นิพนธ์ต้นฉบับ

α-Glucosidase Inhibitory Activity of Water Soluble Extract from Thai Mimosaceous Plants

ฤทธิ์ยับยั้งเอนไซม์แอลฟา-กลูโคซิเดสของสารสกัดส่วนละลายน้ำจากพืชวงศ์กระถิน

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Abstract

The polar part of twenty species of Thai plants in Mimosaceae family was studied for their α-glucosidase inhibition activity by spectrophotometry. The result showed that most water soluble parts of *Albizia lebbeck* (L.) Benth branch, *Xylia xylocarpa* (Roxb.) Taub. bark, *Archidendron jiringa* I.C. Nielsen seed coat, *Albizia lebbeck* (L.) Benth. branch bark and *Parkia speciosa* Hassk pericarb showed high α-glucosidase inhibition with 77.90, 75.62, 74.09, 68.10 and 61.86 percent respectively whilst their half inhibition concentrations (IC_{50}) were 0.1554, 0.3143, 0.3829, 0.3965 and 0.4104 mg/ml respectively.

Keywords: α-Glucosidase inhibition, Half inhibition concentrations (IC_{50}), Mimosaceous plant

บทคัดย่อ

สารสกัดส่วนละลายน้ำของพืชวงศ์กระถิน จำนวน 20 ชนิดถูกนำมาศึกษาฤทธิ์ในการยับยั้งเอนไซม์แอลฟา-กลูโคซิเดส โดยวิธีสเปคโตรโฟโตเมตรี ผลการศึกษาแสดงให้เห็นว่า สารสกัดส่วนละลายน้ำของกิ่งก้านจามจุรีสีทอง เปลือกไม้แดง เปลือกหุ้มเมล็ดลูกเนียง เปลือกกิ่งก้านจามจุรีสีทอง และฝักสะตอ แสดงฤทธิ์ยับยั้งเอนไซม์แอลฟา-กลูโคซิเดส สูงสุดที่ 77.90, 75.62, 74.09, 68.10 และ 61.68 เปอร์เซนต์ ตามลำดับ โดยมีค่าความเข้มข้นในการยับยั้งเอนไซม์ที่ร้อยละ 50 ปริมาณเท่ากับ 0.1554, 0.3143, 0.3829, 0.3965 และ 0.4104 มิลลิกรัมต่อมิลลิลิตร ตามลำดับ

คำสำคัญ : ฤทธิ์ยับยั้งเอนไซม์แอลฟา-กลูโคซิเดส ความเข้มข้นในการยับยั้งเอนไซม์ที่ร้อยละ 50 (IC_{50}) พืชวงศ์กระถิน

Introduction

Herbal medicine, also called botanical medicine or phytomedicine, refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Long practiced outside of conventional medicine, herbal treatments are. It is becoming more interesting as up-to-date analysis and research show their value in the treatment and prevention of disease. Plants had been used for medicinal purposes long before recorded history. Recently, the World Health Organization (WHO) estimated that 80% of people worldwide rely on herbs. Increasing public dissatisfaction with the cost of prescription medications, combined with an interest in returning to natural or organic remedies, has led to an increase in the use of herbal medicines[1]. For most herbs, the bioactive substance that causes a therapeutic effect is not known. Whole herbs contain many bioactive compounds, and it is likely that they work together to produce the desired medicinal effect. The herbs available use in several different types: syrups, oils, liquid extracts, tinctures and dry extracts (pills or capsules). Tea is simply dried herbs left to soak for a few minutes in boiling water. Most Thai people use tea for medicinal treatments. There are several plants for ethnomedical use for blood glucose treatment.

α-Glucosidase is the amylase enzyme for digestion of polysaccharide and oligosaccharide to monosaccharides[2], its contributing starch and glycogen metabolisms in plant and animal tissue is characterized by the variety in substrate recognition. Inhibition of α-glucosidase decreases the blood glucose levels via delaying digestion of poly- and oligosaccharides to absorbable monosaccharides[3]. Thus 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 cyanidin-3-galactoside, a natural anthocyanin was an α -glucosidase inhibitor and could be used in combination with acarbose for treatment of diabetes[4]. Some substances have been developed to phamarceutical, such as Acarbose (Glucobay®) from α-Glucosidase microbial bacteria *Actinoplane* sp.[5], *Voglibose (basen*®) from *Streptomyces hygroscopicus* var. *limoneus*[6] and Miglitol (Glyset®) from *S. roseochromogenus*[7].

