Determination of α-glucosidase inhibitory activity from selected Fabaceae plants

Sukanya Dej-adisai* and Thanet Pitakbut
Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences,
Prince of Songkla University, Hat-Yai, Songkhla, Thailand

Abstract: Nineteen plants from Fabaceae family, which were used in Thai traditional medicine for treatment of diabetes, were determined of α-glucosidase inhibitory activity via enzymatic reaction. In this reaction, α-glucosidase was used as enzyme, which, reacted with the substrate, *p*-nitrophenol-D-glucopyranoside (*p*NPG). After that the product, *p*-nitro phenol (*p*NP) will be occurred and observed the yellow colour at 405 nm. In this study, acarbose was used as positive standard which, inhibited this enzyme with IC_{50} as 331±4.73 µg/ml. *Caesalpinia pulcherrima* leaves showed the highest activity with IC_{50} as 436.97±9.44 µg/ml. Furthermore, *Bauhinia malabarica* leaves presented moderately activity with IC_{50} as 745.08±11.15 µg/ml. However, the other plants showed mild to none activity of α-glucosidase inhibition. Accordingly, this study can support anti-diabetes of these plants in traditional medicine and it will be the database of the biological activity of Fabaceae plant.

Keywords: α-glucosidase inhibitory activity, anti-diabetes, Fabaceae, Thai traditional medicines,

INTRODUCTION

Diabetes is a popular common chronic disease in many countries and the significant increasing numbers of diabetes are concerned. In lately report, the worldwide adult diabetic patients are increasing from 6.4% in 2010 (285 million adults) to 7.7% in 2030 (439 million adults). For developing countries, the patients are mounting around 60% from 2010 to 2030. India is the first country in Asia that has the most numbers of diabetic patients and second country is China (Shaw *et al.*, 2010). Diabetes can be classified into 2 major types. Type 1 is an insulin defection and the most common is a type 2 that is an insulin resistance. It is imbalance between insulin secretion and insulin sensitivity that comes from a lot of substrate consumption and it induces the target cells resistance with insulin (Kuzaya *et al.*, 2002; Giaccair *et al.*, 2009). α- Glucosidase is a key enzyme in carbohydrate digestion. It hydrolyzes glycosidic bond in polysaccharide chains to monosaccharide as glucose. Thereby, acarbose as a alpha glucosidase inhibitor can be used in diabetic therapy (Hakamata *et al.*, 2009). In Asia, medicinal plants have an effect on treatments in many diseases including diabetes (Hung *et al*., 2012). Fabaceae plants are the large one group of medicinal plants. They consist of 490 species, which spread widely around the world including tropical zone, especially in Thailand. They have been used in traditional medicine in Asia such as Thailand, China, Japan and India and many pharmaceutical products are also used from these plants. For folk medicine, these plants can be cured infection, inflammatory, pain and diabetes (Kaewamatawong, 2008; Gao *et al.*, 2010). The chemical constituents, which reported from Fabaceae plants are flavonoids, stilbene derivatives, phenolic compounds, steroids and terpenoids. Many biological activities of these plants were also reported such as anti-oxidant, anti-nociceptive, anti-inflammatory and anti-diabetes activities (Kaewamatawong, 2008; Filho, 2009; Tanzila *et al.*, 2012). Some of Fabaceae plants showed the potential anti-diabetic activity in animal model with lacking the mechanism of action. So, α-glucosidase inhibitory activity is the one of the mechanism of action to reduce blood sugar levels that we are interesting to find out for the Fabaceae plants, which were used for anti-diabetic in folk medicines.

Plant materials

Nineteen plants from Fabaceae family were collected from Thailand in Surat Thani province, Songkla province at Prince of Songkla University and Southern Thai Literary Botanical Garden, Narathiwat province at Hala-bala forest and Ratchaburi province at Central Thai Literary Botanical Garden. The specimens were identified by comparison with authentic samples and are stores as reference at Department of Pharmmacognosy and Pharmaceutical Botany, Faulty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand. Nineteen plants were separated to the part used to become twenty-five samples for this test. All plant materials were washed and dried in the oven at temperature 50°C and were encoded to the number as showed in table 1.

Plant extraction

Plant materials were chopped and blended into small pieces. They were extracted repeatedly with ethanol at room temperature 3 days (x 3 times). The filtrates were

**Corresponding author:* e-mail: sukanya.d@psu.ac.th

pooled and evaporated under reduced pressure at temperature not exceeding 40°C to yield the ethanol extract and were kept at 4°C until examination.

