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Original Research Article

The Potency of Java Apple (*Syzygium samarangense*) AS *α*-Glucosidase and α-Amylase Inhibitor: An In-Silico Approach

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ABSTRACT

Diabetes remains one of the health issues in Indonesia. The number of diabetes patients is increasing each year. The number of diabetes patients also impacts the use of diabetes medications, increasing the demand for diabetes drugs. Acarbose is commonly used to manage diabetes by inhibiting the α-glucosidase and α-amylase. However, acarbose has side effects such as diarrhea, abdominal bloating, and borborygmus. Therefore, an alternative with a similar mechanism to acarbose is needed. As reported that Java apple (*Syzygium samarangense*) has the potency to inhibit α-glucosidase and α-amylase. This study aimed to analyze the potency of the ethyl acetate extract of Java apple stem bark as an α-glucosidase and α-amylase inhibitor using an in silico approach. The types of α-glucosidase used are human maltase-glucoamylase (MGAM) and human pancreatic α-amylase (HPA) as α-amylase. From 61 compounds presented in *S. samarangense* ethyl acetate extract, 17 compounds showed good inhibition and docked at the same active site as acarbose (control drug), indicating that the compounds serve as α-glucosidase and α-amylase inhibitors. The binding affinity of these compounds ranges from -8.4 to -10.8 kcal/mol. Three compounds (epigallocatechin, isoengeletin, and kaempferol-3-*O*-rhamnoside) showed good drug-likeness and drug score value. The drug-likeness value is 0.31525, 1.8995, and 1.9289; the drug score value is 0.82, 0.79, and 0.77, respectively. The toxicity of these compounds was not detected. Therefore, epigallocatechin, isoengeletin, and kaempferol-3-*O*-rhamnoside are promising drug candidates.

***Keywords*:** *Syzygium samarangense*, α-glucosidase, α-amylase, in silico

Introduction

Diabetes mellitus is one of the health problems in Indonesia. It is categorized as a non-communicable disease and possesses a significant health challenge in the country. In 2019, the number of diabetes patients in Indonesia reached 10.7 million, making Indonesia to be the seventh country with the highest number of diabetes patients.[1] According to the Ministry of Health Republic of Indonesia's research in Basic Health Research (Riskesdas) in 2018, the prevalence of diabetes mellitus in Indonesia based on a doctor's diagnosis among individuals above 15 years is 2%. This value indicates an increase compared to the prevalence of diabetes in the same age group in 2013, which was 1.5%.[2] α-Glucosidase and α-amylase are essential enzymes in carbohydrate metabolism. α-Amylase work to degrade complex carbohydrates from food into disaccharides and oligosaccharides. The α-glucosidase is responsible for converting disaccharides and oligosaccharides into monosaccharides. The intestine then absorbs the released glucose, leading to postprandial hyperglycemia. Inhibiting α-glucosidase and α-amylase is crucial in managing type 2 diabetes mellitus by delaying the hydrolysis of complex carbohydrates.[3]

Plants and microorganisms are rich sources of α-glucosidase and α-amylase inhibitors.[3,4] One of the inhibitor agents commonly used in diabetes management is acarbose. However, acarbose has side effects, such as diarrhea, abdominal bloating, and borborygmus.[5]

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Therefore, natural inhibitor agents are crucial in diabetes management as they have no side effects and are more cost-effective than synthetic drugs.[6]

With its natural wealth, the plants growing in Indonesia have potency as medicinal agents, especially in antidiabetic treatments. Plants from the *Syzygium* genus, such as *S. samarangense*, *S. cumini*, *S. malaccense*, *S. polyanthum*, *S. aqueum*, and *S. aromaticum*, have the potential as α-glucosidase and α-amylase inhibitors. The roots of *S. samarangense* have been found to possess antioxidant, antimicrobial, antidiabetic, and anti-inflammatory properties.[7] The stems and wood bark have antifungal activity.[8] The leaves are traditionally used for cracked lips, asthma, fever, anti-hyperglycemic, and bronchitis.[9] Also, the leaf extract of the plant significantly reduced blood glucose levels in mice.[10] Meanwhile, the fruit of the plant is used to heal mouth sores, improve blood circulation, alleviate fever, soothe sore throat[7], antidiabetic, and antihyperglycemic.[11] Another plant from *Syzygium* genus also possess antidiabetic activity and enzyme inhibition. Extracts from *S. cumini* can inhibit α-glucosidase and maltase.[3] Furthermore, ursolic and oleanolic acids isolated from *S. cumini* leaf strongly inhibited α-glucosidase dan α-amylase.[12] The active compounds of *S. aqueum* showed α-glucosidase inhibition activity.[13] The ethanol extract of *S. myrtifolium* leaves also showed α-glucosidase inhibition.[13] Epigallocatechin gallate and myricitrin isolated from *S. polyanthum* showed α-glucosidase inhibition.[12] This study tried to explore the potency of *S. samarangense* as an alternative α-glucosidase and α-amylase inhibitor. The study utilizes an in silico approach to analyze the potency of the ethyl acetate extract of the plant stem bark as an inhibitor of these enzymes. Based on the explanations, this study aimed to conduct an initial screening of compounds found in the ethyl acetate extract as inhibitors of α-glucosidase and α-amylase using an in silico approach.

The method used in this research is in silico, which uses computer-assisted predictions to analyze the interaction between drug compounds and the receptor.[14] Molecular Docking offers various tools utilized in drug design and discovery. It facilitates the visualization of molecular

structures in databases for medicinal chemists. Additionally, it accurately predicts the binding of ligands within receptors, enhancing the drug design process. Molecular docking is time-saving and cost-effective, making it valuable for novel drug development. It proved to be highly beneficial for future medicinal chemists in exploring novel drug designs and the drug development process.[15] This method is also used for screening of antihyperglycemic, antidiabetic, and enzyme inhibition activity.[16–20]

Methods

Materials

The materials used in this study are *S. samarangense* stem bark, ethyl acetate (≤ 100%, Merck, Germany), distilled water (Bratachem Chemicals, Indonesia), the protein structure of α-glucosidase (PDB ID 3TOP) and α-amylase (PDB ID 2QV4) (downloaded from https //www.rcsb.org/), ligand structures (downloaded from https //pubchem.ncbi.nlm.nih.gov/). *S. samarangense* stem bark was collected from Papar, Kediri, East Java, Indonesia (-7.6950161,112.0779763) in October 2018. The plant materials were deposited at LIPI-Herbarium, Purwodadi, East Java, Indonesia, under voucher specimen number 1498/IPH.06/HM/X/2018.

