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Chemical Profiling, Molecular Docking, and Antidiabetic Activity of *Basilicum polystachyon* Leave Methanol Extract

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Abstract

Diabetes mellitus (DM) has become a significant global health concern due to its rapidly rising prevalence, coupled with the considerable side effects and risks associated with antidiabetic medications. Natural products are the main source of drug discovery which are believed to have lower side effects and toxicity than synthetic drugs in clinical treatments. The objective of this study was to reveal the antidiabetic activity of *Basilicum polystachyon* (Lamiaceae) as a potential medicinal plant, along with its chemical profile to solve the problem of this disease. Based on these results, 103 individual compounds were identified in the methanol extract of this plant. Molecular docking simulations revealed several major compounds with good binding energies, indicating that these compounds were potential as α-glucosidase inhibitors. While *in vitro* enzymatic assay showed that the methanol extracts had weak antidiabetic activity with IC_{50} value 289.61 µg/mL. However, considering the long-term effects of synthetic drugs in the body, this plant could be a potential source of a natural antidiabetic agent.

Keywords: Antidiabetic, α-glucosidase, molecular docking, chemical profiling, *Basilicum polystachyon*.

1. Introduction

Diabetes mellitus is a non-communicable and complex degenerative disease characterized by metabolic disorders in the form of increased blood glucose levels [1]. Diabetes mellitus is becoming a major health problem and is gradually becoming a global epidemic disease. Worldwide, approximately 536.6 million people are living with diabetes mellitus, according to the 2021 International Diabetes Federation estimate [2]. From this, it can be predicted, that without effective and successful treatment, diabetes prevalence will continue to increase significantly, reaching 12.2% (783.2 million) in 2045 [3]. This disease is spreading globally and includes several types. Type 1 diabetes is an autoimmune condition that results in the total absence of insulin marked by destroying pancreatic β-cells [4]. Type 2 diabetes results from insulin resistance, where insulin fails to effectively transport glucose from the bloodstream to the interstitial tissues, a condition commonly referred to as insulin resistance [5, 6]. The most dangerous type of diabetes is type 2 diabetes mellitus (T2DM), according to prevalence data and the number of patients. According to the (WHO), T2DM is a metabolic abnormality that impacts more than 422 million people globally and is responsible for 1.6 million annual fatalities, according to estimates. Unfortunately, Asia is the epicentre of the global epidemic of T2DM [7].

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The α-glucosidase is a hydrolase enzyme found in the digestive system in the human body, specifically in the small intestine [8]. The enzyme plays a vital role in diabetes growth then followed by progressing many types of complications pathology. Targeted therapy directed inhibition α-glucosidase has emerged as a promising treatment strategy for controlling blood glucose levels by competitive inhibition [9]. Acarbose, miglitol, and metformin are synthetic medications used to manage blood sugar levels by inhibiting glucose absorption and improving the sensitivity of insulin, but these clinical medications with significant risks and side effects for patients. Diarrhea, bloating, liver, stomach cramps, gastrointestinal disorders, and drug resistance are common side effects found in diabetes patients [10, 11]. Due to these challenges in treating diabetes, exploring effective and safe antidiabetic agents is pushing researchers to discover novel drugs based on natural products. Natural products are the main source of drug discovery which are believed to have lower side effects and toxicity than synthetic treatments [12, 13].

The *B. polystachyon* is a member of the Lamiaceae found in Asia, Africa, and India [14]. This plant is often used in medicine as an antioxidant, antibacterial, and cytotoxic agent [15]. According to several reports, the plant is rich in secondary metabolites including flavonoid, steroid, terpene, saponin, glycoside, alkaloid, phenolic, and coumarin [16, 17]. The plant contains potential compounds identified as phytoconstituents that contribute to antidiabetic activity, such as rosmarinic acid [18], caryophyllene oxide [19], quercetin [20], *p*-coumaric acid [21], dan gallic acid [22]. These secondary metabolites are very beneficial, especially in medicine, for example, as antidiabetic agents [23]. Because of technological and scientific advances, the chemical profile of the plant must be completely identified and its potential as an antidiabetic agent revealed through *in silico* and *in vitro* assessments. This study aimed to identify chemical compounds using Liquid Chromatography-Mass Spectroscopy (LC-MS) and evaluate the antidiabetic activity of the plant leaf extract as a novel α-glucosidase inhibitor using molecular docking simulations and *in vitro* enzymatic assays. The combination of LC-MS and molecular docking simulations are promising strategy in drug discovery for uncovering the potency of phytochemical compounds. *In vitro* assays on crude extracts should be performed as a preliminary stage for further research.

