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ORIGINAL ARTICLE

Chemical Constituents of *Litsea elliptica* and their Alpha-Glucosidase Inhibition with Molecular Docking

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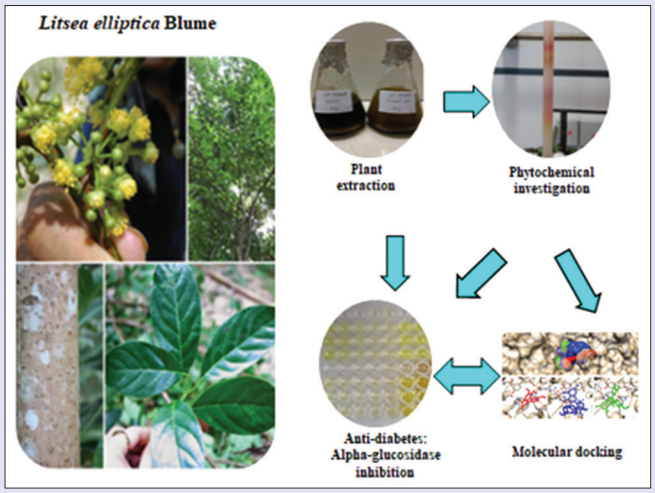
ABSTRACT

Background: *Litsea elliptica* (LE) is an edible plant in Thailand, which has been used as vegetables, flavoring materials, and traditional herbal medicine. Some species of *Litsea* have been reported on anti-diabetic activity. However, LE has not been reported of chemical constituents and their alpha-glucosidase activity. **Objectives:** The aim was to study on chemical constituents and anti-diabetic activity through *in vitro* models of LE. **Materials and Methods:** Chromatographic and colorimetric enzyme methods with computer molecular docking were used in this research. **Results:** A new quercetin-diglycoside (1) and six compounds (2-7) were isolated from LE which were elucidated as quercetin-3-*O*-α-rhamnopyrano side-*O*-(1→2)-α-apiofuranoside (1), quercetin-3-*O*-α-rhamnopyranoside (2), quercetin-3-*O*-β-glucopyranoside (3), uridine (4), isoboldine (5), reticuline (6) and β-sitosterol-D-glucopyranoside (7). Four isolated compounds exhibited anti-alpha glucosidase activity by inhibition concentration at 50% (μg/mL) values as 5 (243.25) <3 (332.19) <1 (335.15) <2 (751.79), respectively. Furthermore, the molecular docking experiment was graphically computed for binding energy between effective compounds and the enzyme. The result suggested that the structure of compound 5 exhibited the lowest binding energy (−7.2 kcal/mol), which interacted at non-catalytic domain at the entrance of the active site. In addition, the mechanism of action of effective compounds could be predicted by the binding sites as non-competitive inhibitor (1 and 5) and competitive inhibitor (3). To sum up, the seven compounds which were isolated of LE are significantly reported for the first time in biological and phytochemical studies, and compound 1 is considerably interpreted as a new compound which presents anti-alpha glucosidase activity.

Key words: Alpha-glucosidase inhibition, chemical constituents, herbal medicine, *Litsea elliptica,* molecular docking

SUMMARY

- Phytochemical and biological study on alpha-glucosidase inhibition of *Litsea elliptica* (LE) were reported for the first
- A new quercetin-diglycoside; quercetin-3-*O*-α-rhamnopyranoside-*O*-(1→2)-α-apiofuranoside was isolated from LE
- Four isolated compounds as isoboldine, quercetin-3-*O*-β-glucopyranoside, quercetin-3-*O*-α-rhamnopyranoside-*O*-(1→2)-α-apiofuranoside, quercetin-3-*O*-α-rhamnopyrano-side exhibited alpha-glucosidase inhibition
- The mechanism of action of these compounds was predicted by using *in silico* study which showed noncompetitive inhibition of quercetin-3-*O*-α-rhamnopyranoside-*O*-(1→2)-α-apiofuranoside and isoboldine; competitive inhibition of quercetin-3-*O*-β-glucopyranoside.



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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INTRODUCTION

Diabetes is a chronic metabolic syndrome which is characterized by permanent hyperglycemia effect. The chronic metabolic syndrome associated with diabetes disease is critically increasing the risk of macro-and microvascular complications. It causes organ damage, cardiovascular disease, artery disease, diabetic kidney failure, and retinal disease, etc.[1,2]

In general, diabetes is majorly categorized into two types as type 1 and type 2 diabetes. Type 1 diabetes is mentioned to insulin-dependent diabetic mellitus (IDDM), which absented or insufficient insulin due to the destruction of pancreatic. Type 2 diabetes is referred to nonIDDM causing by lacking insulin production.[3] The main purpose of diabetes therapeutics is focused on reducing blood glucose levels through various mechanisms such as decrease hepatic gluconeogenesis, stimulate insulin

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secretion, decrease glucose absorption, increase insulin sensitivity, and prolong GLP-1 action.

The medicinal plants have been played a crucial role in diabetic treatment for a long history. Natural compounds that were investigated from natural resources, including micro-organisms and medicinal plants, were significantly reported of anti-alpha glucosidase activity and exhibited high potential effects such as terpenes, alkaloids, quinones, phenolics (flavonoids, phenols, phenylpropanoids), steroids and other compounds.[4] The mechanisms of action from natural compounds that inhibited the enzyme activity were wildly presented in numerous pathways by studying both *in vitro* and *in vitro* models such as the inhibition of alpha-glucosidase, the effects on glucose uptake and glucose transporters, the enhancement of insulin secretion and pancreatic-cell proliferation, the inhibition of protein tyrosine phosphatase 1B activity and the antioxidant activity.[5] Discovering some new potential therapeutic agents from natural compounds are become a key to diabetes study.

