Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Phytochemical Investigation of *Bauhinia winitii* Based on Alpha-Glucosidase Inhibitory Effect and Molecular Docking Affirmation

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Submitted: 07-May-2021 Revised: 31-May-2021 Accepted: 26-Jul-2021 Published: 24-Jan-2022

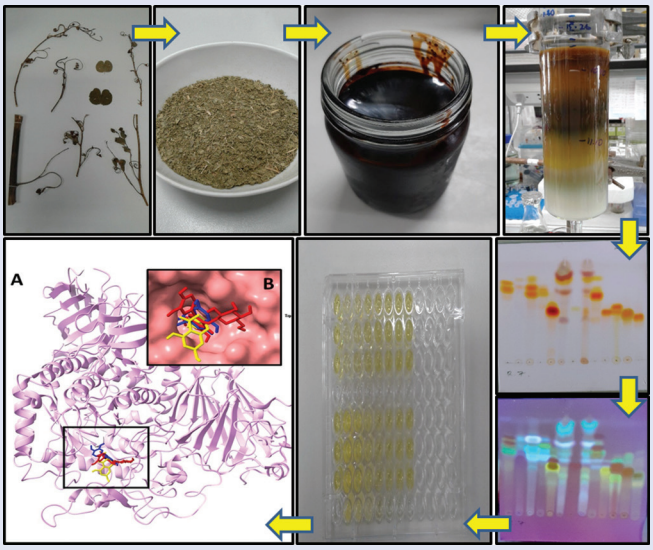
ABSTRACT

Background: *Bauhinia winitii* (BW) (Fabaceae) plant revealed poor phytochemical investigation and anti-diabetic report. As one of the *Bauhinia* family which known with abundant flavonoids content, contrary, BW was only used for the treatment of diarrhea in Thai conservative remedy. **Objectives:** The aim of this study was to investigate the phytochemical constituents and anti-alpha glucosidase activity of BW. **Materials and Methods:** Phytochemical study was based on the alpha-glucosidase inhibitory effect. Isolated compounds were determined by chromatographic and spectroscopic techniques. The alpha-glucosidase enzymatic inhibitory test was then connected with molecular docking affirmation. **Results:** The ethyl acetate and ethanol extracts from leaves and woods exhibited the strongest activity with above 60% inhibition. Six compounds were isolated from these extracts, naringenin (1), luteolin (2), isoquercitrin (3), griffonilide (4), lithospermoside (5), and epi-β amyrin (6). The alpha-glucosidase inhibitory test showed that flavonoids performed potential results with naringenin as the highest IC_{50} on 0.41 mM. The docking ensured the flavonoids activated at the same binding of alpha-glucosidase enzyme active site. **Conclusion:** This study would be the first report of chemical constituents from BW with its isolated compounds was presented anti-diabetic activity as alpha-glucosidase inhibitors.

Key words: Alpha-glucosidase inhibitor, anti-diabetes, *Bauhinia winitii*, molecular docking, phytochemical investigation

SUMMARY

- Phytochemical and biological studies on alpha-glucosidase inhibition of *Bauhinia winitii* were reported for the first
- Six compounds were isolated from BW as naringenin (1), luteolin (2), isoquercitrin (3), griffonilide (4), lithospermoside (5), and epi-β amyrin (6)
- The alpha-glucosidase inhibitory test showed that flavonoids performed potential results with naringenin as the highest IC50 on 0.41 mM
- The docking ensured the flavonoids activated at the same binding of alpha-glucosidase enzyme active site.



Abbreviations used: A°: Angstrom; IC_{50}: Inhibition concentration at 50%; Std: Standard; 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: Part per million.

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DOI: 10.4103/pm.pm_204_21

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INTRODUCTION

Diabetes is defined as a metabolic disease in variable etiology of hyperglycemia such as carbohydrate, fat, and protein disturbances caused by impaired insulin role. It results in long-term clinical effects of various organs multiple disfunction.[1] The category of diabetes has undergone changes since 1980. Categorized as type 1 and type 2 diabetes based on insulin dependency, in fact, expanding with the addition of new criteria named as impaired glucose tolerance and gestational diabetes mellitus. This additional classification indicates the increasing number and the variety of diabetes patients. In diabetic treatment, researchers are interested in finding a new potential drug discovery including from natural resources.[2]

Cite this article as: Dej-adisai S, Rais IR, Wattanapiromsakul C, Pitakbut T. Phytochemical investigation of *Bauhinia winitii* based on alpha-glucosidase inhibitory effect and molecular docking affirmation. Phcog Mag 2021;17:666-72.

In the last decade, we are working on Fabaceae plants to find out the potential natural medicine. From the previous study, *Bauhinia* as one genus of selected Fabaceae plants, which has potent activity close to the standard acarbose on the alpha-glucosidase inhibitory test.[3] This group contains chemical substances as flavonoids, stilbenes, phenolics, steroids, and terpenoids. From the previous report, these chemical constituents presented alpha-glucosidase inhibitory mechanism.[4] *Bauhinia winitii* (BW) as one of the Fabaceae plants, in Thailand, is used as traditional for the treatment of diarrhea.[5] Actually, this plant species has no phytochemical investigation and less anti-diabetic report. Since, from bioactivity screening tests, this plant showed the potent alpha-glucosidase inhibition. Hence, this present study would be gift new additional phytochemical investigation and alpha-glucosidase inhibitory report on this plant.

