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ORIGINAL ARTICLE / ÖZGÜN MAKALE

IN SILICO AND *IN VITRO* ANTIDIABETIC ACTIVITY STUDIES ON THE ETHANOL EXTRACT OF *DESMANTHUS VIRGATUS* (L.) LEAVES

DESMANTHUS VİRGATUS (L.) YAPRAKLARININ ETANOL EKSTRESİ ÜZERİNE İN SİLİKO VE İN VİTRO ANTİDİYABETİK AKTİVİTE ÇALIŞMALARI

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ABSTRACT

Objective: *In vitro and in silico studies using α-glucosidase activity inhibition aimed at identifying the potential antidiabetic properties of ethanol extracts of D. virgatus leaves.*
Material and Method: *Compounds in the ethanol extract of D. virgatus leaves were identified by LC-MS. In vitro and in silico methods were used to determine the antidiabetic activity.*
Result and Discussion: *The protein codes (PDB ID) used were 3AJ7 and 3W37, then acarbose was used as a positive control. By using LC-MS, 160 compounds were identified from the extract. All of the identified compounds were tested in silico. There are 18 compounds (with 3AJ7 protein) and 10 compounds (with 3W37 protein) that fulfill Lipinski's rule with binding affinity values lower than acarbose, namely ≤ -8.2 kcal/mol for 3AJ7 protein and ≤ -8.1 kcal/mol for 3W37. In addition, α-glucosidase inhibition was used to assess the antidiabetic effect of the ethanol extract of D. virgatus leaves in vitro, resulting in IC_{50} values of 144.11 ppm for the extract and 3.78 ppm for acarbose. The IC_{50} values of the extract were included in the medium category.*
Keywords: *Antidiabetic, Desmanthus virgatus, ethanol extract, in silico, in vitro*

ÖZ

Amaç: *Bu çalışma, in siliko ve in vitro (α-glukozidaz inhibisyonu) yöntemlerle D. virgatus bitkisinin yapraklardan elde edilen etanol ekstresinin antidiyabetik etki potansiyelini belirlemeyi amaçlamaktadır.*
Gereç ve Yöntem: *LC-MS kullanılarak D. virgatus yapraklarının etanol ekstresindeki bileşikler tanımlanmıştır. Antidiyabetik aktivite in silico ve in vitro olarak belirlenmiştir.*
Sonuç ve Tartışma: *Kullanılan protein kodları (PDB ID) 3AJ7 ve 3W37 olup, akarbose pozitif kontrol olarak kullanılmıştır. LC-MS kullanılarak ekstrakttan 160 bileşik tanımlanmıştır. Tanımlanan bileşiklerin tümü silikoda test edilmiş. (3AJ7 proteini ile) 18 bileşik ve (3W37 proteini ile) 10 bileşik Lipinski kuralını karşılamakta ve bağlanma afinitesi değerleri akarbose'dan daha düşük olmak üzere, 3AJ7 için ≤ -8.2 kcal/mol ve 3W37 için ≤ -8.1 kcal/mol olarak bulunmuştur. Ayrıca, ekstre ve akarbozun α-glukozidaz inhibitörü etkisi in vitro olarak test edilmiştir, elde edilen IC_{50} değeri sırasıyla 144.11 ppm'dir ve 3.78 ppm'dir. Ekstrenin etkisi orta kategoriye sokulabilir.*

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Anahtar Kelimeler: *Antidiyabetik, Desmanthus virgatus, etanol ekstresi, in siliko, in vitro*

INTRODUCTION

Chronic metabolic disease known as diabetes mellitus is indicated by increased blood sugar levels [1,2]. These chronic disorders have a negative effect on the metabolic system and are involved in the pathophysiology of diabetic complications [3]. The defining characteristic of diabetes mellitus (DM) is increased blood glucose levels brought on by either resistance to insulin or impaired insulin synthesis [4]. Various micro- and macro-vascular problems due to uncontrolled hyperglycemia lead to cardiovascular disease, retinopathy [5], neuropathy, nephropathy, and ulceration with high morbidity and mortality worldwide [6,7]. In the 10th edition Atlas at the end of 2021, the International Diabetes Federation (IDF), reported that one of the 21st century's most rapidly expanding international health emergencies is diabetes [8]. 537 million people all over the world had diabetes in 2021. By 2030 and 2045, there will be 643 million and 783 million more people in this number, respectively [9].

Type 1 diabetes mellitus (T1DM) and type 2 diabetes (T2DM) are the two kinds of diabetes [10]. T1DM is a metabolic disease brought on by apoptosis and an autoimmune or idiopathic mechanism that destroys pancreatic β-cells [11,12]. This causes the pancreas to not produce enough insulin or none at all so the patient has to rely on insulin from the outside [13]. In people with T2DM, the pancreas can still produce insulin, but the insulin produced is unable to incorporate glucose into the cells, causing blood glucose to rise [14]. T2DM can also be caused by insulin resistance [15] as well as pancreatic beta cell failure that contributes to impaired glucose tolerance [14].

Treatment of diabetes can use a therapeutic approach by inhibiting an enzyme responsible for glucose uptake such as α-glucosidase [16]. This strategy, which is present in pancreatic and salivary gland secretions, is crucial to the therapeutic process [17]. The α-D-(1,4)-glycosidic linkages in starch are broken down by this enzyme, which is an endoamylase family member, to produce monosaccharides. Inhibiting the enzyme is a useful method for reducing blood sugar [18].

Over the past few years, there have been significant developments in diabetes prevention. However, there is no curative drug that can reduce the risk of diabetes. Patients with diabetes have been treated with a variety of synthetic pharmaceuticals, including acarbose, biguanides, thiazolidinediones, and sulfonylureas [19]. Diabetes control is still a major issue despite significant advancements in the field of drug discovery and the availability of numerous synthetic medications of different kinds that efficiently combat hyperglycaemia. This is because synthetic substances have evident negative effects [6]. Continuous consumption of synthetic anti-diabetic drugs can cause side effects on the body and psychology of patients [20] such as gastrointestinal disturbances of nausea and diarrhea [21], liver disease [22], followed by an increased risk of hypoglycemia, and more serious side effects such as liver damage or cardiovascular problems [23]. The presence of these side effects necessitates the discovery of new drug entities that are relatively safer and cheaper derived from natural sources compared to synthetic drugs. One of the primary sources for finding new drugs that are helpful for treating a variety of disorders is traditional medicine. In addition, a variety of secondary metabolites found in plant-based traditional remedies act on a variety of targets and may provide additional benefits for the treatment of polygenic disorders like diabetes or obesity. The anti-hyperglycaemic properties of plant extracts and herbal medicine that may be employed as anti-diabetics have been supported by numerous research. Herbal remedies are frequently thought to have fewer adverse effects than manufactured medications [2]. Other than that, when it comes to human intake, traditional medications are comparatively safer than modern oral hypoglycaemic drugs [24].