2. Materials and Methods

2.1 Materials

This study utilized the following materials: methanol (≤ 99%, Merck, Germany), the protein receptor structure of α-glucosidase (PDB ID: 3A4A) (retrieved from https://www.rcsb.org/), ligand conformers (retrieved from https://pubchem.ncbi.nlm.nih.gov/), α-glucosidase enzyme with 1 U/mL activity (Sigma Aldrich, USA), *p*-nitrophenyl-α-D-glucopyranoside substrate at a concentration of 1 M (Sigma Aldrich, USA), DMSO (Sigma Aldrich, USA) and Na_2CO_3 (Sigma Aldrich, USA). The *B. polystachyon* leaves were collected from Tuban, East Java, Indonesia in August 2023. The plant materials were identified at Generasi Biologi Indonesia under specimen number 361/02.Genbinesia/2023.

2.2 Equipment and Instruments

The equipment employed for this research involved a volumetric flask (Pyrex, USA), beaker glass (Pyrex, USA), extraction chamber, spatula, Whatman filter paper, micropipette Eppendorf tubes, Buchner funnel (Haldenwanger, Germany), vacuum pump (VE2100N, Value, Poland), vacuum rotary evaporator (R-300, Buchi, Switzerland), Shimadzu LC-MS instrument (8040 Type, Shimadzu, Japan), incubator, 96-well microplate, and microplate

reader. The docking study was performed using Toshiba Windows 10, Intel(R) Dual Core (TM) @2.16 GHz 2.16 GHz, 4,00 GB RAM, and a 64-bit operating system. The software used involved BIOVIA Discovery Studio Visualizer 2021 Client (Dassault Systèmes Biovia Corp., Vélizy-Villacoublay, France), AutoDock4.2 software package (The Scripps Research Institute, La Jolla, CA, USA), and MarvinSketch software (ChemAxon, Budapest, Hungary).

2.3 Plant Collection and Extraction

Leaf samples of B. polystachyon were collected from Tuban, East Java, Indonesia. The leaves were air-dried for three days, and then ground into a using a grinder, resulting in 150 g of powdered material. This powder sample was macerated using 750 mL of methanol solvent by stirring several times and repeated 3 times. The rest is done in the same way. After that, filtering was carried out with a Buchner funnel assisted by a vacuum pump and obtained methanol filtrate. The ethanol filtrate was evaporated using a vacuum rotary evaporator to obtain a thick greenish-black extract of as much as 36.38 grams.

2.4 Identification of Secondary Metabolites Contained in the Extract

The secondary metabolites present in the methanol extract of *B. polystachyon* leaves were detected using an LC-MS instrument (Shimadzu 8040 Type) completed with a Shimadzu Pack FC-ODS capillary column (2 mm×150 mm id, 3 µm particle size) with a 1 µL injection volume. The instrument that has an Electrospray Ionization (ESI) source with the following parameters: capillary voltage 3,0 kv; column chromatography temperature 35 ºC; flow rate 0.5 mL/min; methanol solvent; MS focused ion mode type [M]+; ionization using ESI; mobile phase isocratic mode; and run time 80 minutes. The secondary metabolites present in the extract were identified by comparing their molecular mass spectra and retention times from the chromatogram with data from NIST database libraries.

2.4 In Silico Molecular Docking Simulation

The α-glucosidase enzyme crystalline structure (PDB ID: 3A4A, 1.6 A resolution) of isomaltase from *S. cerevisiae* as a docking receptor was retrieved from the Protein Data Bank web server (https://www.rcsb.org/). Water molecules and native ligands were sterilized from the enzyme structure. The α-glucosidase structure was subsequently prepared with AutoDock by adding hydrogen polar atoms and Kollman charges. Molecular docking was performed on the major compound from the LC/MS results as ligands. The 3D conformers of the ligands were retrieved from the PubChem web server (https://pubchem.ncbi.nlm.nih.gov/) and energy-minimized through the use of MMFF94 (Merck Molecular Force Field) from the conformers tool which is available in the MarvinSketch program [24].

Molecular docking simulations were conducted using AutoDock4.2 software on the active site of the α-glucosidase based on native ligand position, with a grid center of x = 21.389, y = −7.720, and z = 23.987, grid dimensions of 60 × 60 × 60 Å, and spacing of 0.375 Å [25, 26]. The Lamarckian genetic algorithm was operated to find the optimum ligand conformation and binding position with a GA run of 100. The docking procedure was validated through RMSD value from the reduced native ligands on the enzyme target, with an RMSD value of less than 2 Å [27, 28]. After the process, compounds with binding energy values less than those of the positive control drug (acarbose) were selected as potential α-glucosidase inhibitors. The docking results of the optimal conformation were exported and analyzed using BIOVIA Discovery Studio Visualizer to visualize ligand-receptor interaction patterns and determine the residues of amino acids that play a role in the binding complex.