Mimosaceae is a plant family usually characterized by bipinnate compound leaves and flowers dominated by the stamens. Mimosaceae includes some fruits and vegetables in culinary plants such as *Parkia speciosa* Hassk., *Pithecellobium dulce* Benth., *Neptunia oleracea* Lour. The screening of plants which have high potential for research development of diabetes mellitus (type II)

treatment or blood glucose level controlling in human can be done by studying α-glucosidase inhibitory activity in water extract. So, twenty species of Thai plants in the Mimosaceae family were extracted with solvents and water.

Materials and Methods

Sample collection

Twenty Mimocaceous plants were collected from Suansamunpri (Ratchaburi and Rayong) Pathumthani garden, botanical gardens (Mahidol University, Nakhonpathom and Pharmaceutical Sciences, Chulalongkorn University, Bangkok) and markets (Samyan, Jatujuk) whilst two medicinal plants were purchased from traditional drug store (Vejchapong, Bangkok). All plant samples were identified by botanical classification of plants using macroscopic characteristics.

Sample preparation

The plants were separated into basic parts such as twig, leaves, stem and bark and they were air dried. They were grounded and stored at room temperature. Ten to thirty grams of dried powdered plants were weighed. All samples were extracted using soxhlet apparatus with 500 ml of petroleum ether, dichloromethane, ethanol for at least six hours or until colourless of each solvent. After that water extraction were done with 500 ml of distilled water with continuous shaking at 60°C for 8 hours. The water soluble parts were evaporated to dryness in water bath at 60°C. The plant extract yields were weighed, recorded and stored at -20°C before assays.

α-Glucosidase Inhibition activity and Half Inhibition Concentrations (IC_{50}) Assays

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 U/ml in each incubated (4µl) in 1 mg/ml of plant extracts in distilled water (1µl) and α-glucosidase (4µl) 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 positive control in this study (Figure 1).

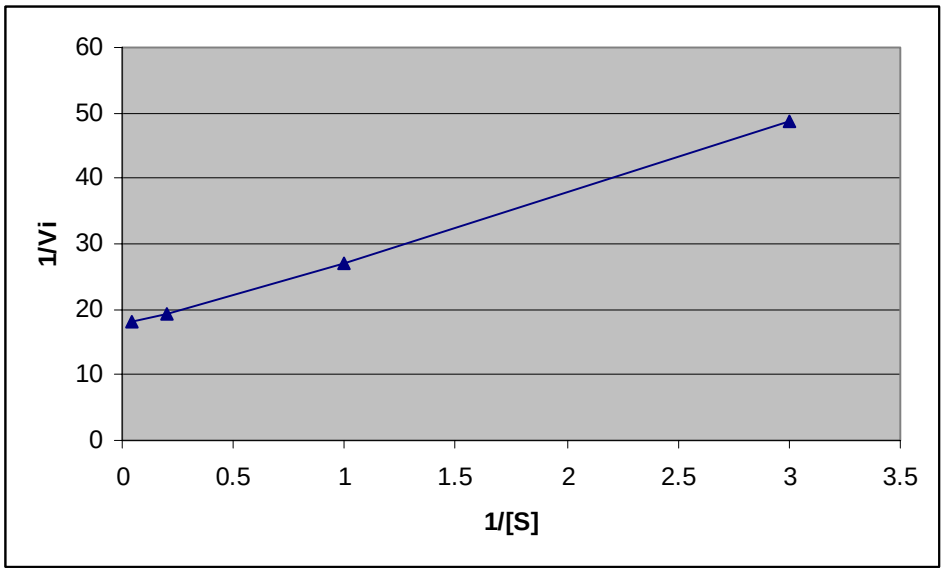

Figure 1 The Lineweaver-Burk plot of α-glucosidase activity ; when
[S] = concentration of substrate at 1, 5, 25, 75 mM
Vi = velocity of reaction proportional to concentration of product
= Δ OD at 405 nm/min

Half Inhibition Concentrations (IC_{50}) of the extracts were determined by constructing a dose-response curve and examining the concentrations that inhibited 50% of enzyme activity and were expressed as mean (mg/ml, *n*=3). IC_{50} is less corresponding to show high α-glucosidase inhibition**.** Percent inhibition was determined by the following equation:

$$\% \text{ Inhibition} = 100 - [(A_{(s)} - A_{(b)})/A_{(c)}] \times 100$$

Where A is the absorbance at 405 nm, $A_{(s)}$ is the absorbance of sample or positive control, $A_{(b)}$ is the absorbance of blank or buffer and $A_{(c)}$ is the absorbance of water.

