JSMARTech
Journal of Smart Bioprospecting and Technology **p-ISSN**: 2686-0805 **e-ISSN**: 2714-7894
October 2022, VOL 03 NO 02 **DOI**:https://doi.org/10.21776/ub.jsmartech.2022.003.02.43

In Silico Analysis of the Effectiveness of α-glucosidase Inhibitors (AGIs) on *Phaseolus vulgaris* L. as Antidiabetic Agents

Alfi Tsuraya[1], Ainun Anugrah[1], Nur Aini[1], Salsabila Najah[1], Siti Sainidah[1], and Yoravika Dwiwibangga[2] , Nia Kurniawan[1], and Fatchiyah Fatchiyah[1,3,*]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Jalan Veteran, Malang, East Java, Indonesia, 65145
[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Brawijaya University, Jalan Veteran, Malang, East Java, Indonesia, 65145
[3]Research Center of Smart Molecule of Natural Genetics Resource, Brawijaya University, Jalan Veteran, Malang, East Java, Indonesia, 65145

Submission: 21 May 2022; **Revised**: 21 September 2022; **Accepted**: 06 October 2022

***Corresponding author**: Fatchiyah Fatchiyah; **e-mail**: fatchiya@ub.ac.id

ABSTRACT. *Diabetes mellitus is a metabolic disorder characterized by high blood sugar levels accompanied by impaired carbohydrate, lipid, and protein metabolism. Alpha-glucosidase inhibitor is an agent that can inhibits the action of the α-glucosidase enzyme, but the synthetic α-glucosidase inhibitors caused various side effects, therefore the search for AGI from natural active compounds is needed. The objective of this study was to determine the effectiveness of the active compound α-glucosidase inhibitor contained in Phaseolus vulgaris as an antidiabetic agent by in silico methods. Flavonoid compounds such as rutin, isoquercitrin, hyperoside, isorhamnetin-3-glucoside, and acarbose (control), were downloaded from the NCBI PubChem database. Human α-glucosidase (PDB ID: 5KZW) 3D structure was obtained from RCSB PDB. Bioactivity predicted using PASSOnline. ADMET and Drug-Likeness with SwissADME and ProTox II. Molecular docking was conducted using HEX 8.0 and visualized with Discovery Studio. This study showed that all of these compounds have sites that interact with the active sites of the α-glucosidase enzyme, and have several similarities compared to acarbose. All compounds have good potential as α-glucosidase inhibitors. Isorhamnetin-3-glucosidase is the most potential characteristic as an α-glucosidase inhibitor. It has amino acid residues HIS717 (hydrogen), TYR360 (hydrogen and hydrophobic), and ARG608 (hydrogen) which shows higher number of residues in common with acarbose and active sites that also shows fairly high stability. Based on ADME prediction, Isorhamnetin-3-glucoside most likely has the best characteristics based on LogS, %Abs, and also LogKp compared to the other compounds, and has much higher %Abs (40%) than acarbose (-1,8%). It even only has 2 violations of Lipinski rules compared to acarbose that has 3 violations. Thus, overall flavonoid group compounds in P. vulgaris, especially have good potential as a glucosidase inhibitors as antidiabetic agents.*

Keywords: *antidiabetic agent, α-glucosidase inhibitor, Phaseolus vulgaris, flavonoid, molecular docking*

INTRODUCTION

Diabetes mellitus (DM) is a serious, chronic, and complex metabolic disorder that is characterized by high blood sugar levels accompanied by impaired carbohydrate, lipid, and protein metabolism as a result of the body's inability to produce the hormone insulin or due to ineffective use of insulin production. Diabetes mellitus consists of two types namely, type 1 diabetes which is caused by heredity, and autoimmune disease that occurs when the body cannot produce the hormone insulin which is in charge of helping the absorption of sugar in the blood into energy, and type 2 diabetes which is caused by a lifestyle when blood glucose levels rise due to high blood sugar level less than optimal production or absorption of insulin by the body. In general, almost 80% of the prevalence of diabetes mellitus is type 2 [1]. During the development of diabetes, the body's cells cannot metabolize sugar properly due to a lack of insulin action on target tissues due to insensitivity or deficiency of insulin (peptide hormone that regulates blood glucose). Diabetes causes long-term damage, dysfunction, and failure of various organ systems (heart, blood vessels, eyes, kidneys, and nerves), leading to disability and premature death [2].

Alpha-glucosidase inhibitor (AGI) is an antidiabetic agent that works by inhibiting the action of the α-glucosidase enzyme. Reducing the absorption of carbohydrates from food by the intestine is a therapeutic approach for postprandial hyperglycemia. Complex polysaccharides will be hydrolyzed by the enzyme amylase to dextrin and further hydrolyzed into glucose by the enzyme α-glucosidase before entering the blood circulation through the absorption of the epithelium. Synthetic amylase-glucosidase and α-glucosidase inhibitors, such as acarbose, have been widely used for the treatment of patients with type II

diabetes but these drugs have also been reported to cause various side effects [3].

The 10th edition of the International Diabetes Federation (IDF) Atlas reveals that currently at least 1 in 10 people or as many as 537 million people in the world live with diabetes. If there is no intervention, this figure is projected to increase, reaching 643 million in 2030 and 784 million in 2045. Based on the prevalence of diabetes patients in Indonesia, the use of anti-diabetic drugs has increased to affect the prevalence of side effects. Based on a study on the use of anti-diabetic drugs, it is known that the side effects of anti-diabetic drugs are a serious problem that should be overcome [2].

Alpha-glucosidase inhibitors, such as acarbose and miglitol, are among the main types of drugs used to control hyperglycemia. However, the side effects of this drug are sometimes severe and include abdominal distension, flatulence, and diarrhea. Oxidative stress is a known pathological feature of diabetes as well as many other diseases, such as coronary heart disease and cancer. Protection against oxidizing agents or free radicals can be enhanced by an adequate intake of dietary antioxidants. Indeed, epidemiological evidence has shown that eating a diet rich in antioxidants, i.e., antioxidant-rich fruits and vegetables are significantly associated with disease avoidance. Thus, the active compounds from these vegetables and medicinal plants are of great interest in the development of new diabetes treatments [2]. In addition, various types of therapy have been tried by many diabetic patients, but the current paradigm of society regarding diabetes treatment tends toward traditional medicine so the development of traditional health services using herbs or medicinal plants is currently growing rapidly [11].

