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Research Article

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Alpha-Glucosidase Inhibitory Activity of Compounds from *Bauhinia strychnifolia*

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ABSTRACT

This study has investigated the α-glucosidase inhibitory activity of Thai medicinal plant (Bauhinia strychnifolia). The colorimetric method and chromatographic technique were carried out for bioassay and phytochemistry. The ethanol extract of B. strychnifolia exhibited good inhibitory activity against α-glucosidase activity with an IC_{50} value of 160 µg/mL which was higher than the value of positive control, acarbose, (α-glucosidase drug, IC_{50}=189 µg/mL). From bioassay-guided isolation, the water fraction was observed to separate into two compounds namely; 3,5,7-Trihydroxychromone-3-O-α-L-rhamnopyranoside (1) and 3,5,7,3',5'-pentahydroxy-flavanonol-3-O-α-L-rhamnopyranoside (2). Among the tested samples, 3,5,7-Trihydroxychromone-3-O-α-L-rhamnopyranoside (1) showed the highest activity against α-glucosidase with an IC_{50} value of 540 µg/mL similar to that of the positive control, acarbose (α-glucosidase drug, IC_{50}=550 µg/mL). This was followed by 3,5,7,3',5'-pentahydroxy-flavanonol-3-O-α-L-rhamnopyranoside (2) which gave an IC_{50} value of 980 µg/mL. From the present study, the findings would be the first report on the anti α-glucosidase activity of compounds from Bauhinia strychnifolia.

Keywords: *Bauhinia strychnifolia; α*-Glucosidase inhibitor; Traditional medicine; Chromone; Flavanonol

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INTRODUCTION

Diabetes mellitus is one of the most prevent diseases in the group of non-communicative diseases (NCD). According to the International Diabetes Federation, about 366 million people are living with diabetes and this figure is projected to increase to 552 million by the year 2030 [1]. Diabetes mellitus has rapidly become a physical and mental burden that lowers the quality of life, leading to high rates of mortality and disability. Commercial α-glucosidase inhibitors such as acarbose and voglibose have been used for medicinal purposes as well as for

treatment of diabetes. These commercial inhibitors exhibit some side effects including liver disorders, flatulence, abdominal pain, renal tumors, hepatic injury, acute hepatitis, abdominal fullness, and diarrhea [2,3].

Alpha-glucosidase is known to be involved in a carbohydrate metabolism process via hydrolysis of the α-glycosidase bond among polysaccharide chain and the release of a single glucose molecule which would be absorbed into the blood stream. Inhibition of this enzyme can slowdown the carbohydrate metabolism and could result into a reduction in blood sugar level in the end [4]. *Bauhinia strychnifolia* Craib (Fabaceae) is known in Thai as Yanang Dang or Khayan**.** In Thai traditional medicine, the stem and root have been used to treat fever, *alcohol intoxication*, allergy and constipation [5].

EXPERIMENTAL SECTION

Reagent and Chemicals

Sephadex LH-20 (20–100 mm, Sigma, St. Louis, MO). Thin-layer chromatography (TLC) was performed on precoated sheets of silica gel 60 F254 (20 x 20 cm^2, 0.25 mm, Merck, New York, USA). The microtiter plate was purchased from Corning Life Sciences (Union City, CA). *p*-Nitrophenyl-alpha-D-glucopyranoside (*p*-PNPG) was purchased from Sisco (Maharashtra, India). Acarbose was purchased from Sigma (St. Louis, USA). Alpha-glucosidase enzyme was purchased from Sisco (Maharashtra, India). All solvents were of laboratory reagent grade and were purchased from commercial sources.

Plant Material

Bauhinia strychnifolia Craib (Fabaceae) is known in Thai as Yanang Dang or Khayan**.** In Thai traditional medicine, the stem and root have been used to treat fever, *alcohol intoxication*, allergy and constipation [5]. *B. strychnifolia* stems were collected in 2010 at the Suan Ya Thai Thongnoppakhun herbal garden in Chonburi province and were identified by a Thai traditional doctor, Mr. Sraupsin Thongnoppakhun. A voucher specimen (SKP 072021901) was kept at the Herbarium of the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand.