2.5 In Vitro Enzymatic Assay

The α-glucosidase inhibitory assay protocol of the extracts was carried out following the method in previous research with slight modification and optimization according to the assay conditions [29]. Amounts of 50 µL of samples (extracts) with various concentrations of 15.625 µg/mL to 1000 µg/mL were mixed with 10 µL of the α-glucosidase (1 U/mL) and incubated for 20 minutes at 37 ºC and 125 µL of 0.1 M phosphate buffer (pH 6.8). After incubation was completed, 20 µL of 1 M *p*-nitrophenyl-α-D-glucopyranoside (substrate) was added and then incubated for 30 minutes. Na_2CO_3 0.1 N (50 µL) was added to terminate the enzymatic reaction. Next, the absorbance was measured at a wavelength of 410 nm using a microplate reader instrument with triplicate measurement. The positive control, acarbose, was tested at various concentrations ranging from 0.3125 µg/mL to 20 µg/mL. The formula was employed to calculate the analysis results, which were shown as percent inhibition, and IC_{50} values were determined from nonlinear regression evaluation based on the dose-response curves [30, 31].

Activity (% inhibition) = (Ac-As)/Ac × 100 %

where Ac = Absorbance of the control; As = Absorbance of the experimental sample

3. Result and Discussion

3.1 Identification of Secondary Metabolites from Methanol Extract of B. polystachyon Leaf

The identification of secondary metabolites was performed using LC-MS, with the results displayed in its chromatogram (Figure 1). From these results, 103 compounds can be identified in the methanol extract of this plant.

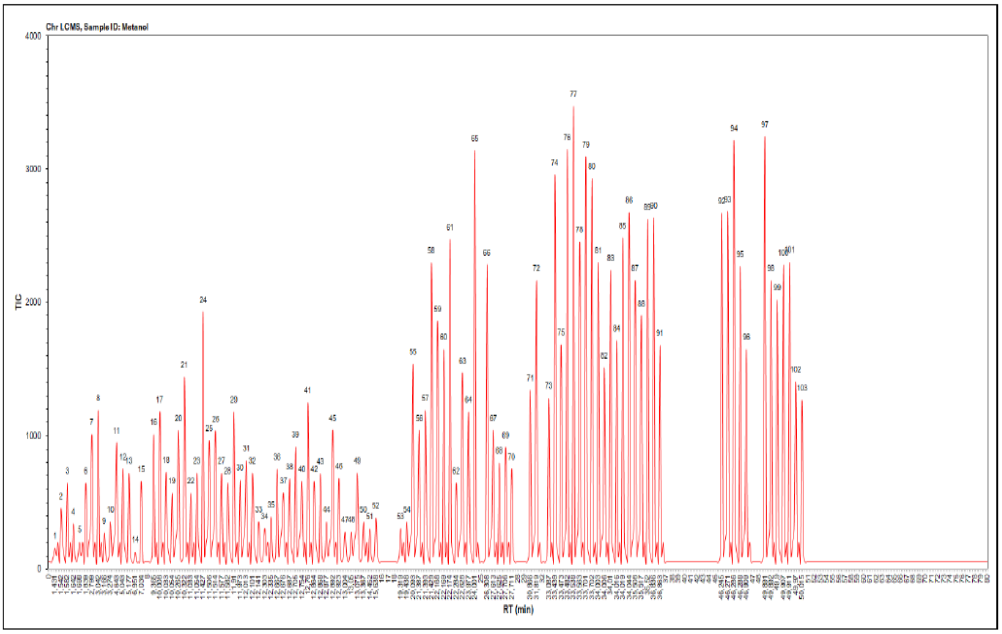


Figure 1: LC-MS chromatogram of methanol extract of the *Basilicum polystachyon* leaves.

As known that methanol solvents can be able to extract all the compounds contained in plant leaves, ranging from polar to non-polar, such as phenolics, flavonoids, glycosides, alkaloids, steroids, terpenoids, phenylpropanoids, polyketides, coumarins, and lignans. Phenolic acids, and glycosides are examples of polar phenolic compounds that are frequently extracted using methanol [32]. The phenolic compounds dominate the extract composition, which considers the presence of hydroxyl groups that are easily extracted with polar solvents such as methanol via hydrogen bonds [33]. The major compounds in the extract identified with a high percentage of peak area are listed in Table 1.