The kidney red beans (*Phaseolus vulgaris* L.) are a source of protein, complex carbohydrates, minerals, vitamins, and phenolic compounds. Phenolic compounds can be classified as phenolic acids, flavones, flavanones, isoflavones, flavanols, anthocyanins, and condensed tannins. These phenolic compounds have a high potential to inhibit enzymes associated with carbohydrate metabolism and reduce the availability of α-amylase and α-glucosidase [5]. Various previous studies have shown that kidney bean seeds have α-amylase inhibitors that inhibit α-amylase activity in animals and insects, are associated with controlling type-2 diabetes mellitus by inhibiting DPP-IV, and that a lectin called phytohaemagglutinin can adjust the activity of peptides such as glucagon GLP-1 (peptides such as glucagon-1). Currently, the treatment of type-2 diabetes involves the use of enzyme-inhibiting drugs in the stomach to reduce the absorption of glucose in the intestine [6]. Therefore, this study aimed to determine the effectiveness of the active compound α-glucosidase inhibitor present in kidney red beans (*Phaseolus vulgaris* L.) as an antidiabetic agent through the in-silico methods.

RESEARCH METHODS

Data Collection

The dataset used in this study was composed of 11 bioactive compounds from the flavonoid group (Isorhamnetin-3-glucoside, Myricetin-3-rhamnoside, Isoquercitrin, Hyperoside, Isoorientin, Rutin, Orientin, Myricetin, Quercetin, and Luteolinn) from extract red bean (Phaseolus vulgaris L.) identified in the published literature [6]. Acarbose compounds identified in the published literature [7] were used as control compounds. The Three-dimensional (3D) structure data file (sdf) of flavonoid compounds were collected from the NCBI PubChem database (https://pubchem.ncbi.nlm.nih.gov/) as ligands. The protein structure of human α-glucosidase was collected from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank database, with PDB ID: 5KZW (https://www.rcsb.org/) as receptor.

Bioactivity Prediction

Prediction of bioactivity using the online tools PASS Online (http://way2drug.com/passonline/index.php). The bioactivity analysis process (α-glucosidase inhibitor) was carried out by inputting the SMILES format of the flavonoid bioactive compound which was previously obtained from the NCBI PubChem database. Prediction results of compound bioactivity using PASS Online are based on the probability values of the Pa (Probable active) and Pi (Probable inactive) scores with a range of values between 0 and 1. Pa (Probable active) which is close to 1 indicates that the compound belongs to the subclass of active compounds, and Pi (Probable inactive) indicates an inactive compound [8]. From the total of 11 bioactive compounds analyzed, there were 4 compounds with Pa values (Rutin (0.858), Isoquercitrin (0.842), Hyperoside (0.842), and Isorhamnetin-3-glucoside (0.799)) and Acarbose as control which indicated the presence of α-glucosidase inhibitor bioactivity as an antidiabetic type II.

ADMET and Drug-Likeness Prediction

Absorption, Distribution, Metabolism, And Excretion-Toxicity (ADMET) analysis was performed using online tools, SwissADME (http://www.swissadme.ch/) and ProTox II

(https://tox-new.charite.de/protox_II/). SwissADME predicts the pharmacokinetic behavior of a compound based on computational physicochemical properties. The ADMET analysis process was carried out by inputting the SMILES format of flavonoid compounds previously obtained from the NCBI PubChem database. Determination of the oral bioavailability of pharmacologically active compounds or not based on Lipinski's Rule of Five which focuses on the provisions, (i) molecular weight (< 500 daltons), (ii) lipophilicity (MlogP) (< 5), (iii) number H-bond donors (–OH and –NH groups) (< 5), (iv) number H-bond acceptors (mainly N or O atoms) (< 10) and (v) rule violations (<2) [9]. Based on drug-likeness, where the rule violation is zero (0) which indicates the compound has drug similarity. Drug likeness will qualitatively assess the probability of a compound to be used as an oral drug related to bioavailability [10].

Preparation of Protein and Ligand for Molecular Docking Analysis

The conformational three-dimensional (3D) ligands (sdf) (Isorhamnetin-3-glucoside (CID 44258009), rutin (CID 5280805), Isoquercitrin (CID 5280804), Hyperoside (CID 5281643), and Acarbose (CID 41774)) were prepared using PyRx version 8.0 software to minimize ligand energy which aims to simplify the docking process so that the time required for the docking process becomes shorter. The ligand preparation result file is saved in. pdbqt format using 'Open Babel' in PyRx version 8.0 software. The downloaded 3D structure (sdf) receptor (α-glucosidase (5KZW)) was prepared using Discovery Studio software to remove water molecules, native ligands, and unnecessary complexes present in the structure (receptor). The receptor preparation result file is saved in .pdbqt format.

Molecular Docking

Ligands (Isorhamnetin-3-glucoside (CID 44258009), Rutin (CID 5280805), Isoquercitrin (CID 5280804), Hyperoside (CID 5281643), and Acarbose (CID 41774)) were added to the receptor (α-glucosidase (5KZW)). The interaction and bond energy formed between receptor and ligand were calculated using rigid docking type HEX 8.0 software. Confirmation of docking results in the form of Root Mean Square (RMS) value. RMS will measure the strength of chemical bonds at the binding sites. RMS (-1.00), indicates that the docking results are valid, good, and there are no errors in the program [11, 12]. The visualization of the docking results was analyzed using the Discovery Studio Visualizer. The main parameters observed were non-bond interactions such as hydrogen bonds. The hydrogen bond is the strongest bond because it has a short bond distance and is an intermolecular force that plays a role in stabilizing biomolecules [13].

