α-GLUCOSIDASE INHIBITORY ACTIVITY OF THAI MIMOSACEOUS PLANT EXTRACTS

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ABSTRACT: Twenty species of Thai plants in Mimosaceae family were exhausively extracted with petroleum ether, dichloromethane and ethanol respectively. The plant extracts were studied for their α-glucosidase inhibition activity by spectrophotometry using 1-deoxynojirimycin as a positive control. The result showed that most percentage yields of the plants were in ethanol extract. *Entada rheedii* seed coat, *Archidendron jiringa* seed coat, *Albizia lebbeck* branch bark, *Parkia speciosa* pericarp and *Albizia lebbeckoides* bark exhibited very high inhibition with IC_{50} of 0.0043, 0.0054, 0.0397, 0.0581 and 0.0702 mg/ml respectively. Moreover, the stem, leaves, branch and bark of *Xylia xylocarpa* also displayed high α-glucosidase inhibitory activity. The most fractions that showed the promising activity are 95 % ethanolic extracts. It is of interest that these species should be further studied for the treatment diabetes mellitus (type II) as the glucosidase inhibitor and the lowering of blood glucose level.

Keywords: α-glucosidase inhibition, Mimosaceous plant

INTRODUCTION: There are 2,000 plants species as ingredients in traditional medicinal prescriptions and more than 300 plants are frequently used since early Rattanakosin period. At present, most of people interested in herbal healthcare and treatment. There are several etnomedical use of plants for decreasing of blood glucose with some scientific supports such as *Drynaria quercifolia* Linn.[1-3]. α-Glucosidase is the intrational enzyme for digestion of polysaccharide and oligosaccharide to monosaccharides[4]. α -Glucosidase inhibitory testing is useful for screening plants which should be used for blood glucose treatment. The previous studies showed α -glucosidase inhibitory activity from substances which developed to phamarceutical substances used such as Acarbose (Glucobay®) from microbial bacteria *Actinoplane* sp.[5], *Voglibose (basen*®) from *Streptomyces hygroscopicus* var*. limoneus*[6] and Miglitol (Glyset®) from *S. roseochromogenus*[7]. This study aimed to screen α - glucosidase inhibitory activity from Thai plant extracts of Mimocaceae family**.**

MATERIALS AND METHODS:

Sample Collection

Twenty Mimocaceous plants were collected from Ratchaburi, Rayong, Pathumthani, Nakhonpathom and Bangkok. All plant samples were identified by Ruangrungsi N. and deposited at the Faculty of Pharmaceutical Sciences Herbarium, Chulalongkorn University.

Sample Preparation

The plants were separated in each part such as twig, leaves, stem and bark and each plant parts were air dried. They were grounded and stored at room temperature.

Solvent Extraction

Dried powdered plant parts were weighed 10 – 30 mg and exhaustive extracted with 500 ml of petroleum ether, dichloromethane and ethanol for at least 8 hours or colourless of each solvent by soxhlet apparatus. The solvent extracts were evaporated to dryness by rotary evaporartor and water bath evaporation respectively. The extracts yields were weighed, recorded and stored at -20°C.

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α-Glucosidase Inhibition Activity

1-Deoxynojirimycin and α-glucosidase from *Saccharomyces cerevisiae* were purchased from Sigma Chemical Co. Ltd (St. Louis, MO). All other chemicals were analytical grade. α-Glucosidase activity assay was developed according to Adisakwattana *et al*[8]. α-Glucosidase was assayed using 0.1 M phosphate buffer at pH 6.9 and 1 mM *p*-nitrophenyl-α-D-glucopyranoside (PNP-G) 950 µl was used as substrate. The concentration of the enzyme was 1 Unit / ml in each incubated (4 µl) in 1 mg/ml of plant extracts in dimethylsulfoxide (1µl) and α-glucosidase (4 µl) was added to the mixture. The reaction was carried out at 37°C for 20 minute and then 1 M Na_2CO_3 (100 µl) was added to terminate the reaction. Enzymatic activity was quantified by measuring the absorbance at 405 nm. One unit of α-glucosidase is defined as the amount enzyme liberating 1.0 µmole of *p*-nitrophenol (PNP) per minute under the conditions specified. 1mM 1-deoxynorijimycin was used as the positive control in this study.