MATERIALS AND METHODS

Plant extract preparation

BW leaves and woods were collected in January 2017 at Central Thai Literary Botanical Garden, Ratchaburi, Thailand. BW was identified and kept as herbarium specimen (SKP 072 02 23 01) at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla Province, Thailand.

The fresh BW leaves (L) and woods (W) were washed and dried in hot air oven at 50°C, then chopped and blended into small pieces. Dried powder of each part of the plant was extracted by sequential maceration using hexane (H), ethyl acetate (EA), ethanol (ET), and boiled water (W), continuously for 3 times, repeated each in 3 days. The solvent was removed under reduced pressure evaporation to give an extract and was kept at 4°C. These plant fraction samples were named HLBW, EALBW, ETLBW, WLBW; HWBW, EAWBW, ETWBW, WWBW.

Phytochemical screening

Eight fractions of BW were screened by alpha-glucosidase inhibitory assay to know the potential active fraction. Percentage on alpha-glucosidase inhibition of each fraction was used for guidance the compounds isolation process. Compounds were isolated by chromatographic methods such as thin-layer, classical column, and gel filtration chromatography. Isolated compounds were interpreted by spectroscopic methods such as ^{1}H-NMR, ^{13}C-NMR, and high-resolution mass spectrometry.

General equipment

Thin-layer chromatography was used Silica gel 60 F_{254} (Merck, Germany). Classical column chromatography was used Silica Flash® P60 (Ultrapure Silica Gel, SiliCycle®, Canada) and gel filtration was used Sephadex® LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Fourier Transform nuclear magnetic resonance (NMR) Spectrometer was performed on ^{1}H-NMR 500 MHz and ^{13}C-NMR 125 MHz, UNITY INNOVA. TOF/Q-TOF mass spectrometer with Dual AJS ESI detector was worked on high-resolution mass spectrometry (Agilent Technologies®, USA). Spectroscopic observation was done at the Scientific Equipment Centre, Prince of Songkla University, Thailand.

Alpha-glucosidase inhibitory assay

Alpha-glucosidase inhibitory test was determined by following the previously reported assay.[3] The reaction was observed from enzymatic degradation of substrate *p*-nitrophenol-D-glucopyranoside (*p*NPG) by glucosidase enzyme which resulted in yellow product, *p*-nitrophenol (*p*NP). The *p*NP was performed and monitored at 405 nm every 30 s for 5 min by microplate reader. Briefly, samples and standard acarbose were suspended with 20% Dimethyl sulfoxide in water. The alpha-glucosidase enzyme from *Saccharomyces cerevisiae* (EC 3.2.1.20) and *p*NPG substrate were diluted in 0.1 M phosphate buffer (pH 7), augmented with bovine serum albumin and sodium azide phosphate-buffered saline (PBS). Thus, 50 µl of PBS, samples or standard acarbose, and enzyme were placed into 96-well plates. The mixture was then incubated at 37°C for 2 min. The completion, 50 µl of 4 mM *p*NPG was added and incubated again for 5 min with kinetic parameter measurement as described above. The velocity was determined by the linear relationship equation between absorbance and time as following equation (1). The percent inhibition was calculated for the highest velocity as following equation (2).

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

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

Measurement was then continued by IC_{50} calculation with using calibration curve equation between percentages of inhibition and sample concentration at five different levels of concentration.

Computerized molecular docking

Three available human alpha-glucosidase structures namely maltase (PDB ID: 2QMJ), glucoamylase (PDB ID: 3TOP) and isomaltase (PDB ID: 3 LPP) and one baker's yeast alpha-glucosidase (PDB ID 3A4A) were downloaded from the RCSB protein databank (https://www.rcsb.org/) and used in this study. Chimera version 1.11.2[6] was applied here to perform the multiple structures and sequences alignments.

Human isomaltase and barker's yeast alpha-glucosidase, as mentioned earlier, were selected as the target proteins. These two proteins were prepared for docking by using AutodockTools version 1.5.6.[7] Whereas, the chemical structures of isolated flavonoids from this study were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and these structures were optimized geometrically and energetically, MMFF94 force field, accordingly by Avagadro version 1.2.0.[8] The native ligand from each protein was used as a navigator to locate the active site and the gridbox was set as a size of 16 Å ×16 Å ×16 Å. Finally, the coordination of the binding pocket of yeast alpha-glucosidase was described in three dimensions, which had a center of x = 21.5, y = −7.4, and z = 24.1, respectively. Whereas the binding site of human isomaltase was set at a center of x = 39.8, y = 58.5, and z = 78.8, consequently.

Autodock Vina version 1.1.2 was chosen to perform the molecular docking in this study.[9] Only exhaustiveness value was adjusted up to 32 while the other parameters were set as a default value. The established docking protocols were validated by redocking the extracted native ligand back into its original binding pocket before applying these protocols here in this study. Furthermore, the structural alignment of the docked and original pose from the native ligand must be less than 3.5 Å when measured in root mean square deviation to indicate the reliability of the establishment protocols.[10] Viewdock package from chimera version 1.11.2[6] was used to analyze the docking result. Finally, the interaction diagram was generated from the target protein and the best conformation of each experiment by applied Ligplot plus.[11]

RESULTS

Fractionation and isolation screening based on bioactivity

Alpha-glucosidase inhibitory test as the bioactivity screening which was applied to classify the most active extract from BW and guidance the compounds isolation priority as listed in Table 1.