RESULTS AND DISCUSSIONS

Bioactivity, ADMET, and Drug-likeness Prediction

Bioactivity prediction is required to obtain the expected biological activity profile of the desired compound for the computer-designed virtual structure prior to synthesis [19]. Prediction of the biological activity of *P. vulgaris* flavonoid compounds was carried out to validate the suitability of these compounds as α-glucosidase enzyme inhibitors. Inhibition of α-glucosidase activity using α-glucosidase inhibitors (AGI) has an impact on inhibiting the hydrolysis (digestion) process of carbohydrates and food starch into glucose which will be absorbed by the intestines, so that there is no increase in blood glucose levels. Flavonoids have an antidiabetic or antihyperglycemic effect which is possible by several mechanisms, namely the inhibition of glucose absorption in the small intestine, stimulating insulin secretion or acting like insulin, and regulating the work of enzymes that play a role in carbohydrate metabolism [6].

Various previous studies have shown that kidney bean seeds have α-amylase inhibitors that inhibit α-amylase activity. Some of the results of the isolation of the main phytoconstituents in *P. vulgaris* can be divided into 3 groups, there are phenolic acid, flavonoids, and proteins [20]. The screening was carried out by bioactivity analysis which showed the potential for α-glucosidase inhibition. The results showed that in general, the flavonoid group had the highest activity (Pa) compared to the phenolic group. Therefore, the flavonoid group compounds were selected for further analysis.

Based on the screening results, there are several compounds that show high biological activity related to α-glucosidase inhibitors, including orientin, rutin, isoorientin, hyperoside, isoquercitrin, myricetin-3-rhamnoside, isorhamnetin-3-glucoside. Inhibition by these compounds showed Pa value greater than Pi and Pa value > 0.7. The biological activity of rutin, hyperoside, and isoquercitrin as an α-glucosidase inhibitor showed Pa value > 0.8, which was not much different from acarbose as control with Pa > 0.9 (Figure 2).

The ADMET properties and drug-likeness of the compounds with the best bioactivity predicted by SwissADME are presented in Table 1.

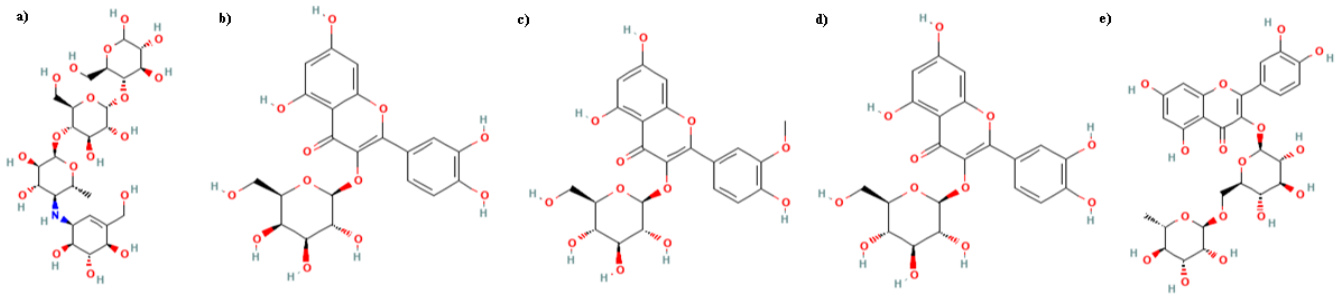


Figure 1. The molecular structure of: (a) acarbose; (b) hyperoside; (c) isorhamnetin-3-glucoside; (d) isoquercitrin; and (e) rutin. The molecular structures were adapted from PubChem database [14-18].

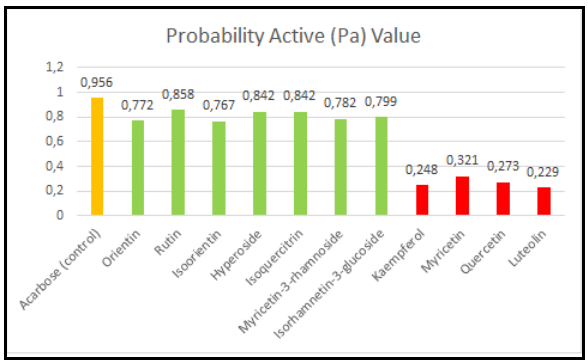


Figure 2. Bioactivity predictions of α-glucosidase inhibitors from *P. vulgaris* flavonoid compounds predicted by PASS Online

Log S value was used as a predictor of solubility value [21]. The value of Log S of the selected compound ranges from 2.7 to 3.2. Log P or ILOGP shows the partition coefficient of the drug design using the GB/SA approach [10]. Based on the predicted LogP value, it is concluded that all of the selected compounds lie within the range value of 0.4 to 1.6. The percent absorption (% Abs) of the ligands was calculated using the reported formula % Abs = 109 – 0.345 TPSA [22]. The selected compounds overall have higher %abs than the control. Skin permeability (log Kp) is important in the development of transdermal drug delivery systems and other topical drug formulations [11]. The more positive the log Kp, (cm/s), the higher the ability of the compound to pass through the skin [22]. The highest skin permeability was owned by the control and the other compounds were lower in the range of -8 to -10. Isorhamnetin-3-glucoside most likely has the best characteristics based on LogS, %Abs, and also LogKp. Based on this, isorhamnetin-3-glucoside has the best ADME property compared to the other seven compounds.

One of the most important criteria for selecting a chemical as a therapeutic candidate is its lack of toxicity [23]. The toxicity of the compound is predicted based on the organ toxicity (hepatotoxicity) and the toxicity endpoint (carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity) are presented in table 3.

The toxicity prediction results show that isoorientin has the lowest LD50 (159 mg/kg), which shows toxic potential. Orientin also shows quite lower LD50 (1213 mg/kg) compared to other compounds. Based on this, it is considered that flavonoid compounds, rutin, hyperoside, isoquercitrin, and isorhamnetin-3-glucoside proceed to molecular docking as further steps. These compounds show only two toxicological endpoints and also categorized as not fetal according to acute toxicity predictions and classified as slightly toxic.

