

Antioxidant and α -Glucosidase Inhibitory Activities of *Solanum xanthocarpum* Schrad. & Wendl. (Yellow Berried Nightshade) Fruit Extract

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Abstract

Type-2 diabetes (T2D) is a chronic metabolic disease usually caused by defects in insulin secretion. In addition, T2D is a potential risk of developing the initiation of its complications, as a result of prolonged high blood glucose level (hyperglycemia) and overproduction of free radicals. The focus of this study was to evaluate antioxidant and antidiabetic activities of *Solanum xanthocarpum* fruit extract using DPPH free radical scavenging assay and α -glucosidase inhibitor, respectively. The CH₂Cl₂ extract showed the highest antioxidant activities with an IC₅₀ value of 1.142±0.23 mg/mL. In addition, the extracts were further studied for their α -glucosidase inhibitory activity. The MeOH extract selectively inhibited intestinal sucrase with an IC₅₀ value of 9.85±1.21 mg/mL while the CH₂Cl₂ extract inhibited baker's yeast with an IC₅₀ value of 3.25±1.35 mg/mL. These results indicated that fruit of *S. xanthocarpum* would be beneficial in diabetes therapy based on its inhibitory activity against α -glucosidase and antioxidant activity.

Keywords: *Solanum xanthocarpum* Schrad. & Wendl., Antioxidant, α -Glucosidase inhibitor

1. Introduction

Type-2 diabetes (T2D) is a chronic metabolic disease usually caused by defects in insulin secretion. One therapeutic approach to decrease postprandial hyperglycemia is to retard absorption of glucose of α -glucosidase. Delaying glucose absorption after meals is known to be beneficial in therapy disease [1]. α -Glucosidase inhibitors are used as drugs for diabetes therapy. However, they have side effects such as bloating, flatulence and diarrhea. Therefore, it is of interest to search for drugs from new sources, which can minimize the side effects [2]. Herbal drugs are prescribed widely because of their effectiveness, fewer side effect and relatively low cost. Nevertheless, long-term of diabetes has risk of many health problems such as cardiovascular disease (CVD), nephropathy, neuropathy, retinopathy and erectile dysfunction, which

contributes to the aforementioned complications [3]. Recent evidence indicates that excess plasma glucose drives overproduction and accumulation of reactive oxygen radicals and oxidative stress. This leads to oxidative damage of cell components such as proteins, lipids, and nucleic acids [4]. In the present study, we evaluated antidiabetic potency of *S. xanthocarpum* through α -glucosidase inhibition and antioxidant activity.

Solanum xanthocarpum Schrad. & Wendl. (Family: Solanaceae, commonly known: Yellow Berried Nightshade or Thai Eggplant) is herb grown as wild plant in many parts of Thailand and has been used as an edible plant. *S. xanthocarpum* are reported to contain several steroidal alkaloids like solanacarpine, solanacarpidine, solancarpine, solasonine and solamargine. Other constituents like caffeic acid coumarins like aesculetin and aesculin, steroids carpesterol, diosgenin, campesterol, daucosterol and triterpenes like cycloartanol and cycloartenol were reported from the fruits [5]. According to many reports on ethnopharmacological use of *S. xanthocarpum*, it can relieve asthma, diabetes, rheumatism, catarrhal, fever, cough, chest pain, stone in the bladder, constipation, toothache and bronchospasm [6]. The aforementioned traditional uses, especially antidiabetic activity was conducted on alloxan induced diabetic rats. Oral administration of the aqueous and methanol extracts of the fruits significantly reduced blood glucose level in both normal and diabetic rats [7] [8]. Nevertheless, there is no report on the constituents from this plant that inhibited the activity of the α -glucosidase. Therefore, this study was to investigate the retarding α -glucosidase and antioxidant activity of *S. xanthocarpum* fruit.

2. Plant material

Fruits of *Solanum xanthocarpum* Schrad. & Wendl. were collected from Thung Song, Nakhon Si Thammarat, southern part of Thailand.

3. Methods

3.1 Plant extraction

Fruits (956 g) were air-dried and ground into powder. The dry powder was extracted with hexane, CH_2Cl_2 and methanol at ambient temperature for 24 hours. The extracts were prepared by concentration the filtrate using a rotary evaporator with the water bath set at 40-50°C. The crude extracts were preserved in cold (4 °C) prior to performing inhibition assay.

3.2 Baker's yeast α -glucosidase inhibitory activity

Assay was performed according to a slightly modified method of Damsud *et.al* [9]. The α -glucosidase (EC 3.2.1.20) was extracted from Baker's yeast. Briefly, 10 μL of the test sample was mixed with α -glucosidase (0.1 U/mL) in 1 mM phosphate buffer (pH 6.9) and incubated at 37 °C for 10 min. Then 40 mL of 0.1 mM *p*-nitrophenyl- α -D-glycopyranoside (PNPG) was added and the mixture was then incubated for 30 min prior to being quenched with the addition of 100 mL 0.1 M Na_2CO_3 . The enzymatic

activity was monitored by following the concentration of released *p*-nitrophenol evaluated at (415 nm). The percent inhibition was determined according to the equation: $[(A_0 - A_1)/A_0] \times 100$, where A_1 and A_0 are the absorbance with and without the sample, respectively. The IC_{50} value was determined from a plot of percentage inhibition on the y-axis against the sample concentration on the x-axis. Acarbose was used as a positive control and the experiment was performed in triplicate.