Phytochemical study

The isolated compounds from BW were six known compounds composed with three flavonoids (compound 1–3), one lactone (compound 4), one cyanoglucoside (compound 5), and one steroid (compound 6). Its isolated compounds were identified as naringenin (1), luteolin (2), isoquercitrin (quercetin-3-*O*-beta-glucopyranoside (3); one lactone compound, one was identified as griffonilide (4), lithospermoside (5), and epi-β amyrin (6). The chemical structures of 1–6 are shown in Figure 1.

Naringenin (1)

White needle crystal (MeOH); UV λ_{max}: 290, 295 nm; IR λ max/cm^{-1} 3350, 2945, 1643, 1450, 1114, 1028, 671-635; $C_{15}H_{12}O_5$; ESI m/z: 272.07 ([M + Na] +); ^{1}H-NMR (500 MHz) (CD_3OD) δ_H/ppm 7.30 (2H, *dt*, *J* = 2.20; 2.68; 8.78, H-2′; H-6′); 6.80 (2H, *dt*, *J* = 2.20; 2.93; 8.78, H-3′; H-5′), 5.89 (1H, *d*, *J* = 2.20, H-6), 5.87 (1H, *d*, *J* = 2.20, H-8), 5.33 (1H, *dd*, *J* = 2.93; 12.93, H-2), 3.09 (1H, *dd*, *J* = 12.93; 17.07, H-3$_{equatorial}$), and 2.68 (1H, *dd*, *J* = 2.93; 17.07, H-3$_{axial}$). The NMR spectra were compared with prior study.[12]

Table 1: Alpha-glucosidase inhibition of *Bauhinia winitii* extracts

Part	Extract	Alpha-glucosidase inhibition (%)±SD (2 mg/mL)	IC_{50} (µg/mL)
Leaves	Hexane (HLBW)	28.56±6.71	-
	Ethyl acetate (EALBW)	39.38±7.28*	592.38
	Ethanol (ETLBW)	68.57±3.54	533.61
	Water (WLBW)	26.69±4.74	-
Woods	Hexane (HWBW)	44.15±2.08	-
	Ethyl acetate (EAWBW)	64.54±2.54	885.72
	Ethanol (ETWBW)	96.14±3.96	320.88
	Water (WWBW)	20.28±2.44	-
Standard	Acarbose	86.09±1.54	229.36

*Final concentration 1 mg/mL. SD: Standard deviation; IC_{50}: Inhibition concentration at 50%; HLBW: Hexane Leaves B. winitii; EALBW: Ethyl acetate Leaves *B. winitii*; ETLBW: Ethanol Leaves *B. winitii*; WLBW: Water Leaves *B. winitii*; HWBW: Hexane Woods *B. winitii*; EAWBW: Ethyl acetate Woods *B. winitii*; ETWBW: Ethanol Woods *B. winitii*; WWBW: Water Woods *B. winitii*

Luteolin (2)

Yellow amorphous powder (MeOH); UV λ_{max}: 348 nm; IR λ max/cm^{-1} 3400, 2948, 1654, 1450, 1113, 1023, 719; $C_{15}H_{10}O_6$; ESI m/z: 286.05 ([M + H] +); ^{1}H-NMR (500 MHz) (CD_3OD) δ_H/ppm 7.38 (1H, *dd*, *J* = 2.2, 8.8 Hz, H-6›), 7.37 (1H, *d*, *J* = 2.2 Hz, H-2›), 6.90 (1H, *d*, *J* = 8.8 Hz, H-5›), 6.53 (1H, *s*, H-3), 6.43 (1H, *d*, *J* = 1.95 Hz, H-8), and 6.20 (1H, *d*, *J* = 1.95 Hz, H-6). The NMR spectra were compared with prior study.[13]

Isoquercitrin (quercetin-3-O -beta-glucopyranoside) (3)

Yellow amorphous powder (MeOH); UV λ_{max}: 292, 357 nm; IR λ max/cm^{-1} 3350, 1654, 1458, 1114, 1027, 671-661, 2945-2832 (sugar); $C_{21}H_{20}O_{12}$; ESI m/z: 464.1 ([M + Na] +); ^{1}H-NMR (500 MHz) (CD_3OD) δ_H/ppm 7.70 (lH, *d*, *J* = 2.2 Hz, H-2›), 7.58 (lH, *dd*, *J* = 2.2, 8.5 Hz, H-6›), 6.86 (lH, *d*, *J* = 8.5 Hz, H-5›), 6.39 (lH, *d*, *J* = 2.0 Hz, H-8), and 6.20 (lH, *d*, *J* = 2.0 Hz, H-6). Proton of sugar moieties δ_H/ppm 5.23 (lH, *d*, *J* = 7.5 Hz, H-1››), 3.70 (1H, *d*, *J* = 2.4; 12.0 Hz, H-6››), 3.54 (1H, *d*, *J* = 5.3; 12.0 Hz, H-6››), 3.47 (lH, *dd*, *J* = 7.8; 9.3 Hz, H-2››), 3.41 (lH, *dd*, *J* = 8.8; 17.8 Hz, H-3››), 3.34 (lH, *d*, *J* = 9.8 Hz, H-4››), and 3.20 (lH, *m*, H-5”). The NMR spectra were based on data comparison with prior study.[14,15]

Griffonilide (4)