One of the plants that has anti-diabetic potential is *Desmanthus virgatus* (L.). The absence of publications related to information on phytochemical contents and the use of *D. virgatus* leaves as anti-diabetics make this research important. Thus, this study aimed to conduct phytochemical analysis including phytochemical identification through LC-MS instruments, and determining the anti-diabetic potential of the leaves using *in vitro* and *in silico* methods.

MATERIAL AND METHOD

Materials

The study's materials included were *D. virgatus* (L.) leaves, ethanol (98%, Merck, Germany), structure protein receptor of α-glucosidase (PDB ID: 3AJ7 and 3W37) (retrieved from https://www.rcsb.org/), ligand conformers (retrieved from https://pubchem.ncbi.nlm.nih.gov/), α-glucosidase enzyme (Sigma Aldrich, USA), acarbose, phosphate buffer, 4-nitrophenyl β-D-glucopyranoside solution, and sodium carbonate (Sigma Aldrich, USA).

The leaves of the plant were collected from Sidoarjo, East Java, Indonesia. The plant materials were determined at Yayasan Generasi Biologi Indonesia, Cerme, Gresik Indonesia with specimen number BT 02/0352/24, March 22, 2024. The specimen was deposited in the Yayasan. In this study, the leaves were used because the leaves are often the part that is rich in secondary metabolites. As research conducted by Sembiring et al. (2018) on various parts of the *Caesalpinia bonduc* (L.) Roxb plant, which is still in the same family as *D. virgatus*, namely Fabaceae, reported that the results of phytochemical tests on the leaves of the plant contained secondary metabolites of flavonoids, saponins, steroids, and tannins. Then, the results of the total phenolic and flavonoid content tests in the leaves were reported at 31.05 ± 0.35 (mgQE/gram) and 146.64 ± 3.94 (mgGAE/gram) [25].

Instruments

The equipment used in this study were beaker glass, extraction chamber, spatula, Whatman filter paper, Buchner funnel, vacuum pump, Shimadzu LC-MS instrument (8040 Type, Shimadzu, Japan), micropipette, 96 well microplate, vacuum rotary evaporator (R-215, Buchi), microplate reader (Multiskan, Thermo Scientific), incubator (Memmert BE500), and Eppendorf tubes. The docking study was performed using Windows 10, Intel(R), 4,00 GB RAM, and a 64-bit operating system. The software used involved Discovery Studio Client 2019, PyRx-0.8, and pyMOL 3.0.

Plant Material Collection and Extraction

D. virgatus leaf samples were collected and air-dried for 3 days in the sun. After drying, the leaves were mashed with a grinder until they became powder, and weighed (3.8 kg). Then, the powdered sample was divided into 2 parts and each part (±1.9 kg) was macerated using 3.4 liters of ethanol by stirring several times, and the process was repeated 4 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 ethanol filtrate was obtained. The ethanol filtrate was evaporated using a vacuum rotary evaporator to obtain a thick greenish-brown extract (478 grams). A small amount of each extract (±0.5 mg) was used for identification of phytochemical content in LC-MS analysis. Ethanol solvent is used because ethanol is a solvent that is safe for human consumption as a solvent for natural ingredients for both food and natural medicines [26]. In addition, ethanol is a semi-polar compound that can dissolve polar and non-polar compounds effectively. This can allow it to extract various secondary metabolites, including phenolics, flavonoids, and alkaloids that are important for antidiabetic activity [27].

Determination of Ethanol Extract's Secondary Metabolites Using LC-MS

Using an LC-MS, secondary metabolites of the ethanol extract of *D. virgatus* leaves were identified. Shimadzu 8040 Type was equipped with a Shimadzu Pack FC-ODS capillary column (2 mm × 150 mm id, 3 µm particle size). The injection volume was 1 µl. The apparatus is equipped with an Electrospray Ionization (ESI) source that has the following specifications: flow rate of 0.5 ml/min; ethanol solvent; focused ion mode MS type [M]+; ionization utilizing ESI; isocratic mobile phase; The eluents used were 0.1% formic acid in acetonitrile and 0.1% distilled water with an isocratic elution system. The isocratic elution system was performed at 0-1 min 95: 5 ratio, 0 min linear gradient elution A from 95% to 5%, 6-7 min isocratic elution 0:100 ratio, 6-7 min linear gradient elution solvent A from 0% to 100%, min 7.5-9 isocratic elution 95: 5 ratio, up to 7.5-9 min elution gradient linear A solvent from 95% to 5%; capillary voltage of 3.0 kv; and run time of 80 minutes. Using the NIST database library and the compounds' retention time and molecular mass spectra from the chromatograms, secondary metabolites present in the extracts were identified.

In Silico Study with Protein 3AJ7 and 3W37

Data generated from the LC-MS analysis were identified using the PubChem web database to determine the compound's name and chemical structure. Then, each of these compounds was tested for Lipinski Druglikeness and pharmacokinetics to determine its potential as a bioactive compound with Lipinski's five rules through the website http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp. Biocomputational molecular docking analysis was used to assess the antidiabetic activity of compounds that fulfilled the Druglikeness and pharmacokinetic tests. The receptor used *in silico* study was α-glucosidase with PPDB code 3AJ7 and 3W37. Preparation was done using PyMOL to obtain sterile protein. Furthermore, PyRx was used to carry out a molecular docking test and determine the binding affinity value. After molecular docking, the compound was interacted and visualized using PyMOL and Discovery Studio to determine the position and type of interaction formed.

In Vitro Study with α-Glucosidase Inhibition Assay

With minor modifications, the *in vitro* α-glucosidase inhibition of the ethanol extract was determined using Etsassala et al.'s (2020) method [28]. 50 µl 0.1 M phosphate buffer (100 mM, pH = 6.8), 10 µl α-glucosidase (1 U/ml), and 10 µl test samples (7.81; 15.63; 31.25; 62.50; 125.00; 250.00; and 500.00 ppm) were mixed, respectively and put into a 96-well plate. The entire mixture was incubated at 37 °C for 15 minutes. Afterward the pre-incubation phase, each well was filled with 20 µl of a 5 mM 4-nitrophenyl β-D-glucopyranoside solution, and the enzymatic reaction was activated. The plate was then incubated for 20 minutes at 37ºC. The reaction was halted after 20 minutes by adding 50 µl of 0.1 M sodium carbonate. Then, the absorbance was measured at a wavelength of 405 nm using a microplate reader. The positive control used in this assay is acarbose with various concentrations of 0.7813; 1.5625; 3.1250; 6.2500; 12.5000; 25.0000; and 50.0000 ppm. The formula was used to determine the level of α-glucosidase inhibition.

$$\text{The percentage of inhibitory activity (\%)} = (1 - A/B) \times 100$$

With A represents the sample absorbance and B represents the control absorbance.