Drug likeness is a parameter described as a means to correlate the physicochemical aspect of a compound with its biopharmaceutical aspect in the human body, especially its influence in the bioavailability of the peroral route [21]. Except for control compounds and rutin, all selected compounds had a 2-violation in Lipinski's rule with the same bioavailability value or score of 0.17 (Table 3). Lowest violations showed that compounds have better potential as a drug (higher drug-likeness).

Molecular docking

Molecular docking was carried out to assess the interaction between α-glucosidase with acarbose as control and several flavonoid compounds from *P. vulgaris* (Hyperoside, Isorhamnetin-3-glucoside, Isoquercitrin, andRutin). To assess how the tested compound inhibits the α-glucosidase enzyme through binding, docking was designed between α-glucosidase (GAA) – acarbose (control), α-glucosidase – Hyperoside, α-glucosidase – Isorhamnetin-3-glucoside, α-glucosidase – Isoquercitrin, and α-glucosidase – Rutin. Some of these compounds were selected through the screening and prediction of ADME and their bioactivity, reviewed through several aspects that could support these compounds as α-glucosidase

inhibitors (AGI). Table 4 shows the interactions between these complexes.

Acarbose has the highest number of hydrogen bond (Table 4). Hydrogen bonds are very important in ligand-enzyme interactions because they stabilize the ligand in the binding pocket [24;25]. They also contribute to the specificity of the ligand-enzyme interactions [26]. The number of hydrogen bonds from the highest to the lowest are acarbose > Isorhamnetin-3-glucoside > isoquercitrin > rutin > hyperoside. Acarbose as a control showed that it had the highest number of hydrogen bonds. However, the highest binding affinity value was shown by the routine compound (-352.37 kJ/mol) which exceeded the acarbose affinity value (-332.08 kJ/mol). The binding affinity values sequentially from the highest to the lowest are rutin > acarbose > hyperoside > isorhamnetin > isoquercitrin. Nevertheless, this binding affinity still showed that all compounds has low affinity which indicate that they have strong binding energy.

Based on the literature, the active site of the target protein using similar model (99% similarities compared to 5KZW) are: GLU174, THR175, AR178, GLU196, THR197, PRO198, ARG199, VAL200, HIS201, SER202, ARG203, ALA204, PRO205, GLN352, LEU355, ASP356, VAL357, VAL358, GLY359, TYR360, ARG608, VAL718, and ALA719 [34]. Amino acid residue binding points on acarbose as a control included ARG178, ASP356, LEU355, HIS717, which are hydrogen bond, and TYR360 (other bond).

However, acarbose has been identified as a competitive inhibitor of α-glucosidase. Meanwhile, several studies mention that several active compounds from plant extracts are classified as non-competitive inhibitors, example is quercetin.

Table 1. Predictions of bioactivity, physicochemical, and pharmacokinetics of *P. vulgaris* flavonoid compounds

Active Compound	Bioactivity and ADMET Properties				
	Pa	Log S	Log P	%Abs	Log Kp
Acarbose (control)	0.956	2.13	0.63	-1.8	-16.29
Orientin	0.772	-2.7	1	39.56	-9.14
Rutin	0.858	-3.3	0.46	16.05	-10.26
Isoorientin	0.767	-2.7	1.6	39.56	-9.14
Hyperoside	0.842	-3.04	1.45	36.37	-8.88
Isoquercitrin	0.842	-3.04	0.94	36.37	-8.88
Myricetin-3-rhamnoside	0.782	-3.2	0.92	36.37	-8.77
Isorhamnetin-3-glucoside	0.799	-3.26	1.21	40.16	-8.73

Table 2. Organ toxicity and toxicity endpoints of *P. vulgaris* flavonoid compounds

Active Compound	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity	LD50 (mg/kg)
Acarbose (control)	0.65	-0.84	0.99	-0.76	-0.7	24000
Orientin	-0.81	-0.72	0.52	0.52	-0.87	1213
Rutin	-0.8	-0.91	0.98	-0.88	-0.64	5000
Isoorientin	-0.81	-0.72	-0.65	0.52	-0.87	159
Hyperoside	-0.82	-0.85	0.66	-0.76	-0.69	5000
Isoquecitrin	-0.82	-0.85	0.66	-0.76	-0.69	5000
Myricetin-3-rhamnoside	-0.73	0.5	0.98	-0.71	-0.93	5000
Isorhamnetin-3-glucoside	-0.83	-0.9	0.93	-0.65	-0.58	5000

Table 3. Drug-likeness: Lipinski's rule dan bioavailability score of *P. vulgaris* flavonoid compounds

Active Compound	PubChem ID	Lipinski Rule of 5				Violations	Bioavailability Score
		MW (g/mol)	mLogP	nON	nOHNH		
Acarbose (control)	41774	645.60	-6.94	19	14	3	0.17
Orientin	5281675	448.38	-2.51	11	8	2	0.17
Rutin	5280805	610.52	-3.89	16	10	3	0.17
Isoorientin	114776	448.38	-2.51	11	8	2	0.17
Hyperoside	5281643	464.38	-2.59	12	8	2	0.17
Isoquercitrin	5280804	464.38	-2.59	12	8	2	0.17
Myricetin-3-rhamnoside	5281673	464.38	-2.32	12	8	2	0.17
Isorhamnetin-3-glucoside	44258009	478.40	-2.37	12	7	2	0.17

Table 4. Interaction of the best binding compounds with α-glucosidase binding site residues