Results

The result of α-glucosidase inhibitory activity of *Albizia lebbeck* (L.) Benth. branch, *Xylia xylocarpa* (Roxb.) Taub. bark, *Archidendron jiringa* I.C. Nielsen seed coat, *Albizia lebbeck* (L.) Benth. branch bark and *Parkia speciosa* Hassk. pericarb were 77.90 %, 75.62 %, 74.09 %, 68.10 % and 61.86 % respectively (Table 1). Moreover, the half concentrations (IC_{50}) of the plants in water extract with enzyme inhibitory activities more than 50% varied from 0.1554 to 0.7462 mg/ml (Table 2). The IC_{50} of *Albizia lebbeck* (L.)

Benth. branch, *Xylia xylocarpa* (Roxb.) Taub. bark, *Archidendron jiringa* I.C. Nielsen seed coat, *Albizia lebbeck* (L.) Benth. branch bark and *Parkia speciosa* Hassk. pericarp were 0.1554, 0.3143, 0.3829, and 0.4104 mg/ml respectively.

Table 1 Yield and α-Glucosidase Inhibitory Activity of Plant Extracts

Scientific name of plants	**% α-Glucosidase inhibition[a] (1mg/ml)**
Acacia catechu (L.f.) Willd.[1]	
leaves	58.11 ± 10.29
branch	2.39 ± 3.58
Acacia farnesana (Linn.) Willd.[2]	
twig	41.93 ± 6.81
Acada pennata (L.) Willd.[2]	
twig	0.00 ± 0.00
Acacia rugata Merr.[2]	
leaves	0.00 ± 0.00
pericarp	11.24 ± 1.81
Adenanthera microsperma Teijsm.[2]	
leaves	7.53 ± 1.87
branch	7.33 ± 4.74
Adenanthera pavonina Linn.[2]	
leaves	13.31 ± 6.77
branch	19.81 ± 5.05
pericarp	22.64 ± 2.89
Albizia lebbeck (L.) Benth.[2]	
leaves	0.00 ± 0.00

Scientific name of plants	**% α-Glucosidase inhibition[a] (1mg/ml)**
Cathormion umbellatum (Vahl) Kosterm.[2]	
leaves	56.43 ± 15.22
branch	0.00 ± 0.00
bark	47.50 ± 16.40
Entada rheedii Spreng.[2]	
seed coat	18.38 ± 3.62
cotyledon	11.92 ± 0.64
pericarp	18.38 ± 3.62
Leucaena glauca Benth.[4]	
twig	0.00 ± 0.00
pericarp	0.00 ± 0.00
Mimosa pudica Linn.[4]	
twig	29.39 ± 12.64
Neptunia oleracea Lour.[2]	
twig	0.00 ± 0.00
Parkia speciosa Hassk.[2]	
seed	4.00 ± 3.75
pericarp	61.86 ± 3.85
Pithecellobium dulce Benth.[2]	

Table 1 Yield and α-Glucosidase Inhibitory Activity of Plant Extracts

Scientific name of plants	% α-Glucosidase inhibition[a] (1mg/ml)	Scientific name of plants	% α-Glucosidase inhibition[a] (1mg/ml)
branch	77.90 ± 4.39	leaves	1.70 ± 2.94
branch bark	68.10 ± 10.39	stem bark	0.00 ± 0.00
Albizia lebbeckoides (DC.) Benth.[2]		*Samanea saman* (Jacq.) Merr.[2]	
leaves	54.12 ± 8.74	leaves	0.00 ± 0.00
branch	17.49 ± 5.49	branch	23.21 ± 6.09
bark	13.52 ± 5.50	bark	37.31 ± 8.99
*Albizzia myriophyll*a Benth.[1]		*Xylia xylocarpa* (Roxb.) Taub.[5]	
leaves	16.37 ± 3.69	leaves	34.77 ± 2.18
branch	9.65 ± 2.34	stem	40.72 ± 9.34
Albizia procera (Roxb.) Benth.[2]		branch	57.55 ± 14.86
stem bark	50.82 ± 7.31	bark	75.62 ± 6.37
Archidendron jiringa I.C. Nielsen.[2]			
seed	12.05 ± 5.16		
seed coat	74.09 ± 6.99		