Statistical Analysis

The results were reported as the mean IC_{50} value. Statistical analysis was performed using IBM SPSS Statistics 25. The IC_{50} differences were examined using parametric statistical methods with the One Way Anova test followed by the Games-Howell test. Results were considered significantly different when $p < 0.05$.

RESULT AND DISCUSSION

Determination of Ethanol Extract's Secondary Metabolites Using LC-MS

160 compounds were identified by LC-MS as the secondary metabolites of *D. virgatus* ethanol extract. The extract's LC-MS chromatogram is displayed in Figure 1.

Ethanol is semi-polar, which can attract compounds that are polar and non-polar [29]. The dominant compounds of ethanol extract are flavonoids including 7,4'-dihydroxyflavone, 5,7-dihydroxyisoflavone, demethyltorosaflavone C, calycosin, kaempferol, and naringenin. As known, flavonoids provide hypoglycemic effects in diabetics [30] by enhancing antioxidant status, lipid profile, and controlling glucose levels [31]. In addition, flavonoids have anti-cancer, anti-inflammatory, anti-oxidant, anti-bacterial, anti-thrombotic, anti-fungal, and anti-viral effects [32]. Furthermore, there are also phenylpropanoids, phenolic acids, glycosides, and their derivatives, besides aldehydes, ketones, terpenoids, alkaloids, and tannins in small amounts. Alkaloids are thought to be the primary inhibitors of the enzymes α-glucosidase and α-amylase, which are the best methods for reducing blood glucose levels [33]. On the other hand, tannins can lower hyperglycaemia after meals and stop or postpone the absorption of glucose by blocking α-glucosidase activity [34].

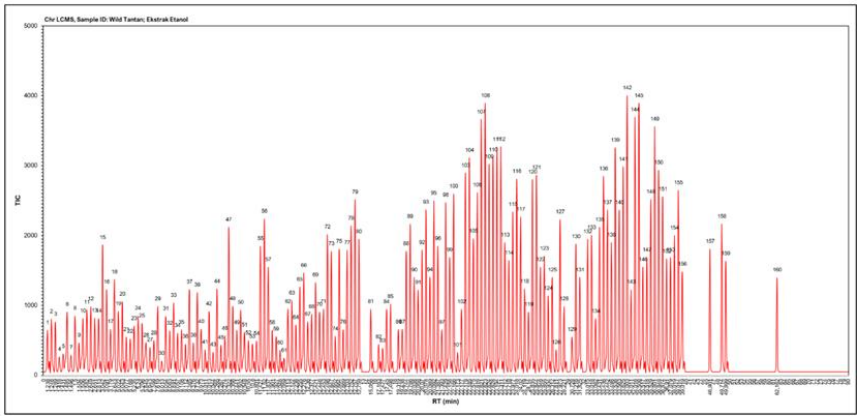


Figure 1. LC-MS chromatogram of the ethanol extract of *D. virgatus* leaves

In Silico Study with Protein 3AJ7 and 3W37

Of the 160 compounds from LC-MS, an evaluation of 5 Lipinski's rules was carried out and 65 compounds that fulfilled Lipinski's rules were obtained. Then, the 65 compounds that fulfill Lipinski's rules will be continued for *in silico* testing. According to the results of the *in silico* study, 18 compounds were determined that fulfill Lipinski's rule with binding affinity values lower than acarbose (as positive control) and have molecular interactions similar to the control. These compounds are 5-hydroxy-7-[2-(4-hydroxyphenyl)ethenyl]-2,2-dimethyl-2H-1 benzopyran, 7,4'-dihydroxyflavone, apigenin, 4-(3-methyl-but-1-enyl)-3,4',5-trihydroxystilbene, genistein, biochanin A, fustin, kaempferol, 2-(2,3-dihydroxy-3 methylbutyl)-5-(2-phenylethenyl)-1,3-benzenediol, artocarpanone, (2*S*)-5,7-dimethoxy-3',4'-methylenedioxyflavanone, glyceollin II, tricin, quercetin 3,5,3'-trimethylether, caesalpinin K, neocaesalpin H, caesalpinin C, and bonducellpin C.

First, the physicochemical tests were carried out in *in silico* study. Based on Lipinski's rule of five, the following physicochemical parameters are determined: logP values less than 5, molar refractivity (MR) values between 40 and 130 cm^3/mol, molecular weights under 500 dalton, hydrogen bonds no less than 5, and acceptor bond donors no less than 10 [35]. LogP value less than 5 represents hydrophobicity [36] and can be used to quantify the degree of lipophilicity of substances ingested by the human body [37,38]. Due to the molar refractivity value varying between 40 to 130 cm^3/mol, the molecule will engage with receptors with ease and have good steric characteristics [39]. Then, the molecular weight of coumarin and its derivatives have met the rules where the molecular weights are less than 500 daltons. The results of the Lipinski test of 18 potential compounds are presented in Table 1.

After the physicochemical test is done, the docking simulation was carried out. Before doing that, it is necessary to prepare the receptor. In general, protein structures in the Protein Data Bank (PDB) contain solvent molecules in the form of water and other residues (Figure 2). Therefore, it is necessary to remove water molecules so as not to interfere when docking simulations are carried out and to ensure that what really interacts is the ligand and receptor. In addition, it is also necessary to add hydrogen. In order to get the docking condition closer to pH 7, hydrogen atoms are also added [40].

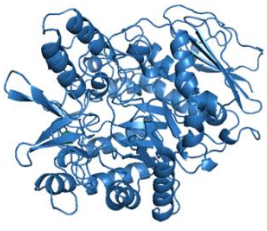


Figure 2. Receptor α-glucosidase (PDB code: 3AJ7)

Table 1. The results of the physicochemical test of 18 potential compounds

No	Compound name	Molecular mass (<500) Dalton	LogP (<5)	Hydrogen bond donor (<5)	Hydrogen bond acceptors (<10)	Molar refractivity (40-130) cm^3/mol
1.	7,4'-dihydroxyflavone	254	2.7140	2	4	69.1491
2.	5-hydroxy-7-[2-(4- hydroxyphenyl)ethenyl]-2,2-dimethyl-2H-1-benzopyran	294	4.4524	2	3	88.8826
3.	4-(3-methyl-but-1-enyl)-3,4',5-trihydroxystilbene	296	4.6230	3	3	90.6784
4.	Apigenin	270	2.4196	3	5	70.8139
5.	Genistein	270	2.4196	3	5	70.8139
6.	Biochanin A	284	2.7226	2	5	75.7011
7.	Fustin	288	1.4807	4	6	71.5847
8.	Kaempferol	286	2.3053	4	6	72.3857
9.	Artocarpanone	302	2.5185	3	6	76.7469
10.	(2*S*)-5,7-dimethoxy-3',4'-methylenedioxyflavanone	328	3.1390	0	6	84.4273
11.	2-(2,3-dihydroxy-3- methylbutyl)-5-(2-phenylethenyl)-1,3- benzenediol	314	2.9425	4	4	91.1502
12.	Glyceollin II	338	3.2901	2	5	91.0506
13.	Tricin	330	2.4368	3	7	83.9179
14.	Quercetin 3,5,3'-trimethyl ether	344	2.7053	2	7	88.2051
15.	Caesalpinin K	376	3.4253	2	5	100.0566
16.	Neocaesalpin H	350	3.2014	2	5	90.3346
17.	Caesalpinin C	416	3.6731	1	6	109.2898
18.	Bonducellpin C	420	2.3457	2	7	105.4656

Validation of the docking method is done by re-docking the natural ligand acarbose (code: 3AJ7) using the prepared α-glucosidase receptor. Root Mean Square Deviation (RMSD) statistics will appear during docking validation. The RMSD expresses the change of protein-ligand interaction in the crystal structure before and after the docking procedure to calculate the deviation values [41]. If the RMSD value is less than 2Å, the docking approach is considered legitimate and can be applied to compound docking tests. This indicates that the docking parameters utilized were appropriate [40].