Compounds	Interaction	Chemistry Bond	Binding energy (kJ/mol)
Acarbose (as a control)	**A:ARG178:HH21** - :LIG1:O	Hydrogen Bond	-332.08
	:LIG1:H - :LIG1:O	Hydrogen Bond	
	:LIG1:H - A:ASP356:O	Hydrogen Bond	
	:LIG1:H - A:LEU355:O	Hydrogen Bond	
	:LIG1:H - A:HIS717:O	Hydrogen Bond	
	A:ASP356:CA - :LIG1:O	Hydrogen Bond	
	:LIG1:H - A:ASP356:OD2	Hydrogen Bond	
	:LIG1:H - A:ASP356:O	Hydrogen Bond	
	:LIG1:H - A:LEU355:O	Hydrogen Bond	
	:LIG1:H - :LIG1:O	Hydrogen Bond	
	:LIG1:H - :LIG1:O	Hydrogen Bond	
	:LIG1:H - :LIG1:O	Hydrogen Bond	
	:LIG1:O - A:TYR360	Other	
Hyperoside	**A:TYR360:HN** - :LIG1:O	Hydrogen Bond	-305.85
	A:VAL867:HN - :LIG1:O	Hydrogen Bond	
	:LIG1:H - :LIG1:O	Hydrogen Bond	
	:LIG1:H - :LIG1:O	Hydrophobic	
	:LIG1 - A:ARG608	Hydrophobic	
Isorhamnetin-3-glucoside	**:LIG1:H** - :LIG1:O	Hydrogen Bond	-299.07
	:LIG1:H - A:ARG608:O	Hydrogen Bond	
	A:HIS717:CE1 - :LIG1:O	Hydrogen Bond	
	:LIG1:H - A:HIS717:NE2	Hydrogen Bond	
	A:TYR360:HN - :LIG1	Hydrogen Bond	
	A:ARG608:HE - :LIG1	Hydrogen Bond	
	A:ARG608:NH2 - :LIG1	Electrostatic	
	A:TYR360 - :LIG1	Hydrophobic	
	:LIG1 - A:ARG608	Hydrophobic	
Isoquercitrin	**:LIG1:H** - :LIG1:O	Hydrogen Bond	-296.61
	:LIG1:H - A:TYR360:O	Hydrogen Bond	
	:LIG1:H - A:PRO361:O	Hydrogen Bond	
	:LIG1:H - A:HIS717:NE2	Hydrogen Bond	
	:LIG1 - A:VAL588	Hydrophobic	
	:LIG1 - A:ARG608	Hydrophobic	
Rutin	**A:ARG608:HE** - :LIG1:O	Hydrogen Bond	-352.37
	A:VAL867:HN - :LIG1:O	Hydrogen Bond	
	:LIG1:H - :LIG1:O	Hydrogen Bond	
	A:HIS584 - :LIG1	Hydrophobic	
	A:HIS717 - :LIG1:C	Hydrophobic	
	:LIG1 - A:ARG608	Hydrophobic	
	:LIG1 - A:ARG608	Hydrophobic	
	:LIG1- A:ARG594	Hydrophobic	
	:LIG1 - A:PRO595	Hydrophobic	

The tested compounds that were used in this experiment have not been identified with certainty the type of inhibition. Therefore, it is recommended to further identify the inhibition kinetics. A study has been conducted on a compound with a non-competitive inhibitory type, which is docked on the allosteric site of the human α-glucosidase enzyme. Possible allosteric binding sites of enzymes include ARG608, HIS717, GLU869, VAL867, GLU866, LEU865, HIS584, ARG 585 [27].

Alpha-glucosidase interaction with Hyperoside has three amino acid residues, which site in common with the control is TYR 360 (hydrogen bond). Hyperoside was stabilized by three hydrogen bond and two hydrophobic interactions. The interaction of the receptor with hyperoside showed binding to 3 types of amino acid residues, with the same amino acid residues as the control: TYR 360 (hydrogen bond), the common binding amino acid residues with the active site: ARG608 (hydrophobic) and TYR360 (hydrogen bond), and common with the allosteric sites were VAL 867 (hydrogen bond) and ARG608 (hydrophobic). In addition, hyperoside showed strong anti-α-glucosidase properties [28]. As the result of molecular modeling docking, hyperoside also exhibited the high affinity with α-glucosidase and, thus, displayed potential inhibitory activity against α-glucosidase.