Equipments, Instruments, and Tools

The equipments used for this study included extraction chamber, vacuum pump (VE2100N, Value, Poland), Buchner flask (Pyrex, USA), Buchner funnel (Haldenwanger, Germany), filter paper, Beaker glass (Pyrex, USA), spatula, Buchi Rotavapor (R-300, Buchi, Switzerland), Shimadzu LC-MS (8040 LC/MS, Shimadzu, Japan), and LC-MS column (Shimadzu Shim Pack FC-ODS (2 mm x 150 mm, 3 µm)). Docking study was perform used Lenovo, Windows 11 Home Single Language, Intel(R) Core(TM) i3-10110U CPU @ 2.10GHz 2.59 GHz, 8,00 GB RAM, and 64-bit operating system. The used software included Biovia Discovery Studio 2016 Client (Dassault Systèmes, Vélizy-Villacoublay, France), ChemDraw 15.1 (PerkinElmer Informatics, Inc., USA), PyRx 0.8 (Sargis Dallakyan, The Scripps Research Institute, USA), OSIRIS DataWarrior 5.5.0 software (Acetilion Pharmaceuticals Ltda., Rio de Janeiro, Brazil), and OSIRIS Property Explorer (Acetilion Pharmaceuticals Ltda., Rio de Janeiro, Brazil).

Identification of Compounds Consisted of The Extract

The compounds presented in the ethyl acetate extract of Java apple stem bark were identified using a LC-MS instrument (Shimadzu LC-MS – 8400 LC/MS) equipped with a capillary column (Shimadzu Shim Pack FC-ODS). The diameter of the column is 2 mm×150 mm×3 µm with injection volume 1 µl. The instrument was set as follows: capillary voltage 3 kv; column temperature 35 ºC; flow rate 0,5 mL/min; solvent is methanol 90% with water; MS focused ion mode type $[M]^+$; collision energy 5,0 V; desolvation gas flow 60 mL/hr; desolvation temperature 350 ºC, scanning 0,6 sec/scan (mz 10-1000); mobile phase mode isocratic; source temperature 100 ºC; and run time 80 minutes. Secondary metabolites were identified using a database based on molecular mass spectra and peaks from the LC-MS chromatogram.

Molecular Docking and Visualization

The protein used is human maltase-glucoamylase (MGAM) as α-glucosidase (PDB ID: 3TOP)[21] and α-amylase (PDB ID: 2QV4)[22] downloaded from the Protein Data Bank (https://www.rcsb.org/). Water and native ligand were removed from the protein structure using Biovia Discovery Studio 2016 (Dassault Systèmes, Vélizy-Villacoublay, France). The chemical structures of the identified compounds from Java apple were searched using PubChem (https://pubchem.ncbi.nlm.nih.gov/). The 3D structures of the compounds were downloaded in .sdf (Structure Data Format) format. The succesfully downloaded compounds were energy-minimized compounds using OpenBabel which is available in PyRx version 0.8 software. The docking process was performed using PyRx version 0.8 with Autodock Vina. The grid position was set at x = 12,384745; y = 48,136073; z = 26,209218 for 2QV4 and x = -53,337000; y = 9,738273; z = -64,733545 for 3TOP. The grid box size for both proteins was set at x = 25,0000; y = 25,0000; dan z = 25,0000. After docking process, compounds with a binding affinity smaller than the control drug (acarbose) were selected.[6] The interactions between the ligands and the proteins were analyzed in 2D and 3D using Biovia Discovery Studio 2016.

Drug-likeness

The ethyl acetate extract of Java apple stem bark was analyzed for its drug-likeness potency based on Lipinski's rule of five, which states that a compound is likely to be drug-like if it meets the following criteria: molecular weight (MW) < 500, clogP ≤ 5, hydrogen bond acceptor (HBA) ≤ 10, hydrogen bond donor (HBD) ≤ 5, and number of rotatable bonds (n-ROTB) ≤ 10.[23] Drug-likeness score was carried out using OSIRIS DataWarrior 5.5.0 software (Acetilion Pharmaceuticals Ltda., Rio de Janeiro, Brazil).

Risk of Toxicity and Drug Scoring Analysis

Toxicity analysis was performed using the OSIRIS DataWarrior 5.5.0 software. This application can calculate toxicology parameters to determine tumorigenicity, mutagenicity, effects on reproduction, and irritating effects.[24] Drug scoring analysis was evaluated using OSIRIS Property Explorer (Acetilion Pharmaceuticals Ltda., Rio de Janeiro, Brazil).[25]

Results aAnd Discussion

LC-MS Results

The LC-MS chromatogram of the ethyl acetate extract of Java apple is shown in Figure 1. From Figure 1, it can be seen that there are 61 compounds identified in the extract. These identified compounds are screened as potential inhibitors of α-glucosidase and α-amylase using in silico.

Molecular Docking Analysis

All identified compounds were subjected to docking with the α-glucosidase and α-amylase. Then, compounds with binding affinity values smaller than acarbose were selected. The results of molecular docking for compounds with binding affinity values smaller than acarbose are presented in Table 1. In α-glucosidase (3TOP), acarbose has a binding affinity value of -8.2 kcal/mol, with the amino acid residues that bind to acarbose being LYS 1164, LYS 1460, ASP 1526, ARG 1510, ASP 1420 (H-bond), TYR 1251 (pi-sigma), and PHE 1560 (pi-alkyl). In α-amylase (2QV4), the binding affinity value for acarbose is -8.3 kcal/mol, with the amino acid residues TRP 59, ASP 300, HIS 305, GLN 63 (H-bond), TRP 59 (pi-sigma), and TYR 62 (pi-alkyl) binding to acarbose. There are 17 active compounds in the extract with lower binding affinity values than acarbose. The binding affinity value is related to the strength of the bond between the ligand and the receptor, where a lower binding affinity value indicates a more stable bond between the ligand and the receptor[26], and the reaction between the ligand and receptor can occur spontaneously.[27] The binding affinity values of these compounds range from -8.4 to -10.8 kcal/mol. The docking results showed negative values, indicating that the 17 compounds exhibited good inhibition against the α-glucosidase and α-amylase.