Validation of α-glucosidase with acarbose receptor resulted in a binding affinity value of -8.2 kcal/mol with an RMSD value of 0.0 Å. This indicates that the docking method used is valid and the parameter settings used fulfill the validation criteria, so that the parameters can be used further for docking test of compounds. From this, validation will be used for docking simulation on potential compounds. To identify the areas of the ligand and receptor proteins that interact, docking analysis was used. Based on the principle of binding energy, this interaction becomes easier to bind the smaller the energy value [42]. Table 2 displays the binding affinity values for 18 candidate compounds.

In addition to binding energy, the interactions that occur between the ligand and the receptor also need to be considered to guarantee that the ligand is bound to the active site of the receptor surface. Discovery Studio 2019 was used to create the visualization needed to see this interaction. Table 3 displays the outcomes of the molecular interactions.

Based on the binding affinity values in Table 2, the test compound with the lowest binding affinity value is Glyceollin II with a value of -10.1. While the highest binding affinity value is Apigenin at -8.2. While other compounds are included in the range. Judging from the data above, 18 test compounds are considered potential in inhibiting α-glucosidase. This is reinforced by previous research, where in the research of Ali et al. (2024) kaempferol and quercetin act as multi-target antidiabetic agents, leading to glucose homeostasis; both compounds show their ability to inhibit and regulate several important targets in the development of diabetes [43].

Table 2. The results of the physicochemical test of 18 potential compounds from *in silico* test with 3AJ7 protein

No	Compound name	Smile	CAS Number	Binding affinity (kcal/mol)
1.	7,4'-dihydroxyflavone	C1=CC(=CC=C1C2=CC(=O)C3 =C(O2)C=C(C=C3)O)O	2196-14-7	-8.7
2.	5-hydroxy-7-[2-(4-hydroxyphenyl)ethenyl]-2,2-dimethyl-2H-1-benzopyran	CC3(C)/C=C\c2c(O)cc(/C=C/c 1ccc(O)cc1)cc2O3	1114548-08-1	-8.6
3.	4-(3-methyl-but-1-enyl)-3,4',5- trihydroxystilbene	CC(C)/C=C/c2c(O)cc(/C=C/c1ccc(O)cc1) cc2O	87320-15-8	-8.9
4.	Apigenin	C1=CC(=CC=C1C2=CC(=O)C3=C (C=C(C=C3O2)O)O)O	8002-66-2	-8.2
5.	Genistein	C1=CC(=CC=C1C2=COC3=CC(=C C(=C3C2=O)O)O)O	446-72-0	-8.4
6.	Biochanin A	COC1=CC=C(C=C1)C2=COC3=C C(=CC(=C3C2=O)O)O	491-80-5	-8.3
7.	Fustin	C1=CC(=C(C=C1[C@@H]2[C@H](C(=O)C 3=C(O2)C=C(C=C3)O)O)O)O	4382-36-9	-8.8
8.	Kaempferol	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C (C=C3O2)O)O)O)O	520-18-3	-8.3
9.	Artocarpanone	COC1=CC(=C2C(=O)C[C@H](OC2=C1)C 3=C(C=C(C=C3)O)O)O	520-25-2	-8.5
10.	(2*S*)-5,7-dimethoxy-3',4'-methylenedioxyflavanone	COC1=CC2=C(C(=O)C[C@H](O2)C3= CC4=C(C=C3)OCO4)C(=C1)OC	133464-45-6	-9.1
11.	2-(2,3-dihydroxy-3-methylbutyl)-5-(2-phenylethenyl)-1,3-benzenediol	[H][C@@](O)(Cc2c(O)cc(/C=C/c1ccccc1)cc2O)C(C)(C)O	1114548-07-0	-8.5
12.	Glyceollin II	CC1(C=CC2=CC3=C(C=C2O1)OC[C @@]4([C@H]3OC5=C4C=CC(=C5)O)O)C	67314-98-1	-10.1
13.	Tricin	COC1=CC(=CC(=C1O)OC)C2=CC (=O)C3=C(C=C(C=C3O2)O)O	520-32-1	-8.4
14.	Quercetin 3,5,3'-trimethyl ether	COC1=CC(=CC2=C1C(=O)C(=C (O2)C3=CC(=C(C=C3)O)OC)OC)O	13459-09-1	-8.4
15.	Caesalpinin K	[H][C@]3(OC(C)=O)CCC(C)(C)[C@]4(C)C [C@]([H])([O+2]#C)[C@]2([N+2](#C)#C)[C@] ([H])(Cc1occc1[C@@]2(C)[N+]#C)[C@@]34C	468733-36-0	-8.4
16.	Neocaesalpin H	[H][C@]3(C)/C1=C/C(=O)O[C@]1(O)C[C@]4([H])[C@@]25CCC[C@@](C)(C(=O)O)[C@]2([H])CC[C+]34([H])C5	538357-65-2	-9.3
17.	Caesalpinin C	CC(=O)O[C@H]1C[C@H](C([C@]2([C@]1([C@H]3CC4=C(C=CO4) C(=C)[C@@H]3CC2)C)O)(C)C)OC(=O)C	817167-15-0	-8.9
18.	Bonducellpin C	CC(=O)O[C@H]1CCC([C@]2([C@] 1([C@H]3CC4=C(C=CO4)[C@H]([C@@H]3[C@H](C2)O)C(=O)OC)C)O)(C)C	197781-84-3	-9.1

Furthermore, tricin, which belongs to the flavonoid class of secondary metabolites, showed a protective role against high glucose-induced heart damage in diabetic cardiomyopathy (DCM) cell models. By reducing oxidative stress and inflammation and inhibiting the TLR4-MYD88-NF-κB pathway, tricin showed significant therapeutic potential for the treatment of DCM [44].