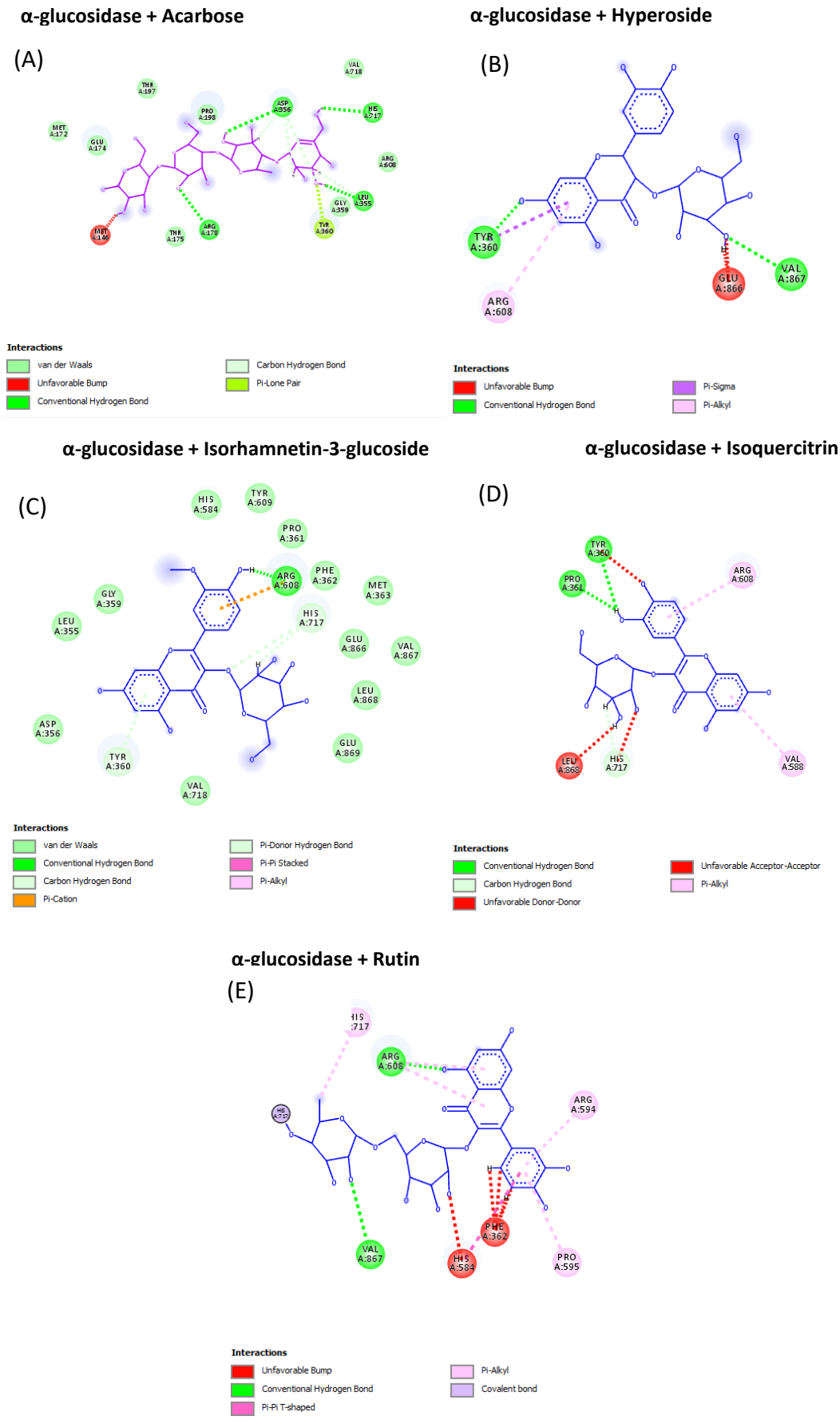


Figure 3. Interactions between the (A) acarbose; (B) Hyperoside; (C) Isorhamnetin-3-glucoside;(D) Isoquercitrin; and (E) Rutin with the binding site residues of α-glucosidase as visualized in DS Visualizer

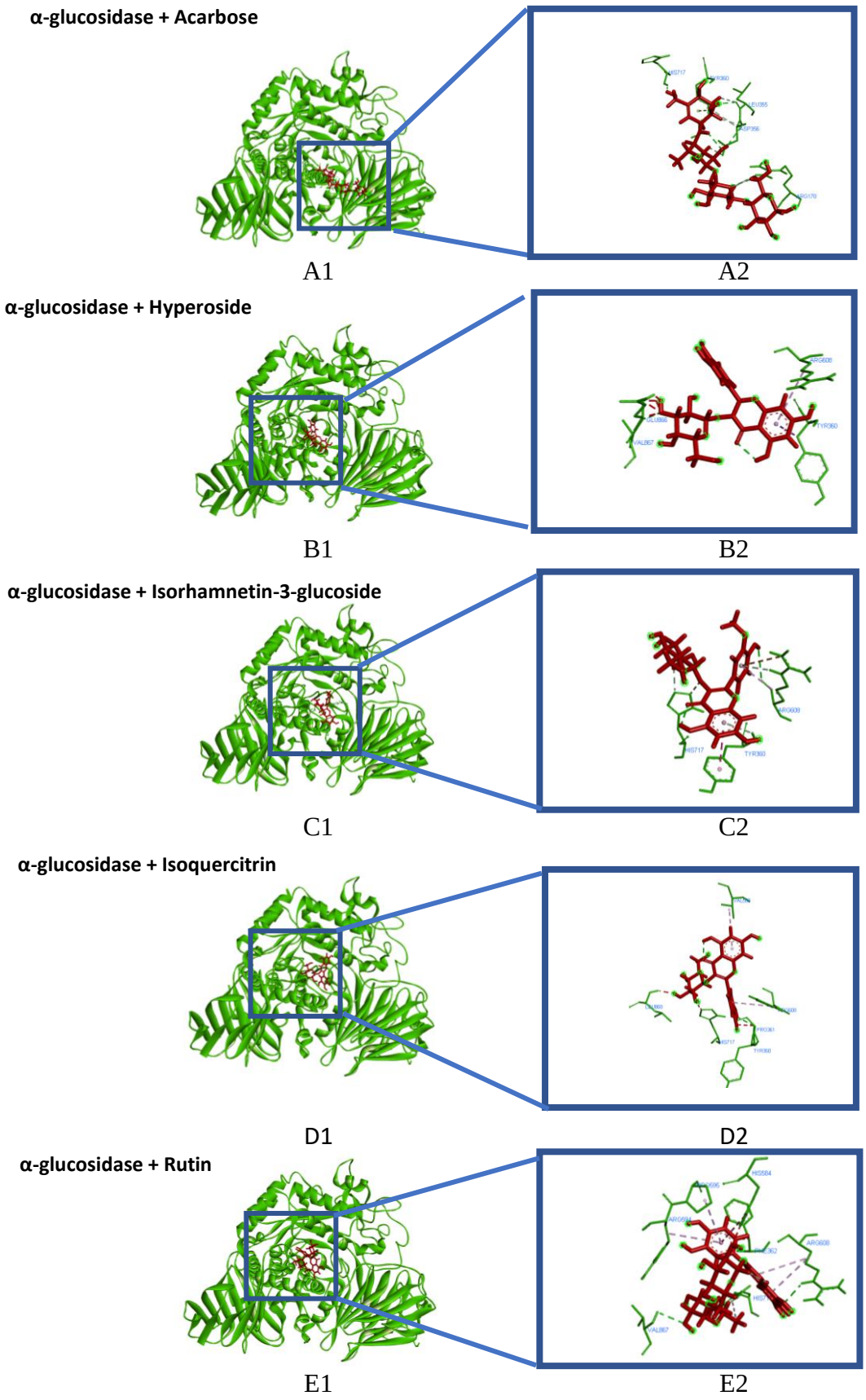


Figure 4. Interaction of α-glucosidase with target compounds (A1) conformation of α-glucosidase interacted with acarbose (A2) 3D structure. (B1) conformation of α-glucosidase interacted with Hyperoside (B2) 3D structure. (C1) conformation of α-glucosidase interacted with Isorhamnetin-3-glucoside (C2) 3D structure (D1) conformation of α-glucosidase interacted with Isoquercitrin (D2) 3D structure (E1) conformation of α-glucosidase interacted with Rutin (E2) 3D structure