The visualization of the molecular docking results toward the 17 compounds is shown in Figure 2, and the amino acid residues are presented in Table 2. These figures illustrated the interactions between the α-glucosidase and α-amylase with the ligands. In the α-glucosidase, most ligands bind to identical amino acid residues, namely TYR1251, PHE1560, TRP1355, and TRP1369. Most ligands also bind to identical amino acid residues as acarbose (TYR1251 and PHE1560). These findings were consistent with previous research indicating that the active site of the α-glucosidase (PDB ID: 3TOP) included TRP1418, TYR1251, TRP1355, TRP1369, PHE1560, and TRP1523.[28] From the results of this research, it was evident that the tested ligands bind to the active site of the enzyme.[29] Several compounds such as biflorin, campesterol glucoside, epigallocatechin, kaempferol, kaempferol-3-*O*-rhamnoside, and pinocembrin bind to the same amino acid residue as acarbose (ASP1526) through hydrogen bonding.[16] Meanwhile, isoengeletin bind to the amino acid residue LYS1460.[18]

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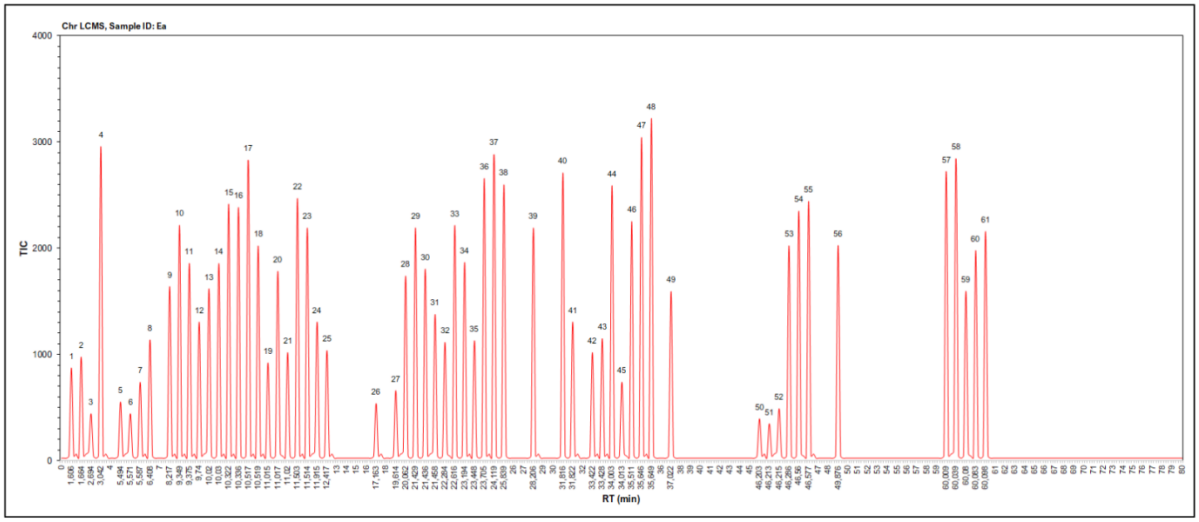


Figure 1: LC-MS chromatogram of the ethyl acetate extract of Java appl

Table 1: The binding affinity values of the ligands with each protein and the drug-likeness values according to Lipinski's rule of five

Compounds	3TOP	2QV4	MW	clogP	HBA	HBD	n-ROTB	Violation of lipinski's rule
Rule			<500	≤5	≤ 10	≤ 5	≤10	≤1
Acarbose	-8.2	-8.3	645.605	-7.1771	19	14	9	Yes (3)
(-)-strobopinin	-8.8	-8.8	270.283	2.8453	4	2	1	No (0)
(+)-6-8-di-C-methylpinocembrin-5-methyl ether	-8.7	-8.9	298.337	3.4649	4	1	2	No (0)
7-hydroxy-5-methoxy-6-8dimethylflavanone	-9.4	-8.8	298.337	3.4649	4	1	2	No (0)
beta-sitosterol	-9.9	-9.5	414.715	7.8552	1	1	6	No (1)
beta-sitosterol-D-glucoside	-8.4	-9.7	576.855	6.0181	6	4	9	No (1)
Betulin	-8.5	-9.2	442.725	6.7202	2	2	2	No (1)
Biflorin	-9.5	-8.7	354.31	-1.0269	9	6	2	No (1)
Campesterol glucoside	-9.4	-10.8	562.829	5.5637	6	4	8	No (1)
Demethoxymatteucinol	-8.9	-8.6	284.31	3.1892	4	2	1	No (0)
Epibetulinic acid	-8.7	-8.6	456.708	6.3706	3	2	2	No (1)
Epigallocatechin	-8.9	-9.0	306.269	1.163	7	6	1	No (1)
Isoengeletin	-9.4	-9.0	434.396	0.3932	10	6	3	No (1)
Kaempferol	-8.7	-9.1	286.238	1.8359	6	4	1	No (0)
Kaempferol-3-*O*-rhamnoside	-9.1	-8.2	432.38	0.9255	10	6	3	No (0)
Kaempferol-7-rhamnoside	-9.7	-9.4	432.38	0.7733	10	6	3	No (0)
Lupeol	-8.5	-9.7	426.726	7.6469	1	1	1	No (1)
Pinocembrin	-8.8	-8.4	256.256	2.5014	4	2	1	No (0)

3TOP - PDB ID of human maltase-glucoamylase (MGAM); 2QV4 - PDB ID of human pancreatic α-amylase (HPA); MW – molecular weight; clogP - Consensus octanol-water partition coefficient; HBA - hydrogen bond acceptor; HBD - hydrogen bond donor; n-ROTB - number of rotatable bond.

Similarly to α-glucosidase, the molecular docking results toward the 17 compounds with α-amylase also shown that the ligands bind to the same amino acid residues (TRP59, TYR62, and HIS305). The binding of ligands to the same amino acid residues as acarbose indicated that the tested ligands bind to the enzyme's active site.[20] Most ligands even bind to the same amino acids as acarbose (TRP59 and TYR62) through conventional H-bonds and hydrophobic interactions. The most influencing factors for ligand binding to the receptor are hydrophobic interactions, hydrogen bonding, and electrostatic interactions.[29,30] When ligands exhibit these interactions, they can significantly contribute to inhibiting an enzyme. These interactions play a crucial role in forming stable complexes between the ligands and the enzyme's active site, thus inhibiting the enzyme's activity and potentially serving as effective inhibitors for therapeutic purposes.