$$\% \text{ Inhibition} = 100 - \frac{(\text{Abs of sample} - \text{Abs of blank}) \times 100}{\text{Abs of control}}$$

Abs = Absorbance at 405 nm

Control = DMSO (dimethylsulfoxide),

Blank = buffer

Half Inhibition Concentrations (Ic_{50})

Half Inhibition Concentrations (IC_{50}) of the extracts were determined by constructing a dose-response curve and examined the concentrations of concentration that inhibited 50% of enzyme activity. IC_{50} is less corresponding to show high α-glucosidase inhibition.

RESULTS: The percentage yield of each plant extracts were shown in table 1. Mostly, the higher yields were from ethanolic extracts. This study showed that mostly α-glucosidase inhibitory activity of plant extracts were in ethanolic extracts which *Entada rheedii* Spreng. seed coat, *Archidendron jiringa* I.C. Nielsen seed coat, *Cathormion umbellatum* (Vahl) Kosterm. stem bark, *Albizia lebbeck* (L.) Benth. branch bark, *Albizia procera* (Roxb.) Benth. stem bark, *Xylia xylocarpa* (Roxb.) Taub. branch, and *Pithecellobium dulce* Benth. stem bark exhibited very high activity whilst *Parkia speciosa* Hassk. pericarp, *Cathormion umbellatum*. (Vahl) Kosterm. leaves, *Albizia lebbeckoides* (DC.) Benth. bark, *Acacia catechu* (L.f.) Willd. leaves and *Samanea saman* (Jacq.) Merr**.** bark showed high enzyme inhibitory activity activity. Moreover, The stem, leaves and bark of *X. xylocarpa* (Roxb.) Taub**.** displayed also high α-glucosidase inhibitory activity (table 2).

Half Inhibition Concentrations (IC_{50}) of the plant extracts which high enzyme inhibitory activity more than 80 % were varied from 0.0043 to 0.1796 mg/ml (table 3). The IC_{50} of *Entada rheedii* Spreng. seed coat, *Archidendron jiringa* I.C. Nielsen seed coat, *Albizia lebbeck* (L.) Benth. branch bark, *Parkia speciosa* Hassk. pericarp and *Albizia lebbeckoides* (DC.) Benth. bark were 0.0043, 0.0054, 0.0397, 0.0581 and 0.0702 mg/ml respectively whilst IC_{50} of 1-deoxynojirimycin was 5.0540 mg/ml (25.27 mM).

DISCUSSION: Plants have played a crucial role in maintaining human health and improve quality of life as foods, fruits, beverages, cosmetics, dyes and medicines for thousand years. Mimosaceae is one of 34 families as well as some fruits and vetgetables in culinary plants such as *Parkia speciosa* Hassk., *Pithecellobium dulce* Benth., *Neptunia oleracea* Lour. and *Acacia rugata* Merr**.** In this sense, some plants contain phytochemicals as alkaloids, phenolics, turpinoids or glycosides that are useful to maintenance of human health. This study showed that plants in Mimocaceae family were exhibited very high enzyme inhibition activity such as *Entada rheedii* Spreng. seed coat, *Archidendron jiringa* I.C. Nielsen seed coat and *Albizia lebbeck* (L.) Benth. branch bark which IC_{50} were less than 5 µg/ml. Moreover, most parts (leaves, stem, branch and bark) of *X. xylocarpa* (Roxb.) Taub. also showed high enzyme inhibition which IC_{50} range were 100-180 µg/ml. No previous data of *Entada rheedii* Spreng., *Archidendron jiringa* I.C. Nielsen and *X. xylocarpa* (Roxb.) Taub. showed this biological activity but there were *Albizia lebbeckoides* (DC.) Benth. stem bark could decrease blood glucose level in white rat[9] and had no toxicity by oral or subcutaneous routs[10]. *Parkia speciosa* Hassk. seed was as a purgative and could decrease blood glucose level by β-sitosterol and sigmasterol[11,12] whilst empty pods showed hypo-glycemic effect from stigmast-4-en-3-one[13]. This study showed its seed less enzyme inhibition activity than pericarp. *X. xylocarpa* (Roxb.) Taub. is the one of economic plant for building and its seed is as food[14-15] but high enzyme inhibitory activity would be interested for increasing economic value. It should be further studied of removing tannin and re-test enzyme activity and confirming with other assays.

