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ORIGINAL ARTICLE

A New Stilbene Derivative and Isolated Compounds from *Bauhinia pottsii* var. *pottsii* with their Anti-alpha-glucosidase Activity

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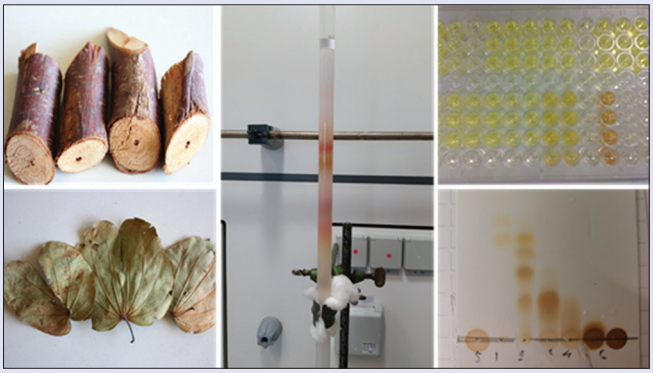
ABSTRACT

Background: Natural products are becoming important alternative medicines for diabetes treatment. Many *Bauhinia* plants in Thailand have been used in Thai traditional medicines for diabetic patients. *Bauhinia* plants have been reported on anti-alpha-glucosidase activity. However, *Bauhinia pottsii* var. *pottsii* G. Don which is a local plant in Southern Thailand has not been reported on antidiabetic activity and phytochemistry. Hence, it was selected for further study. **Objectives:** Isolation of chemical constituents from *B. pottsii* var. *pottsii* and determination of their biological activity on alpha-glucosidase inhibition. **Materials and Methods:** Chromatographic techniques, enzymatic assay, and molecular docking were done in this study. **Results:** A new stilbene derivative, 1,3-benzenediol-5-(-2-phenylethyl)-6-(2′,3′-butanediol)-3′, methyl (1) and five known compounds such as 3,4-dihydroxybenzoic acid (2), quercetin (3), 3-*O*-methylquercetin (4), a mixture of beta-sitosterol and stigmasterol (5), and 3,4,5-trimethoxyphenyl-beta-D-glucopyranoside (6) were isolated from *B. pottsii* var. *pottsii*. The plant extracts and isolated compounds were determined for anti-alpha-glucosidase activity. The results showed that the compounds 2, 3, and 4 were inhibited alpha-glucosidase enzyme activity with inhibition concentration at 50% (IC_{50}) values of 3.637 mM, 0.486 mM, and 0.292 mM, respectively. While positive control, acarbose exhibited IC_{50} value as 0.166 mM. The mechanism of action was proved using computer molecular docking. Compound 2 was predicted as mixed-type inhibitor, whereas compounds 3 and 4 were predicted as competitive inhibitors. **Conclusion:** This study will be the first report of phytochemical and biological study from *B. pottsii* var. *pottsii* including the first report of new compound.

Key words: Anti-alpha-glucosidase activity, *Bauhinia pottsii*, molecular docking, phytochemistry, stilbene derivative

SUMMARY

- Chemical constituents from *B. pottsii* var. *pottsii* and determination of their biological activity on alpha-glucosidase inhibition were reported as the first time
- A new stilbene derivative; 1,3-benzenediol-5-(-2-phenylethyl)-6-(2′,3′-butanediol)-3′, methyl was isolated from *B. pottsii* var. *pottsii*
- Three isolated compounds as protocatechuic acid and two flavonoids, quercetin and 3-*O*-methylquercetin, exhibited alpha-glucosidase inhibition
- The mechanism of action of these compounds was predicted using *in silico* study which showed mixed-type inhibition and competitive inhibition of protocatechuic acid and two flavonoids, respectively.



Abbreviations used: A°: Angstrom; IC_{50}: Inhibition concentration at 50%; ESI: Electrospray ionization; *m/z*: Mass-to-charge ratio; MHz: Megahertz; NMR: Nuclear magnetic resonance, ^{1}H NMR: proton NMR, ^{13}C NMR: Carbon-13 NMR; ppm: Parts-per million.

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INTRODUCTION

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.[1] Type 2 diabetes is referred to non-insulin-dependent diabetic mellitus that pancreatic beta-cells lost some ability to produce and secrete insulin, including insulin-resistant is also one of the causes of type 2 diabetes.[2]

Type 2 diabetes is a metabolic disease that can be prevented through lifestyle modification, diet control, and control of overweight and obesity.[3] The antidiabetic drugs which are used for Type 2 diabetes were treated through various mechanisms, increase insulin secretion, increase insulin sensitivity, increase glucose uptake to muscle, and decrease glucose absorption. In this research, we studied how to decrease glucose absorption using an anti-alpha-glucosidase activity model. Alpha-glucosidase or maltase is the most important enzyme which is located in the small intestine. The enzyme is used for digesting

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polysaccharide or sugar into monosaccharide, especially glucose molecules. Hence, the inhibition of this enzyme activity will decrease glucose absorption in intestine and bloodstream.