Molecular docking analysis not only offered crucial details about the structural relationship between the enzyme and the metabolites, but also provides the binding energy so that certain compounds can be considered favorable α-glucosidase inhibitors [45]. The interaction visualization demonstrated that acarbose interacts with active site residues TYR 158, SER 240, GLU 332, PRO 312, and HIS 280 by conventional hydrogen bonding. Some potential compounds such as 7,4'-dihydroxyflavone, artocarpanone, (2S)-5,7 dimethoxy-3',4'- methylenedioxyflavanone, glyceollin II, tricin, caesalpinin,

and caesalpinin C showed the same interaction. Some ligands having the same Pi-Alkyl bond are TYR158, HIS 280, and LYS 156. Pi interactions are often associated with stability.

Table 3. Visualization of ligan-receptor interaction of potential compound

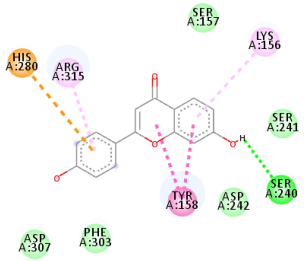
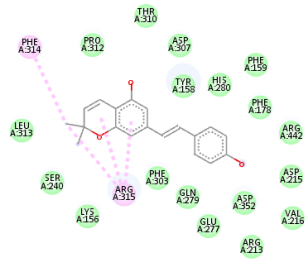
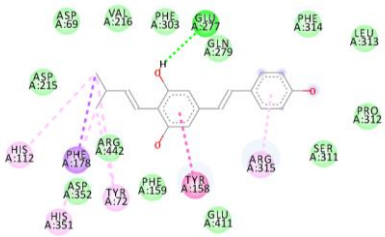
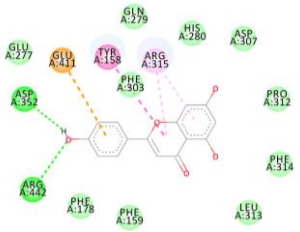
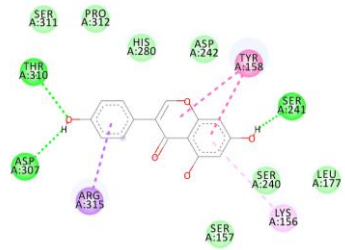
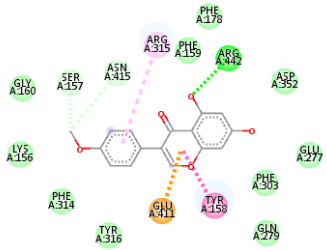
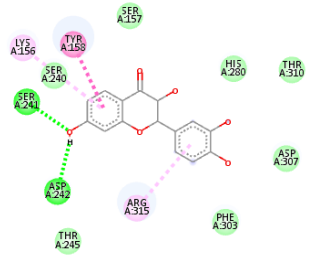
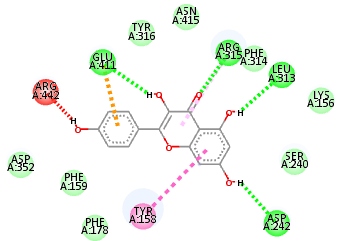
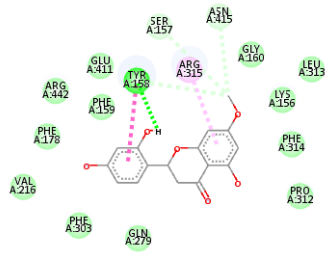
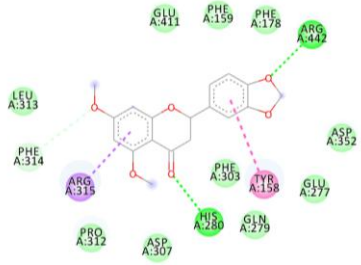
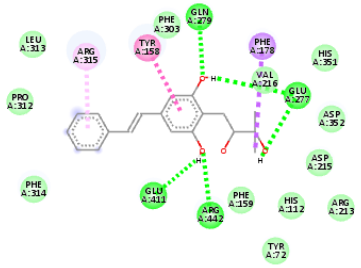
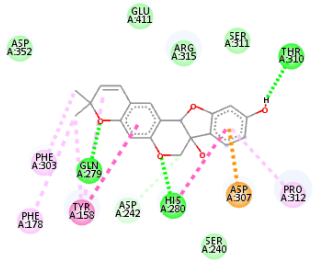
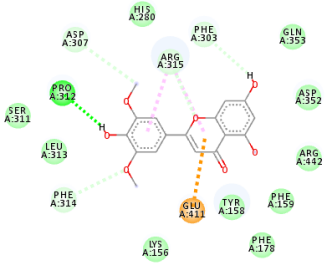
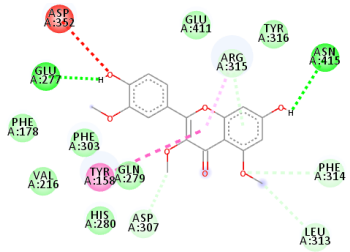
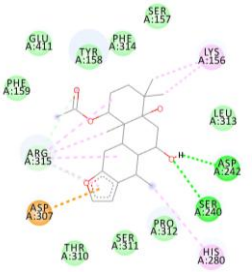
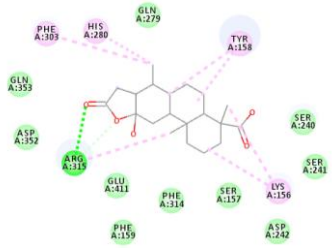
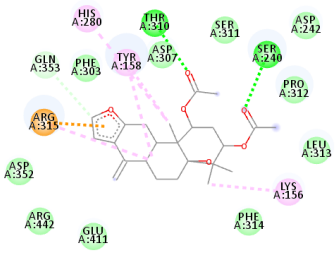
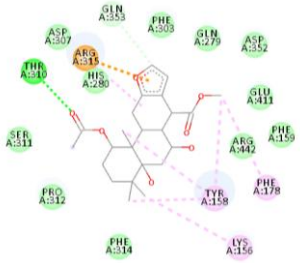
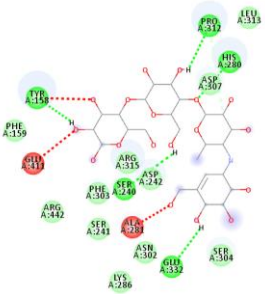
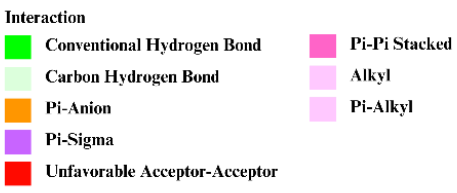
 7,4'-dihydroxyflavone	 5-hydroxy-7-[2-(4-hydroxyphenyl)ethenyl]-2,2- dimethyl-2H-1-benzopyran
 4-(3-methyl-but-1-enyl)-3,4',5- trihydroxystilbene	 Apigenin
 Genistein	 Biochanin A
 Fustin	 Kaempferol
 Artocarpanone	 (2*S*)-5,7-dimethoxy-3',4'- methylenedioxyflavanone

Table 3 *(continue)*. Visualization of ligan-receptor interaction of potential compound

 2-(2,3-dihydroxy-3- methylbutyl)-5-(2- phenylethenyl)-1,3- benzenediol	 Glyceollin II
 Tricin	 Quercetin 3,5,3'-trimethyl ether
 Caesalpinin K	 Neocaesalpin H
 Caesalpinin C	 Bonducellpin C
 Acarbose	

Charge transfer is involved in a lot of Pi-sigma (Pi-alkyl and Pi-sulfur) interactions, which aids in the drug's intercalation at the receptor binding site [46]. The way ligands attach to receptors is influenced by a variety of factors, including electrostatic and hydrophobic interactions as well as hydrogen bonding. The ligand-receptor complex's binding energy and strength are significantly influenced by these interactions [47].