The Isolated Compounds from *B. strychnifolia*

A dried powder of *B. strychnifolia* stems (4.5 kg) was extracted twice with EtOH (28 L) at room temperature. The solvent was removed under reduced pressure to give an EtOH extract of 993.5 g in weight and was stored at 4°C. The EtOH extract (993.5 g) was successively partitioned in order to obtain hexane (5.2 g, 0.5%w/w), chloroform (153.9 g, 15.5%w/w), ethyl acetate (58.6 g, 5.9%w/w), water (458.9 g, 46.2%w/w) and a precipitate from the ethyl acetate: water fractions (49.0 g, 4.9%w/w), respectively. The water and chloroform fractions which showed appreciable α-glucosidase inhibitors effects were further isolated to obtain the pure compounds.

The water fraction with a weight of 40.0 g was chromatographed on a Dianion HP-20 using water, water/methanol and methanol to afford 6 fractions (F1-F6). Fraction F6 (6.0 g) was separated by silica gel column chromatography using 10% methanol in ethyl acetate to give six subfractions (F6/1a–F6/6a). Subfraction F6/2a (3.47 g) was purified by column chromatography on silica gel using 10% methanol in ethyl acetate to give subfraction F6/1b-F6/3b. The Subfraction F6/3b was purified by column chromatography on silica gel using 20% methanol in chloroform to obtain 3,5,7-trihydroxychromone-3-α-L-rhamnopyranoside (1) (white solid, 5.3 mg, 0.01325% w/w) and 3,5,7,3',5'-pentahydroxyflavanonol-3-O-α-L-rhamnopyranoside (2) (white solid, 65 mg, 0.1625% w/w) (Figure 1).

Pure compounds (1-2) were isolated by chromatographic techniques and the spectroscopic data of pure compounds were compared with those previously reported earlier [6,7].

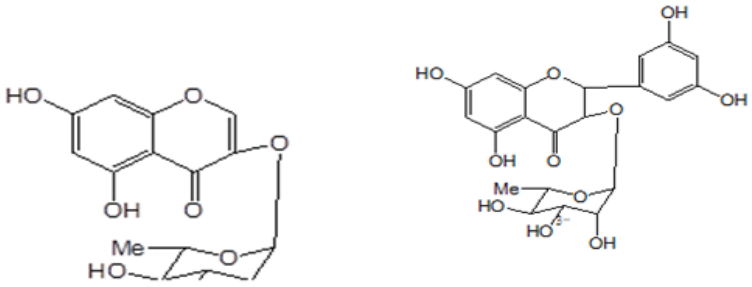

Figure 1. Chemical structures of compounds 3,5,7-Trihydroxychromone-3-O-α-L-rhamnopyranoside (1) and 3,5,7,3′,5′-Pentahydroxyflavanonol-3-O-α-L-rhamnopyranoside (2)

Determination of α-Glucosidase Activity

Alpha-glucosidase inhibition was determined for all extracts according to previously reported assays [8]. This protocol would be described briefly subsequently. After hydrolysis reaction, a substrate such as p-nitrophenyl-alpha-D-glucopyranoside (*p*NPG) turned into *p*-nitrophenol as a product which had a yellow color and could be detected at 405 nm. Firstly, 50 µL of 8 mg/mL sample solution were mixed in the wells with both 50 µL of phosphate buffer solution containing 2 mg/mL of bovine serum albumin, 0.2 mg/mL sodium azide (PBS) and 50 µL 1 unit/ml of α-glucosidase enzyme in PBS. In the control well, 5% of DMSO solution was used. However, 8 mg/mL of acarbose solution replaced the sample solution in the positive control wells. All the wells were incubated at 37°C for 2 min before adding the 4 mM of *p*NPG into the mixed wells. Subsequently, the entire reaction was detected at 405 nm every half minutes for 5 min by micro-plate reader. The α-glucosidase inhibitory activity was calculated as percentage inhibition. The IC_{50} value was defined as the concentration of samples tested that provided 50% inhibition of α-glucosidase activity.