At present, natural products are becoming important alternative medicines for diabetes treatment. Over 170 different natural medicines are used for combating diabetes. However, only a fraction of these products has reliable clinical evidences of effectiveness. Herbal formulae containing multiple herbs may have synergistic effects.[4] Thus, searching for antidiabetic drugs from nature will find out new sources of lead compounds which can be used in drug discovery and diabetic treatment.

Many *Bauhinia* plants in Thailand have been used in Thai traditional medicines for diabetic patients. From our previous report,[5] the plants in genus *Bauhinia* have been reported on anti-alpha-glucosidase activity. However, *Bauhinia pottsii* var. *pottsii* G. Don which is a local plant in southern Thailand has not been reported on antidiabetic activity and phytochemistry. Hence, this will be the first report concerning anti-alpha-glucosidase and its isolated compounds of this plant.

MATERIALS AND METHODS

General experimental procedures

One-dimensional and two-dimensional NMR spectrometry were observed by Fourier Transform NMR Spectrometer (^{1}H-NMR 500 MHz and ^{13}C-NMR 125 MHz), model UNITY INNOVA, Varian. LC-MS/MS was analyzed on a Finnigan LCQ-Duo ion trap mass spectrometer with an ESI source (ThermoQuest) coupled to a Finnigan Surveyor HPLC system with an EC 150/3 Nucleodur 100-3 C18ec column (Macherey-Nagel). A gradient of water and acetonitrile with 0.1% formic acid each was applied from 2% to 70% acetonitrile in 60 min at 30°C. The flow rate was 0.5 ml/min. The injection volume was about 20 µL. NMR and LC-MS/MS experiments were observed at the Institute of Pharmacy and Molecular Biotechnology, Department of Pharmaceutical Biology, Heidelberg University, Germany. High-resolution mass spectrometry was observed using positive mode of TOF/Q-TOF mass spectrometer combined with Dual AJS ESI detector (Agilent Technologies®, USA) at Scientific Equipment Centre, Prince of Songkla University, Thailand.

Phytochemical investigation was done using classical column chromatography. The normal stationary phase was performed using Silica Flash® P60 (Ultrapure Silica Gel, particle size 40–63 µm, SiliCycle®, Canada). Sephadex® LH-20 (bead size 25–100 µm, GE Healthcare Bio-Sciences AB, Uppsala, Sweden) was used for column chromatography to purify the compounds. Silica gel 60 F_{254} (Merck, Germany) was used for thin-layer chromatography.

Plant preparation

The wood and leaf of *B. pottsii* var. *pottsii* G. Don were collected from the swamp forest and identified by the botanist of The Pikun Thong Royal Development Study Centre, Narathiwat, Thailand. The plant specimen was deposited at the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand, with herbarium number as SKP 072 02 16 01.

The dried samples were macerated with various solvents for 72 h. The filtrate was evaporated with a rotary evaporator under reduced pressure at 40°C. The crude extracts were kept at 4°C. The marc was repeated for 3 times of extraction with the same solvent. After that, the marc was macerated with the other solvents, ethyl acetate and ethanol, respectively. Finally, the marc was boiled with water at 70°C for 6 h. The filtrate was also evaporated with a rotary evaporator under reduced pressure at 40°C. The crude extracts were kept at 4°C until examination.

Phytochemical study techniques

Chromatographic techniques such as column chromatography, thin-layer chromatography, size-exclusion chromatography and reversed phase RP-18 chromatography were used for isolation and purification of chemical constituents of this plant. After that, the isolated compounds were interpreted using NMR and LC-MS/MS techniques.

Assay on anti-alpha-glucosidase activity

The assay on anti-alpha-glucosidase activity was followed by previously reported.[5] The enzyme activity was measured using the colorimetric method. The yellow color of the product was observed using UV-spectrometry at 405 nm. The color of reaction produced from hydrolysis reaction between the substrate (*p*-nitrophenyl-alpha-D-glucopyranoside) and alpha-glucosidase enzyme.

Briefly, the tested samples and positive standard (acarbose) were dissolved in 20% of dimethyl sulfoxide in water. Alpha-glucosidase enzyme from *S. cerevisiae* (EC 3.2.1.20) and *p*-nitrophenyl-alpha-D-glucopyranoside were dissolved in 0.1 M phosphate buffer pH 7, which was supplemented with bovine serum albumin and NaN_3. 50 µL of phosphate buffer, enzyme, and sample were added to the 96-well plate and incubated for 2 min. Then, 50 µL of the substrate was added and the enzyme activity was measured following the kinetic parameter at 405 nm (interval time 30 min and 20 cycles).