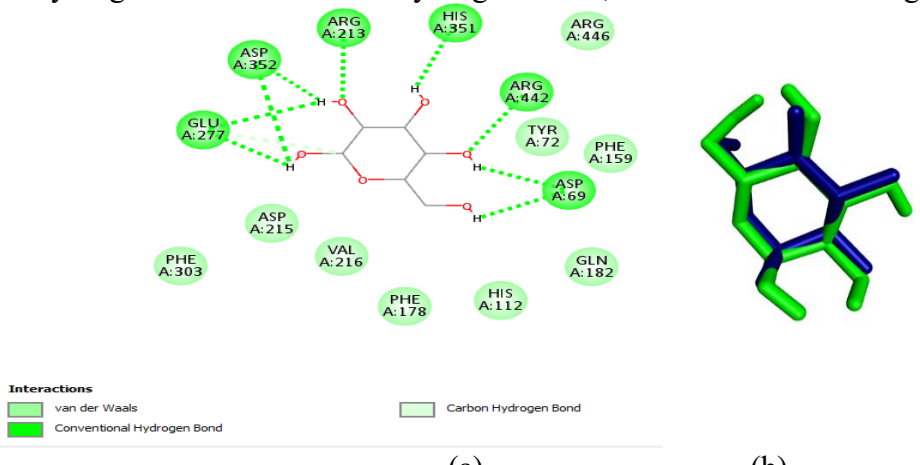
Table 1: The binding energy values and inhibition constants of the ligands with receptors.

Compound	Concentration (%)
Quercetin-3-glucoside	2.29
Apigenin 7-O-rutinoside	2.16
Apigenin-7-(6”-*p*-coumarylglucoside)	2.30
Luteolin-7-apiosyl (1→2) glucoside	2.54
Apigenin-7-[rhamnosyl (1→2) galacturonide]	2.26
Acacetin-7-rutinoside	2.14
Kaempferol-3-glucoside-2”-rhamnoside-7-rhamnoside	2.35
Quercetin 3-O-sophoroside	2.37

The phenolic acid is present in many types, namely gallic acid, ascorbic acid, vanillic acid, ferulic acid, *p*-coumaric acid, caffeic acid, and rosmarinic acid. This study also confirmed the results of a previous study on compounds contained in *B. polystachyon*, as shown by the high quantity of phenolic compounds [17]. The extract contains free phenolic compounds, including quercetin, myricetin, kaempferol, apigenin, acacetin, luteolin, hispidulin, hesperetin, velutin, and salvigenin. Additionally, methanol also contains methyl hydrophobic groups that can bind with non-polar compounds, such as steroids and terpenoids, like stigmasterol, α-amyrin, and squalene, but very small concentrations indicated that these compounds have a low solubility in methanol solvents [34, 35]. Alkaloids, phenylpropanoids, polychetides, coumarins, and lignans were identified in the extracts but as minor compounds with small percentages.

3.2 Molecular Docking Simulation

Molecular docking simulations were conducted on the major compounds identified by LC-MS to identify their potential as α-glucosidase inhibitors. The major compounds, which are highly concentrated in the extract, have a significant role in the pharmacological activity compared to the minor compounds [36]. The enzyme target used in this study was the crystal structure of isomaltase from *S. cerevisiae* (3A4A), which has a high similarity of 71.92% with the α-glucosidase structure from *S. cereviseae* in Swiss-Prot (P53341) [37]. A control docking parameter was performed using the redocked native ligand, α-D-glucose on receptor protein 3A4A. The re-docked conformation of the native ligand overlapped with the original conformation before molecular docking simulation and the root mean square deviation (RMSD) was determined to be 0.783 Å, indicating that the established docking parameters were capable of reproducing the native conformation. The binding energy of native ligand α-D-glucose towards the enzyme is -5.67 kcal/mol with six amino acid residues interactions via conventional hydrogen bonds and carbon hydrogen bonds, which are shown in Figure 2.



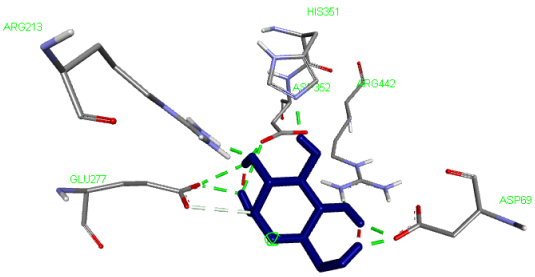
(a) (b)

Figure 2: (a) Visualization of 2D interactions and (b) superimposition of the re-docked native ligand.

