ฤทธิ์ยับยั้งเอนไซม์แอลฟากลูโคซิเดสของสารสกัดไมยราบ

α-Glucosidase inhibitory activity of extracts from *Mimosa pudica* L.

อำภา คนซื่อ[1], ชยันต์ พิเชียรสุนทร[2], ชูศรี ตลับมุข[3]

Ampa Konsue[1], Chayan Picheansoonthon[2], Chusri Talubmook[3]

บทคัดย่อ

สารยับยั้งเอนไซม์แอลฟากลูโคซิเดส สามารถนำมาใช้เพื่อการรักษาโรคเบาหวานชนิดไม่พึ่งพาอินซูลิน (DM Type 2) ได้ การนำสารยับยั้งเอนไซม์แอลฟากลูโคซิเดสจากธรรมชาติ เช่น ไมยราบ มาใช้จะก่อให้เกิดประโยชน์แก่ผู้ป่วยเบาหวาน (Type 2)ได้ ดังนั้น งานวิจัยครั้งนี้ จึงได้ทำการศึกษาฤทธิ์ยับยั้งเอนไซม์แอลฟากลูโคซิเดสของสารสกัดไมยราบที่สกัดด้วยตัวทำละลายที่แตกต่างกัน ผลการศึกษาพบว่า สารสกัดจากแอลกอฮอล์ 50% มีฤทธิ์ยับยั้งการทำงานของเอนไซม์แอลฟากลูโคสซิเดสได้ดีที่สุดโดยมีค่า IC_{50} เท่ากับ 0.008±0.0.005 mg/ml รองลงมาคือ สารสกัดจากน้ำมีค่า IC_{50} เท่ากับ 0.021±0.013 mg/ml สารสกัดจากแอลกอฮอล์ 80% มีค่า IC_{50} เท่ากับ 0.019±0.005 mg/ml ตามลำดับ จากผลการทดลองแสดงให้เห็นว่า สารสกัดจากไมยราบที่สกัดด้วยตัวทำละลายทั้ง 3 แบบ มีฤทธิ์ยับยั้งเอนไซม์แอลฟากลูโคซิเดสได้ดีกว่า อะคาโบส (Acarbose®) ซึ่งเป็นสารมาตรฐานที่มี IC_{50} เท่ากับ 0.649±0.026 mg/ml ข้อมูลนี้สามารถนำไปใช้ในการศึกษาวิจัยต่อไป เพื่อประยุกต์ใช้ในการบำบัดและป้องกันโรคเบาหวานต่อไป

คำสำคัญ: ฤทธิ์ยับยั้งเอนไซม์แอลฟากลูโคซิเดส เบาหวาน ไมยราบ อะคาโบส®

Abstract

α-glucosidase inhibitors are used in the treatment of non insulin–dependent diabetes mellitus (DM Type 2). Utilization of the α-glucosidase inhibitors from natural source such as *Mimosa pudica* L. will be useful for the patients with diabetes mellitus Type 2. This study was aimed to investigate the α-glucosidase inhibitory activity of *M. pudica* extracted by using different solvents. The results revealed that the 50% ethanolic extract (MPHE) exhibited the highest α-glucosidase inhibitory activity with IC_{50} of 0.008±0.0.005 mg/ml. The aqueous extract (MPA) with IC_{50} of 0.021±0.013 mg/ml and 80 % ethanolic extract (MPE) exerted the equal inhibitory activity with IC_{50} of 0.019±0.005 mg/ml. The studied extracts showed the potent α-glucosidase inhibitors comparable to authentic drug, Acarbose®, with IC_{50} of 0.649±0.026 mg/ml. The data are useful for further study in order to utilize for treatment and prevention of diabetes.

Keywords: α-glucosidase inhibitors activity, Diabetes Mellitus, *Mimosa pudica* L., Acarbose®

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[1] *นิสิตปริญญาเอก, สาขาวิทยาศาสตร์สุขภาพ คณะแพทยศาสตร์ มหาวิทยาลัยมหาสารคาม อำเภอเมือง จังหวัดมหาสารคาม 44000*

[2] *ศาสตราจารย์, คณะแพทยศาสตร์ มหาวิทยาลัยมหาสารคาม อำเภอเมือง จังหวัดมหาสารคาม 44000*

[3] *ผู้ช่วยศาสตราจารย์, ภาควิชาชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหาสารคาม อำเภอกันทรวิชัย จังหวัดมาหาสารคาม 44150*

[1] *Ph.D Students (Health Science), Faculty of Medicine, Mahasarakham University, Mueang District, Maha Sarakham 44000*

[2] *Professor., Faculty of Medicine Mahasarakham University, Mueang District, Maha Sarakham 44000*

[3] *Assistant Prof., Department of Biology Faculty of Science, Mahasarakham Universiry, Maha Sarakham, 44150*

Introduction

Diabetes mellitus is a metabolic disorder disease characterized by hyperglycemia resulting from defects in insulin secretion followed by dysfunction and failure of organs especially the eyes, kidneys, nerves, heart and arteries[1].