In addition to *in silico* testing using protein 3AJ7, it was also carried out with protein 3W37 where the molecular docking results obtained 10 potential compounds that fulfill the Lipinski rule with a binding affinity value lower than acarbose (Table 4). Of the 10 compounds, there are 4 compounds that are the same in the test with protein 3AJ7 (Table 2). Variations in the three-dimensional (3D) structure can affect the active site and interaction with the ligand. In addition, amino acid residues around the binding site on proteins 3AJ7 and 3W37 can change the way the compound binds to the protein.

Table 4. The results of the physicochemical test of 10 potential compounds from *in silico* test with 3W37 protein

No	Compound name	Smile	CAS Number	Binding affinity (kcal/mol)
1.	5-hydroxy-7-[2-(4-hydroxyphenyl)ethenyl]-2,2-dimethyl-2H-1-benzopyran	CC3(C)/C=C\c2c(O)cc(/C=C/c1ccc(O)cc1)cc2O3	1114548-08-1	-8.3
2.	Luteolin	O=c2cc(c1ccc(O)c(O)c1)oc3cc(O)cc(O)c23	491-70-3	-8.2
3.	Fustin	C1=CC(=C(C=C1[C@@H]2[C@H](C(=O)C3=C(O2)C=C(C=C3)O)O)O)O	4382-36-9	-8.2
4.	Glyceollin I	O[C@]12[C@](C=3C(OC1)=C4C(=CC3)OC(C)(C)C=C4)(OC=5C2=CC=C(O)C5)[H]	57103-57-8	-8.9
5.	Glyceollin II	CC1(C=CC2=CC3=C(C=C2O1)OC[C@@]4([C@H]3OC5=C4C=CC(=C5)O)O)C	67314-98-1	-8.9
6.	Glyceofuran	CC(C)(O)c2cc1cc3c(cc1o2)OC[C@@]5(O)C[C@@H]3Oc4cc(O)ccc45	78873-52-6	-8.6
7.	Demethyltorosaflavone C	CC1=C(O)C(=O)C2OC3=CC4=C(C(=O)C=C(O4)C4=CC=C(O)C(O)=C4)C(O)=C3C12	145194-15-6	-8.5
8.	Neocaesalpin H	[H][C@]3(C)/C1=C/C(=O)O[C@]1(O)C[C@]4([H])[C@@]25CCC[C@@](C)(C(=O)O)[C@]2([H])CC[C+]34([H])C5	538357-65-2	-8.3
9.	Pulcherrin B	[H][C@@H]4C[C@@]2(O)C(C)(C)[C@@]([H])(OC(=O)c1ccccc1)CC[C@]2(C)[C@@]5([H])Cc3occc3[C+]6([H])(C)[C@]45[N+3]6(#C)#[O+]	1132772-55-4	-9.1
10.	Chebulagic acid	*=C6O[C@]%11(C)O[C@@H]3COC(=O)c1cc(O)c(O)c(O)c1c2c(O)c(O)c(O)cc2C(=O)[O+5]%12(#C)(C)O/C/5=C(O)\C[C@]\4%10/C=N/[C@]3(OC4=I/[C@@]9(O)C(=O)Oc8c(O)c(O)cc(C5=*\c7cc6cc(O)c7O)c8[C@@]9%10C=C)[C@H](C)[C@H]%11%12	23094-71-5	-9.6

In Vitro α-Glucosidase Inhibition Assay

Antidiabetic testing can generally use α-glucosidase and α-amylase inhibition. However, in this study, α-glucosidase inhibition was used. The use of α-glucosidase inhibitors is because it is more effective in controlling glucose levels compared to α-amylase. This is because α-glucosidase works at the final stage of carbohydrate digestion by hydrolysing oligosaccharides and disaccharides in the wall of the small intestine, thus helping to prevent a spike in glucose levels after a meal. By inhibiting this enzyme, glucose absorption can be significantly delayed [48]. In addition, research by Poongunran et al. (2016) showed that α-glucosidase inhibition has a lower IC_{50} value of 28.2 µg/ml compared to the α-amylase inhibitor of 39.9 µg/ml for the same extract fraction [49].

In vitro antidiabetic studies determined the levels of α-glucosidase activity inhibition by comparing the amount of p-nitrophenyl produced to the initial enzyme activity [50]. The primary enzyme involved in the digestion of carbohydrates, along with lipids, is called α-glucosidase [28]. It helps break down complex carbs into simpler, more readily absorbed forms [28] playing a role in the breakdown of complex carbohydrates into simpler and more easily absorbed carbohydrates [51]. Inhibition of this enzyme leads to decreased glucose uptake by the small intestine which results in decreased blood glucose levels [52]. People with diabetes have higher α-glucosidase levels than normal people [53]. In order to stop the post-prandial spike in blood sugar levels, the enzyme inhibitors delay the intestinal absorption of glucose [54]. Therefore, this enzyme is one of the target enzymes for the treatment of T2DM.

The α-glucosidase inhibitory activity was measured at 405 nm due to that's the wavelength at which p-nitrophenol absorbs the strongest (400–410 nm) [55]. Parameters of the α-glucosidase inhibition activity through *in vitro* study are the inhibition concentration (IC_{50}). This is the amount that can stop 50% of the activity of α-glucosidase [56]. The following are categories of several IC50 (ppm) values. IC50 values < 50 ppm, 50 < IC50 < 100 ppm, 100 < IC50 < 150 ppm, 150 < IC50 < 200 ppm, and IC50 > 200 ppm are categorized as very strong, strong, moderate, weak, and very weak, respectively [57]. This inhibition activity was tested by inhibiting α-glucosidase using *p*-NPG substrate and α glucosidase where α-glucosidase will hydrolyze *p*-NPG substrate into α-D-glucose and p-nitrophenol (yellow color). Using a microplate reader, the yellow color of the reaction served as an indicator of the enzyme inhibitory activity. If the amount of yellow color in the final solution is less than in the inhibitor-free solution, it means the inhibitor has a higher capacity to impede the α-glucosidase reaction [55].