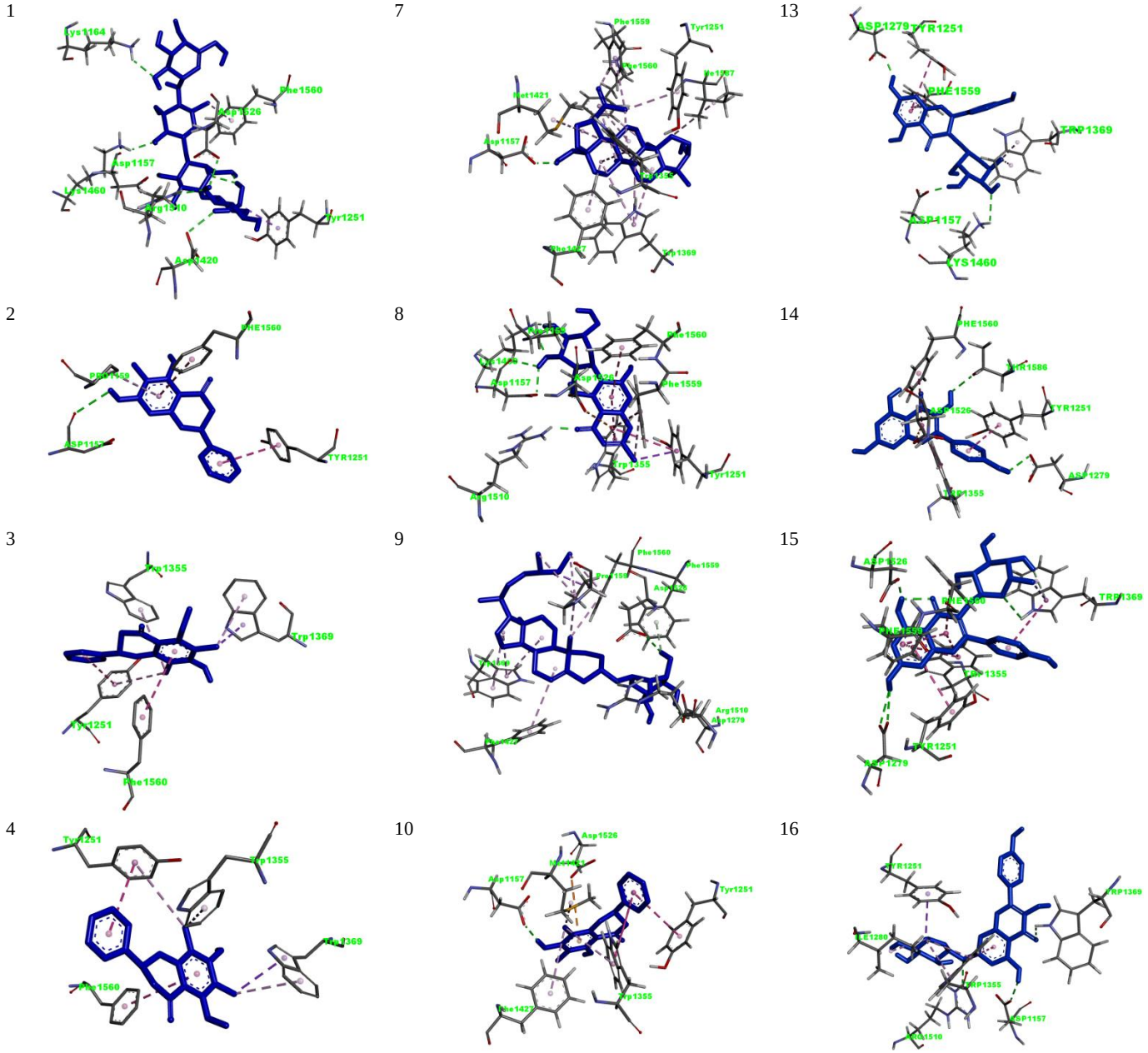
Acarbose is a diabetes medication used as a digestive inhibitor to treat type II diabetes. Acarbose inhibits the α-glucosidase found in intestinal enterocytes and α-amylase in the pancreas. The function of α-amylase is to digest starch into oligosaccharides, which are then converted by α-

glucosidase into monosaccharides. Acarbose has a high binding affinity to α-amylase, which prevents α-amylase from binding to starch and thus prevents the digestion of starch into monosaccharides. The inability of digestive enzymes to hydrolyze acarbose is due to the presence of an imine bridge that digestive enzymes cannot break down. This characteristic becomes a critical element of enzyme inhibition, as acarbose effectively interferes with the enzymatic breakdown of carbohydrates, preventing the conversion of starch into monosaccharides.[5]

Acarbose was used as a control drug in this study because previous research has shown that acarbose exhibits excellent inhibitory activity. Acarbose demonstrated a binding affinity of -7.4 kcal/mol towards α-glucosidase[31] and -7.3 kcal/mol towards α-amylase.[21] Meanwhile, other studies have also shown that the binding affinity towards α-glucosidase is -6.7 kcal/mol and towards α-amylase is -7.7 kcal/mol.[32] The values from these results are lower than previous research, showing -8.2 kcal/mol for α-glucosidase and -8.3 kcal/mol for α-amylase.

The compounds in the extract showed good inhibitory activity, as indicated by their lower binding affinity values than the control drug. Previous studies have also shown that compounds found in the plant, such as pinocembrin, have the potency for anti-inflammatory properties.[30] In diabetes, inflammation, and oxidative stress play a crucial role.[33] Other in silico screening studies have mentioned that compounds from the *Syzygium* genus plants have the potency as antidiabetic agents. D-(+)-Catechin, a flavonoid compound from *S. cumini* var. album, showed high affinity towards the α-glucosidase with a binding affinity value -5.94 kcal/mol.[34] Moreover, delphinidin-3-gentiobioside and isoquercitrin from *S. cumini* demonstrated significant inhibition of the α-glucosidase enzyme (both -8.5 kcal/mol).[35] In addition to inhibiting the α-glucosidase, compounds from the *Syzygium* genus also showed inhibitory activity against the α-amylase. E-β-caryophyllene found in the essential oil of *S. cumini* demonstrated α-amylase inhibitory activity with a binding affinity value of -5.61 kcal/mol.[36] This study contributes to the current research on the bioactivity of the *Syzygium* genus, particularly its inhibitor activity against α-glucosidase and α-amylase, with a special emphasis on *S .samarangense*.