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Table 1 Yield of Plant Extractions

Mimosaceous plants	% Yield of Plant Extractions (w/w)*		
	Petroleum ether	Dichloro methane	Ethanol
***Acacia catechu* (L.f.) Willd.**[1]			
leaves	2.60	5.00	16.40
branch	0.60	19.90	3.80
***Acacia farnesiana* (Linn.) Willd.**[2]			
twig	2.60	6.73	11.53
***Acada pennata* (L.) Willd.**[2]			
twig	1.04	2.12	14.52
***Acacia rugata* Merr.**[2]			
leaves	3.47	3.00	12.60
pericarp	4.03	0.93	22.76
***Adenanthera microsperma* Teijsm.**[2]			
leaves	34.00	11.00	11.30
branch	0.87	0.27	3.67
***Adenanthera pavonina* Linn.**[2]			
leaves	9.15	2.20	17.30
branch	0.87	0.67	4.07
***Albizia lebbeck* (L.) Benth.**[2]			
leaves	3.40	1.60	12.00
branch	0.73	8.40	4.13
branch bark	2.07	1.33	4.73
***Albizia lebbeckoides* (DC.) Benth.**[3]			
leaves	1.50	1.00	7.50
branch	4.00	0.60	2.60
bark	1.80	0.28	11.48
***Albizia myriophylla* Benth.**[1]			
leaves	15.62	2.87	13.12
branch	20.62	1.87	5.12
***Albizia procera* (Roxb.) Benth.**[2]			
stem bark	0.40	0.37	6.17
***Archidendron jiringa* I.C. Nielsen**[2]			
seed	0.50	0.30	4.75
seed coat	0.60	0.20	14.40
***Cathormion umbellatum* (Vahl) Kosterm.**[2]			
leaves	1.55	1.00	22.45
branch	1.00	0.20	5.67
bark	1.07	0.67	15.33
***Entada rheedii* Spreng.**[2]			
seed coat	0.12	0.05	5.17
cotyledon	8.12	2.50	4.47
pericarp	0.55	0.20	5.10
***Leucaena glauca* Benth.**[4]			
twig	1.72	1.96	10.28
pericarb	0.50	0.20	5.95
***Mimosa pudica* Linn.**[4]			
twig	2.95	1.00	12.60
***Neptunia oleracea* Lour.**[2]			
twig	1.90	8.05	11.00
***Parkia speciosa* Hassk.**[2]			
seed	23.60	3.93	10.37
pericarb	2.40	0.53	2.63
***Pithecellobium dulce* Benth.**[2]			
leaves	5.73	5.73	12.47
stem bark	0.48	0.48	2.64
***Samanea saman* (Jacq.) Merr.**[2]			
leaves	4.54	3.27	14.82
branch	1.50	1.92	6.83
bark	5.64	1.50	45.14
***Xylia xylocarpa* (Roxb.) Taub.**[5]			
leaves	4.73	2.00	22.40
stem	0.85	0.50	5.50
branch	0.48	13.39	18.78
bark	0.86	0.46	30.56

*Percentage yield (w/w) was calculated as (dry extract weight/dry starting material dry weight) × 100.
[1]Rayong [2]Bangkok [3]Nakhonpathom [4]Pathumthani [5]Ratchaburi