[1]Rayong [2]Bangkok [3]Nakhonpathom [4]Pathumthani [5]Ratchaburi

[a] Percentage of inhibition was calculated at time = 20 min as 100 − % reaction, whereby the % of reaction = (α-glucosidase in sample/α- glucosidase in control) × 100,
control = distilled water

Table 2 IC_{50} of high α-Glucosidase Inhibitory Activity of Plants Extracts.

Plant extractions	IC_{50} (mg/ml)
Albizia lebbeck (L.) Benth. branch	0.1554
Xylia xylocarpa (Roxb.) Taub. bark	0.3143
Archidendron jiringa I.C. Nielsen seed coat	0.3341
Albizia lebbeck (L.) Benth. branch bark	0.3965
Parkia speciosa Hassk. pericarp	0.4104
Acacia catechu (L.f.) Willd. leaves	0.4977
Cathormion umbellatum (Vahl) Kosterm. leaves	0.5247
Xylia xylocarpa (Roxb.) Taub. branch	0.5660
Albizia lebbeckoides (DC.) Benth. leaves	0.5819
Albizia procera (Roxb.) Benth. stem bark	0.7462

Discussions

Many plants synthesize active substances that are useful to the maintenance of health in humans and other animals. These include phytochemical compounds such as phenolics that are known for high polarity and high solubility in polar solvents of which water has the most polarity one. This study showed that some plants in Mimocaceae family exhibited high α-glucosidase inhibition such as *Albizia lebbeck* (L.) Benth. branch and branch bark which showed α-glucosidase inhibition activity at 77.90% and 68.10% with half inhibition concentrations (IC_{50}) at 0.1554 and 0.3965 mg/ml whilst its leaves showed no activity. Ethanolic extracts of its bark showed high enzyme inhibition and antioxidant activities[9, 10] as did *X.* (Roxb.) Taub. bark, *Archidendron jiringa* I.C. Nielsen seed coat, and *Parkia speciosa* Hassk. pericarb. The previous study showed soponins of *Albizia lebbeck* (L.) Benth. bark had effects on reproductive system of male albino rat[11]. *X. xylocarpa* (Roxb.) Taub., is a tree that resembles walnut and its seed is used as food[12,13] whilst *Archidendron jiringa* I.C. Nielsen is a tree that edible plant that seed that has high protein and djenkolic acid which showed cytotoxicity and kidney toxicity in animal testing[14]**.** *Parkia speciosa* Hassk**.** is kitchen and cooking herb which seeds are eaten as vegetable which has a rather peculiar smell but is an acquired taste. It is popular in southern Thailand, Myanmar, Malaysia,

Indonesia and North-eastern India and the ethnomedical used for anti-hyperglycemia[15,16]. Results from the screening showed that some mimosaceous plants have a good α-glucosidase inhibition potential. Therefore further investigation will be carried out to study for separation and identification of the extracts and confirming with other assays before clinical study or toxicity testing in animals.

Conclusion & Recommendation

Mimosaceous plants are common trees and native to tropical southern Asia, Southeast Asia. They are popular food in Myanmar, Indonesia and also consume in Malaysia such as *Archidendron jiringa* I.C. Nielsen and *Parkia speciosa* Hassk.. *Acacia catechu* (L.f.) Willd. and *Albizia procera* (Roxb.) Benth. are ethnomedical Thai plants. There are not previous studies on α-glucosidase inhibitory activity of mimosaceous plants. From this study, all the extracts tested with α-glucosidase inhibition, 10 samples exhibited a high to moderate enzyme inhibitory activity. The extracts from branch and bark of *Albizia lebbeck* (L.) Benth. *and Xylia xylocarpa* (Roxb.) Taub. showed a remarkable activity. Plants continue to be a major source of medicines. Some medicinal plants have long been recognized and widely used, while new uses for medicinal plants have been discovered and popularized. Sustainability has increasingly become Recommendation of medicinal plants intake which has scientific reports to support the good clinical evidences of high potential and safety should be encouraged. Studies are in progress to identify the bioactive components via *in vitro* bioassay[17].

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