Anti-alpha glucosidase activity is a typical model which has been used for studying anti-hyperglycemic activity. Alpha-glucosidase naturally locates in the small intestine, which functionalizes the digestion of polysaccharides and oligosaccharides. Glucose is a small product from the digestion system, which suddenly absorbed into blood circulation through the small intestine. Thus, delaying the enzyme activity by using natural product inhibitors were dramatically become a key role in decreasing glucose absorption.[6]

Litsea elliptica Blume (LE) is an edible plant that belongs to the Lauraceae family. It is called Tham Mang as Thai name. LE can be found in southern Thailand. The leaves have been used for cooking and producing the flavor for food additive, while the barks have been recorded in traditional Thai medicine for anti-flatulence. However, there were plenty of scientific reports which had been published on phytochemistry and biological activities. Numerous chemical constituents of *Litsea* species such as alkaloids[7] and volatile oils[8] have been found from bark and leaf, respectively. The methanolic root and stem extracts of LE presented anti-oxidant activity, whereas anti-microbial activity was exhibited a slight effect from stem root and inner bark extracts.[9] LE plant was clinically studied in Sprague-Dawley rats for acute toxic effect and toxic effect on red blood cells of its essential oil.[10,11] In addition, the methanolic leaf extract of *Litsea petiolata* significantly exhibited anti-mutagenicity activity[12] and few of *Litsea* plants had been investigated on anti-diabetic activities via *in vitro* and *in vivo* models such as *Litsea glutinosa*, *Litsea coreana* and *Litsea monopetala*.[8,13,14] Therefore, LE is expected to exhibit anti-diabetic activities and provide a new source of active compounds.

Due to the literature reviews, *Litsea* plants had been reported on anti-diabetic activities via *in vitro* and *in vivo* models. Therefore, LE is expected to exhibit anti-diabetic activities and provide a new source of active compounds.

MATERIALS AND METHODS

Plant material

LE plant was collected from Trang province, Thailand. The plant was identified by a botanist; Mr. Pachock Puudjaa, and permanently deposited at Office of the Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Bangkok, Thailand. The herbarium was similarly compared to the plant reference number as BKF No. 180678.

Extraction

The fresh plant was categorically separated into two parts as leaf and stem. Next, the plant was cleaned and dried at room temperature for 24 h. Then, it was cut into small pieces and dried in the hot air oven at 50°C for 48 h. The dried plant was ground into coarse-grained powder by the blender. The powdered samples were macerated with various solvents following the solvent's polarity from non-polar to polar solvents as hexane, ethyl acetate, ethanol, and water, respectively. The chemical constituents of LE were extracted and grouped following the polarity of each solvent.

The dried samples were initially extracted with hexane for 72 h. The solution was filtered by filter paper and evaporated with a rotary evaporator at 40°C. The residues were repeatedly extracted for 3–4 times with the same solvent to increase the extracted yield. Afterward, the residues were continually extracted with the other solvents following by ethyl acetate and ethanol, respectively, which were repeatedly extracted for 3–4 times each. Finally, the residues will be boiled with distilled water at 70°C for 6 h to get the water extract. Finally, the extracts were kept at 4°C until the study.

Phytochemical study and structure elucidation techniques

The plant extracts were isolated to obtain the pure compounds by using column chromatographic techniques, vacuum liquid chromatography, and classical column chromatography. Size exclusion chromatography was performed using Sephadex® LH-20. high-resolution spectroscopy. The spectroscopic techniques were used for the structure elucidation of the isolated compounds.

1D and 2D NMR were observed by Fourier Transform NMR Spectrometer (^{1}H-NMR 500 MHz and ^{13}C-NMR 125 MHz), model UNITY INNOVA, Varian. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis was performed using Finnigan LCQ-Duo ion trap mass spectrometer with an ESI source (ThermoQuest) coupled to a Finigan Surveyor high-performance liquid chromatography system with an EC 150/3 Nucleodur 100-3 C18EC column (Macherey-Magel). 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 20 µL. These experiments were completed at the Institute of Pharmacy and Molecular Biotechnology, Department of Pharmaceutical Biology, Heidelberg University, Germany.

High-resolution electrospray ionization mass spectra were determined by TQF/Q-TOF Mass spectrometer (Agilent, USA) at Scientific Equipment Centre, Prince of Songkla University, Thailand.

Anti-alpha glucosidase activity

The enzymatic reaction was determined using the colorimetric method. The hydrolysis between the substrate (*p*-nitrophenyl-alpha-D-glucopyranoside) and the alpha-glucosidase enzyme will be produced the yellowish solution that will be detected at the absorbance 405 nm. Consequently, the absorption of the color significantly related to the potential of the inhibitors.[7,15]

Briefly, the test samples and positive standard (acarbose) were clearly dissolved in 20% of DMSO in water. The phosphate buffer pH 7 consisted of $NaH_2PO_4.H_2O$ and Na_2HPO_4, which was supplemented with 0.2% of bovine serum albumin and 0.02% of NaN_3. Alpha-glucosidase enzyme isolated from *Saccharomyces cerevisiae* (EC 3.2.1.20) and *p*-nitrophenyl-alpha-D-glucopyranoside were purchased from Sigma®. The enzyme and substrate were generally dissolved in cold buffer.

Initially, 50 µL of phosphate buffer was added into test-well, while 100 µL was added into blank-well. Then, 50 µL of test sample and standard were added into each well. After that, 50 µL of the enzyme solution was added into the test-wells and incubated at 37°C for 2 min. Afterward, 50 µL of the substrate was added to each well. Finally, the enzymatic reaction

was measured by following the kinetic parameter; interval time: 30 s for 20 cycles, absorbance at 405 nm. The anti-alpha glucosidase activity was calculated by using the velocity of the reaction, which was computed by equation (1) and equation (2) as provided below.