White amorphous crystal (MeOH); UV λ_{max}: 285 nm; IR λ max/cm^{-1} 3399, 2909, 1733, 1636, 1461, 1081, 796-651; $C_8H_8O_4$; ESI m/z: 168.04 ([M + Na] +); ^{1}H-NMR (500 MHz) (CD_3OD) δ_H/ppm 6.62 (1H, *dd*, *J* = 2.6; 9.7, H-4), 6.27 (1H, *dd*, *J* = 2.2; 9.7, H-5), 5.88 (1H, *d*, *J* = 1.5, H-2), 4.89 (1H, *dd*, *J* = 1.9; 10.5, H-8), 4.32 (1H, *dt*, *J* = 2.2; 3.1; 8.1, H-6), and 3.52 (1H, *dd*, *J* = 8.1; 10.5, H-7). ^{13}C-NMR (CD_3OD): δ_C/ppm 175.8 (C-1), 164.8 (C-3, *s*), 144.2 (C-5, *s*), 120.6 (C-4, *s*), 112.5 (C-2, *s*), 85.2 (C-8, *s*), 80.0 (C-7, *d*), 73.6 (C-6, *s*). The NMR spectra were compared with prior study.[16]

Lithospermoside (5)

White amorphous powder (H_2O); UV λ_{max}: 286 nm; IR λ max/cm^{-1} 3434, 2957, 1634, 1543, 692, specific peak at 2221.7 cm^{-1} for (C ≡ N); $C_{14}H_{19}NO_8$; ESI m/z: 329.13 ([M + Na] +); ^{1}H-NMR (500 MHz) (D_2O)

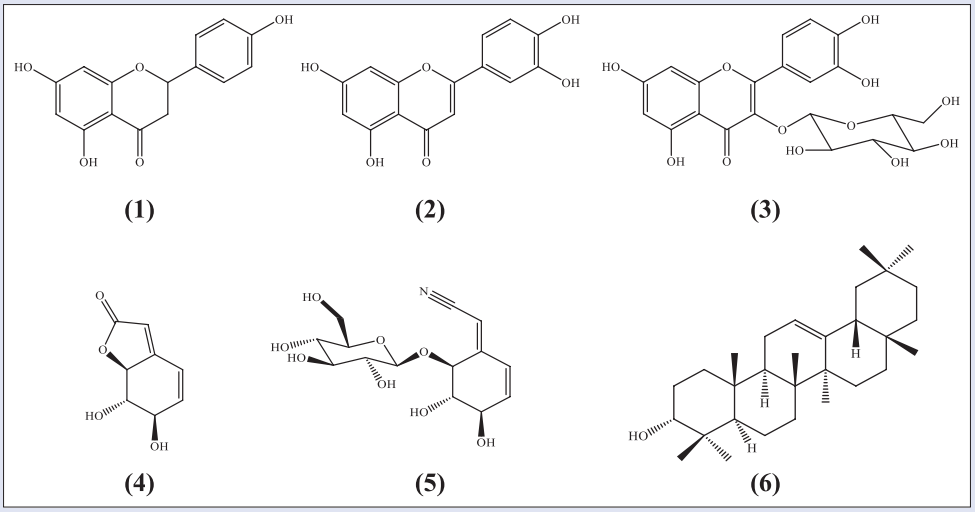

Figure 1: The chemical structure of naringenin (1), luteolin (2), isoquercitrin (quercetin-3-O-beta-glucopyranoside (3), griffonilide (4), lithospermoside (5), and epi-β amyrin (6)

δ_H/ppm 6.36 (1H, *dd*, *J* = 1.7; 10.3, H-2), 6.14 (1H, *dd*, *J* = 3.2; 9.8, H-3), 5.64 (1H, *brd s*, H-7), 4.87 (1H, *dd*, *J* = 1.7; 8.3, H-6), 4.32 (1H, *dt*, *J* = 2.4; 2.9; 5.4, H-4), and 3.97 (1H, *dd*, *J* = 6.1; 8.1, H-5). Sugar spectra at 4.90 (1H, *dd*, *J* = 2.4; 5.6, H-1›), 3.92 (1H, *dd*, *J* = 2.2; 12.4, H-6›), 3.75 (1H, *dd*, *J* = 5.6; 12.4, H-6›), 3.53 (1H, *dt*, *J* = 2.4; 6.8; 12.4, H-2›), 3.52 (1H, *dt*, *J* = 2.7; 6.6; 12.4, H-5›), 3.46 (1H, *m*, H-3'), and 3.44 (1H, *m*, H-4'). ^{13}C-NMR (D_2O): δ_C/ppm 158.1 (C-1), 138.9 (C-3, *s*), 129.8 (C-2, *s*), 120.5 (C-8, *s*), 99.9 (C-7, *s*), 78.7 (C-6, *s*), 76.7 (C-5, *d*), 72.7 (C-4, *s*); sugar moiety at δ_C/ppm 105.4 (C-1', *s*), 78.9 (C-3', 5, *d*), 78.6 (C-5', *s*), 75.6 (C-2', *s*), 72.5 (C-4', *s*), 63.7 (2C-6', s). The NMR spectra were compared with prior study.[17,18]

Epi-β amyrin (6)