The inhibition of enzyme activity was measured by calculating the velocity of the reaction. The UV absorption was plotted using linear regression between the difference of absorbance and time as following Eq. (1). The highest velocity (V) from the initial reaction of each sample was collected and calculated the percentage of inhibition by Eq. (2) as follows:

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

$$\%\text{Inhibition} = \frac{(\text{V control-blank}) - (\text{V sample-blank})}{(\text{V control-blank})} \quad \text{Eq. (2)}$$

Computer molecular docking of alpha-glucosidase enzyme

The crystal structure of alpha-glucosidase from *S. cerevisiae*, PDB: 3a4a,[6,7] was downloaded from the RCSD Protein Data Bank (http://www.rcsb.org) and this targeted protein was prepared for docking experiment by AutoDockTools version 1.5.6.[8] Whereas, the 3D structures of the interesting compounds were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and these structures were optimized by the geometry and GAFF optimizations from Avogadro version 1.2.0[9] before subjection to AutoDockTools for the docking preparation process. Glucose as a native ligand was used to indicate the active site, which was described as a grid box in the 3D structure as the center of X-axis = 21.1, the center of Y-axis =-7.4, and center of Z-axis = 24.2, respectively. Sizes of this grid box were presented as 16 Å ×16 Å ×16 Å. Moreover, the native ligand was extracted and redocked for the docking validation method.

AutoDock Vina version 1.1.2[10] was selected to proceed the docking experiment in this study. All parameters in this experiment were set up as default values except the exhaustiveness was adjusted as 20. Eventually, ViewDock package from Chimera version 1.11.2[11] was performed to analyze all results from this experiment. The best confirmation of the interesting compounds was selected from the outcomes based on the combination of the lowest binding affinity and the alignment of those structures. For the validation protocol, RMSD was measured between