α-glucosidase



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α-glucosidase

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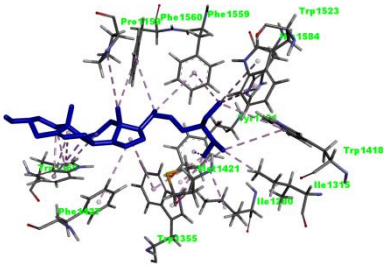

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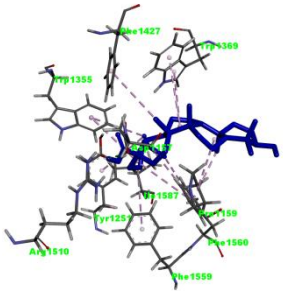

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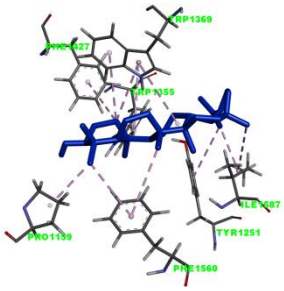

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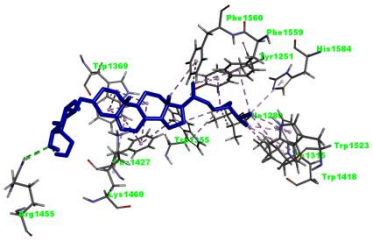

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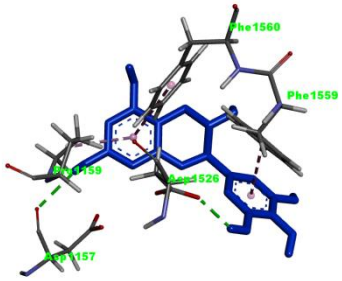

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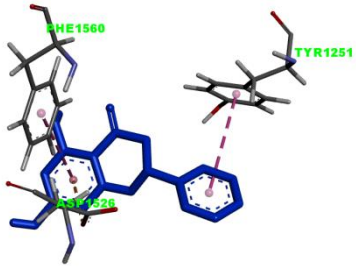

α-amylase

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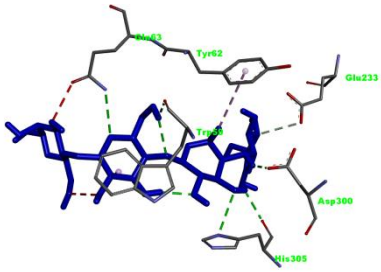

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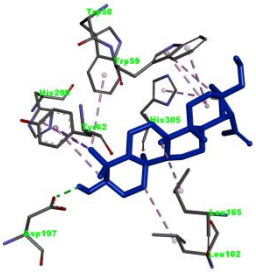

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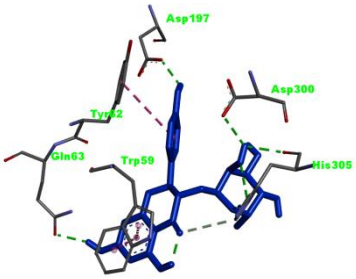

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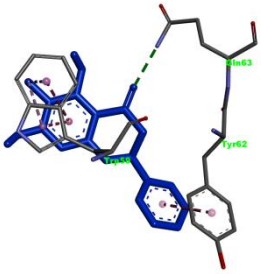

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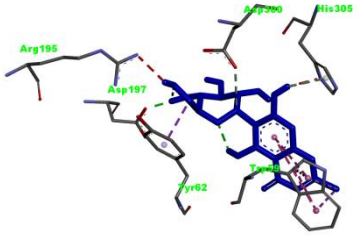

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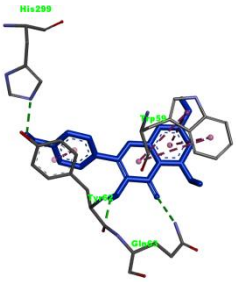

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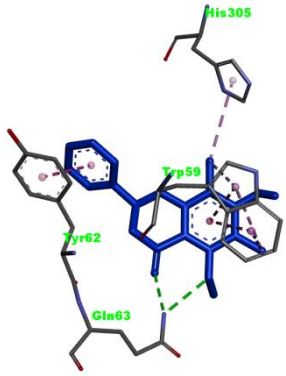

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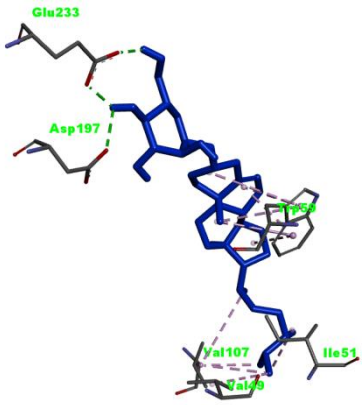

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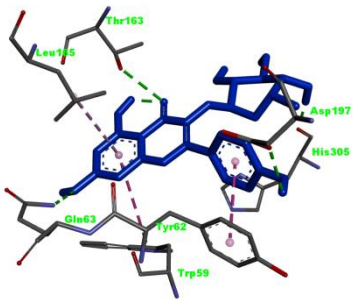