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

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

Computer molecular docking

The crystal structure of baker yeast's alpha-glucosidase (PDB: 3a4a) from the earlier studies was obtained from the RCSD Protein Data Bank (http://www.rcsb.org). Thus, the target protein was properly prepared by AutodockTools version 1.5.6.[8,16] The isolated compounds from LE extracts were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), if possible, or drawn by ACD ChemSketch (Free version) in case of not possible to obtained from the database. Avogadro version 1.2.0[9,17] was used for structural energy minimization. Geometric optimization and General Amber Force Field were also applied in this step. Only optimized ligands were subjected to perform the molecular docking. To find the active site on the enzyme, glucose as the native ligand was used as a guideline. Grid box was created based on the native molecule in the size of 17 A° ×17 A° × 17 A°, which had a center of X-axis = 21.1, center of Y-axis = −7.4 and centre of Z-axis = 24.2, respectively.

In this study, the docking experiment was performed by Autodock Vina version 1.1.2[10,18] and most of the parameters were set as default except the exhaustiveness value was adjusted up to 24. Significantly, the docking protocol was validated by re-docking the native ligand into the active site of the alpha-glucosidase, and root-mean-square deviation (RMSD) of the re-docked ligand should be accepted to confirm the reliability of this method before proceeding the experiment.[11,19] In the postdocking analysis, Viewdock package from Chimera version 1.11.2[12,20] was selected to visualize the molecular interactions and analyst the binding energy. The best conformation of all docked ligands was decided by the combination of the lowest binding affinity and the structural alignment of those structures.

To obtain more information, the refinement and rescoring of the docked poses were performed. Begin with, the best conformation from Autodock Vina was extracted by vina split program, and the extracted conformation together with alpha-glucosidase was together subjected to perform single-point energy estimation from Autodock 4.2.6.[11,19] All parameters were set as a default, and the center of the ligand was used as the centre of the grid box.

RESULTS

Phytochemical investigation of *Litsea elliptica* extracts

Seven compounds, as shown in Figure 1 were isolated from leaves, and woods of LE extracts were categorized as flavonoid glycosides, alkaloids, and steroid compounds. The compound 1 was interpreted as a new flavonoid glycoside.

Compound 1

Compound 1 obtained as a yellow powder and dissolved in methanol. Molecular formula: $C_{26}H_{28}O_{15}$. UV absorption was observed by using photodiode array (PDA) of LC-MS/MS, which showed at 250 and 331 nm. Low-resolution mass (ESI) was detected by LC-MS/MS showing the mass peaks at *m/z* 580.99 ([M + H] +) [Figure 2].

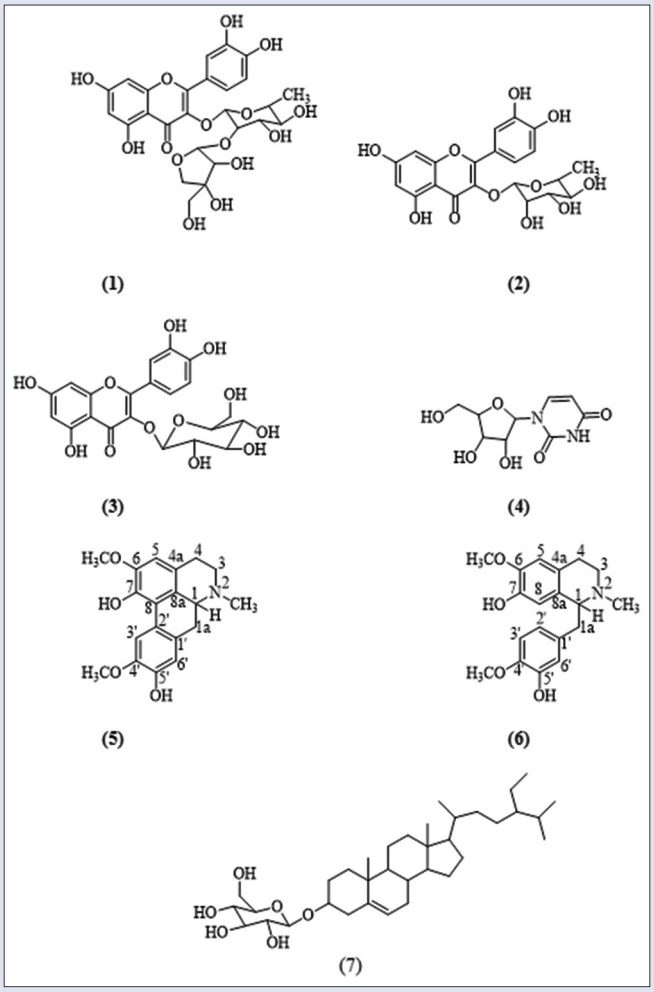

Figure 1: The isolated compounds (1-7) from *Litsea elliptica*

The ^{1}H NMR spectrum (500 MHz in MeOH-*d*4) showed the characteristic of quercetin structure by exhibiting the proton signals (δ_H) at 6.19 (*d*, *J* = 2.10 Hz, H-6), 6.35 (*d*, *J* = 2.10 Hz, H-8), 7.32 (*d*, *J* = 2.10 Hz, H-2'), 6.91 (*d*, *J* = 8.30 Hz, H-5') and 7.28 (*dd*, *J* = 8.30, 2.10 Hz, H-6'). The sugar was identified as rhamnopyranoside by showing the proton signals (δ_H) at 5.40 (*d*, *J* = 1.70 Hz, H-1"), 4.17 (*dd*, *J* = 3.40, 1.80 Hz, H-2"), 3.85 (*dd*, *J* = 9.70, 3.40 Hz, H-3"), 3.29 (*m*, H-4"), 3.56 (*dd*, *J* = 9.60, 6.15 Hz, H-5") and 0.97 (*d*, *J* = 6.20 Hz, H-6"). The second sugar was identified as apiofuranoside by showing the proton signals (δ_H) at 5.11 (*d*, *J* = 2.60 Hz, H-1""), 3.91 (*d*, *J* = 2.60 Hz, H-2""), 3.81 (*d*, *J* = 9.80 Hz, H-4""), 3.68 (*d*, *J* = 9.80 Hz, H-4"") and 3.53 (*s*, H-6""). The coupling constant between first anomeric proton at 5.40 ppm (H-1") and 4.17 ppm (H-2") presented alpha configuration of rhamnopyranoside. The coupling constant between second anomeric proton at 5.10 ppm (H-1"") and 3.91 ppm (H-2"") presented alpha configuration of apiofuranoside.