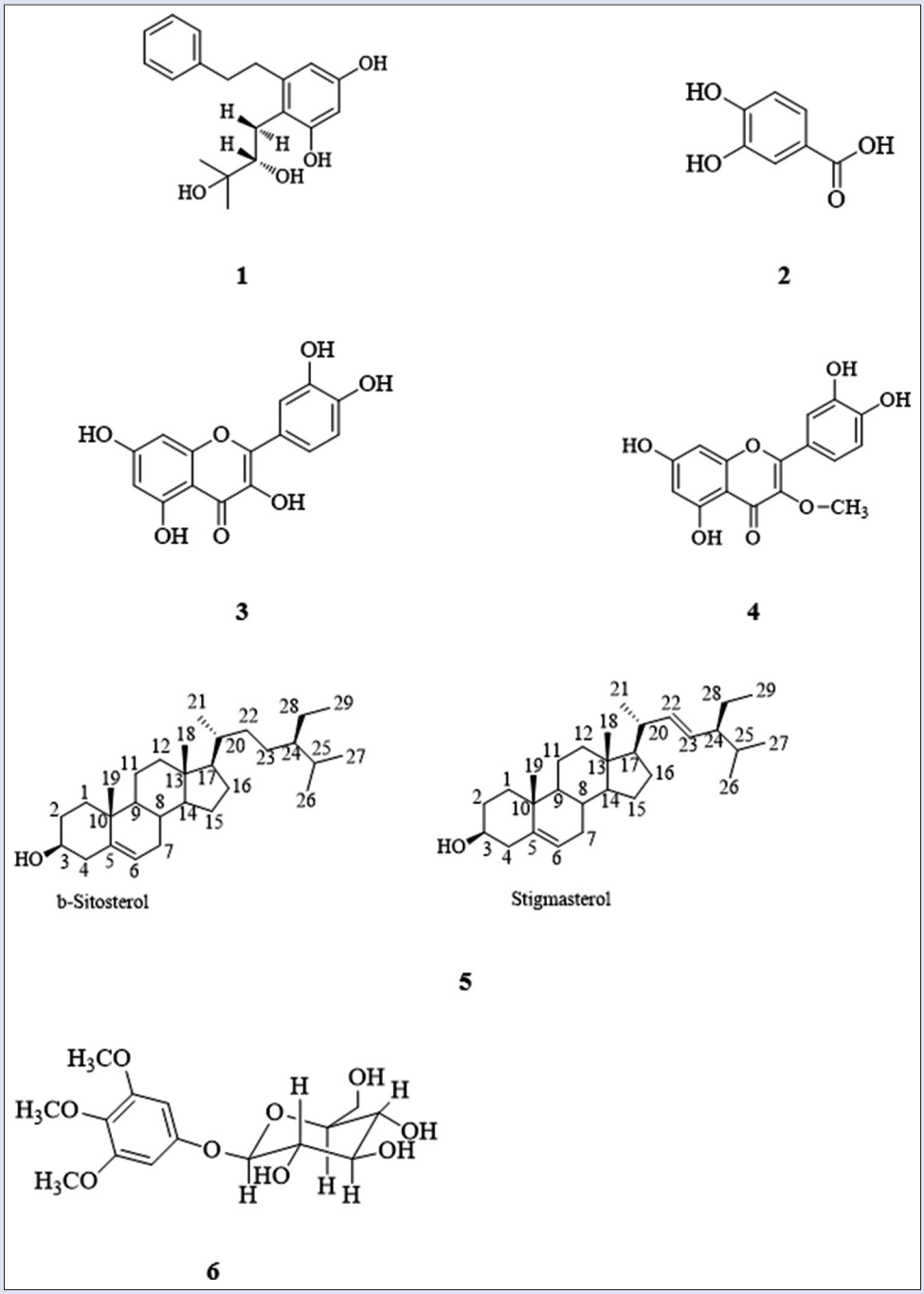

Figure 1: The structure of isolated compounds from *Bauhinia pottsii* var. *pottsii*. 1 = 1,3-benzenediol-5-(-2-phenylethyl)-6-(2′,3′-butanediol)-3′, methyl, 2 = 3,4-dihydroxybenzoic acid; 3 = quercetin, 4 = 3-*O*-methylquercetin, 5 = a mixture of beta-sitosterol and stigmasterol, 6 = 3,4,5-trimethoxyphenyl-beta-D-glucopyranoside

the native ligand and redocked ligand to confirm the reliability of this experiment.[12]

RESULTS AND DISCUSSION

Phytochemical investigation of *Bauhinia pottsii* var. *pottsii*

Six compounds were isolated from *B. pottsii* var. *pottsii*. Compound 1 is a new compound, a stilbene derivative named as 1,3-benzenediol-5-(-2-phenylethyl)-6-(2′,3′-butanediol)-3′, methyl. This is the first time to report its structure and bioactivity of this compound. Five known compounds were interpreted as 3,4-dihydroxybenzoic acid (protocatechuic acid) (2), quercetin (3), 3-*O*-methylquercetin (4), a mixture of beta-sitosterol and stigmasterol (5), and 3,4,5-trimethoxyphenyl-beta-D-glucopyranoside (6) [Figure 1].

Compound 1 (A new stilbene derivative)

Compound 1 was obtained as a yellow amorphous and dissolved in methanol. Chemical formula: $C_{19}H_{24}O_4$ and ultraviolet (UV) λ_{max}: 217, 236, and 279 nm. The ^{1}H nuclear magnetic resonance (NMR)

spectrum (500 MHz in MeOH-*d*4) showed the chemical shifts of aromatic protons at 6.19 ppm (*d*, *J* = 2.51 Hz, H-2), 6.22 ppm (*d*, *J* = 2.45 Hz, H-4), 7.19 ppm (*dd*, *J* = 8.30, 1.5 Hz, H-2"), 7.24 ppm (*ddd*, *J* = 7.54, 7.06, 1.61 Hz, H-3"), 7.15 ppm (*tt*, *J* = 6.68, 1.33 Hz, H-4"), 7.24 ppm (*ddd*, *J* = 7.54, 7.06, 1.61 Hz, H-5"), and 7.19 ppm (*dd*, *J* = 8.30, 1.50 Hz, H-6"). The chemical shifts of -CH_2-CH_2- that linked between two aromatic rings showed at 2.81 ppm (*m*, H-7) and 2.82 ppm (*m*, H-8). The substitution which identified as butanediol showed the signals at 2.85 ppm (*dd*, *J* = 14.60, 10.8 Hz, H-1'), 2.57 ppm (*dd*, *J* = 14.50, 10.50 Hz, H-1'), 3.45 ppm (*d*, *J* = 10.50, 1.80 Hz, H-2'), 1.23 ppm (*d*, *J* = 2.90 Hz, H-4'), and 1.23 ppm (*d*, *J* = 2.90 Hz, H-5').

The ^{13}C spectrum (125 MHz in MeOH-*d*4) showed the chemical shifts of aromatic carbons at 156.43 (C-1), 100.88 (C-2), 155.82 (C-3), 107.91 (C-4), 142.18 (C-5), 116.23 (C-6), 141.87 (C-1"), 128.06 (C-2"), 127.95 (C-3"), 125.44 (C-4"), 127.95 (C-5"), and 128.06 (C-6"). The chemical shifts of -CH_2-CH_2- that linked between two aromatic rings showed at 35.54 (C-7) and 35.37 (C-8). The chemical shifts of butanediol showed at 27.18 (C-1'), 80.13 (C-2'), 72.44 (C-3'), 23.43 (C-4'), and 24.32 (C-5').

The positive ESI/HR/MS spectrum of compound 1 showed an $[M^+ Na]^+$ ion peak at m/z 339.1581 [M = 316.1687] supporting the molecular formula $([C_{19}H_{24}O_4]+Na)^+$ (calcd. for $C_{19}H_{24}O_4$, 316.1675, diff −3.83 ppm). ^1H-^1H and ^1H-^{13}C from COSY and HMBC correlations at the position C-7, C-8, C-1', C-2', and C-3' were the important correlations.