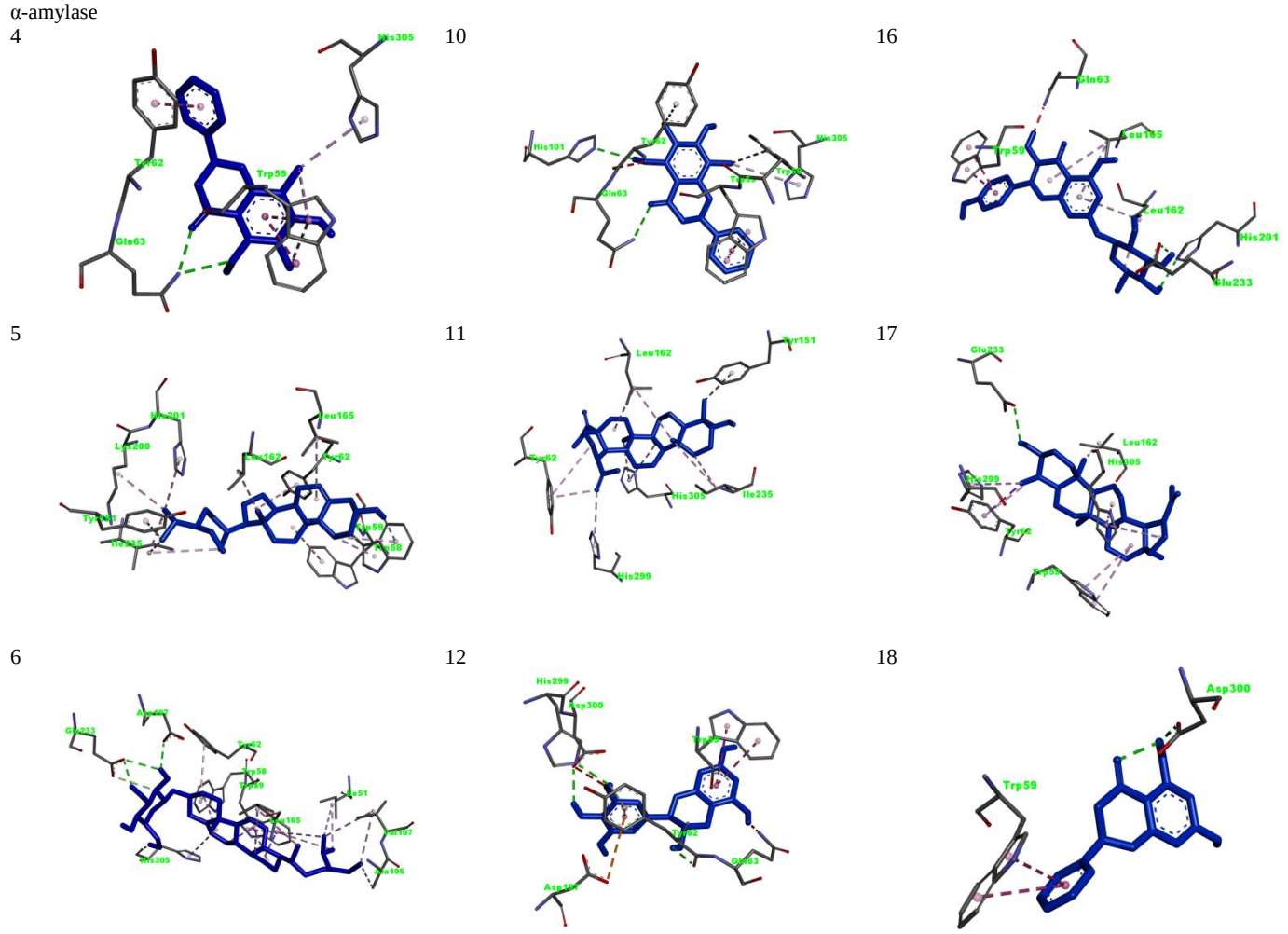

Figure 2: The visualization of the molecular docking results of acarbose and the 17 active compounds from the extract. which have the potency to inhibit (I) α-glucosidase and (II) α-amylase.
The amino acid residues involved in the binding of each ligand; 1. Acarbose; 2. (-)-Stropopinin; 3. (+)-6.8-Di-C-methylpinocembrin-5-methylether; 4. 7-Hydroxy-5-methoxy-6-8 dimethylflavanone; 5. beta-sitosterol; 6. beta-sitosterol-D-glucoside; 7. Betulin; 8. Biflorin; 9. Campesterol glucoside; 10. Demethoxymatteucinol; 11. Epibetulinic acid; 12. Epigallocatechin; 13. Isoengeletin; 14. Kaempferol; 15. Kaempferol-3-*O*-rhamnoside; 16. Kaempferol-7-rhamnoside; 17. Lupeol; and 18. Pinocembrin.

Drug-likeness Analysis
Drug-likeness analysis using Lipinski's Rule of Five stated that orally active compounds should not violate more than one of the following criteria: MW (molecular weight) < 500, clogP (logP) ≤ 5, HBA (hydrogen bond acceptors) ≤ 10, HBD (hydrogen bond donors) ≤ 5, and nROTB (number of rotatable bonds) ≤ 10.[23,37] The results of the rule analysis are shown in Table 1. All selected compounds meet these criteria, indicating that all compounds are suitable for drug candidate development.[38]
In addition to the drug-likeness analysis using the rule, drug-likeness analysis was also conducted computationally with OSIRIS DataWarrior. The results of the drug-likeness scores are shown in Table 4. The drug-likeness score is related to the suitability of a compound as a drug candidate. The compound may be a drug candidate if the drug-likeness score is positive.[39] The drug-likeness approach is based on common substructure fragments in commercial drugs. A positive drug-likeness score indicates that the molecule contains some of the fragments commonly found in commercial drugs, suggesting that the compound resembles drugs and has a high chance of success in developing antidiabetic drugs.[40] There are only four compounds having positive drug-likeness values, namely epigallocatechin (0.31525), isoengeletin (1.8995), kaempferol-3-*O*-rhamnoside (1.9289), and kaempferol-7-rhamnoside (1.8856).

Toxicity and Drug Score Analysis
After conducting the drug-likeness analysis, toxicity analysis was performed using OSIRIS DataWarrior. The measured toxicity values include mutagenic (M), tumorigenic (T), reproductive effect (RE), and irritant (I) with the same software. Meanwhile, the drug score was calculated using the OSIRIS property explorer software. The drug score combines the results of drug-likeness, cLogP, logS, molecular weight, and toxicity to provide a value that can be used to assess the potency of a compound as a drug.[25] Toxicity and drug score analysis showed in Table 3.
The four compounds previously considered to be potential drugs (epigallocatechin, isoengeletin, kaempferol-3-*O*-rhamnoside, and kaempferol-7-rhamnoside) showed different results in the analysis. Epigallocatechin showed a drug-likeness value of 0.31525. Although this value is the smallest among the four compounds, it is still higher than the drug-likeness value of acarbose (-7.4039). Furthermore, the drug score of epigallocatechin is high (0.82). Epigallocatechin also demonstrated no toxicity (negative results for mutagenicity, tumorigenicity, reproductive effects, and irritant).