The ^{13}C spectrum (125 MHz in MeOH-*d*4) showed the characteristic of quercetin structure by exhibiting the carbon signals (δ_C) at 157.80 (C-2), 134.94 (C-3), 178.19 (C-4), 161.75 (C-5), 98.44 (C-6), 166.14 (C-7), 93.52 (C-8), 157.08 (C-9), 104.39 (C-10), 121.46 (C-1'), 115.49 (C-2'), 145.03 (C-3'), 148.36 (C-4'), 115.03 (C-5') and 121.35 (C-6'). The sugar was identified as rhamnopyranoside by showing the carbon signals (δ_C) at 101.43 (C-1"), 77.94 (C-2"), 70.29 (C-3"), 72.25 (C-4"), 70.56 (C-5") and 16.33 (C-6"). The

second sugar was identified as apiofuranoside by showing the carbon signals (δ_C) at 110.86 (C-1""), 76.45 (C-2""), 78.98 (C-3""), 73.60 (C-4"") and 64.17 (C-5"").

HMBC long-range coupling presented the correlation of an anomeric proton at 5.40 ppm (H-1") of rhamnopyranoside correlated to aromatic carbon at 134.94 ppm (C-3) in ring C. Moreover, the second sugar, which identified as apiofuranoside exhibited the correlation of anomeric proton at 5.10 ppm (H-1""), which correlated to rhamnopyranoside at 77.94 ppm (C-2"). This correlation was confirmed the relationship apiofuranoside and rhamnopyranoside, which were connected by-*O*-(1→2) linkage [Figure 3].

The positive ESI/HR/MS spectrum of compound 1 presented an [M + Na] + ion peak at *m/z* 603.1337 (M = 580.1446) supporting the molecular formula ([$C_{26}H_{28}O_{15}$] +Na) + (Calcd for $C_{26}H_{28}O_{15}$, 580.1428, Diff 3.15 ppm. First, the quasi-molecular ion was fragmented by lossing apiofuranoside and showing the mass peak at *m/z* 449.06. Then, the structure was fragmented, a rhamnofuranoside which found the mass peak at *m/z* 303.28. This compound was named as quercetin-3-*O*-alpha-rhamno pyra noside-(1→2)-*O*-alpha-apiofuranoside.

Compound 2

Compound 2 obtained as a yellow powder and dissolved in methanol. Molecular formula: $C_{21}H_{20}O_{11}$. UV absorption was observed by using PDA of LC-MS/MS, which showed at 231, 255, and 349 nm. Low-resolution mass (ESI) was detected by LC-/MS showing the mass peaks at *m/z* 448.87 ([M + H] +). The ^{1}H NMR spectrum (500 MHz in MeOH-*d*4) showed the characteristics of the quercetin structure by exhibiting the proton signals (δ_H) at 6.19 (*d*, *J* = 2.10 Hz, H-6), 6.36 (*d*, *J* = 2.10 Hz, H-8), 7.33 (*d*, *J* = 2.10 Hz, H-2'), 6.90 (*d*, *J* = 8.30 Hz, H-5') and 7.29 (*dd*, *J* = 8.30, 2.20 Hz, H-6'). The sugar was identified as rhamnopyranoside by presenting the proton signals (δ_H) at 5.34 (*d*, *J* = 1.60 Hz, H-1"), 4.21 (*dd*, *J* = 3.40, 1.70 Hz, H-2"), 3.74 (*dd*, *J* = 9.40, 3.40 Hz, H-3"), 3.32 (*t*, *J* = 9.50 Hz, H-4"), 3.44 (*dd*, *J* = 9.70, 6.40 Hz, H-5") and methyl protons at 0.94 (*d*, *J* = 6.00 Hz, H-6"). The coupling constant between anomeric proton at 5.34 (H-1") and methine proton at 4.21 (H-2") presented the alpha configuration of rhamnopyranoside.

The ^{13}C spectrum (125 MHz in MeOH-*d*4) showed the characteristic of quercetin structure by exhibiting the carbon signal (δ_C) at 158.51 (C-2), 136.24 (C-3), carbonyl carbon at 179.64 (C-4), 163.18 (C-5), 99.81 (C-6), 165.82 (C-7), 94.72 (C-8), 159.30 (C-9), 105.92 (C-10), 123.00 (C-1'), 116.97 (C-2'), 149.76 (C-3'), 146.38 (C-4'), δ116.37 (C-5'), δ122.86 (C-6'). The sugar was identified as rhamnopyranoside by showing the carbon signals (δ_C) at 103.52 (C-1"), 71.91 (C-2"), 72.14 (C-3"), 73.28 (C-4"), 72.01 (C-5") and methyl carbon at 17.63 (C-6").

The ^{1}H and ^{13}C spectra of compound 2 were similarly compared to the chemical shifts from a previous report, which was mentioned to quercetin-3-*O*-alpha-rhamnopyranoside.[13,21]

Compound 3

Compound 3 obtained as a yellow powder and dissolved in methanol. Molecular formula: $C_{21}H_{20}O_{12}$. UV absorption was observed by using PDA of LC-MS/MS, which showed at 251 and 347 nm. Low-resolution mass (ESI) was detected by LC-/MS showing the mass peaks at *m/z* 464.89 ([M + H] +). The ^{1}H NMR spectrum (500 MHz in MeOH-*d*4) showed the characteristic of quercetin structure by exhibiting the proton signals (δ_H) at 6.20 (*d*, *J* = 2.10 Hz, H-6), 6.40 (*d*, *J* = 2.10 Hz, H-8), 7.83 (*d*, *J* = 2.20 Hz, H-2'), 6.86 (*d*, *J* = 8.50 Hz, H-5'), 7.58 (*dd*, *J* = 8.50, 2.20 Hz, H-6'). The sugar was identified as glucopyranoside which showed the proton signals (δ_H) at 5.15 (*d*, *J* = 7.80 Hz, H-1"), 3.81 (*dd*, *J* = 9.70, 7.80 Hz, H-2"), 3.56 (*dd*, *J* = 7.00, 2.40 Hz, H-3"), 3.84 (*dd*, *J* = 3.40, 1.00 Hz, H-4"), 3.47 (*td*, *J* = 6.20, 1.10 Hz, H-5") and 3.64 (*dd*, *J* = 11.20, 6.00 Hz, H-6") and 3.55 (*dd*, *J* = 10.70, 6.00 Hz, H-6"). The coupling constant between anomeric proton at 5.15 (H-1") and methine proton at 3.81 (H-2") presented the beta configuration of glucopyranoside.