Due to the Karplus equation,[13] the coupling constant at the chiral proton showed the correlation at 3.45 ppm (*d*, *J* = 10.50, 1.80 Hz, H-2') which coupled with methylene proton at 2.85 and 2.57 ppm that could be used to explain the configuration of this structure. The chiral proton was coupled with methylene protons at 2.85 ppm (H_β) with Θ nearly to 180° which showed the *J* = 10.50 Hz and coupled with 2.57 ppm (H_α) with Θ nearly to 60° which showed the *J* = 1.80 Hz [Figure 2].

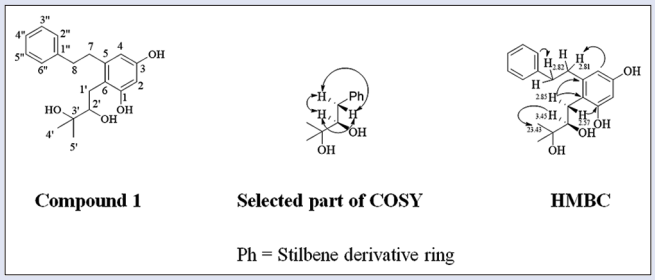

Figure 2: COSY and HMBC correlation of compound 1

The result of ESI-positive mass spectroscopy of compound 1 showed the molecular ion peak at 317.06 m/z ($[M^+ H]^+$) [Figure 3]. There were several main fragments from ESI-mass which showed at 299.12, 281.16, and 227.19 m/z. These fragments were broken down from the main structure by losing of water molecules and substitution. The benzenediol derivative fragment showed at 135.09 m/z. The spectrum of compound 1 was interpreted as a new stilbene derivative which was substituted with butanediol. The compound was named as 1,3-benzenediol-5-(-2-phenylethyl)-6-(2',3'-butanediol)-3', methyl [Figure 2].

Compound 2 (Protocatechuic acid)

Compound 2 was obtained as brown needle crystals and dissolved in methanol. Chemical formula: $C_7H_6O_4$. ESI mass: 155.06 m/z ($[M^+ H]^+$). UV λ_{max}: 250 nm. The ^1H NMR spectrum (500 MHz in MeOH-*d*4) showed the chemical shifts of aromatic protons at 7.44 ppm (*d*, *J* = 2.00 Hz, H-2), 6.81 ppm (*d*, *J* = 8.10 Hz, H-5), and 7.42 ppm (*dd*, *J* = 8.10, 2.00 Hz, H-6). The ^{13}C spectrum (125 MHz in MeOH-*d*4) showed the chemical shifts of aromatic carbons at 121.77 (C-1), 116.38 (C-2), 144.61 (C-3), 150.06 (C-6), 114.44 (C-5), and 122.62 (C-6). The carbonyl of carboxylic acid showed at 168.98 (C-7). The chemical shifts of compound 2 were compared to a previous report as 3,4-dihydroxybenzoic acid.[14]

Compound 3 (Quercetin)

Compound 3 was obtained as yellow powder and dissolved in methanol. Chemical formula: $C_{15}H_{10}O_7$. ESI mass: 303.27 m/z ($[M^+ H]^+$). UV λ_{max}: 250 and 346 nm. The ^1H NMR spectrum (500 MHz in MeOH-*d*4) showed the chemical shifts of quercetin structure at 6.17 ppm (*d*, *J* = 1.50 Hz, H-6), 6.38 ppm (*d*, *J* = 1.60 Hz, H-8), 7.74 ppm (*d*, *J* = 2.00, H-2'), 6.88 ppm (*d*, *J* = 8.50 Hz, H-5'), and 7.64 ppm (*dd*, *J* = 8.50, 2.10 Hz, H-6'). The

Table 1: Anti-alpha-glucosidase activity of *Bauhinia pottsii* var. *pottsii* extracts

Number	Part	Plant extract	Percentage inhibition at 2 mg/mL	IC_{50} (μg/mL)
1	Leaf	Hexane	6.04±1.48	-
2		Ethyl acetate	24.46±0.78	-
3		Ethanol	74.51±1.13	1,260
4		Water	84.27±2.67	520
5	Wood	Hexane	6.28±1.91	-
6		Ethyl acetate	44.97±0.64	2,160
7		Ethanol	81.16±0.40	1,280
8		Water	68.08±0.22	1,330
9	Positive standard	Acarbose	92.79±0.14	88.75