3.3 Rat intestinal α -glucosidase inhibitory activity

Assay was performed according to a slightly modified method of Damsud *et.al* [9]. α -Glucosidase was extracted from rat intestinal crude enzymes consisting of maltase and sucrase. Initially, the powder (1 g) was homogenized with 0.9% NaCl (30 ml) and centrifugation (12,000 g) for 30 min to obtain the supernatant. The bioassay was performed as follows. Briefly, 10 μ L of the test sample was added to 0.1 M phosphate buffer (pH 6.9, 30 μ L), 20 μ L of the substrate solution (maltose: 10mM, 20 μ L; sucrose: 100 mM) in 0.1 M phosphate buffer (pH 6.9), glucose kit (80 μ L) and crude enzyme solution (20 μ L). The reaction mixture was then incubated at 37°C for maltose (20 min) or sucrose (60 min). The concentration of glucose released from the reaction mixture was detected by the glucose oxidase method using a glu-kit (Human, Germany). The percentage inhibition of activity was calculated using a similar expression described in section 3.2.

3.4 Free radical scavenging activity

Hydrogen radical donating activity of fruit extracts was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The DPPH is a stable radical; its ethanolic solution showed an absorbency maximum at 517 nm. The test sample reduced DPPH radical and thus decreased its absorbance at 517 nm. The bioassay can be carried out as follows. Briefly, the reaction mixture contained 0.5 ml of sample, 0.5 ml of 0.1 mM DPPH (in methanol). After standing for 30 minutes, the absorbance of the mixture was measured at 517 nm and expressed as the inhibition percentage of free radicals by the samples after calculation using the expression in section 3.2.

4. Results and discussion

4.1 Free radical scavenging activity

The evaluation of antioxidant properties of plant materials was performed by DPPH radical scavenging assay. The IC_{50} values of *S. xanthocarpum* extract are reported in Table 1. The CH_2Cl_2 extract showed highest inhibition with IC_{50} values of 1.14 ± 0.23 mg/mL, while the MeOH extract showed the inhibition with IC_{50} values of 5.98 ± 0.78 mg/mL. The standard ascorbic acid displayed scavenging activity with IC_{50} value of 0.21 ± 0.25 mg/mL.

Table 1. Antioxidant activity of crude extracts

Crude extracts	DPPH scavenging (IC ₅₀) ^a mg/mL
MeOH	5.98±0.78
CH ₂ Cl ₂	1.14±0.23
Hexane	50.25±1.25
Ascorbic acid	0.21±0.25

^aThe IC₅₀ value is defined as the inhibitory concentration to inhibit 50% of enzyme activity

The highest antioxidant CH₂Cl₂ extract activity of is possibly contributed to the presence of high contents of flavonoid, ascorbic and phenolic compounds *S. xanthocarpum* fruit are rich in flavonoids and alkaloids. Tanins, terpenoids and saponins showed distributory effect among the various polar and non polar extracts of fruit. It is apparent that non-polar and polar fractions accounted for low flavonoid content while extracts derived in me-dium polar solvents showed higher content of flavonoids. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process. [10] [11]. *S. xanthocarpum* fruit showed source of alternative antioxidant can be used as a medicine plants against the disease caused by free radicals.

4.2 Inhibitory activity of crude extracts against α -glucosidase

The crude extracts were investigated for α -glucosidase inhibitory activity by using baker's yeast and rat intestine (maltase and sucrose) as a source of enzyme. In Table 2, the CH₂Cl₂ extract inhibited yeast α -glucosidase with an IC₅₀ value of 10.25±1.52 mg/mL. On the other hand, the MeOH extract revealed highly potent inhibition against rat intestinal in sucrose and maltase with an IC₅₀ values of 3.25±1.35 and 9.85±1.21 mg/mL, respectively. The enhanced inhibition of *S. xanthocarpum* was likely to be contributed by its highest antioxidant activity (Table 1.). It was indicated that increasing polar of solvent led to increase inhibitory activity. In addition, *S. xanthocarpum* could be effective in controlling fasting and postprandial blood glucose levels in diabetic animal models. This hypothesis was supported by the reports of D.M.kar. *et al.* showed that oral administration of aqueous extract of the fruit to diabetic rats significantly decreased plasma glucose levels [7] while, the research of M. Antitha *et al.* the ethanolic extracts (400mg/kg) produced significant reduction in elevated blood glucose level by the 28th day of STZ-induced diabetes [12]. In addition, it showed multiple biological activities such as anti-oxidative effect, anti-urolithiatic, and anti-microbial affect, which is a great potential in therapy and prevention of several major human diseases including cancer and diabetes. Therefore, *S. xanthocarpum* was selected for further to isolate and structure elucidation of compound.

Table 2. α -Glucosidase inhibition of crude extracts

Crude extracts	IC ₅₀ ^a (mg/mL)		
	yeast α -glucosidase	Maltase	Sucrase
MeOH	NI ^b	9.85±1.21	3.25±1.35
CH ₂ Cl ₂	10.25±1.52	NI	NI
Hexane	NI	NI	NI
Acarbose	2.78±2.45	0.49±0.12	1.25±0.25

^aThe IC₅₀ value is defined as the inhibitory concentration to inhibit 50% of enzyme activity

^bNo inhibition

5. Conclusion

Solanum xanthocarpum Schrad. & Wendl. fruit showed that the crude extracts inhibited DPPH radical and α -glucosidase. Determination of the dual inhibitory effect of *S. xanthocarpum* extract will help to develop new alternative drug candidates for antidiabetic agent and prevention of related complications. However, pharmacological, phytochemical and toxicological studies are needed to confirm its efficiency and safety.

6. Acknowledgements

This project was supported by Program of Applied Biology and Faculty of Science and Technology, Rajamangala university of Technology Sivijaya, Nakhon Si Thammarat Thailand.

7. References

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