water	ND
Fractions c(Mitragynine)	ND
Fractions b	ND
Fractions a	ND
Acarbose	15.74
ND: Not Detected	

Research Conclusion

From solution hexane, dichloromethane, ethyl acetate, ethanol, methanol and water and mitragynine and substance divided by column chromatography found three fractions which are a, b, and c. Fractions c and test its identity by Nuclear Magnetic Resonance (NMR) (CDCl_3) and Fourier transform infrared spectroscopy (FTIR) from chemical shift found that proton (^1H) and carbon (^{13}C) at different positions and FTIR spectrum of fractions c conform with structure of mitragynine with has the number and position of ^1H and ^{13}C conformed with previous research⁷⁻⁸. However, fractions a and b, found chemical shifts that proton (^1H) and carbon (^{13}C) and FTIR spectrum are not conformed with the structure of mitragynine. In this research, acarbose is used as a positive control factor. It found that the concentration of crude extract in 10 ml test of *Mitragyna speciosa* from ethyl acetate and inhibit the activity of α -glucoside at $61.52 \pm 0.47\%$ (IC_{50} 17.28 mg/mL) which is closed to acarbose $78.94 \pm 2.97\%$ (IC_{50} 15.74 mg/mL) which has the least concentration of inhibition. Acarbose 0.1562 mg/mL, $4.95 \pm 5.96\%$, hexane 2.5 mg/mL, 0.78 ± 11.52 , dichloromethane 2.5 mg/mL, $4.40 \pm 7.73\%$, ethyl acetate 0.3125 mg/mL, $0.1 \pm 5.20\%$, ethanol 1.25 mg/mL, $0.29 \pm 2.54\%$, methanol 0.625 mg/mL, $0.71 \pm 0.49\%$, fractions c (mitragynine) 5 mg/mL, $1.54 \pm 4.50\%$, water, fractions a and b found no inhibition activity. This shows that *Mitragyna speciosa* can inhibit the activity of α -glucosidase with solution ethyl acetate. This is another way to treat and mitigate the unpleasant side effect of diabetes type-2 which is derived from the old science and tradition of local doctors in the treatment of diabetes which is complied with the research of Ankit Saneja in 2009⁹ found that the leave of *Mitragyna speciosa* triggers the delivery of glucose to muscle cell and affects the mitigation of sugar in the blood. In 2014 Lee¹⁰ made the experiment of *Mitragyna speciosa* with ethanol found the effect in suppressing α -glucosidase 20.83% but it has the concern in usage because in Thailand *Mitragyna speciosa* is an illegal drug as in the law of 1979¹¹ and there are researches about the impact of consumption of *Mitragyna speciosa* about the nervous system of mitragynine found that the nerve system has the same level of cocaine caused vomit¹²⁻¹³ and the crude extracts of *Mitragyna speciosa* are toxic to liver and kidney and the treatment of diabetes with *Mitragyna speciosa* must be studied further.

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