The α-glucosidase enzyme is located in the brush border of the small intestine and is required for the breakdown of carbohydrates to absorbable monosaccharides. The α-glucosidase inhibitors delay, but do not prevent the absorption of ingested carbohydrates, reducing the post- prandial glucose and insulin peaks. α-glucosidase inhibitors are among the available glucose-lowering medications[2] and are used in the treatment of non insulin–dependent diabetes mellitus (DM). Utilization of the α-glucosidase inhibitors from natural source such as *Mimosa pudica* L. will be useful for the patients with DM Type 2.

Mimosa pudica L. (Mimosaceae) is a common plant found in moist waste ground, lawns, open plantations and weedy thickets. Leaves and stems of the plant have been reported to contain an alkaloid mimosine. Leaves also contain mucilage and root contains tannins.[3] The plant is used traditionally in the treatment of many diseases arising from corrupted blood and bile, billious fever, piles, jaundice, leprosy, ulcers, and small pox[4]. It has been traditionally used in Indian system of medicine for the treatment of diabetes.[2] The *M. pudica* invites attention of the researchers worldwide for its pharmacological activities such as anti-inflammatory and analgesic[5], diuretic property[6], hypolipidemic[7], hepatoprotective[4], wound healing[8] anti-diarrhoeal[9], cytotoxicity, antimicrobial and antioxidant[10] activities as well. Antidiabetic activity of the ethanolic leaf extract of *M. pudica* in alloxan (150 mg/kg)–induced diabetic rats was investigated and compared to Metformin (500 mg/kg), a standard diabetic drug. The extract significantly decreased serum glucose level.[11] However, a controversial results of the activities of this plant extract was found when the extract of *M. pudica* leaves at a dose of 250 mg/kg, possessing significant hyperglycemic effects both in normal and glucose loaded mice has also been reported.[12]

The inhibitory effect on α-Glucosidase underlying antidiabetic activity of the leaf extract from *M. pudica* has not been reported. The present study was therefore, carried out to investigate the α−Glucosidase Inhibitory activity of *M. pudica* extracted by different solvents, in-vitro.

Materials and methods

Plant materials

Mimosa pudica L. grown in weedy thickets from Kalasin Province, Northeast Thailand was used in this study. It was identified by the Botanist at the Department of Biology, Faculty of Science, Mahasarakham University, Thailand. The voucher specimens (code: MP0001/AK. MED) were deposited in the same University.

Preparation of plant extract

The fresh whole plants with flowers and fruits were collected, washed with tap water and dried in hot air oven at a temperature of 60 °C. The dried plants were then pulverized by an electric blender. The plant powder was extracted using different solvents (distilled water, 50% or 80% ethanol) to obtain aqueous extract (MPA), 50% ethanolic extract (MPHE) and 80% ethanolic extract (MPE).

MPHE and MPE were prepared by macerating the plant powder in 50% and 80% ethanol for 7 days. MPA was prepared by boiling 1 kg of plant powder in 10 L of distilled water for 15 min. The residue powder was excluded by using the filter papers. The filtrate was evaporated followed by freeze-dried to obtain dark brown extract. The extracts were kept in the fridge until be used.

α-glucosidase Inhibitory Activity

α-glucosidase inhibitory activity of the extracts from *M. pudica* L. was assessed by using Dong *et al.* assay.[13] with slight modifications. Briefly, a volume of 60 µl of sample solution and 50 µl of 0.1 M phosphate buffer (pH 6.8) containing α-glucosidase solution (0.2 U/ml) was incubated in 96 well plates at 37 ºC for 20 min. After pre-incubation, 50 µl of 5 mM p-nitrophenyl-α-D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) was added to each well and incubated at

37 ºC for another 20 min. Then the reaction was stopped by adding 160 µl of 0.2 M $NaCO_3$ into each well, and absorbance was readings (A) were recorded at 405 nm by micro-plate reader and compared to a control which had 60 µl of buffer solution in place of the extract. For blank incubation (to allow for absorbance produced by the extract), enzyme solution was replaced by buffer solution and absorbance recorded. The α-glucosidase inhibitory activity was expressed as inhibition (%) and was calculated as follows.

$$\% \text{ inhibition} = \left(\frac{\text{Ablank - Asample}}{\text{Ablank}}\right) \times 100$$

A_{blank} = absorbance of control without test solution
A_{sample} = absorbance of sample with test solution

IC_{50} values were calculated by the graphic method.

Statistical analysis

All data were expressed as mean ± standard error of mean (SEM). Statistical analysis was carried out using F-test (One-Way ANOVA) followed by Duncan's test. The criterion for statistical significance was a p-value less than 0.05.