Determination of α-glucosidase inhibitory activity

The assay for determination of α-glucosidase inhibitory activity was modified from previously reported assays (Walker *et al.*, 1995; Kim *et al.*, 2008; You *et al.*, 2011).

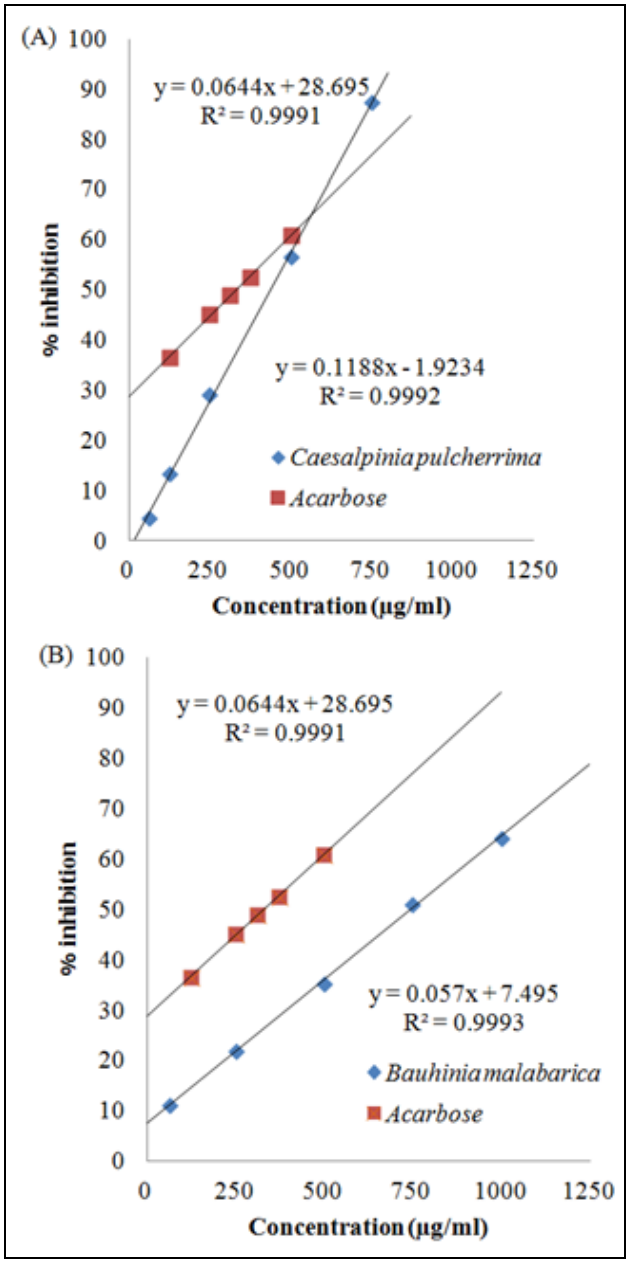

Fig. 1: Linear regression equations of IC_{50} between (A) acarbose and *Caesalpinia pulcherrima* leaves and (B) acarbose and *Bauhinia malabarica* leaves.

The determination of α-glucosidase reaction is observed the yellow product, *p*-nitrophenol (*p*NP) that is produced from the substrate, *p*-nitrophenol-D-glucopyranoside (*p*NPG) by glucosidase enzyme. In brief, 50µl of 10 mM phosphate buffer solution (pH 7), which was containing 2 mg/ml of bovine serum albumin and 0.2 mg/ml of sodium azide (PBS) was added into well plate. Besides, 50 µl of 1 Unit/ml of α-glucosidase from *Saccharomyces cerevisiae* (Type I, lyophilized powder, Sigma, EC 3.2.1.20) and 50 µl of 8 mg/ml sample solution were mixed with PBS. In solvent control, 5% DMSO solution was used while positive control was replaced with 8 mg/ml of acarbose in well. The mixtures were incubated at 37°C for 2 minutes. After that, 50 µl of 4 mM *p*NPG was added in the well finally. The mixture was required to incubate again for 5 minutes at same condition. The *p*NP was performed and monitored at 405 nm every 30 seconds for 5 minutes by micro plate reader. The velocity was determined by linear relationship equation between absorbance and time as following equation (1).

$$\text{Velocity} = \frac{\Delta\text{Absorbance at 405 nm}}{\Delta\text{Time}} \quad (1)$$

The highest velocity (V) from initial reaction of each sample was collected and calculated percentage of inhibition by equation (2) as showed below.

$$\%\ \text{Inhibition} = \frac{\text{V control} - \text{V sample}}{\text{V control}} \times 100 \quad (2)$$

Determination of the half maximal inhibitory concentration (IC_{50}) from the active extracts

The samples that showed the potential α-glucosidase inhibitory activity (more than 70% inhibition) were selected for determination the IC_{50} by using the concentration of sample and positive standard, acarbose as 0.5-0.0625 mg/ml and 0.5-0.125 mg/ml, respectively. The IC_{50} was decided by calibration curve equation between percentages of inhibition and sample concentration (at least five concentrations).