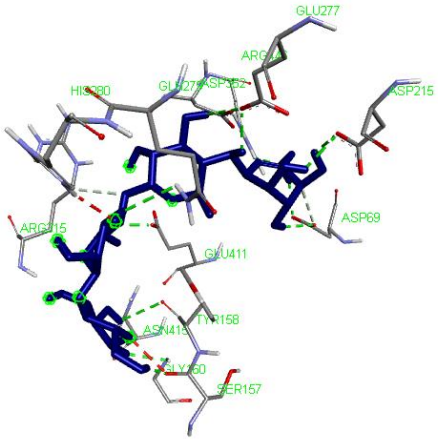
In molecular docking simulation toward α-glucosidase, acarbose as the standard drug has a binding energy value of -9.09 kcal/mol, with receptor amino acid residues that interact with acarbose including Asp215, Asp352, and Glu277 which are the three catalytic residues of α-glucosidase used to hydrolyze oligosaccharides into glucose molecule. These results are similar to those of a previous study where acarbose interacted with residues Asp69, Gln277, Gln279, Asp352, and Glu411 [38]. The docking score from this simulation was lower compared to earlier studies with binding energy -7.78 and -8.7 kcal/mol [39, 40]. The amino acid residues on the active site of the enzyme also contribute to the interaction between ligands and receptors, particularly Asp69, His112, Gln279, and Glu411. The interactions of these compounds with amino acids present in the catalytic site may decrease catalytic activity and inhibit the α-glucosidase as a functional protein [41, 42]. There were four major compounds in the methanol extract with more negative binding energies and lower inhibition constant values than those in the positive control acarbose, indicating that the compounds exhibited promising inhibitory activity towards α-glucosidase. Table 2 displays the binding energies of several ligands towards α-glucosidase via binding to the catalytic site. The binding energy values of these major compounds ranged from approximately -6.39 to -11.73 kcal/mol. A more negative binding energy typically indicates stronger binding and greater stability of the ligand-receptor complex [43, 44, 45]. Thus, the inhibitory activity is more effective and these compounds can prohibit oligosaccharide substrates from entering the enzyme catalytic sites.

Table 2: The binding energy values and inhibition constants of the ligands with receptors.

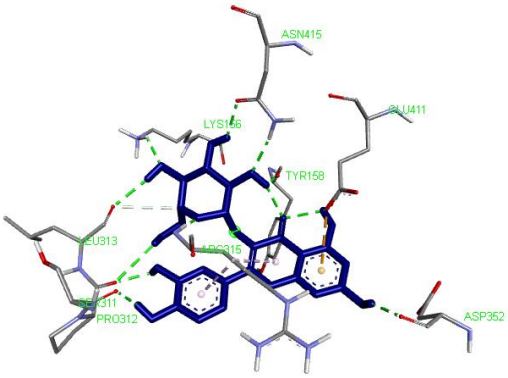
Compound	Free Energy of Binding (kcal/mol)	Inhibition Constant (Ki) (nM)
α-D-glucose (native)	-5.67	69750
Acarbose (control)	-9.09	218.92
Quercetin-3-glucoside	-8.00	1360
Apigenin 7-O-rutinoside	-10.22	32.06
Apigenin-7-(6”-*p*-coumarylglucoside)	-11.73	2.52
Luteolin-7-apiosyl (1→2) glucoside	-9.51	106.80
Apigenin-7-[rhamnosyl (1→2) galacturonide]	-8.71	413.49
Acacetin-7-rutinoside	-8.95	275.37
Kaempferol-3-glucoside-2”-rhamnoside-7-rhamnoside	-9.13	203.46
Quercetin 3-O-sophoroside	-6.39	20640



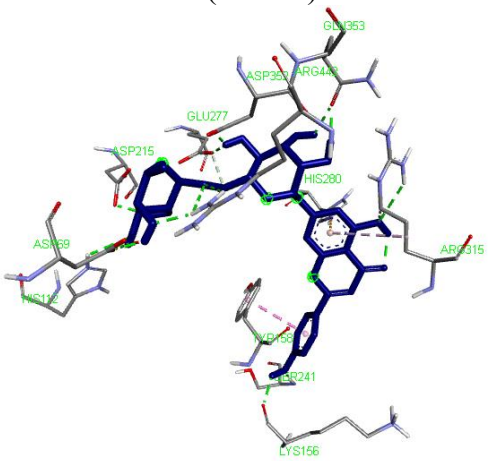
α-D-glucose (native)