Table 2 α-Glucosidase inhibitory activity of plant extracts

Mimosaceous plants	% α-Glucosidase inhibition*		
	Petroleum ether	Dichloro methane	Ethanol
***Acacia catechu* (L.f.) Willd.**			
leaves	3.06 ± 2.89	2.51 ± 2.20	**82.28 ± 1.03**
branch	0.92 ± 1.38	0.68 ± 1.18	64.35 ± 1.14
***Acacia farnesiana* (Linn.) Willd.**			
twig	29.04 ± 14.51	33.11 ± 9.61	77.52 ± 8.33
***Acada pennata* (L.) Willd.**			
twig	12.21 ± 7.30	14.65 ± 6.25	5.81 ± 8.66
***Acacia rugata* Merr.**			
leaves	20.38 ± 19.17	16.50 ± 8.31	13.69 ± 11.86
pericarp	34.88 ± 11.47	30.43 ± 13.65	17.02 ± 10.55
***Adenanthera microsperma* Teijsm.**			
leaves	7.81 ± 6.99	5.27 ± 9.12	1.34 ± 2.33
branch	33.25 ± 13.78	32.41 ± 10.72	33.18 ± 17.36
***Adenanthera pavonina* Linn.**			
leaves	5.32 ± 5.45	2.80 ± 4.85	
branch	13.46 ± 9.72	14.18 ± 9.14	27.65 ± 4.80
pericarp	3.95 ± 4.96	4.23 ± 1.89	10.74 ± 6.70
***Albizia lebbeck* (L.) Benth.**			
leaves	24.64 ± 15.27	20.59 ± 17.81	18.51 ± 18.03
branch	47.85 ± 18.90	42.96 ± 14.75	33.10 ± 12.12
branch bark	19.97 ± 11.46	12.82 ± 8.44	**93.85 ± 0.98**
***Albizia lebbeckoides* (DC.) Benth.**			
leaves	2.06 ± 3.57	4.26 ± 5.09	36.34 ± 6.28
branch	6.21 ± 6.81	4.47 ± 3.95	10.01 ± 3.98
bark	4.91 ± 4.89	4.47 ± 3.95	**83.98 ± 7.59**
***Albizia myriophylla* Benth.**			
leaves	20.22 ± 8.04	14.56 ± 13.36	33.17 ± 10.34
branch	33.45 ± 11.77	24.86 ± 8.81	28.28 ± 10.46
***Albizia procera* (Roxb.) Benth.**			
stem bark	45.81 ± 18.83	33.31 ± 7.19	**92.01 ± 2.31**
***Archidendron jiringa* I.C. Nielsen**			
seed	25.57 ± 12.67	18.14 ± 12.19	12.51 ± 6.13
seed coat	35.20 ± 15.95	37.18 ± 11.82	**95.67 ± 0.92**
***Cathormion umbellatum* (Vahl) Kosterm.**			
leaves	24.53 ± 12.69	21.64 ±17.87	**85.04 ± 7.31**
stem	23.71 ± 17.79	21.02 ± 10.57	12.27 ± 8.45
bark	53.04 ± 12.65	32.97 ± 14.13	**94.00 ± 2.34**
***Entada rheedii* Spreng.**			
seed coat	41.02 ± 20.42	35.35 ± 20.29	**98.73 ± 0.46**
cotyledon	25.33 ± 13.57	24.15 ± 13.20	28.08 ± 11.28
pericarp	31.53 ± 6.41	27.09 ± 8.44	74.01 ± 2.02
***Leucaena glauca* Benth.**			
twig	5.29 ± 9.17	0.80 ± 1.39	3.42 ± 3.98
pericarb	24.36 ± 15.15	8.48 ± 1.96	16.31 ±11.27
***Mimosa pudica* Linn.**			
twig	16.58 ± 14.36	16.03 ± 13.91	33.55 ± 16.33
***Neptunia oleracea* Lour.**			
twig	20.72 ± 7.33	10.14 ± 9.26	15.23 ± 15.20
***Parkia speciosa* Hassk.**			
seed	10.24 ± 9.00	10.57 ± 9.21	45.72 ± 5.67
pericarp	18.62 ± 13.07	21.93 ± 6.82	**89.46 ± 5.12**
***Pithecellobium dulce* Benth.**			
leaves	21.80 ± 12.25	15.18 ± 14.40	36.55 ± 5.13
stem bark	35.10 ± 4.71	39.96 ± 9.12	**90.13 ± 2.60**
***Samanea saman* (Jacq.) Merr.**			
leaves	21.63 ± 14.22	15.61 ± 9.74	26.36 ± 3.68
branch	26.22 ± 12.39	11.92 ± 5.24	14.96 ± 1.77
bark	40.88 ± 17.07	30.65 ± 8.58	**81.59 ± 3.40**
***Xylia xylocarpa* (Roxb.) Taub.**			
leaves	27.99 ± 18.93	42.22 ± 45.31	**89.99 ± 1.88**
stem	11.71 ± 5.86	19.27 ± 10.15	**87.72 ± 2.77**
branch	30.55 ± 8.13	35.82 ± 15.23	**91.56 ± 3.48**
bark	31.72± 9.29	28.04 ± 3.94	**82.87 ± 3.46**