The ^{13}C spectrum (125 MHz in MeOH-*d*4) showed the characteristic of quercetin structure by exhibiting the carbon signals at 157.34 (C-2), 134.33 (C-3), 178.90 (C-4), 161.58 (C-5), 98.63 (C-6), 164.90 (C-7), (δ_C) 93.52 (C-8), 157.04 (C-9), 104.13 (C-10), 121.46 (C-1'), 116.34 (C-2'), 144.39 (C-3'), 148.51 (C-4'), 114.73 (C-5'), and 121.56 (C-6'). The sugar was identified as glucopyranoside, which showed the carbon signals (δ_C) at 103.99 (C-1"), 71.75 (C-2"), 723.68 (C-3"), 68.60 (C-4"), 75.77 (C-5"), and 60.52 (C-6").

The ^{1}H and ^{13}C spectra of compound 3 were similarly compared to the chemical shifts from a previous report, which was mentioned to quercetin-3-*O*-beta-glucopyranoside (isoquercitrin).[14,22]

Compound 4

Compound 4 obtained as brown-yellow powder and dissolved in methanol. Molecular formula: $C_9H_{12}NO_6$. UV absorption was observed by using PDA of LC-MS/MS, which showed at 226 and 260 nm. Low-resolution mass (ESI) was detected by LC-/MS showing the mass peaks at *m/z* 244.83 ([M + H] +). The ^{1}H NMR spectrum (500 MHz in MeOH-*d*4) showed the characteristic of pyrimidine ring by exhibiting the

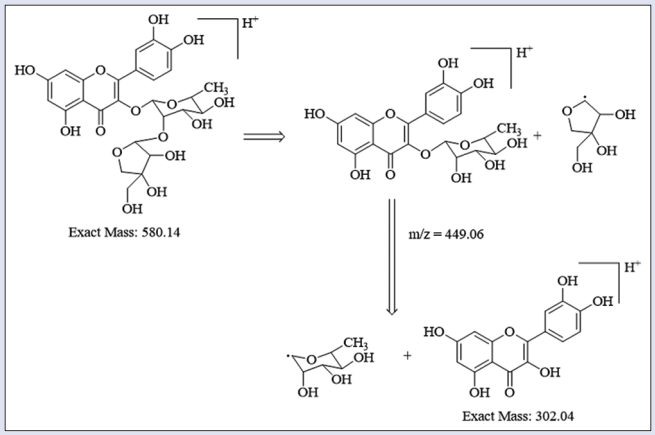

Figure 2: Mass fragmentation of compound 1

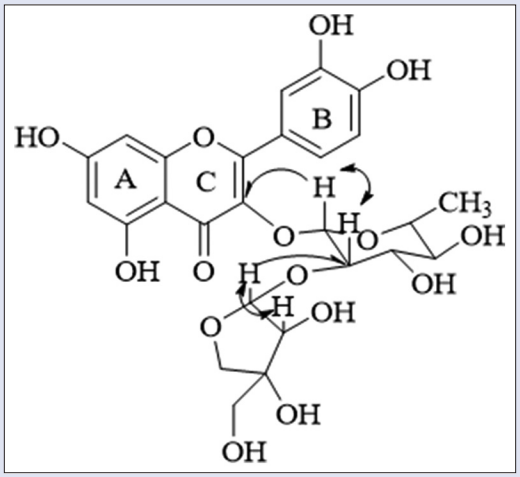

Figure 3: HMBC (→) and COSY (↔) correlation of compound 1

proton signals (δ_H) at 5.96 (*d*, *J* = 8.10 Hz, H-5), 8.00 (*d*, *J* = 8.10 Hz, H-6). The sugar was identified as ribofuranoside which showed the proton signals (δ_H) at 5.89 (*d*, *J* = 4.60 Hz, H-1'), 4.17 (*t*, *J* = 5.00 Hz, H-2'), 4.14 (*t*, *J* = 5.00 Hz, H-3'), 4.00 (*dt*, *J* = 3.00 Hz, H-4'), 3.73 (*dd*, *J* = 12.20, 3.20 Hz, H-5') and 3.82 (*dd*, *J* = 12.2, 2.8 Hz, H-5').

The ^{13}C spectrum (125 MHz in MeOH-*d*4) showed the characteristic of pyrimidine by exhibiting the carbon signals (δ_C) at 151.06 (C-1), 164.78 (C-4), 101.23 (C-5), 141.28 (C-6). The sugar was identified as ribofuranoside, which showed the carbon signals (δ_C) at 89.31 (C-1'), 74.29 (C-2'), 69.89 (C-3'), 84.95 (C-4') and 60.86 (C-5').