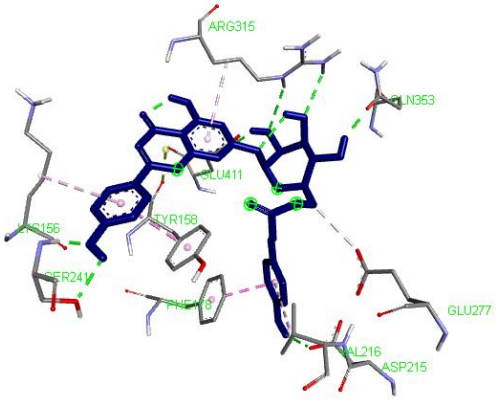
Acarbose (control)



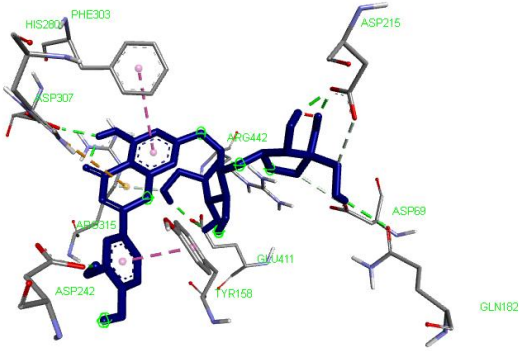
Quercetin-3-glucoside



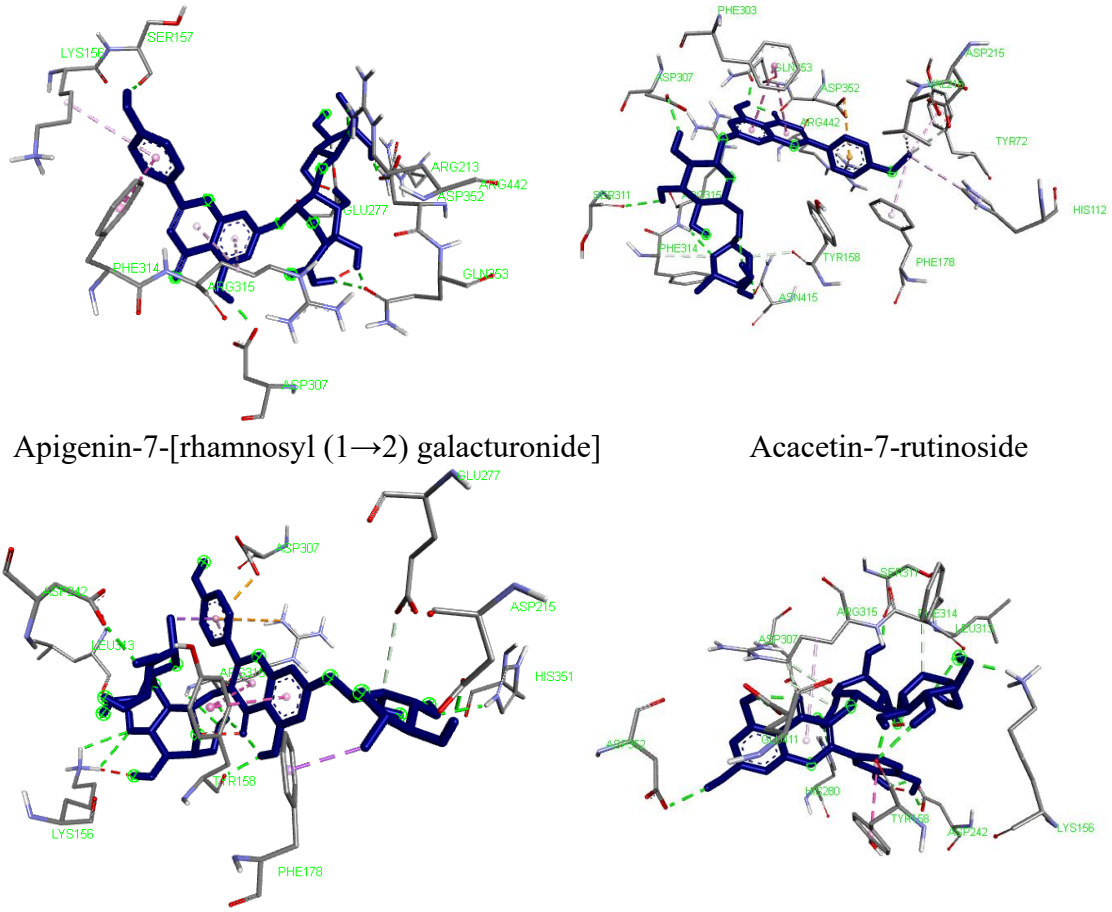
Apigenin 7-O-rutinoside



Apigenin-7-(6”-p-coumarylglucoside)