White amorphous powder ($CDCl_3$); UV λ_{max}: 290, 338 nm; IR λ max/cm^{-1} 3019, 2976, 2936, 1215, 1046; $C_{30}H_{50}O$; ESI m/z: 426.38 ([M + H] +); ^{1}H-NMR (500 MHz) ($CDCl_3$): 3 parts of β amyrin identical peaks which were olefinic proton signal at δ_H/ppm 5.61 (1H, *d*, *J* = 6.1, H-12), hydroxyl neighborhood proton at δ_H 3.44 (1H, *brd*, s, H-3), 8 methyl protons as singlet peaks around δ_H 0.82–1.13 ppm. ^{13}C-NMR (D_2O): identical carbon spectra were at δ_C/ppm 122.1 (C-12, *s*), 76.3 (C-3, *s*), 34.5-16.2 (C-23 to C-30, 8CH_3, *s*). The NMR spectra were compared with prior study.[19,20]

DISCUSSION

Alpha-glucosidase inhibitory assay

From the results, most of the solvent extracts from BW were showed alpha-glucosidase inhibitory activity of more than 60% except hexane and water extracts [Table 1]. Since, the IC_{50} value of ethanol wood extract Ethanol Woods *B. winitii* (ETWBW) was exhibited the highest activity with 320.88 µg/ml nearly to the standard, acarbose as 229.36 µg/ml. Hence, further study was to investigate the active compounds that responded for this effect by using chromatographic techniques.

The isolated compounds were three flavonoids as naringenin, luteolin, and isoquercitrin (quercetin-3-*O*-beta-glucopyranoside); one lactone as griffonilide; a cyanoglucoside as lithospermoside; and steroid as epi-β amyrin. From the results in Table 2, naringenin exhibited the highest α-glucosidase inhibitory activity which followed by luteolin and isoquercitrin. The other compounds which were steroids showed the lower potential effect and even more for cyanoglucoside and lactone, respectively.

Generally, flavonoids are founded mostly distributed in plants have been utilized as natural compounds with high pharmacological activities.[19] As much as 103 flavonoids with many structural types were reported as alpha-glucosidase inhibitors.[20] This makes flavonoids as enchanting modulators of alpha-glucosidase inhibitor. From the flavonoid chemical structure, intended effect was given by OH group's number and position as the determinant factor.

Table 2: Alpha-glucosidase inhibition test of isolated compounds

Compounds	Extract	IC_{50} (mM)
Naringenin	EALBW	0.41
Luteolin	EALBW	0.57
Isoquercitrin	ETWBW	0.52
Griffonilide	ETLBW	65.62
Lithospermoside	ETLBW	9.99
Epi-β amyrin	EALBW	3.99
Acarbose	-	0.19

IC_{50}: Inhibition concentration at 50%; EALBW: Ethyl acetate Leaves *B. winitii*; ETWBW: Ethanol Woods *B. winitii*; ETLBW: Ethanol Leaves *B. winitii*

From the result, naringenin was the most active alpha-glucosidase inhibitor with IC_{50} 0.41 mM, slightly stronger than acarbose and luteolin. Currently, it was accepted that hydroxyl group modification or elimination in flavonoids would be reducing the alpha-glucosidase inhibitory effect.[21] Even though naringenin showed less hydroxylation at 3′-position of ring B; and the absence of the double bond of C2=C3 in ring C, it did not show any contrary for the insignificant difference in IC_{50} potency between the flavonoids.

Computerized molecular docking

Molecular docking is a sophisticated computational approach that has been used as a tool to provide a better understanding of the interaction between a small molecule and the target protein. Recently, the role of molecular docking has increased since the corona outbreak to help scientists to find the drug candidate. Therefore, in this study, the cutting-edge technology had been applied here to have a closer look into the molecular level of the promising anti-glucosidase metabolite from BW such as naringenin, luteolin, and isoquercitrin.

To evaluate the inhibitory possibility of the isolated flavonoids from BW in human glucosidase, the available protein structures of glucosidase from both human and bakers' yeast were aligned as shown in Figure 2. The multiple structures and sequences alignments revealed high similarity among human glucosidases but a moderate similarity among human and baker's yeast glucosidases, which was accepted to select one of the human glucosidases to evaluate the possibility. Therefore, human isomaltase (PDB: 3 LPP and called human glucosidase from now on) was chosen due to its smaller molecule of the native ligand, which would provide a smaller time in the validation step for the docking experiment.

Based on the evolutionary point of view,[22] the study suggested that yeast glucosidase could not be used as a good model for human glucosidase. However, our data here showed differently. As shown in Figure 2, the multiple structural alignments exhibited a good fit in most parts among these protein structures including the binding site as shown in the red box from Figure 2, especially, the catalytic domain [the blue box in Figure 2]. WIDMNE domain was reported as the conserved catalytic region that plays an important role in the hydrolysis reaction of human glucosidase[23] and two of these residues were identical in baker's yeast glucosidase, xIDxxx, which was normally found only one amino acid that was identical.[22] Moreover, the positive correlation between human and yeast glucosidase was known since 1995.[24] However, this does not mean that strong yeast glucosidase inhibitors would always inhibit firmly with human glucosidase. Therefore, the inhibitory evaluation of human glucosidase is necessary to proceed.

The bioactive flavonoids that were isolated from BW were docked with yeast glucosidase. The docking results were compared to the outcomes from our *in vitro* study to validate the docking results and the mode of binding was examined before evaluation the possible inhibitory activity on human glucosidase. As expected, the docking experiment from yeast glucosidase was in good agreement with the *in vitro* study as described earlier. However, most likely, two out of three flavonoids (luteolin and isoquercitrin) could inhibit the activity from human glucosidase.