The interaction of Isorhamnetin-3-glucoside has three amino acid residue sites, with two sites in common with the control are HIS 717 (hydrogen bond) and TYR 360 (hydrogen and hydrophobic). The common binding amino acid residues with active sites: ARG608 (hydrogen bond) and TYR360 (hydrogen bond), and common with allosteric sites were HIS 717 (hydrogen bond) and ARG608 (hydrogen bond). Isorhamnetin-3-glucosidase was stabilized by six hydrogen bond, two hydrophobic and one electrostatic interaction. In addition, the interaction between the ligand and this receptor has a fairly high binding energy value of -3-299.07 kJ/mol. Isorhamnetin-3-glucoside has shown potential as anti-diabetic agent [34]. Isorhamnetin could be used as an alternative or potential complementary treatment in T2DM [35].

Interaction the Isoquercitrin complex and the receptor produced five amino acid residue sites, with two sites the same as the control are HIS 717 (hydrogen bond) and TYR 360 (hydrogen bond). The common binding amino acid residues with active sites: ARG608 (hydrophobic) and TYR360 (hydrogen bond), and common with allosteric sites were HIS 717 (hydrogen bond) and ARG 608. The interaction between isoquercitrin to α-glucosidase was stabilized by four hydrogen bonds plus two hydrophobic interactions. Isoquercitrin, a nearly identical quercetin-3-monoglucoside. Technically the two are different (isoquercetin has a pyranose ring whereas isoquercitrin has a furanose ring), but functionally the two molecules are indistinguishable. It was reported to have an inhibitory effect against intestinal α-glucosidases in in vivo rat models and in vitro intestinal homogenates of rats [29].

Meanwhile, in the interaction with Rutin, there were six amino acid residue sites with one site common as the control is HIS 717 (hydrophobic). The common binding amino acid residues with active sites is ARG608 (hydrogen and hydrophobic), and common with allosteric sites were HIS 584 (hydrophobic), HIS717 (hydrophobic), VAL867 (hydrogen bond) and ARG608 (hydrogen and hydrophobic). The binding energy of the -glucosidase complex – Rutin showed the lowest value (-352.37 kJ/mol) even lower than the control value (-332.08 kJ/mol) which the more negative the affinity value, the stronger the bond. It was found that rutin was stabilized by three hydrogen bond and four hydrophobic interactions. There was also reported that Rutin showed a significant inhibition of alpha-glucosidase [30, 31].

In a brief, based on amino acid residues that common to control and alpha-glucosidase active sites also based on its interaction, potential compound from the highest to lowest are: isorhamnetin-3-glucosidase which has HIS717 (hydrogen), TYR360 (hydrogen and hydrophobic), and ARG608 (hydrogen); isoquercitrin which has HIS 717 (hydrogen bond), TYR 360 (hydrogen bond), and ARG608 (hydrophobic); rutin which has ARG608 (hydrogen and hydrophobic) and HIS 717 (hydrophobic); and hyperoside which has TYR 360 (hydrogen bond), and ARG608 (hydrophobic). As mentioned before, hydrogen bond is the strongest category of binding interaction, followed by hydrophobic bond, and other bonds. The more residues, more binding with active sites, highest number of strong binds, the compounds are more potential.

The conformation of the 2d and 3d structures is shown by Figures 3 and 4. Based on the molecular interaction, the tested compound has a fairly high binding affinity. Structure map of the α-glucosidase showed that all of the compounds have sites that interact with the active sites of the α-glucosidase enzyme (two amino acid residues), and have several similarities compared to acarbose. These findings show that in general, all compounds have good potential as α-glucosidase inhibitors. This can be seen from the value of binding affinity, binding site compared, and the similarity of the location of the bond, and the number of strong bonds. In addition, based on bioactivity analysis using PASS, PASS has an average accuracy of 95% (Mun'im et al., 2013). The tested compounds have a high Pa value (Pa>0,7) which means that these compounds have high potency based on α-glucosidase inhibitory bioactivity.

However, in this case, compounds that were used in this experiment have not been identified with certainty the type of inhibition. To define the mode of inhibition, further studies of the enzyme kinetics should be conducted in order to obtain specific information and to conduct the most suitable method. It is also recommended to use software or tools that have specific docking capabilities so that they can target more specific active sites and produce more precise results. Besides, fit also suggested future research to find out how this work and how they mechanism in the human body.

Nevertheless, it still can be predicted that, based on the prediction results of ADME and bioactivity as well as on the results of molecular docking, it is estimated that the compound that has the most effective potential to become an inhibitor of the α-glucosidase enzyme or become a potential compound as AGIs is isorhamnetin-3-glucosidase. This result is based on amino acid residues that in common with acarbose and alpha-

glucosidase active sites, also its interaction and bonds category. Isorhamnetin-3-glucosidase has HIS717 (hydrogen), TYR360 (hydrogen and hydrophobic), and ARG608 (hydrogen) which shows higher number of residues in common with acarbose and active sites that also shows fairly high stability. As well as in the ADME prediction, Isorhamnetin-3-glucoside most likely has the best characteristics based on LogS, %Abs, and also LogKp compared to the other compounds, and has much higher %Abs (40%) than acarbose (-1,8%). It even only has 2 violations of Lipinski rules compared to acarbose that has 3 violations.

Based on this study, it suggested that all tested compounds which belong to the *P. vulgaris* flavonoid group have good potential as alpha-glucosidase inhibitors. This discovery is strengthened by the in vivo and in vitro evidence of α-glucosidase inhibitory activity by *P. vulgaris*. A study conducted by Nunez-Aragon et al. (2019), showed that the total hydrolyzate and <1 kDa fraction of *P. vulgaris* had antihyperglycemic activity, by intestinal glucose absorption and inhibition of the α-glucosidase enzyme and was safe up to 5000 mg kg− 1 [32]. Screening of Indonesian medicinal plants with α-glucosidase inhibitors carried out by Mun'im et al. (2013) also showed that *P. vulgaris* had a very low IC50 value, which was 4.83 ppm [33]. The lower IC50 indicates the more effective the bioactivity of the compound. Thus, flavonoid compounds in *P. vulgaris,* especially isorhamnetin-3-glucosidase, have good potential as α-glucosidase inhibitors as antidiabetic agents.