Luteolin-7-apiosyl (1→2) glucoside



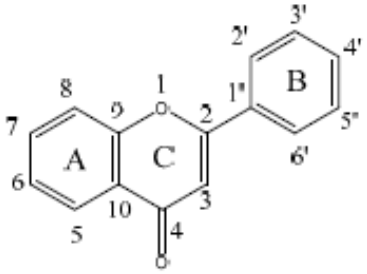
Apigenin-7-[rhamnosyl (1→2) galacturonide] Acacetin-7-rutinoside

Kaempferol-3-glucoside-2”-rhamnoside-7-rhamnoside Quercetin 3-O-sophoroside

Figure 2: The visualization of the ligand-receptor interactions of native, acarbose, and major compounds from the extract with the α-glucosidase enzyme.

Figure 2 shows that most ligands interact with the identical residues of amino acids at the catalytic site. Several compounds, such as quercetin-3-glucoside, apigenin-7-rutinoside, apigenin-7-[rhamnosyl (1→2) galacturonide], and quercetin 3-O-sophoroside, bind to the same amino acid residue (Asp352) via hydrogen bonding as acarbose. Similar amino acid residues that form hydrogen bonds are Asp215, Glu277, and Asp352, according to other research [46]. The hydrophobic interactions occur between hydrophobic groups in ligands with hydrophobic groups in the α-glucosidase structure, in which the amino acid residue contains aromatic rings, including Tyr72, His112, Tyr158, Phe178, His280, and Phe303, similar to Pi-alkyl, Pi-sigma, and π-π T shaped. On the other hand, charged amino acids in protein chains, such as Asp352, Glu411, and Arg442 form electrostatic interactions with several ligands. Another study confirmed that this amino acid residue is one of the catalytic sites of the 3A4A protein and plays an essential role in the hydrolysis process [47]. For a bioactive compound to function as a therapeutic agent must bind to a specific catalytic site with significant interactions [48]. Hydrogen bonding, electrostatic, and hydrophobic interactions are the main factors influencing ligand binding to the receptor. These interactions play a valuable role in the binding energy and strength of the ligand-receptor complex [49].

Table 3: The chemical structures and binding energy values of major compounds



Compound	3	4	5	6	7	2”	3’	4’	5’	Binding energy
1	O-Glu		OH		OH		OH	OH		-8.00
2			OH		O-Rut			OH		-10.22
3			OH		*p*-coumaryl glucoside			OH		-11.73
4			OH		apiosyl glucoside			OH	OH	-9.51
5			OH		rhamnosyl galacturonide			OH		-8.71
6			OH		O-Rut			OCH_3		-8.95
7	O-Glu		OH		rhamnoside-2”-rhamnoside			OH		-9.13
8	O-Sop		OH		OH		OH	OH		-6.39

(Glu, glucoside; Sop, sophoroside; Rut, rutinoside)

Based on the molecular docking results, the relationship between the structure and inhibitory activity of the compounds can be known as shown in Table 3. Apigenin-7-(6"-*p*-coumarylglucoside) had the lowest binding energy value, indicating the most optimal inhibitory activity. Previous studies that *p*-coumaric substituents on flavonoid glycosides can increase α-glucosidase inhibitory activity [50]. The *p*-coumarin group can interact with the catalytic residues of the enzyme, particularly Glu277, Asp215, and Phe178. The compounds quercetin-3-glucoside, kaempferol-3-glucoside-2"-rhamnoside-7-rhamnoside, and quercetin 3-O-sophoroside have the same glycosylation position but with different types of sugar, it appears that sophorose substituents decrease the inhibitory activity, while the addition of sugar groups at 7-position of A ring in the flavone skeleton increases the inhibitory activity. Replacement of the -OH group with -OCH_3 in compounds 2 and 6 resulted in a decrease in inhibitory activity, similar to previous studies [51]. The rutinose sugar substituent is more favorable for its inhibitory activity than rhamnosyl (1→2) galacturonide, as shown in compounds 2 and 5, which exhibited significant differences in compound activity.

Ultimately, the molecular docking accomplished in this research predicted patterns of interactions and potential compounds extracted by methanol from *B. polystachyon* leaves, which has higher glucosidase inhibitory activity than standard acarbose. These compounds must be isolated for further investigation of their activity as α-glucosidase inhibitors.

3.3 In Vitro α-Glucosidase Inhibition Assay

Targeted inhibition of α-glucosidase has emerged as a promising treatment strategy for controlling blood glucose levels by competitive inhibition. Table 4 presents the inhibitory activity by methanol extracts and acarbose shown by IC_{50} values.