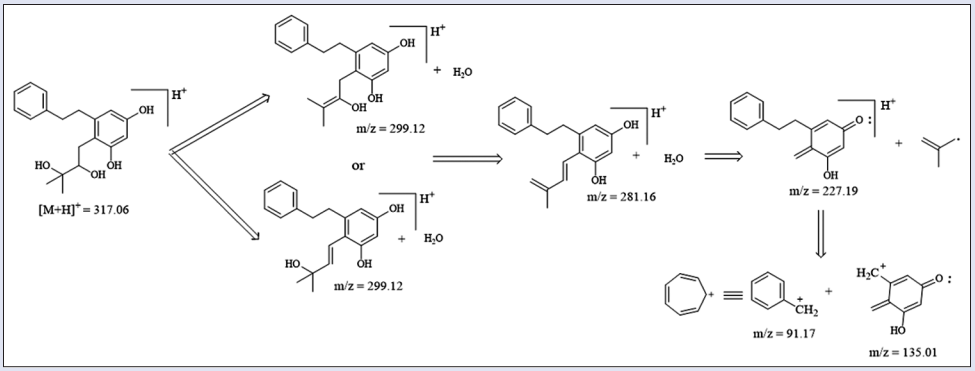

Figure 3: Mass fragmentation of a new stilbene derivative from *Bauhinia pottsii* var. *pottsii*

Table 2: Anti-alpha-glucosidase activity of isolated compounds

Number	Compound	IC_{50} (μg/mL)	IC_{50} (mM)
1	Compound 1	Inactive	Inactive
2	Compound 2	560.27	3.637
3	Compound 3	146.97	0.486
4	Compound 4	92.34	0.292
5	Compound 5	Inactive	Inactive
6	Compound 6	Inactive	Inactive
	Acarbose	107.19	0.166

^{13}C spectrum (125 MHz in MeOH-*d*4) showed the chemical shifts of quercetin at 146.54 (C-2), 137.39 (C-3), 179.44 (C-4), 161.11 (C-5), 98.27 (C-6), 164.58 (C-7), 92.99 (C-8), 156.55 (C-9), 103.11 (C-10), 120.18 (C-1'), 114.71 (C-2'), 144.77 (C-3'), 149.23 (C-4'), 114.76 (C-5'), and 120.26 (C-6'). The chemical shifts of compound 3 were compared to previous report as quercetin.[15]

Compound 4 (3-O-methoxyquercetin)

Compound 4 was obtained as yellow needle crystals and dissolved in methanol. Chemical formula: $C_{16}H_{12}O_7$. ESI mass: 317.20 m/z ([M$^+$ H]$^+$). UV λ_{max}: 251 nm. The ^{1}H NMR spectrum (500 MHz in MeOH-*d*4) showed the chemical shifts of quercetin structure at 6.38 ppm (*d*, *J* = 2.00 Hz, H-6), 6.19 ppm (*d*, *J* = 2.00 Hz, H-8), 7.61 ppm (*d*, *J* = 2.20 Hz, H-2'), 6.89 ppm (*d*, *J* = 8.50 Hz, H-5'), and 7.52 ppm (*d*, *J* = 8.50, 2.20 Hz, H-6'). The substitution at ring C which identified as methoxy showed the signal at 3.77 ppm (*s*, 3-OCH_3). The ^{13}C spectra (125 MHz in MeOH-*d*4) showed the chemical shifts of quercetin at 156.99 (C-2), 138.10 (C-3), 178.56 (C-4), 161.65 (C-5), 93.28 (C-6), 164.58 (C-7), 98.35 (C-8), 156.55 (C-9), 104.40 (C-10), 121.49 (C-1'), 115.03 (C-2'), 145.03 (C-3'), 148.52 (C-4'), 114.98 (C-5'), and 120.87 (C-6'). The substitution at ring C which identified as methoxy showed the signal at 59.08 (C-3-OCH_3). The chemical shifts of compound 4 were compared to a previous report as 3-*O*-methylquercetin.[16]

Compound 5 (a mixture of beta-sitosterol and stigasterol)

Compound 5 was obtained as white needles. It was soluble in chloroform. Molecular formula: $C_{29}H_{50}O$ (beta-sitosterol) and $C_{29}H_{48}O$ (stigmasterol)*. The ^{1}H NMR spectrum (500 MHz in $CHCl_3$-*d*) showed the chemical shifts of olefinic protons of beta-sitosterol and stigmasterol* at 5.35 (*d*, *J* = 5.20 Hz, H-6), 5.01* (*dd*, *J* = 15.10, 8.6 Hz, H-22), and 5.15* (*dd*, *J* = 15.21, 8.6, H-23). The methyl protons of beta-sitosterol and stigmasterol were assigned at 0.69 and 0.70* (H-18), 1.01 (H-19), 0.92 (H-21), 0.81-0.82 (H-26 and 27), and 0.83 (H-29), respectively. The ^{13}C spectrum (125 MHz in $CHCl_3$-*d*) showed the chemical shifts of olefin carbons at 140.73 (C-5) and 121.73 (C-6), 129.25* (C-22), and 138.29* (C-23). The methyl carbons were assigned at 11.85 (C-18), 19.38 (C-19), 18.69 (C-21), 19.80 (C-26), 19.20 (C-27), and 11.97 (C-29). The spectra of compound 5 were compared to a previous report of β-sitosterol and stigmasterol.[17] The proportion of the mixture between beta-sitosterol and stigmasterol approximately calculated the ratio at 4:1 using integration of ^{1}H-NMR at H-18 of methyl protons.