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Table 2: Amino acid residues and types of interactions for each compound in the ethyl acetate extract of Java apple stem bark towards α-glucosidase (PDB ID 3TOP) and α-amylase (PDB ID 2QV4)

Protein	Ligand	Amino acid residues and type of interactions										
			Hydrophobic interaction					Non-classical H-bond		Electrostatic	Unfavorable	
		Conventional H-bond	Pi-sigma	Pi-pi stacked	Pi-Alkyl	Pi-Pi T-shapped	Alkyl	Carbon hydrogen	Pi-donor hydrogen bond	Pi-anion	Donor-donor	Acceptor-aceptor
3TOP	Acarbose	LYS1164. LYS1460. ASP1526. ARG1510. ASP1420	TYR1251	-	PHE1560	-	-	-	-	-	-	ASP1157
	(-)-strobopinin	ASP1157	-	-	PRO1159	PHE1560. TYR1251	-	-	-	-	-	-
	(+)-6-8-di-C-methylpinocembrin-5-methyl ether	-	TRP1369	-	TRP1355	PHE1560. TYR1251	-	-	-	-	-	-
	7-hydroxy-5-methoxy-6-8dimethylflavanone	-	TRP1369	-	TRP1355	PHE1560. TYR1251	-	-	-	-	-	-
	beta-sitosterol	-	TRP1369. TYR1251	-	TRP1369	-	HIS1584. PHE1559. PHE1427. TRP 1355. PRO1159. PHE1560. ILE1280. ILE1315. MET1421.	-	-	-	-	-

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Protein	Ligand	Amino acid residues and type of interactions										
			Hydrophobic interaction					Non-classical H-bond		Electrostatic	Unfavorable	
		Conventional H-bond	Pi-sigma	Pi-pi stacked	Pi-Alkyl	Pi-Pi T-shapped	Alkyl	Carbon hydrogen	Pi-donor hydrogen bond	Pi-anion	Donor-donor	Acceptor-aceptor
							TRP1418. TRP1523					
	beta-sitosterol-D-glucoside	ARG1455	TRP1369. TYR1251	-	PHE1427	-	HIS1584. TRP1523. ILE1315. TRP1418. ILE1280. PHE1559. TRP1355. PHE1560. PHE1427. LYS1460	-	-	-	-	-
3TOP	Betulin	ASP1157	-	-	PHE1559	-	ILE1587. TRP1369. PHE1560. MET1421. TRP1355. PHE1427. PHE1559. TYR1251	-	-	-	-	-
	Biflorin	ARG1510. ASP1526. ASP1157. LYS 1460	TYR1251	-	PHE1559	TYR1251. PHE1559. TRP1355. PHE1560	-	PRO 1159	-	ASP1526	-	-

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Protein	Ligand	Amino acid residues and type of interactions										
			Hydrophobic interaction					Non-classical H-bond		Electrostatic	Unfavorable	
		Conventional H-bond	Pi-sigma	Pi-pi stacked	Pi-Alkyl	Pi-Pi T-shapped	Alkyl	Carbon hydrogen	Pi-donor hydrogen bond	Pi-anion	Donor-donor	Acceptor-aceptor
3TOP	Campesterol glucoside	ASP1526. ASP1279. ARG 1510	PHE1560	-	PHE1427	-	TRP1369. PRO1159. PHE1560	-	-	-	-	-
	Demethoxymatteucinol	ASP 1157	-	-	-	TRP 1355. TYR 1251	PHE 1427. MET 1421. TRP 1355	-	-	ASP1526	-	-
	Epibetulinic acid	ASP1157	-	-	ILE1587. TYR1251. TRP1355	-	TRP 1369. PRO 1159. PHE 1427. TRP 1355. PHE 1559	-	-	-	ARG1510	-
	Epigallocatechin	ASP1157. ASP1526.	-	-	PRO1159	PHE1559. PHE1560	-	-	-	-	-	-
	Isoengeletin	LYS1460. ASP1157. ASP1279	-	-	TRP1369	TYR1251. PHE1559	-	-	-	-	-	-
	Kaempferol	THR1586	-	-	-	TYR1251. PHE1560. TRP1355	-	-	-	ASP1526	-	-

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Protein	Ligand	Amino acid residues and type of interactions										
			Hydrophobic interaction					Non-classical H-bond		Electrostatic	Unfavorable	
		Conventional H-bond	Pi-sigma	Pi-pi stacked	Pi-Alkyl	Pi-Pi T-shapped	Alkyl	Carbon hydrogen	Pi-donor hydrogen bond	Pi-anion	Donor-donor	Acceptor-aceptor
	Kaempferol-3-*O*-rhamnoside	ASP1279. ASP1526. TRP1369	TRP1369	-	-	TRP1369. TYR1251. PHE1560. PHE1559. TRP1355	-	-	TRP1369	-	-	-
	Kaempferol-7-rhamnoside	ASP1157. TRP1369. ARG 1510	TYR1251	-	-	TRP1355	ILE1280. TRP1355	-	-	-	-	-
3TOP	Lupeol	-	-	-	-	-	ILE1587. TYR1251. TRP1369. TRP1355. PHE1560. PHE1427. PRO1159	-	-	-	-	-
	Pinocembrin	-	-	-	-	TYR1251. PHE1560	-	-	-	ASP1526	-	-
2QV4	Acarbose	TRP59. ASP300. HIS305. GLN63	TRP59	-	TYR62	-	-	-	-	-	-	-

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			Hydrophobic interaction					Non-classical H-bond		Electrostatic	Unfavorable	
		Conventional H-bond	Pi-sigma	Pi-pi stacked	Pi-Alkyl	Pi-Pi T-shapped	Alkyl	Carbon hydrogen	Pi-donor hydrogen bond	Pi-anion	Donor-donor	Acceptor-aceptor
2QV4	(-)-stropopinin	GLN63	TRP59	TYR62. TRP59	-	-	-	-	-	-	-	-
	(+)-6-8-di-C-methylpinocembrin-5-methyl ether	GLN63	TRP59	TYR62. TRP59	HIS305. TRP59	-	-	-	-	-	-	-
	7-hydroxy-5-methoxy-6-8dimethylflavanone	GLN63	TRP59	TYR62. TRP59	HIS305. TRP59	-	-	-	-	-	-	-
	beta-sitosterol	-	-	-	LEU165	-	HIS201. LYS200. TYR151. ILE235. LEU162. LEU165. TRP59. TYR62. TRP 58	-	TRP59	-	-	-
	beta-sitosterol-D-glucoside	ASP197. GLU233. HIS 305	TRP59	-	-	-	TRP58. TYR62. ILE51. VAL107. ALA106. LEU165. TRP59	GLU233	-	-	-	-