Table 4: The IC_{50} values for inhibition of α-glucosidase by the methanol extract and acarbose.

Sample	Concentration (µg/mL)	Inhibition (%)	IC_{50} (µg/mL)
Methanol extract	15.625	6.87	289.61
	31.25	16.64	
	62.5	26.00	
	125	41.36	
	250	49.88	
	500	58.08	
	1000	60.65	
Acarbose (control)	0.3125	17.66	1.92
	0.625	31.37	
	1.25	48.63	
	2.5	61.15	
	5	64.07	
	10	71.66	
	20	82.45	

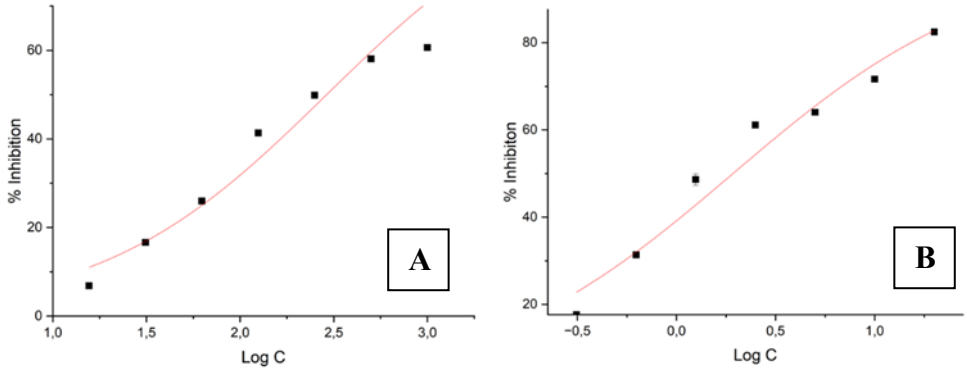


Figure 3. The α-glucosidase inhibitory (% inhibition vs concentration graph) of methanol extract *B. polystachyon* (A) and standard acarbose (B).

The percentage inhibition of *B. polystachyon* methanol extract and acarbose showed concentration dependence. When the methanol extract was evaluated in terms of α-glucosidase inhibitory activity, it was determined that weak inhibitory activity, 60.65% at the highest concentration of extract solution, which is 1000 µg/mL. While the results of the acarbose standard showed very strong activity, with 82.45% inhibition at a concentration of only 20 µg/mL. The methanol extract displayed weak α-glucosidase inhibitory action compared with acarbose, and the IC_{50} value was calculated as 289.61 µg/mL.

These results revealed a poor correlation with the molecular docking simulation results, where many compounds have much better inhibitory activity than acarbose, especially the group of glycosylated compounds, ranging from apigenin-7-rutinoside, apigenin-7-(6"-*p*-coumarylglucoside), and luteolin-7-apiosyl (1→2) glucoside to phenolic compounds and flavonoids, which have been studied for their potential antidiabetic activity, such as quercetin [52], apigenin [53], and kaempferol [54]. Antagonistic interactions between compounds are believed to be the main cause of weak enzyme inhibitory activity. This revealed that there is a possibility of an antagonistic effect on the overall activity of the extract or minor compounds

that may have contributed to lower antidiabetic activity. The antagonistic effects of plant extracts are widely known in phytochemistry, especially regarding interactions between compounds [55]. This is similar to other research findings that reported limited bioactivity as a result of antagonistic interactions between major and minor chemical compounds in the extract [56, 57]. Based on this research, the α-glucosidase inhibitory activity of plant extracts could not be predicted with certainty, even in extracts with high contents of phenolic and glycoside compounds. The inhibitory activity of the plant extract depended on the type of compound content, concentration of major and minor compounds, compound interactions, and environmental conditions of plants.

Conclusions

This study marks the first scientific report on the identification of chemical compounds in the *B. polystachyon* leaves using LC-MS and the investigation of the antidiabetic activity of the plant towards the α-glucosidase using *in silico* and *in vitro* assessment. The identification process revealed that the leaves contained various compounds, including phenolics, flavonoids, glycosides, alkaloids, steroids, terpenoids, phenylpropanoids, polychetides, coumarins, and lignans. The results of *in vitro* assays showed that the methanol extract of this plant has weak inhibitory activity with an IC_{50} value of 289.61 µg/mL, although *in silico* study revealed several major compounds with potential antidiabetic activity. However, further research on the isolation of potential compounds, their combination with nanomaterials, and nanoencapsulation processes could be an option to continue this research as a solution to diabetes problems.

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"Conflict of Interest: The authors declare that they have no conflict of interest"

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