Acarbose is the positive control that works by inhibiting the α-glucosidase activity [54]. It was reported that acarbose is a substrate that inhibits the α-glucosidase activity due to its structural similarity with oligosaccharides [58]. Acarbose is easily absorbed by the active side of the enzyme, which prevents the enzyme from doing its intended function [52]. The inhibitory potential against α-glucosidase enzyme is represented as IC_{50} value [59]. Percentage inhibition of α-glucosidase by acarbose and by the ethanol extract of *D. virgatus* leaves can be found in Tables 5 and 6, respectively.

Table 5. Percentage inhibition of α-glucosidase by acarbose and its IC_{50} value

Conc. (ppm)	Log[Conc.]	%Inhibition	IC_{50} (ppm)
0.7813	-0.1072	20.55 ± 6.72	3.78 ± 0.65
1.5625	0.1938	27.00 ± 5.76	
3.1250	0.4949	39.76 ± 6.40	
6.2500	0.7959	57.10 ± 2.11	
12.5000	1.0969	63.44 ± 3.03	
25.0000	1.3979	64.94 ± 0.65	
50.0000	1.6990	68.36 ± 3.78	

Table 6. Percentage inhibition of α-glucosidase by the ethanol extract of *D. virgatus* leaves and its IC_{50} value

Conc. (ppm)	Log[Conc.]	%Inhibition	IC_{50} (ppm)
7.81	0.89279	4.77 ± 1.76	144.11 ± 6.93
15.63	1.19382	15.30 ± 3.92	
31.25	1.49485	22.88 ± 4.38	
62.50	1.79588	35.66 ± 0.03	
125.00	2.09691	38.10 ± 0.70	
250.00	2.39794	59.38 ± 2.58	
500.00	2.69897	77.24 ± 1.11	

The data from Table 5 showed that the % inhibition value increased with increasing ethanol extract concentration. More inhibition of enzyme activity results from higher percentages of inhibition

resulting from each concentration [50]. From the results of %inhibition and log concentration, a graph was made as shown in Figure 3.

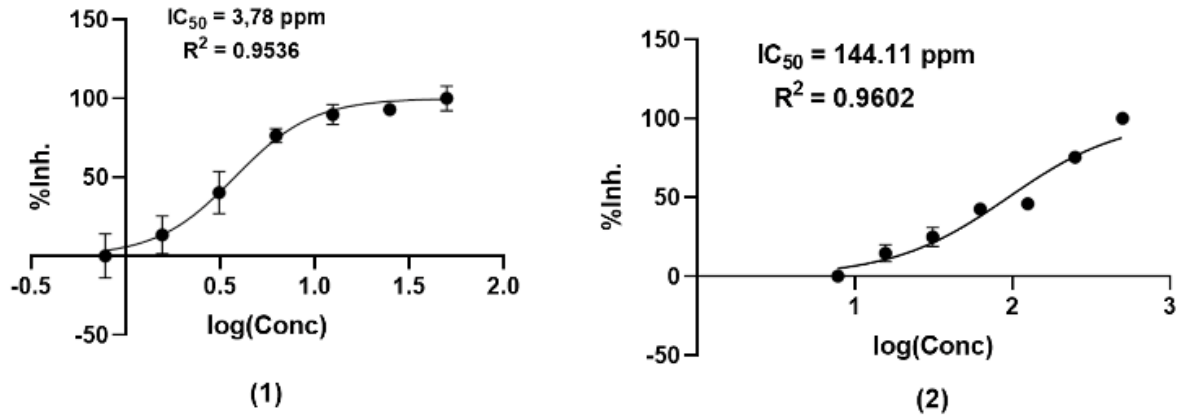


Figure 3. (1) graph of log concentration and % inhibition of acarbose (2) graph of log concentration and % inhibition of ethanol extract of *D. virgatus*

The IC_{50} value of 144.11 ppm was obtained from the testing of α-glucosidase inhibition from ethanol extract using a microplate reader. The ethanol extract of *D. virgatus* leaves can be categorized as medium based on the value obtained. This value is lower than acarbose whose IC_{50} value is 3.78 ppm. This is due to the fact that acarbose is one of the active substances that can successfully inhibit α-glucosidase's activity. In the meantime, it is evident from the ethanol extract sample of *D. virgatus* leaves that it contains a number of active compounds that enable competitive interactions amongst compounds, reducing the effect on the inhibition of α-glucosidase [50]. Based on statistical analysis using SPSS by comparing the IC_{50} value of acarbose with ethanol extract, there is a significant difference with a p value is 0.049 with a mean difference of 48.9065.

CONCLUSION

All of the identified compounds were tested *in silico*, using receptors (PDB ID: 3AJ7) 18 compounds and with (PDB ID: 3W37) 10 compounds were found to fulfill Lipinski's rule with a binding affinity value ≤ -8.2 for 3AJ7 protein and ≤ -8.1 for 3W37, which is similar to acarbose. Meanwhile, the antidiabetic test of the ethanol extract of *D. virgatus* (L.) leaves and acarbose *in vitro* study using α-glucosidase inhibition obtained IC_{50} values of 144.11 ppm and 3.78 ppm, respectively.