CONCLUSION

Four compounds that show high biological activity (Pa value > 0.7) related to α-glucosidase inhibitors with good ADMET characterization results, including rutin, hyperoside, Isoquercitrin, and Isorhamnetin -3-glucoside. This *in silico* study showed that all of these compounds have sites that interact with the active sites of the α-glucosidase enzyme, and have several similarities compared to acarbose. These findings show that in general, all compounds have good potential as α-glucosidase inhibitors. Isorhamnetin-3-glucosidase is the most potential characteristic as a α-glucosidase inhibitor based on amino acid residues that in common with acarbose and alpha-glucosidase active sites, also its interaction and bonds category. It has amino acid residues HIS717 (hydrogen), TYR360 (hydrogen and hydrophobic), and ARG608 (hydrogen) which shows higher number of residues in common with acarbose and active sites that also shows fairly high stability. Based on ADME prediction, Isorhamnetin-3-glucoside most likely has the best characteristics based on LogS, %Abs, and also LogKp compared to the other compounds, and has much higher %Abs (40%) than acarbose (-1,8%). It even only has 2 violations of Lipinski rules compared to acarbose that has 3 violations. Thus, overall flavonoid group compounds in *P. vulgaris*, especially *isorhamnetin-3-glucoside*, have good potential as a glucosidase inhibitors as antidiabetic agents.

ACKNOWLEDGEMENT

We thank Bioinformatic lecturers and Bioinformatics practice assistants 2022 for the guidance to finish this work and proofread our manuscript.

REFERENCES

[1] Putra, R. J. S, A Achmad dan H Rachma, Pharmaceutical Journal of Indonesia, 2017, 2(2), 45-50.
[2] Salehi, B, et al., Biomolecules, 2019, 1-121.
[3] Febrinda, A. E, et al., J. Teknol. dan Industri Pangan, 2013, 24(2), 161-167.
[4] Fitriansyah, M. I dan R. B Indradi, Farmaka, 2018, 16(2), 337-346.
[5] Mojica, L, et al., Food Research International, 2015, 69, 38-48.
[6] Tran, N, B Pham dan L Le., Biology (Basel), 2020, 9(9), 252.
[7] Werdani, Y., & Widyawati, P, Advances in Social Science, Education and Humanities (ASSEHR), 2018, 98, 164-167.
[8] Puri, M, et al., Artificial Neural Network for Drug Design, Delivery and Disposition, 2015, London: CRC Press.
[9] Macabeo, A. P, I. A Pilapil dan K. Y Garcia, Journal Molecules, 2020, 25, 1-9.
[10] Daina, A, O Michielin dan V Zoete, Journal of chemical information and modeling, 2014, 54(12), 3284–3301.
[11] Du, J, et al., Advances in Computational Science and Computing. 2018. New York: Springer.
[12] Gaur, R, T Hohn dan P Sharma, Plant Virus-Host Interaction, 2012, New York: Elsevier.
[13] Coumar, M. S., Molecular Docking for Computer-Aided Drug Design. 2021, London: Academic.
[14] PubChem, 2022, https://pubchem.ncbi.nlm.nih.gov/compound/41774, accessed on 24 April 2022.
[15] PubChem, 2022, https://pubchem.ncbi.nlm.nih.gov/compound/5281643, accessed on 24 April 2022.
[16] PubChem, 2022, https://pubchem.ncbi.nlm.nih.gov/compound/5318645, accessed on 24 April 2022.

[17] PubChem, 2022, https://pubchem.ncbi.nlm.nih.gov/compound/5281673, accessed on 24 April 2022.
[18] PubChem, 2022, https://pubchem.ncbi.nlm.nih.gov/compound/5280805, accessed on 24 April 2022.
[19] Basha, S. A, M Yunoos dan J Ahmed, Indo American Journal of Pharmaceutical Research, 2018, 8(6), 1441-1445.
[20] Rahman, N.,et al, Biomolecules, 2019, 9, 544.
[21] Suhud, F, et al., IOP Conference Series: Earth and Environmental Science, 2019, 293.
[22] Bitew, M, et al., PloS One, 2021, 16(2), e0260853.
[23] Sadeghi, M, et al., In Silico Pharmacology, 2021, 9(1), 1-8.
[24] Kosta, J., Fishbein JC, Heilman JM eds. Amsterdam: Elsevier, 2016, 10, 139–186.
[25] Abelian, A, et al., Twenty-third Edition. Adejare A ed. Amsterdam: Elsevier, 2021, 105-128.
[26] Safitri, A, et al., Journal of Smart Bioprospecting and Technology, 2020, 1(2), 25-30.
[27] Rahman, N.,et al, Biomolecules, 2020, 10(5)
[28] Lin, Y. T, et al., Antioxidants, 2022, 11(2), 320.
[29] Pereira, D. F, et al., Nutrition, 2011, 27(11-12), 1161-1167.
[30] Dubey, S, et al., Future Journal of Pharmaceutical Sciences, 2017, 7(2), 158-162.
[31] Bljajić, K., Brajković, A., Čačić, A., Vujić, L., Jablan, J., Saraiva de Carvalho, I., & Zovko Končić, M., Horticulturae, 2021, 7(2), 15.
[32] Nuñez-Aragón, P. N, M Segura-Campos dan E Negrete-León, J. Sci Food Agric, 2019, 99, 587-595.
[33] Mun'im, A, Katrin dan Azizzahwati, International Journal Aromatic Plants, 2013, 3, 144-150.
[34] Dahae, L. et al, processes, 2021, 9(3)
[35] Kalai et al., 2021, Int. Journal of Molc Sci, 23, 734