The ^{1}H and ^{13}C spectra of compound 4 were similarly compared to the chemical shifts from a previous report as nucleoside, which was named as uridine.[15-17,23-25]

Compound 5

Compound 5 obtained as brown-yellow powder and dissolved in methanol. Molecular formula: $C_{19}H_{21}NO_4$. UV absorption was observed by using PDA of LC-MS/MS, which showed at 246 and 266 nm. Low-resolution mass (ESI) was detected by LC-/MS showing the mass peaks at *m/z* 328.07 ([M + H] +). The ^{1}H NMR spectrum (500 MHz in MeOH-*d*4) showed the characteristic of alkaloid by exhibiting the proton signals (δ_H) at 3.01 ppm (*m*, H-1), 2.53 ppm (*s*, 2-NCH$_3$), 3.06 ppm (*m*, H-3), 2.65 ppm (*m*, H-4), 3.11 ppm (*m*, H-4), 6.61 ppm (*s*, H-5), 3.87 ppm (*s*, H-6, OCH$_3$), 2.45 ppm (*m*, H-1a), 3.01 ppm (*m*, H-1a), 3.86 ppm (*s*, H-4', OCH$_3$), 6.70 ppm (*s*, H-6'), and 8.09 ppm (*s*, H-8').

The ^{13}C spectrum (125 MHz in MeOH-*d*4) showed the characteristic of alkaloid by exhibiting the carbon signals (δ_C) at 62.78 (C-1), 33.48 (C-1a), 42.48 (2-N-CH$_3$), 53.12 (C-3), 27.92 (C-4), 125.96 (C-4a), 108.67 (C-5), 124.16 (C-6), 55.17 (C-6, OCH$_3$), 141.21 (C-7), 119.82 (C-8), 124.16 (C-8a), 128.72 (C-1'), 122.86 (C-2'), 112.79 (C-3'), 145.74 (C-4'), 5.17 (C-4', OCH$_3$), 144.91 (C-5') and 114.19 (C-6').

The ^{1}H and ^{13}C spectra of compound 5 were similarly compared to the chemical shifts from a previous report as isoboldine.[7,18]

Compound 6

Compound 6 obtained as brown-yellow powder and dissolved in methanol. Molecular formula: $C_{19}H_{23}NO_4$. UV absorption was observed by using PDA of LC-MS/MS, which showed at 241 and 271 nm. Low-resolution mass (ESI) was detected by LC-/MS showing the mass peaks at *m/z* 330.21 ([M + H] +). The ^{1}H NMR spectrum (500 MHz in MeOH-*d*4) showed the charateristic of alkaloid by exhibiting the proton signals (δ_H) at 3.69 (*dd*, *J* = 7.50, 5.00 Hz, H-1), 2.44 (*s*, 2-NCH$_3$), 3.13 (*ddd*, *J* = 12.50, 9.40, 5.40 Hz, H-3), 2.70 (*m*, H-3), 2.65 (*ddd*, *J* = 16.30, 5.20, 3.70 Hz, H-4), 2.83 (*ddd*, *J* = 15.90, 9.40, 6.10 Hz, H-4), 6.61 (*s*, H-5), 3.78 (*s*, H-6, OCH$_3$), 6.13 (*s*, H-8), 3.04 (*dd*, *J* = 13.80, 5.00 Hz, H-1a), 2.69 (*dd*, *J* = 13.70, 7.50 Hz, H-1a), 6.60 (*d*, *J* = 2.10 Hz, H-2'), 3.80 (*s*, H-4', OCH$_3$), 6.78 (*d*, *J* = 8.20 Hz, H-5') and 6.51 (*dd*, *J* = 8.20, 2.10 Hz, H-6').

The ^{13}C spectrum (125 MHz in MeOH-*d*4) showed the characteristic of alkaloid by exhibiting the carbon signals (δ_C) at 64.46 (C-1), 39.41 (C-1a), 41.13 (2-NCH$_3$), 46.18 (C-3), 24.51 (C-4), 123.89 (C-4a), 111.18 (C-5), 146.44 (C-6), 54.91 (C-6, OCH$_3$), 143.76 (C-7), 114.27 (C-8), 129.11 (C-8a), 132.29 (C-1'), 116.17 (C-2'), 145.94 (C-3'), 146.09 (C-4'), 55.03 (C-4', OCH$_3$), 111.32 (C-5') and 120.53 (C-6').

^{1}H and ^{13}C spectra of compound 6 were similarly compared to the chemical shifts from a previous report as reticuline.[7,18]

Compound 7

Compound 7 obtained as a white powder and dissolved in chloroform and methanol (9:1). Molecular formula: $C_{35}H_{60}O_6$. The ^{1}H NMR spectrum (500 MHz in DMSO-*d*6) showed the characteristic of beta-sitosterol structure by exhibiting the proton signals (δ_H) at 5.30 (*d*, *J* = 4.9 Hz, H-6, olefinic proton), 0.63 (*s*, CH$_3$-18, methyl protons), 0.94 (*s*, CH$_3$-19, methyl protons), 0.88 (*d*, *J* = 6.5 Hz, CH$_3$-21, methyl protons), 0.79 (*d*, *J* = 6.9 Hz, CH$_3$-26 and 27, methyl protons), 0.80 (*d*, *J* = 7.1 Hz, CH$_3$-29, methyl protons). The sugar was identified as glucopyranoside, which showed the proton signals (δ_H) at 4.20 (*d*, *J* = 7.7 Hz, H-1') and 3.88 (*m*, H-6') The coupling constant of anomeric proton at 4.20 (H-1") presented beta configuration of glucopyranoside.

The ^{13}C spectrum (125 MHz in DMSO-*d*6) showed the characteristic of beta-sitosterol structure by presenting the carbon signals (δ_C) at 37.48 (C-1), 29.98 (C-2), 78.01 (C-3), 39.57 (C-4), 141.71 (C-5), 122.46 (C-6), 30.53 (C-7), 29.05 (C-8), 50.87 (C-9), 36.74 (C-10), 20.98 (C-11), 38.09 (C-12), 43.12 (C-13), 56.69 (C-14), 23.87 (C-15), 26.72 (C-16), 57.43 (C-17), 12.94 (C-18), 20.21 (C-19), 34.61 (C-20), 19.88 (C-21), 32.63 (C-22), 26.72 (C-23), 46.72 (C-24), 29.05 (C-25), 20.21 (C-26), 20.37 (C-27), 21.86 (C-28) and 13.05 (C-29). The sugar was identified as glucopyranoside, which showed the carbon signals (δ_C) at 102.04 (C-1'), 74.73 (C-2'), 78.17 (C-3'), 71.38 (C-4'), δ78.04 (C-5'), and 62.37 (C-6').