Results

α-glucosidase inhibitory activity of different extracts of *M. pudica* using Acarbose as a standard inhibitor showed that these four extracts significantly (p<0.05) inhibited the activity of α-glucosidase enzyme compared to Acarbose. MPHE showed the highest inhibitory activity with IC_{50} of 0.008±0.005 mg/ml followed by MPA (IC_{50} of 0.021±0.0 13 mg/ml) MPE (IC_{50} of 0.019±0.005 mg/ml) and Acarbose ($IC_{50}$of 0.649±0.026 mg/ml), respectively. The details of the results were summarized in Table 1. These extracts exhibited a concentration-dependent inhibition of the enzyme. The highest concentration of 0.078 mg/ml of MPE exerted a maximum inhibition (87.131±0.067%) as shown in Figure 1.

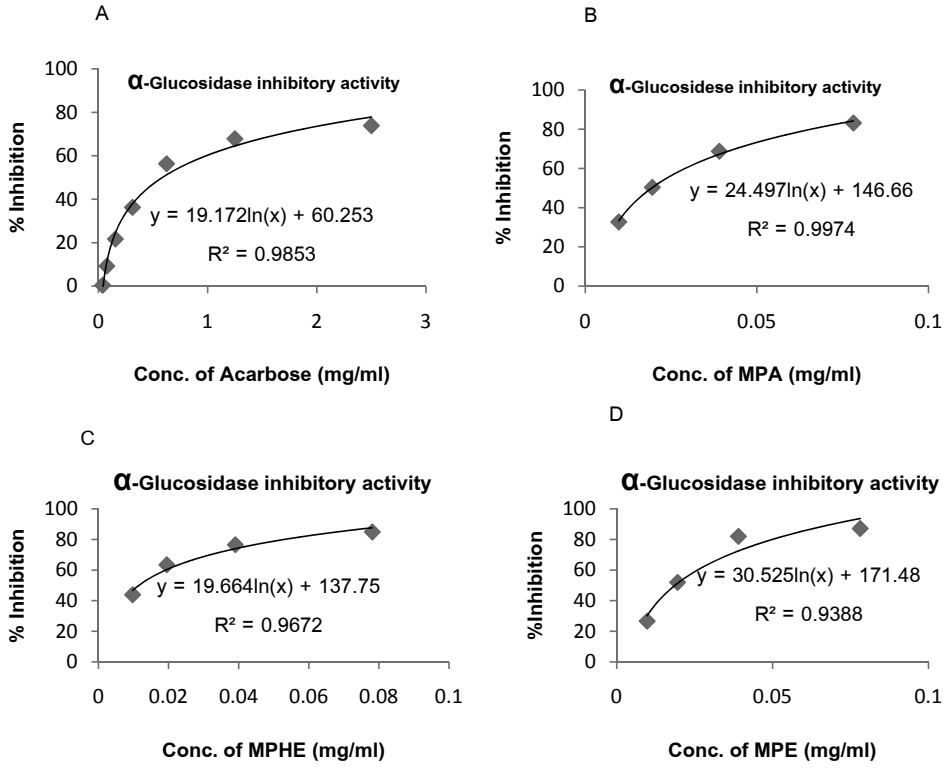

Figure 1 α-Glucosidase inhibitory activity of Acarbose (A) and different extracts of *M. pudica*; MPA (B), MPHE (C), and MPE (D)

Table 1 α-Glucosidase inhibitory activity of different extracts of *M.pudica* compared to Acarbose

Sample	α-Glucosidase inhibition IC_{50} value (mg/mL)
Acarbose	0.649±0.026*
MPA	0.021±0.013
MPHE	0.008±0.005
MPE	0.019±0.005

(*) Different letters indicate statistically significance among the groups ($p<0.05$).

Discussion

The results obtained from this *in-vitro* study clearly indicate that the whole plant extracts of *M. pudica* have strong α- glucosidase inhibitory activity. 50% ethanol extracts exhibited the highest α-glucosidase inhibitory activity with IC_{50} of 0.008 mg/ml.

The inhibitory effects of the extracts from various medicinal plants on α- glucosidase enzyme have been reported. Some Mexican plants used in the treatment of type 2 diabetes showed α-glucosidase inhibitory activity with IC_{50} of 14 µg/ml for *Cecropia*, 21 µg/ml for *Malmea*, and 109 µg/ml for *Acosmium*, which were lower than that of acarbose (128 µg/ml).[14] The methanolic extract from *Tournefortia hartwegiana*, an anti-hyperglycemic agent showed significant inhibition of α-glucosidase activity *in vitro*, in a concentration-dependent manner (IC_{50} of 3.16 mg/ml).[15]

The findings from the present study are in accordance with the study by Afrapoli et al. who found that the flowering aerial parts of *Polygonum hyrcanucum* extracted by methanol show remarkable α- glucosidase inhibitory activity with IC_{50} of 15 mg/ml.[16]

However, it is found that the aqueous and 80% ethanol extracts possessed the similar inhibitory capacity. We can assume that one or more different compounds present in the extracts may play an important role in the α-glucosidase inhibition.

In conclusions, The 50% ethanol extracts from whole aerial parts of *M. pudica* exhibit a notable α-glucosidase inhibitory capacity, suggesting the efficacy of this plant as hypoglycemic agent for control and management of diabetes mellitus.

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