As shown in Figure 3, the molecular docking experiment supported the inhibitory activity of bioactive flavonoids that were found earlier in the *in vitro* experiment. All three flavonoids could bind at the same site, an active site, of yeast glucosidase [Figure 3a and b]. The molecular interactions between the compounds of interest and the target glucosidase were involving either nucleophilic residue, Asp or D, from the conserved catalytic domain, xIDxxx, or acid/base catalytic residues, acted as proton donor. These two groups of residues played a vital role in the hydrolysis reaction of glucosidase. Binding at least one of these residues could potentially inhibit the enzymatic activity.

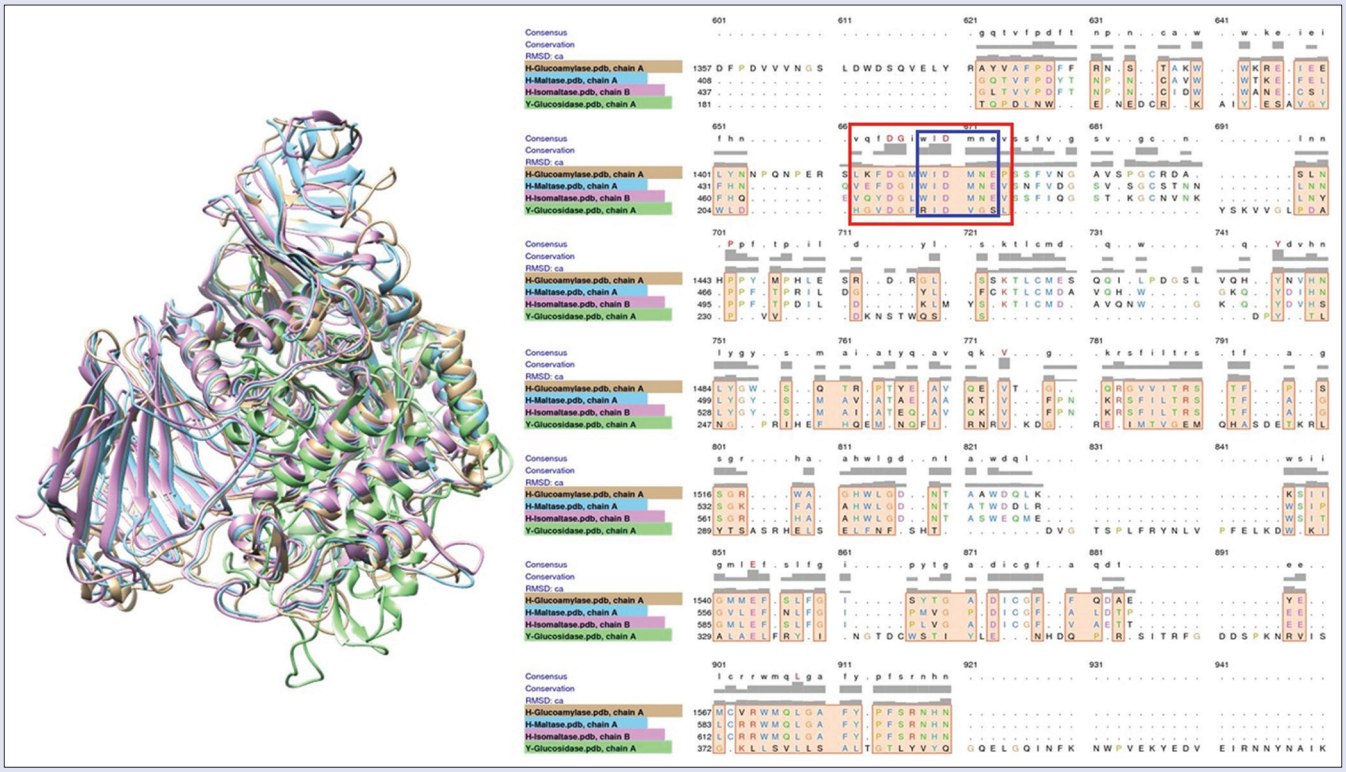

Figure 2: The structural and multiple sequences alignment of three human alpha-glucosidase namely glucoamylase (PDB ID: 3TOP in brown), maltase (PDB ID: 2QMJ in blue) and isomaltase (PDB ID: 3LPP in pink), and yeast alpha-glucosidase (PDB ID: 3A4A in green). The red box indicates the conserved domain and identical residues among these glucosidases