*Percentage of inhibition was calculated at time = 20 min as 100 - % reaction,
whereby the % of reaction = (mean α-Glucosidase in sample/mean α-Glucosidase in control) × 100,
control = DMSO (dimethylsulfoxide)

Table 3 IC_{50} of high α-Glucosidase inhibitory activity plant extracts

Mimosaceous plants	IC_{50} (mg/ml)
Entada rheedii Spreng. seed coat	0.0043
Archidendron jiringa I.C. Nielsen seed coat	0.0054
Albizia lebbeck (L.) Benth. branch bark	0.0397
Parkia speciosa Hassk. pericarp	0.0581
Albizia lebbeckoides (DC.) Benth. bark	0.0702
Cathormion umbellatum (Vahl) Kosterm. bark	0.0704
Albizia procera (Roxb.) Benth. stem bark	0.0772
Pithecellobium dulce Benth. stem bark	0.0842
Acacia catechu (L.f.) Willd. leaves	0.0859
Cathormion umbellatum (Vahl) Kosterm. leaves	0.0908
Xylia xylocarpa (Roxb.) Taub. leaves	0.0998
Xylia xylocarpa (Roxb.) Taub. stem	0.1120
Xylia xylocarpa (Roxb.) Taub. branch	0.1286
Xylia xylocarpa (Roxb.) Taub. bark	0.1432
Samanea saman (Jacq.) Merr. bark	0.1796

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ฤทธิ์ยับยั้งเอนไซม์แอลฟา - กลูโคซิเดสของสารสกัดจากพืชวงศ์ Mimosaceae

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บทคัดย่อ: พืชวงศ์ Mimosaceae จำนวน 20 ชนิดนำมาสกัดด้วย ปิโตรเลียมอีเทอร์ ไดคลอโรมีเทน และ เอทานอล ตามลำดับ นำสารสกัดมาศึกษาฤทธิ์ในการยับยั้งเอนไซม์แอลฟา – กลูโคซิเดส โดยวิธีสเปคโตรโฟโตเมตรี โดยใช้ 1 – ดีออกซีจิริมัยซิน เป็นตัวควบคุมบวก ผลการศึกษาแสดงให้เห็นว่าปริมาณสารสกัดส่วนใหญ่อยู่ในสารสกัดด้วยเอทานอล สารสกัดจากเยื่อหุ้มเมล็ดสะบ้ามอญ เยื่อหุ้มเมล็ดลูกเนียง เปลือกกิ่งคาง ฝักสะตอ และ เปลือกต้นคางแสดงฤทธิ์ยับยั้งเอนไซม์สูง โดยมีค่าความเข้มข้นที่ยับยั้งเอ็นไซม์ได้ร้อยละ 50 เท่ากับ 0.0043, 0.0054, 0.0397, 0.0581 และ 0.0702 มิลลิกรัมต่อมิลลิลิตร ตามลำดับ นอกจากนี้ส่วนต้น ใบ กิ่ง และเปลือกของไม้แดงแสดงฤทธิ์ในการยับยั้งเอนไซม์แอลฟา – กลูโคซิเดสสูงเช่นกัน สารสกัดที่แสดงฤทธิ์ยับยั้งเอนไซม์ส่วนใหญ่ ร้อยละ 95 อยู่ในส่วนสารสกัดเอทานอล เป็นที่น่าสนใจอย่างยิ่งที่จะศึกษาพืชเหล่านี้ต่อไปเพื่อใช้ในการบำบัดรักษาโรคเบาหวาน (ชนิดที่ 2) โดยใช้เป็นตัวยับยั้งเอนไซม์กลูโคซิเดส และทำให้ระดับน้ำตาลในเลือดลดลง

คำสำคัญ: ฤทธิ์ยับยั้งเอนไซม์แอลฟา–กลูโคซิเดส พืชวงศ์ Mimosaceae

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