Compound 6 (3,4,5-trimethoxyphenyl-beta-D-glucopyranoside)

Compound 6 was obtained as brown needle crystals and dissolved in methanol. Chemical formula: $C_{15}H_{22}O_9$. ESI mass: 346.88 m/z m/z ([M$^+$ H]$^+$). UV λ_{max}: 217, 236 and 270 nm. The ^{1}H NMR spectra (500 MHz in MeOH-*d*4) showed the chemical shifts of aromatic protons at 6.48 ppm (*s*, H-2,6). The sugar substitution which was identified as glucopyranoside showed the chemical shifts at 4.80 ppm (*d*, *J* = 7.43 Hz, H-1'), 3.43 ppm (*m*, H-2'), 3.30-3.43 (*m*, H-3',4',5'), 3.65 ppm (*dd*, 12.01, 2.27 Hz, H-6'), and 3.91 ppm (*dd*, 12.06, 6.60 Hz, H-6'). Beta form of sugar was considered from coupling constant value at anomeric proton at H-1'. The ^{13}C spectra (125 MHz in MeOH-*d*4) showed the chemical shifts of aromatic carbons at 154.61 (C-1), 94.68 (C-2,6), 153.35 (C-3,5), and 133.00 (C-4). The anomeric proton of glucopyranoside showed the correlation to C-1 of aromatic carbon which was confirmed by HMBC. The sugar substitution which was identified as glucopyranoside showed the chemical shifts at 101.76 (C-1'), 73.60 (C-2'), 76.99 (C-3' or 5'), 70.27 (C-4'), 76.65 (C-3' or 5'), and 61.29 (C-6'). The chemical shifts of compound 6 were compared to a previous report as 3,4,5-trimethoxyphenyl-beta-D-glucopyranoside.[14]

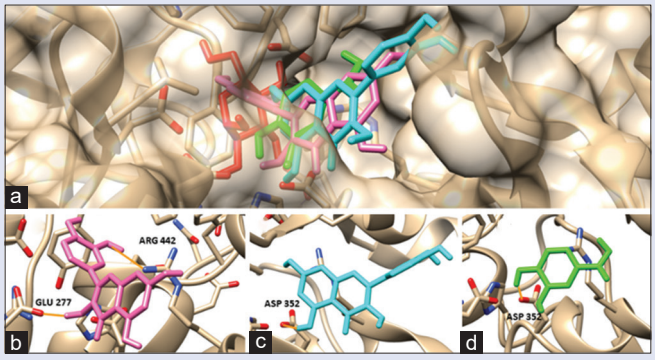

Figure 4: The positions of the best conformation in the active site of alpha-glucosidase (Amber yellow) and the interaction of quercetin (Pink), 3-*O*-methylquercetin (Light blue), 3,4-dihydroxybenzoic acid (Green) and glucose as a native ligand (Red) from docking experiments. (a) An overlays positions of quercetin, 3-*O*-methylquercetin, 2,3-dihydroxy-benzoic acid and glucose in the active site of alpha-glucosidase. (b) H bonds (orange line) between quercetin and alpha-glucosidase. (c) H bond (orange line) between 3-*O*-methylquercetin and alpha-glucosidase. (d) H bonds (orange line) between 3,4-dihydroxybenzoic acid and alpha-glucosidase

Bioactivity on alpha-glucosidase inhibition of plant extracts

Wood and leaf of *B. pottsii* var. *pottsii* were extracted with various solvents as hexane, ethyl acetate, ethanol, and water, respectively to provide eight crude extracts which were continued to determine the bioassay. They were used at the concentration of 2 mg/mL for preliminary screening of anti-alpha-glucosidase activity. Afterward, the extracts which exhibited enzyme inhibitory activity would be figured out the inhibition concentration at 50% (IC_{50}) values. The result of plant extracts is shown in Table 1.

The results of plant extracts were suggested that the ethanol and water extracts from both leaf and wood showed the potential enzyme inhibitory effect. However, the highest potency of alpha-glucosidase inhibition was water extract of the leaf which showed the IC_{50} value as 520 μg/mL.

Bioactivity on alpha-glucosidase inhibition of isolated compounds

Six isolated compounds were determined on anti-alpha-glucosidase activity to find out the active compound using IC_{50} values. The result of their activity is shown in Table 2.

According to the result in Table 2, three compounds which were interpreted as 3,4-dihydroxybenzoic acid (2), quercetin (3), and

3-*O*-methylquercetin (4) exhibited enzyme inhibitory activity with IC_{50} values as 3.637, 0.486, and 0.292 mM, respectively. The highest active compound for alpha-glucosidase inhibition was 3-*O*-methylquercetin (4).