RESULTS

α-Glucosidase inhibitory activity

Twenty-five samples were prepared from nineteen plants for this test. Consequently, the results were showed in table 1. So, twelve samples showed the activity lower than 50% inhibition. Furthermore thirteen samples could inhibit enzyme more than 50%. The branch of *Bauhinia purpurea* and *Senna siamea* and *Clitoria ternatea* leaves, were against this enzyme between 50-60%. The only one of those that showed percentage of inhibition from 60-70% was branches of *Bauhinia acuminate*. Two samples were included in the group that had activity from 70-80% inhibition as *Bauhinia acuminate* branches and *Albizia procera* leaves. Four samples, which showed higher activity than 80-90% inhibition were *Caesalpinia pulcherrima* flowers, *Tamarindus indica* leaves, *Bauhinia variegate* branches and *Bauhiniawinitii* branches. Lately,

Table 1: α-glucosidase inhibition of selected Fabaceae plant extracts.

No.	Scientific name	Plant part	α-glucosidase inhibition (%)	± S.D.
1	*Albizia procera*	Leaves	79.06	3.63
2	*Bauhinia acuminata*	Leaves	62.19	3.40
3	*Bauhinia acuminata*	Branches	74.07	1.28
4	*Bauhinia aureifolia*	Branches	31.31	1.04
5	*Bauhinia malabarica*	Leaves	94.38	2.68
6	*Bauhinia purpurea*	Pods	19.85	1.05
7	*Bauhinia purpurea*	Branches	58.22	1.22
8	*Bauhinia purpurea*	Leaves	27.56	0.47
9	*Bauhinia variegata*	Branches	87.24	0.81
10	*Bauhinia winitii*	Branches	83.79	2.11
11	*Caesalpinia bonduc*	Leaves	21.87	0.82
12	*Caesalpinia bonduc*	Branches	57.07	2.40
13	*Caesalpinia pulcherrima*	Flowers	85.42	2.79
14	*Caesalpinia pulcherrima*	Leaves	96.35	3.97
15	*Cassia fistula*	Leaves	47.16	3.17
16	*Cassia garrettiana*	Branches	26.50	1.83
17	*Clitoria ternatea*	Leaves	57.61	3.47
18	*Dalbergia cochinchinensis*	Branches	44.70	3.49
19	*Leucaena leucocephala*	Seeds	30.05	0.88
20	*Leucaena leucocephala*	Branches	35.25	3.39
21	*Pithecellobium dulce*	Branches	41.98	3.51
22	*Samanea saman*	Branches	10.40	1.34
23	*Senna siamea*	Leaves	19.09	2.43
24	*Senna siamea*	Branches	52.63	0.66
25	*Tamarindus indica*	Leaves	81.95	2.16
Std.	Acarbose		80.00	0.35

Bold letter = activity more than 70%.

leaves of *Caesalpinia pulcherrima* and *Bauhinia malabarica* showed the highest activity group with 95.99% and 94.38% inhibition, respectively. While positive control, acarbose, showed 80% inhibition. Thus the samples that showed the α-glucosidase inhibitory activity more than 70% inhibition were selected for determination of IC_{50} in the next step.

IC_{50} of active extracts

Eight samples from seven plants were chosen for determination of IC_{50}. as showed in table 2. Acarbose was used to be as standard drug and showed a strong activity with IC_{50} at 331±4.73 µg/ml. From the results, they had two samples, which showed the potential activity close to positive standard. The first one with highest activity was *Caesalpinia pulcherrima* leaves, which showed the IC_{50} at 436.97±9.44 µg/ml. The difference between IC_{50} of this sample and acarbose was 0.3 folds and the slope of linear regression equation represented the different rate of this plant, which was higher than acarbose almost 2 times (fig. 1A). It indicated that this sample had higher activity close to the standard. Furthermore *Bauhinia malabarica* leaves also showed the good activity with IC_{50} at 745.08± 11.15 µg/ml. So the difference was 1.25 folds when compared with acarbose (fig. 1B).