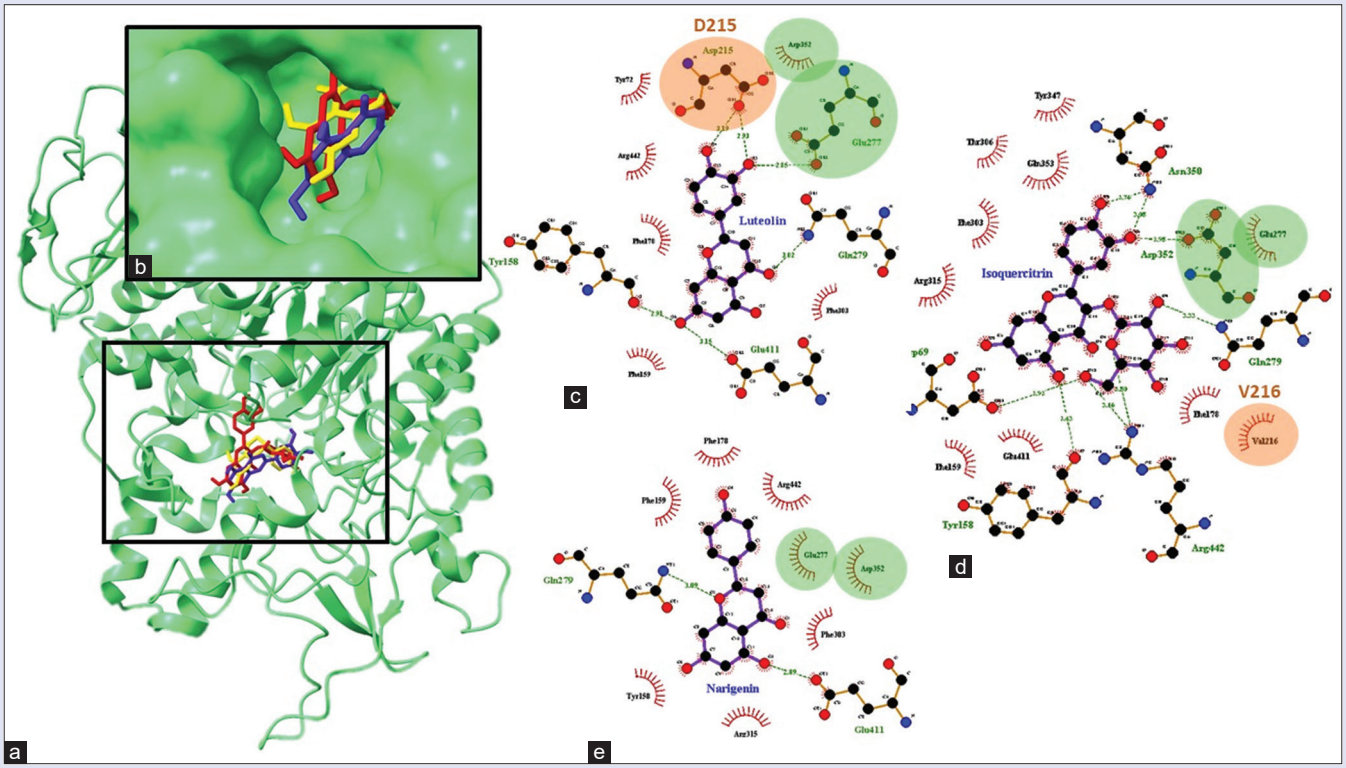

Figure 3: The molecular interaction from the docking experiment between the baker's yeast alpha-glucosidase (green) and flavonoids namely luteolin in blue, naringenin in yellow and isoquercitrin in red. (a) The overview of the flavonoids-glucosidase interaction and (b) the close-up view at the active site. (c-e) showed the interaction diagram of flavonoids-glucosidase complex. Orange circle was indicated the catalytic signature residues in the conserved domain. Whereas, the green circle was indicated the catalytic proton donor residues

Therefore, these were the main focus of this discussion. As presented in Figure 3c, luteolin could form one hydrogen bonding with Asp 215 or D 215 as a catalytic nucleophile (the conserved residual in xxIDxx domain) and other hydrogen bonding was found at Glu 277 as a catalytic proton donor. Furthermore, one hydrophobic interaction was spotted with another catalytic proton donor (Asp 352). On the other hand, isoquercitrin did not form any hydrogen bonding with Asp 215 but rather interact hydrophobically with Val 216 or V 216, the neighbor residue. Moreover, one hydrogen bonding and one hydrophobic interaction were found at Asp 352 and Glu 277, respectively [Figure 3d]. The findings here, at first, seemed to contradict with the previous studies of luteolin and isoquercitrin that reported the noncompetitive behavior, which normally should not bind at the active site.[25,26] However, Blat[27] has shown that sometimes a noncompetitive inhibitor could also bind at the same site as a competitive inhibitor. Therefore, the findings here were most likely in line with earlier studies. Last but not least, naringenin only interacted hydrophobically with catalytic proton donors Asp 352 and Glu 277 as presented in Figure 3e. This was similar to the previous report of naringenin that showed a competitive manner.

Luteolin and isoquercitrin were the most promising candidates that could inhibit human glucosidase based on the molecular docking experiment here whereas naringenin was not. As shown in Figure 4a and b, even all flavonoids could insert themselves into the binding pocket but only luteolin and isoquercitrin could form the hydrogen bounding with the residues inside this pocket [Figure 4c and d]. Conversely, naringenin did not have any hydrogen bonding, only hydrophobic interactions were found. Even one of the interacted residues was a catalytic proton donor Asp 571, but this did not convince that naringenin could potentially inhibit human glucosidase [Figure 4e]. Unlike the previous docking experiment, luteolin could not establish the hydrogen bonding with the catalytic nucleophile but rather interacted with another residue in the active site, Asp 355, and formed the hydrophobic interaction with Asp 472 or D 472 from the catalytic domain (WIDMNE) for instance. Besides, the hydrophobic interaction between luteolin and a catalytic proton donor, Asp 571, was also found as presented in Figure 4c. The same molecular interaction pattern was applied here with isoquercitrin. There was no hydrogen bonding was found, only hydrophobic interactions were formed with one of the residues from the WIDMNE domain as Met 473 or M 473 and the catalytic proton donor, Asp 571 [Figure 4e]. Therefore, based on the results here, it was most likely that luteolin and isoquercitrin could potentially inhibit human glucosidase activity and this was supporting the finding from the meta-analysis of flavonoids and type 2 diabetes.[28] The study reported that flavones and flavonols, which are the group of luteolin and isoquercitrin, respectively, could prevent the development of diabetes and one of the possible mechanisms that have been purposed is reducing the postprandial glucose response, which is directly related to the inhibitory activity of the glucosidase.[29]