The ^{1}H and ^{13}C spectra of compound 7 were similarly compared to the chemical shifts from a previous report which was mentioned to beta-sitosterol-beta-D-glucopyranoside.[19,26]

Anti-alpha glucosidase activity

The LE extracts were tested on anti-alpha glucosidase activity to figure out the fractions, which exhibited the enzyme inhibitory effect as shown in Table 1. From the result showed as the most effective extract of leaf and stem were both ethanol extract. The study was continued to find out the active compounds in this plant. So, the isolated compounds of this plant were studied on the anti-enzyme activity as shown in Table 2. From the result showed as the most effective isolated compound was compound 5.

Table 1: Anti-alpha glucosidase activity of *Litsea elliptica* extracts

n	Plant part	Solvent extract	IC_{50} (μg/mL)
1	Leaf	Hexane	-
2		Ethyl acetate	-
3		Ethanol	530
4		Aqueous	610
5	Wood stem	Hexane	-
6		Ethyl acetate	-
7		Ethanol	150
8		Aqueous	690
SD	Acarbose	Acarbose	88.75

SD: Standard deviation

Table 2: The anti-alpha glucosidase activity of the isolated compounds from *Litsea elliptica* extracts

n	Compound	IC_{50} (μg/mL)
1	Quercetin-3-*O*-α-rhamnopyranoside-*O*-(1→2)-α-apiofuranoside	355.15
2	Quercetin-3-*O*-α-rhamnopyranoside (quercitrin)	751.79
3	Quercetin-3-*O*-β-glucopyranoside (isoquercitrin)	332.19
4	Uridine	inactive
5	Isoboldine	243.25
6	Reticuline	inactive
7	β-sitosterol-D-glucopyranoside	inactive
SD	Acarbose	107.19

SD: Standard deviation

Computer molecular docking of active compounds

The method was confirmed by re-docking of the extracted native molecule and protein, from the parental crystal structure, which was a crucial step which indicated the reliability from the established docking protocol. The overlay structures of redocked and original molecules must evaluate, and the general RMSD value was analyzed. This value exhibited an average distance between atoms of the identical molecule, and the accepted values from the re-docking experiment were <3.50 A°.[11,19]

According to the active compounds of LE plant, there was no report on the molecular interaction level between compound 5 and alpha-glucosidase from yeast on both *in vitro* and *in silico*. The only couple of the studies had reported the anti-alpha glucosidase activity from compound 5[20,21,27,28] as also presented in this study. Hence, here we purposed, from ours *in silico* study, that compound 5 could insert itself into the same pocket, the active site, as known flavonoid, compound 3, as shown in Figure 4.

The results of docking indicated that compound 5 interacted with the non-catalytic domains, TRY 158 and PHE 314, at the entrance region of the active site but not in the catalytic domains (GLU 277 and ASP 352) or the stabilizer domain ARG 442. Consequently, compound 5 also showed the strongest binding with the lowest energy at-7.2 kcal/mol that calculated from the docking experiment, and this result agreed with *in vitro* assay, as shown above.

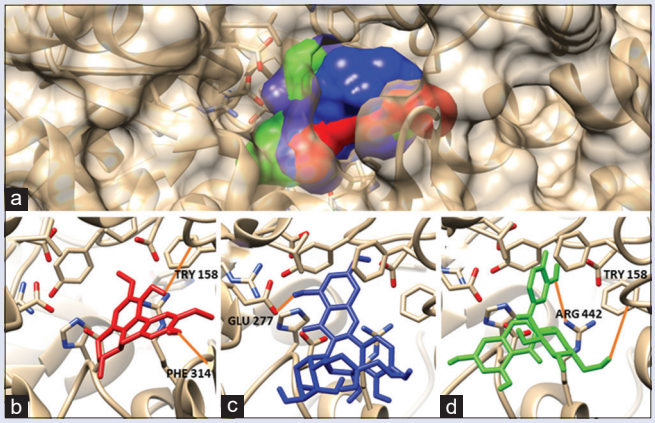

Figure 4: The positions of the best conformation and alpha-glucosidase as showed in a surface (a) or sticks formats (b-d) for compound 5 (Red), compound 3 (Blue) and compound 1 (Green) in the active site of alpha-glucosidase (Amber yellow) and the molecular interactions among these ligands and alpha-glucosidase from docking experiments. (a) An overlays positions of compound 5, 3 and 1 in the active site of alpha-glucosidase. (b) H bonds (Orange line) between compound 5 and alpha glucosidase. (c) H bond (Orange line) between compound 3 and alpha glucosidase. (d) H bonds (Orange line) between compound 1 and alpha glucosidase

To confirm this finding, the refinement and rescoring of the best pose of both compounds was performed by single-point energy estimation from Autodock 4.2.6 as described in the previous section to evaluate the losing hydrophobic property of the new compound 1 and the outcome was shown in Table 3.

As also anticipated and agreed with previous information from the previous report,[22,29] compound 3 and compound 1 showed less stability in van der Waals interaction due to present the higher energy than compound 3, approximately 4.0 kcal/mol, in the term of vdW + Hbond [Table 3], according to the estimation from Autodock4. Losing the van der Waals interaction caused compound 3 and compound 1 to have less stable energy in total intermolecular interaction and in the end, weaker free energy of binding.[22,29]

DISCUSSION

Seven compounds were isolated from LE which were named as Quercetin-3-*O*-α-rhamnopyranoside-*O*-(1→2)-α-apiofuranoside (newcompound)(1),quercetin-3-*O*-α-rhamnopyranoside(quercitrin)(2), quercetin-3-*O*-β-glucopyranoside (isoquercitrin) (3), uridine (4), isobodine (5), reticuline (6) and β-sitosterol-D-glucopyranoside (7).