RESULT AND DISCUSSION

α-Glucosidase Inhibition of Ethanol Extract and Fractions from *B. strychnifolia*

The EtOH extracts of *B. strychnifolia* (stem) were the most potent anti α-glucosidase activity with an IC_{50} value of 160 µg/mL, followed by the water fraction with an IC_{50} value of 240 µg/mL. The chloroform fraction, precipitate ethyl acetate, ethyl acetate fraction and hexane fraction also gave IC_{50} values of 260 µg/mL, 460 µg/mL, 820 µg/mL and 910 µg/mL respectively. The ethanol (EtOH) extract of *B. strychnifolia* exhibited good inhibitory activity against *α*-glucosidase activity with an IC_{50} value of 160 µg/mL which was higher than the value obtained from the positive control, acarbose, (*α*-glucosidase drug, IC_{50}=189 µg/mL) (Table 1).

Table 1. **Alpha-glucosidase inhibitory activity of the ethanol extract and various fractions of *B. Strychnifolia***

Samples	IC_{50} (µg/mL)
Ethanol	160
Hexane fraction	910
Chloroform fraction	260
Ethyl acetate fraction	820

Water fraction	240
Precipitate ethyl acetate: Water	460
Acarbose	189

α-Glucosidase Inhibition of Compounds from *B. strychnifolia* (stem)

B. strychnifolia (stem) ethanol extract was separated to yield two compounds namely; 3,5,7-Trihydroxychromone-3-O-**α**-L-rhamnopyranoside (1) showed the highest activity against α-glucosidase with an IC_{50} value of 540 µg/mL, followed by 3,5,7,3**'**,5**'**-pentahydroxy-flavanonol-3-O-**α**-L-rhamnopyranoside (2) with an IC_{50} value of 980 µg/mL as shown in Table 2 and Figure 1. The 3,5,7-Trihydroxychromone-3-O-**α**-L-rhamnopyranoside (1) showed the highest activity against α-glucosidase with an IC_{50} value of 540 µg/mL similar to that of the positive control**,** acarbose (α-glucosidase drug, IC_{50}=550 µg/mL). It has been reported that 3,5,7-Trihydroxychromone-3-O-**α**-L-rhamnopyranoside (1) exhibited an anti-HT-29, KB and MCF-7 cancer cells. While the 3,5,7,3′,5′-Pentahydroxyflavanonol-3-*O*-α-L-rhamnopyranoside (2) possessed a potent activity against cancer KB, HT-29, MCF-7 and HeLa cancer cells [9]. Moreover, *B. strychnifolia* (stem) showed anti-HIV-1 integrase and anti-allergic activities [10].

Table 2. Alpha-glucosidase inhibitory activity of compounds 1–2 from *B. Strychnifolia*

Compounds	IC_{50} (µg/mL)
3,5,7-Trihydroxychromone-3-O-α-L-rhamnopyranoside (1)	540
3,5,7,3',5'-pentahydroxy-flavanonol-3-O-α-L-rhamnopyranoside (2)	980
Acaebose (positive control)	550

CONCLUSION

This would be the first study on the isolation of compounds from *B. strychnifolia* for anti *α*-glucosidase activity. In addition, it is also the first report on compounds 1 and 2 isolated from *B. strychnifolia* on anti α-glucosidase activity. 3,5,7-Trihydroxychromone-3-O-**α**-L-rhamnopyranoside (1) possessed a potent activity against glucosidase activity similar to that of the positive control**,** acarbose**.** However, 3,5,7,3**'**,5**'**-pentahydroxy-flavanonol-3-O-**α**-L-rhamnopyranoside (2) presented a good activity. These two compounds could be regarded as good candidates for the development of anti α-glucosidase activity agents and their mechanisms of action will be further investigated.

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