The anti-alpha-glucosidase activity of flavonoid compounds was proved by explaining of structure–activity relationship of their structures. The substitution of hydroxyl on ring A and B at C-5, C-7, C-3', and C-4' is significantly enhancing the activity and the methylation at C-3 on ring C is the slightly decreased activity of flavonoid when compared with the other position of the structure.[18]

Computer molecular docking of active compounds

The inhibition of enzyme activity has proved using docking experiment. The crystal structure of alpha-glucosidase from *Saccharomyces cerevisiae*, PDB: 3a4a[6,7] was downloaded from the RCSD Protein Data Bank (http://www.rcsb.org) and this targeted protein was prepared for docking experiment by AutoDockTools version 1.5.6.[8] Whereas, the three-dimensional (3D) structures of the interesting compounds were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) and these structures were optimized by the geometry and General AMBER Force Field (GAFF) optimizations from Avogadro version 1.2.0[9] before subjection to AutoDockTools for the docking preparation process. Glucose as a native ligand was used to indicate the active site, which was described as a grid box in the 3D structure as the center of X-axis = 21.1, the center of Y-axis = −7.4, and center of Z-axis = 24.2, respectively. Sizes of this grid box were presented as 16 Å × 16 Å × 16 Å. Moreover, the native ligand was extracted and redocked for the docking validation method.

AutoDock Vina version 1.1.2 (The Scripps Research Institute, San Diego, California, USA)[10] was selected to proceed the docking experiment in this study. All parameters in this experiment were set up as default values except the exhaustiveness was adjusted as 20. Eventually, ViewDock package from Chimera version 1.11.2[11] was performed to analyze all results from this experiment. The best confirmation of the interesting compounds was selected from the outcomes based on the combination of the lowest binding affinity and the alignment of those structures. For the validation protocol, root-mean-square deviation (RMSD) was measured between the native ligand and redocked ligand to confirm the reliability of this experiment.[12]

The result from the redocking experiment of the extracted native ligand, as a glucose, indicated that this docking method is liable based on the RMSD values which was lower than 3.50 Å (RMSD = 0.92 Å).[12]

All the best conformations, as well as native ligand from the experiment, are shown in Figure 4a. The catalytic domains (GLU 277 and ASP 352) and the stabilizer domain (ARG 442)[6] were involved in the inhibitory mechanism between quercetin, 3-*O*-methoxyquercetin, 3,4-dihydroxybenzoic acid, and alpha-glucosidase enzyme. The hydrogen bonds were found between the hydroxy groups on ring A and B from quercetin and GLU 277 and ARG 442 in the active site of the enzyme [Figure 4b]. Moreover, the hydroxy groups on ring A from 3-*O*-methylquercetin and on the aromatic ring from 3,4-dihydroxybenzoic acid could also bind with ASP 352 in the same pocket [Figure 4c and d]. Quercetin and 3-*O*-methylquercetin showed the same affinity energy at −7.5 Kcal/mol, whereas 3,4-dihydroxybenzoic acid had a lower energy, −5.6 Kcal/mol.

Molecular docking experiment was wildly and internationally used by researchers to study the molecular interaction between the interested ligands and the targeted protein, which provides the better understanding. However, the validation of the protocol is required to ensure that the obtained data are reliable. The result [as shown in Figure 4] from the redocking experiment in this study was accepted due to RMSD ≤3.5 Å.[18] Even though the affinity energy of flavonoids (quercetin and 3-*O*-methoxyquercetin) was identical and this result was a contradiction with the actual anti-alpha-glucosidase test, it could be differentiated the activity between flavonoids and benzoic acid, because they have the different pharmacophore. The position and the interaction between quercetin, 3-*O*-methylquercetin, and glucosidase enzyme indicated the competitive inhibition behavior. This finding strongly agreed with the previously reported.[19] In addition, benzoic acid derivatives were showed the mixed type inhibition,[20] which was also correlated with docking result from 2,3-dihydroxybenzoic acid, which interacted with one of the catalytic domains.

CONCLUSION

Six compounds were isolated from *B. pottsii* var. *pottsii* including a new stilbene derivative and five five known compounds, protocatechuic acid, quercetin, 3-*O*-methoxyquercetin, a mixture of beta-sitosterol and stigmasterol, and 3,4,5-trimethoxyphenyl-beta-D-glucopyranoside. The crude extracts and isolated compounds were studied on antidiabetic activity using *in vitro* and *in silico* models, anti-alpha-glucosidase activity and computer molecular docking, respectively. The ethanolic and water extracts of leaf and wood showed the potential effect on anti-alpha-glucosidase. However, only three isolated compounds as protocatechuic acid and two flavonoids, quercetin and 3-*O*-methylquercetin, exhibited alpha-glucosidase inhibition. The mechanism of action of these compounds was predicted by using *in silico* study which showed mixed-type inhibition and competitive inhibition of protocatechuic acid and two flavonoids, respectively.

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Conflicts of interest

There are no conflicts of interest.

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