Four isolated compounds; 1, 2, 3 and 5 showed the activity against alpha-glucosidase enzyme with inhibition concentration at 50% (μg/mL) values; 5 < 3<1 < 2, respectively. The active compounds could be categorized into two groups as flavonoid glycosides and alkaloid compounds. Flavonoid compounds had numerous reports on anti-diabetic activity through *in vitro* and *in vivo* models. The flavonoids that structured as quercetin had been proved as the strong efficacy of anti-alpha glucosidase. The substitutions in the structure played a key role to decline or enhance the activity of inhibitors, especially; hydroxylation in the structure is the powerful functionality that can enhance the activity. Methylation or methoxylation is affected to the activity depending on the position, but most of methylation and methoxylation are slightly decreased activity. The glycosylation of flavonoids decreases the inhibitory activity on alpha-glucosidase, depending on the substitution site and the class of sugar molecule. The decreasing inhibitory effect on alpha-glucosidase after glycosylation probably causes by the expanding molecular mass and polarity and transfer to the nonplanar structure. After the hydroxyl unit is substituted by a sugar molecule, steric hindrance may take place, which weakens the binding interaction between flavonoids and alpha-glucosidase.

The active compounds were studied on molecular docking to see the structure and functional group of compounds interacting with the active site of the alpha-glucosidase enzyme. The outcome indicated that the new compound 1 was inhibited by weak binding energy at non-catalytic domains TRY 158 and stabilizer domain at ARG 442 showing higher affinity energy at −3.0 kcal/mol whereas compound 3 was attached at the catalytic domain (GLU 277) which more compatible binding at less energy at −5.4 kcal/mol. On the other hand, many

Table 3: The refinement and rescoring of the best pose of isoquercitrin (compound 3) and quercetin-3-*O*-α-rhamnoside-*O*-(1→2)-α-apiofuranoside (compound 1) from Autodock 4.2.6 compare with original scoring from Autodock Vina

Cpd	Autodock 4.2.6								Auto-dock vina
	vdW + Hbond (kcal/mol) (1)	Electro static energy (kcal/mol) (2)	De-solva -tion energy (kcal/mol) (3)	Total inter molecular interaction energy (kcal/mol) (1+2 + 3)	Total internal energy (kcal/mol) (4)	Torsion free energy (kcal/mol) (5)	Un-bound's energy (kcal/mol) (6)	Estimate free energy of binding (kcal/mol) (4+5 + 6)	Affinity (kcal/mol)
3	−14.0229	0.2807	6.4698	−7.2724	−1.57	3.28	0	−5.56	−5.4
1	−10.0046	−0.0991	7.8364	−2.2673	−5.71	4.47	0	−3.51	−3.0

Cpd: Compound; vdW: Van der Waals interaction; Hbond: H-bonding interaction

studies had significantly presented the potential of flavonoids in anti-glucosidase activity and reported the molecular mechanism of those flavonoids.[23,24,30,31] Furthermore, it had already known that glycosylation at 3-OH position in ring C could reduce the inhibitory activity, and the bigger molecule of sugar that was substituted, the less potency will become due to the loss of hydrophobic property. It had clearly proved that hydrophobic property had driven the intermolecular interaction between flavonoids and the enzyme.[22,29] As expected, the compound 1 exhibited the looser binding, due to the higher affinity energy at-3.0 kcal/mol, than compound 3, which could bind more favorably, less energy at-5.4 kcal/mol. Compound 5 interacted with the non-catalytic domains, TRY 158 and PHE 314, at the entrance region of the active site but not in the catalytic domains (GLU 277 and ASP 352) or the stabilizer domain ARG 442 showed strongest binding, the lowest energy at −7.2 kcal/mol, from docking experiment and this agreed with our *in vitro* assay. In addition, the mechanism of action of effective compounds could be predicted by the binding sites as non-competitive inhibitor (1 and 5) and competitive inhibitor (3).

An alkaloid is a group of organic nitrogenous bases which classified by the nature of nitrogen-containing structure such as pyrrolidine, piperidine, quinoline, isoquinoline and indole alkaloids, etc. The isolated alkaloids were interpreted as isobodine and reticuline, which categorized in the group of benzyltetrahydroisoquionoline. Since the various type of alkaloid structures, a few of them had been reported on structue activity relationships via *in silico* experiment. The result suggested that the most effective alkaloid ligand, which presented the highest docking score, should be formed more hydrogen bonds and hydrophobic interactions at the catalytic domain by using hydroxy group in their structures. Ib contrast to the lower docking score, the alkaloid ligand formed less hydrogen bonds and hydrophobic interaction.[32] The report was supported to the reticuline (6) that presented the highest docking score at −7.2 kcal/mol, which related to *in vitro* study.

Conclusively, the characteristics of the effective alpha-glucosidase inhibitors had been focused on these criteria (a) sugar-like structures or substrate-like structure, (b) the capability to bond ionic interaction with nucleophilically catalyzing residues, (c) transition-state-like structures, (d) the ability to make hydrogen bonds with catalytic acid residues (arginine, histidine, aspatic acid, etc), (e) the ability to form ionic and hydrophobic interactions at the other sites than the active site and (f) the ability to make covalent bonds with enzyme through an epoxy or aziridine group.[25,33]

CONCLUSION

In a nutshell, LE was selected to study for phytochemistry, which could be obtained 7 compounds from leaf and wood extracts. In addition, the plant extracts, and its isolated compounds were evaluated for anti-diabetic activity, which was tested on anti-alpha glucosidase activity. The compound 1, 3, and 5 exhibited anti-alpha glucosidase activity. These results will be the first report of LE containing the effective compounds which played a crucial role in anti-diabetic property. The plant and their isolated compounds will be interestingly for further study in *in vivo* model to concern the systemic effect in the animal.

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Conflicts of interest

There are no conflicts of interest.

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