DISCUSSION

Fabaceae plants have been used in traditional medicine for many ailments (Kaewamatawong, 2008; Gao *et al.*, 2010). So, our research aims to proof the traditional use of these plants for diabetes by determination of α-glucosidase inhibitory assay. From the results showed the highest activity of *Caesalpinia pulcherrima* leaves to α-glucosidase inhibition. Thereby this plant is possible a candidate for diabetic treatment. Since both IC_{50} and proportion (0.32 folds) of this plant were nearly too same values as positive control. However, the slope of linear regression equation showed that this plant had the rate of reaction higher than standard, acarbose. Besides this plant is used in traditional medicine for diabetes and other disease such as dysentery and skin disease (Sakong *et al.*, 2011; Krishnaswamy *et al.*, 2013). In recently report, steroid and flavonoids were found in this plant e.g. β-sitosterol, kaempferol, quercitrin, isoquercitrin, afzelin and so on. Both kaempferol and isoquercitrin could inhibit this enzyme. Especially, isoquercitrin showed postprandial hypoglycemia in diabetic mice (Tadera *et al.*, 2006; Kurakane *et al.*, 2011; Park *et al.*, 2013). *Bauhinia malabarica* leaves showed the moderate activity. Despite IC_{50} was more than standard as well as the proportion

Table 2: IC_{50} of selected plants.

No.	Sample	Part use	IC_{50} (μg/ml)	± S.D.	Proportion*
	Acarbose		331.00	4.73	
1	*Albizia procera*	Leaves	924.05	18.61	1.79
3	*Bauhinia acuminata*	Branches	1034.70	14.41	2.13
5	*Bauhinia malabarica*	Leaves	745.08	11.15	1.25
9	*Bauhinia variegata*	Branches	994.14	12.75	2.00
10	*Bauhinia winitii*	Branches	1012.46	9.27	2.06
13	*Caesalpinia pulcherrima*	Flowers	1031.44	13.05	2.12
14	*Caesalpinia pulcherrima*	Leaves	436.97	9.44	0.32
25	*Tamarindus indica*	Leaves	1040.28	13.90	2.14

*Proportion of each sample when compared with standard.

were 1.25 folds compared to standard. This leave will become a good option for diabetic treatment. Because of the lately research reported that this plant could reduce blood sugar and regenerate β cell in diabetic rat (Balasubramanian *et al*., 2012). A part from traditional recipes, this leaves were mixed into the recipes and used for many ailments not only diabetes but also liver disorder, asthma, cholera and skin disease (Johnson *et al.*, 2006; Hasan *et al.*, 2009). From studies, this plant contained flavonoids as same as previous plant. The chemical composition, which was reported from this plant was 5,7-dimethoxy-3',4'-methylenedioxyflavanone, 5,7 dimethoxyflavanone, isobonducellin and bonducellin. So, this plant maybe practically alternative treatment (Srinivas *et al.*, 2003; Rao *et al.*, 2005). In the other hand, flower part had a lower effect than the leaf part. Another plants have mild activity, IC_{50} were higher than 900 μg/ml and difference more than 1.70 folds. However, they could decrease blood sugar level in previous animal studies of *Tamarindus indica* (Maiti *et al.,* 2004; Sagheb *et al.,* 2010) and *Bauhinia variegate* (Azevedo *et al.*, 2006; Kumar *et al.*, 2012) both. So, these reports can support the traditional use for diabetes of some Fabaceae plants. However, *Bauhinia winitii* did not have any reports for diabetes treatment in traditional medicine, but it uses for many disorders including diarrhoea, dysentery, and cough and relief headache (Kaewamatawong, 2008). Plants in Fabaceae family as *Caesalpinia pulcherrima* can treat diabetes in traditional medicine as same as *in vivo* study. This study showed the potential anti-glucosidase activity that is the one of mechanism of action in anti-diabetic drug. So, it could be the mechanism of action for the plants in Fabaceae family, which are using for diabetes in traditional medicines. Nonetheless, more investigation should be done to complete the study of anti-diabetic of this family.

CONCLUSION

α-Glucosidase inhibitory activity of selected plants from Fabaceae family was reported in the first time. These plants are used in traditional medicine for diabetic treatment. So, from the results, the leaves of *Caesalpinia pulcherrima* and *Bauhinia malabarica* showed the potential α-glucosidase inhibitory activity. Thus, these two plants are interesting for further study of active compounds for α-glucosidase inhibitor.

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