CONCLUSION

Six compounds were isolated from BW as naringenin (1), luteolin (2), isoquercitrin (3), griffonilide (4), lithospermoside (5), and epi-β amyrin (6). The alpha-glucosidase inhibitory test showed that flavonoids performed potential results with naringenin as the highest IC_{50} on 0.41 mM. The docking ensured the flavonoids activated at the same binding of alpha-glucosidase enzyme active site. The molecular docking did not only support our *in vitro* alpha-glucosidase inhibitory activity but also suggested the interaction site and binding mode of the compounds of interest. Finally, yet importantly, the molecular docking also revealed

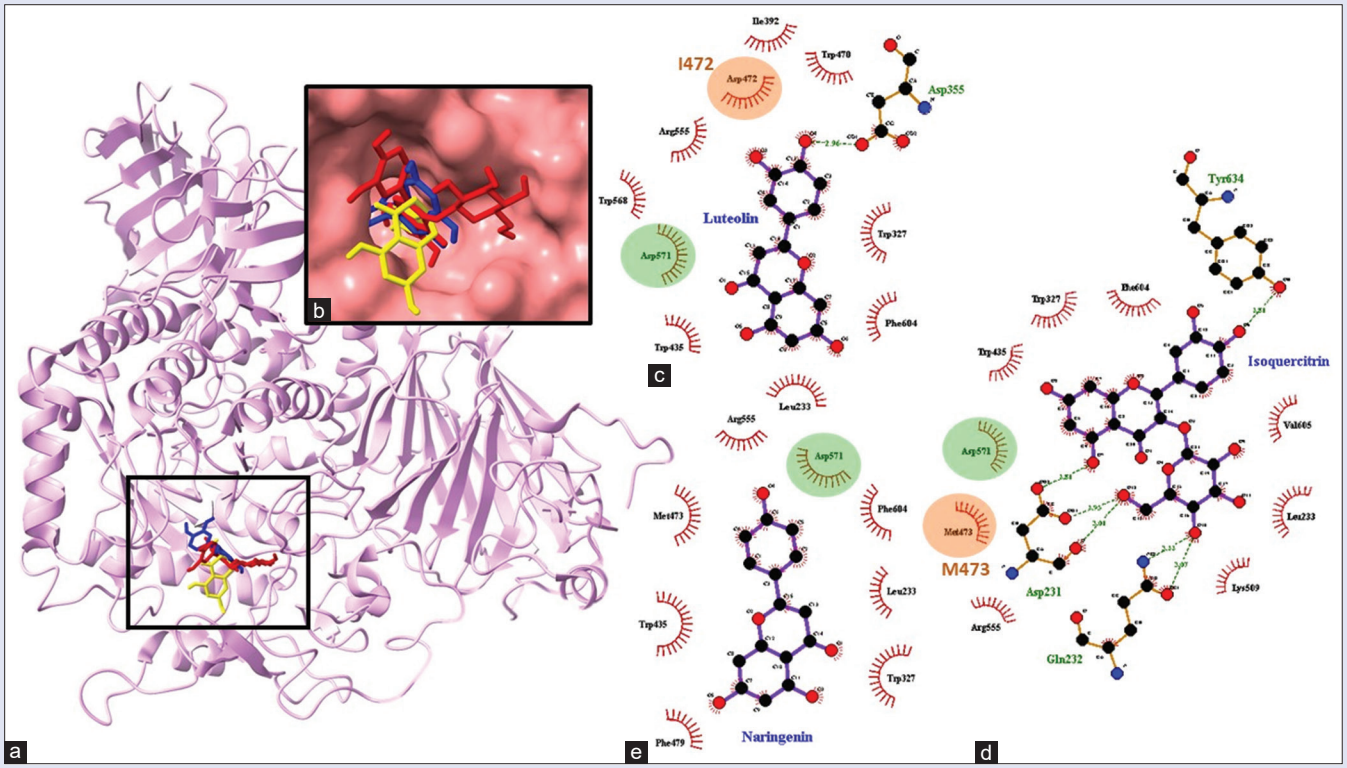

Figure 4: The molecular interaction from the docking experiment between the human isomaltase (pink) and flavonoids namely luteolin in blue, naringenin in yellow and isoquercitrin in red. (a) The overview of the flavonoids-glucosidase interaction and (b) the close-up view at the active site. (c-e) showed the interaction diagram of flavonoids-glucosidase complex. Orange circle was indicated the catalytic signature residues in the conserved domain. Whereas, the green circle was indicated the catalytic proton donor residues

that luteolin and isoquercitrin were expected to be potential inhibitors for human glucosidase. This could explain the claim of the flavonoids in type 2 diabetes. Hence, this study would be the first report of chemical constituents from BW with its isolated compounds was presented anti-diabetic activity as alpha-glucosidase inhibitors.

Acknowledgements

We proudly thank the Department of Pharmacognosy and Pharmaceutical Botany and the Pharmaceutical Laboratory Service Center, Faculty of Pharmaceutical Sciences, Prince of Songkla University for the lab space and equipment.

Financial support and sponsorship

This research was supported by Thailand's Education Hub for ASEAN Countries (TEH-AC 089/2016) scholarship.

Conflicts of interest

There are no conflicts of interest.

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