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Protein	Ligand	Amino acid residues and type of interactions										
			Hydrophobic interaction					Non-classical H-bond		Electrostatic	Unfavorable	
		Conventional H-bond	Pi-sigma	Pi-pi stacked	Pi-Alkyl	Pi-Pi T-shapped	Alkyl	Carbon hydrogen	Pi-donor hydrogen bond	Pi-anion	Donor-donor	Acceptor-aceptor
2QV4	Betulin	ASP197	TYR62	-	-	-	LEU162. HIS299. LEU165. TRP59. HIS305. TRP58. TYR62	-	-	-	-	-
	Biflorin	ASP197	TYR62. TRP59	TRP59	-	-		-	-	-	ARG195. HIS305	
	Campesterol glucoside	GLU253. ASP197	-		TRP59	-	VAL49. ILE51. VAL107. TRP59	-	-	-	-	-
	Demethoxymatteucinol	HIS101. GLN 63	-	TRP 59	TRP58. HIS305. TYR62	-		-	-	-		TYR62
	Epibetulinic acid	-	-	-	TYR62. HIS299	-	HIS305. TYR151. ILE235. LEU162	-	-	-	-	-
	Epigallocatechin	TYR62.	-	TRP59	-	-	-	-	-	ASP197	GLN63	ASP300

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Protein	Ligand	Amino acid residues and type of interactions										
			Hydrophobic interaction					Non-classical H-bond		Electrostatic	Unfavorable	
		Conventional H-bond	Pi-sigma	Pi-pi stacked	Pi-Alkyl	Pi-Pi T-shapped	Alkyl	Carbon hydrogen	Pi-donor hydrogen bond	Pi-anion	Donor-donor	Acceptor-aceptor
		HIS299. ASP300										
	Isoengeletin	HIS305. ASP300. GLN63 ASP 197	-	TRP59. TYR62	-	-	-	-	-	-	-	-
	Kaempferol	GLN63. TYR62. HIS299	-	TRP59. TYR62	-	-	-	-	-	-	-	-
2QV4	Kaempferol-3-*O*-rhamnoside	THR163. HIS305. ASP197. GLN63	-	TRP59. TYR62	LEU165	-	-	-	-	-	-	-
	Kaempferol-7-rhamnoside	GLU233. HIS201	-	TRP59	LEU162. LEU165	-	LEU162	-	-	-	GLN63	-
	Lupeol	GLU 233	TYR62	-	-	-	TYR62. HIS299. LEU162. TRP59. HIS305	-	-	-	-	-
	Pinocembrin	ASP300	-	TRP59	-	-	-	-	-	-		

Table 3: Toxicity, drug-likeness, and drug score of the compounds in the ethyl acetate extract of Java apple stem bark

Compounds	Toxicity				Drug-likeness	Drug score
	M	T	RE	I		
Acarbose	none	none	none	none	-7.4039	0.29
(-)-strobopinin	none	none	none	none	-0.23425	0.79
(+)-6-8-di-C-methylpinocembrin-5-methyl ether	none	none	none	none	-0.087682	0.6
7-hydroxy-5-methoxy-6-8-dimethylflavanone	none	none	none	none	-0.087682	0.6
beta-sitosterol	none	none	none	none	-4.475	0.13
beta-sitosterol-D-glucoside	none	none	none	none	-8.3009	0.12
Betulin	none	none	none	none	-23.933	0.15
Biflorin	none	none	none	none	-8.4272	0.45
Campesterol glucoside	none	none	none	none	-11.881	0.14
Demethoxymatteucinol	none	none	none	none	-0.23425	0.62
Epibetulinic acid	none	none	none	none	-21.49	0.15
Epigallocatechin	none	none	none	none	0.31525	0.82
Isoengeletin	none	none	none	none	1.8995	0.79
Kaempferol	high	none	none	none	-0.082832	0.46
Kaempferol-3-*O*-rhamnoside	none	none	none	none	1.9289	0.77
Kaempferol-7-rhamnoside	high	none	none	none	1.8856	0.46
lupeol	none	none	none	none	-22.172	0.13
Pinocembrin	none	none	none	none	-0.22006	0.83

M – Mutagenicity; T – tumorigenic; RE - reproductive effect; I – irritant

On the other hand, isoengeletin exhibited a drug-likeness value of 1.8995 and a drug score of 0.79. These values indicate that isoengeletin has a high potency as an antidiabetic drug candidate, especially in inhibiting α-glucosidase and α-amylase. Additionally, no toxicity was found for isoengeletin regarding mutagenicity, tumorigenicity, reproductive effects, and irritant, making it a promising drug candidate. In addition, kaempferol-3-O-rhamnoside exhibited the highest drug-likeness value, 1.9289, with a drug score 0.77. Toxicity analysis showed no irritation, tumorigenicity, mutagenicity, or reproductive effects for this compound. Therefore, kaempferol-3-O-rhamnoside is also considered a promising drug candidate. Another compound in the extract, kaempferol-7-rhamnoside, is not recommended as a drug candidate. Despite having a high drug-likeness value (1.8856), this compound showed harmful effects, leading to a low drug score of 0.46. A low drug score indicates it is not recommended as a drug candidate due to its high mutagenicity.

Conclusion

The molecular docking results revealed 17 compounds with potential as inhibitors of α-glucosidase and α-amylase. Most of these compounds bind to the same active site of the enzymes as acarbose (the control drug) and exhibit lower binding affinity. The analysis using Lipinski's rule of five indicated that all compounds have the potency as oral drugs. Further analysis related to drug-likeness and toxicity identified 3 compounds as potential candidates for α-glucosidase and α-amylase inhibitors. These three compounds are epigallocatechin, isoengeletin, and kaempferol-3-O-rhamnoside. Other compounds showing good molecular docking results can be explored for their potential activities. Further research is needed to isolate the compounds and in vitro assay of α-glucosidase dan α-amylase from isolated compounds. Hopefully, this research can be used as a reference for discovering potential antidiabetic drug candidates with inhibition mechanisms on α-glucosidase and α-amylase

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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