AUTHOR CONTRIBUTIONS

Concept: T.T., A.P.W., L.F., S.L.H., R.M.; Design: T.T.; Control: T.T., A.P.W.; Sources: T.T., A.P.W.; Materials: T.T., A.P.W.; Data Collection and/or Processing: L.F., S.L.H., R.M.; Analysis and/or Interpretation: L.F., S.L.H., R.M.; Literature Review: T.T., L.F., S.L.H., R.M.; Manuscript Writing: T.T., A.P.W., L.F.; Critical Review: T.T., A.P.W., L.F., S.L.H., R.M.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. Oliveira, H., Fernandes, A., Brás, N.F., Mateus, N., de Freitas, V., Fernandes, I. (2020). Anthocyanins as antidiabetic agents *in vitro* and *in silico* approaches of preventive and therapeutic effects. Molecules. 25(17), 1-30. [CrossRef]
2. Malik, A., Jamil, U., Butt, T.T., Waquar, S., Gan, S.H., Shafique, H., Jafar, T.H. (2019). *In silico* and *in vitro* studies of lupeol and iso-orientin as potential antidiabetic agents in a rat model. Drug Design, Development and Therapy. 13, 1501-1513. [CrossRef]
3. Yaribeygi, H., Sathyapalan, T., Atkin, S.L., Sahebkar, A. (2020). Molecular mechanisms linking oxidative stress and diabetes mellitus. Oxidative Medicine and Cellular Longevity. 2020, 8609213. [CrossRef]
4. Rachdaoui N. (2020). Insulin: The friend and the foe in the development of type 2 diabetes mellitus. International Journal of Molecular Sciences. 21(5), 1-21. [CrossRef]
5. Mohajan, D, Mohajan, H.K. (2023). Hyperglycaemia among diabetes patients: A preventive approach. International Journal of Innovative Science and research Technology, 2(6), 27-33. [CrossRef]
6. Elhady, S.S., Youssef, F.S., Alahdal, A.M., Almasri, D.M., Ashour, M.L. (2021). Anti-hyperglycaemic evaluation of *Buddleia indica* leaves using *in vitro, in vivo* and *in silico* studies and its correlation with the major phytoconstituents. Plants, 10(11). [CrossRef]
7. Aktas, G., Kocak, M.Z., Bilgin, S., Atak, B.M., Duman, T.T., Kurtkulagi, O. (2021). Uric acid to HDL cholesterol ratio is a strong predictor of diabetic control in men with type 2 diabetes mellitus. Aging Male, 23(5),1098-1102. [CrossRef]
8. Liu, J., Bai, R., Chai, Z., Cooper, M.E., Zimmet, P.Z., Zhang, L. (2022). Low-and middle-income countries demonstrate rapid growth of type 2 diabetes: An analysis based on global burden of disease 1990-2019 data. Diabetologia, 65(8), 253-260. [CrossRef]
9. Khatimah, H. (2023). Analisis tingkat pengetahuan faktor risiko DM dengan status DM pada guru SMA negeri di kelurahan Paccerakang. Jurnal Nursing Update, 14, 86–92.
10. Dirir, A.M., Daou, M., Yousef, A.F., Yousef, L.F. (2022). A review of alpha-glucosidase inhibitors from plants as potential candidates for the treatment of type-2 diabetes. In Phytochemistry Reviews. Springer Netherlands. 21(4). [CrossRef]
11. Eizirik, D.L., Pasquali, L., Cnop, M. (2020). Pancreatic β-cells in type 1 and type 2 diabetes mellitus: Different pathways to failure. Nature Reviews Endocrinology, 16(7), 349-362. [CrossRef]
12. Faida, A.N., Dyah, Y., Santik, P. (2020). Kejadian diabetes melitus tipe I pada usia 10-30 tahun. Higeia Journal of Public Health. 4(1), 33-42.
13. Dafriani, P., Dewi, R.I.S. (2019). Tingkat pengetahuan pada pasien diabetes melitus (DM) tipe 2. Jurnal Abdimas Saintika, 1(1), 45-50.
14. Ayuni, N.M.I. (2020). Efek buah naga merah (*Hylocereus polyrhizus*) terhadap penurunan kadar glukosa darah pada diabetes tipe 2 effect of red dragon fruit (*Hylocereus polyrhizus*) on reducing blood glucose levels in type 2 diabetes. Jurnal Ilmiah Kesehatan Sandi Husada, 11(1), 554-560. [CrossRef]
15. Galicia-garcia, U., Benito-vicente, A., Jebari, S., Larrea-sebal, A. (2020). *Costus ignus*: Insulin plant and it's preparations as remedial approach for diabetes mellitus. International Journal of Molecular Sciences, 21(6275), 1-34.
16. Syachriyani, S., Firmansyah, F. (2022). Potensi antihiperglikemik ekstrak kulit buah semangka (*Citrullus lanatus* linn.) terhadap diabetes mellitus melalui penghambatan aktivitas enzim alfa glukosidase. Jurnal Mandala Pharmacon Indonesia, 8(2), 243-251. [CrossRef]
17. Kalinovskii, A.P., Sintsova, O.V., Gladkikh, I.N., Leychenko, E.V. (2023). Natural inhibitors of mammalian α-amylases as promising drugs for the treatment of metabolic diseases. International Journal of Molecular Sciences, 24(22), 16514.
18. Sameeh, M.Y., Khowdiary, M.M., Nassar, H.S., Abdelall, M.M., Amer, H.H., Hamed, A. (2022). Thiazolidinedione derivatives: *in silico*, *in vitro*, *in vivo*, antioxidant and anti-diabetic evaluation. Molecules, 27(830),1-15.
19. Shivanagoudra, S.R., Perera, W.H., Perez, J.L., Athrey, G., Sun, Y., Wu, C.S. (2019). *In vitro* and *in silico* elucidation of antidiabetic and anti-inflammatory activities of bioactive compounds from *Momordica charantia* L. Bioorganic & Medicinal Chemistry, 27(14), 3097-3109. [CrossRef]
20. Saputra, B.A. (2021). Potensi ekstrak daun sambiloto sebagai obat antidiabetes. Jurnal Penelitian Perawat Profesional, 3(2), 253-260. [CrossRef]
21. Indrianingsih, A.W., Wulanjati, M.P., Windarsih, A., Bhattacharjya, D.K., Suzuki, T., Katayama, T. (2021). *In vitro* studies of antioxidant, antidiabetic, and antibacterial activities of *Theobroma cacao*, *Anonna muricata* and *Clitoria ternatea*. Biocatalysis and Agricultural Biotechnology, 33, 101995. [CrossRef]

22. Mugaranja, K.P., Kulal, A. (2020). Alpha-glucosidase inhibition activity of phenolic fraction from *Simarouba glauca*: An *in-vitro*, *in-silico* and kinetic study. Heliyon. 6(7), e04392. [CrossRef]
23. Adiputra, R. (2023). Efek samping penggunaan obat anti diabetes jangka panjang: Sebuah meta analisis. Jurnal Kesehatan Tambusai. 4(3).
24. Khanal, P., Patil, B.M. (2020). Integration of *in silico*, *in vitro* and *ex vivo* pharmacology to decode the anti-diabetic action of *Ficus benghalensis* L. bark. Journal of Diabetes & Metabolic Disorders, 19, 1325-1337. [CrossRef]
25. Sembiring, E.N., Elya, B., Sauriasari, R. (2018). Phytochemical screening, total flavonoid and total phenolic content and antioxidant activity of different parts of *Caesalpinia bonduc* (L.) Roxb. Pharmacognosy Journal., 10(1), 123-127.
26. Hikmawanti, N.P.E., Fatmawati, S., Asri, A.W. (2021). The effect of ethanol concentrations as the extraction solvent on antioxidant activity of katuk (*Sauropus androgynus* (L.) Merr.) leaves extracts. IOP Conference Series: Earth and Environmental Science, 755(1). [CrossRef]
27. Jitrangsri, K., Chaidedgumjorn, A., Satiraphan, M. (2020). Effect of ethanol percentage upon various extraction methods of peanut based on antioxidant activity with trans-resveratrol and total phenolic contents. Pharmaceutical Sciences Asia, 47(2), 164-172. [CrossRef]
28. Etsassala, N.G.E.R, Badmus, J.A., Marnewick, J.L., Iwuoha, E.I., Nchu, F., Hussein, A.A. (2020). Alpha-glucosidase and alpha-amylase inhibitory activities, molecular docking, and antioxidant capacities of *Salvia aurita* constituents. Antioxidants, 9(11), 1-14. [CrossRef]
29. Sadik, F., Rifqah, A.A.A. (2022). Standarisasi parameter spesifik ekstrak etanol daun pegagan (*Centella asiatica* L.) sebagai antidiabetes. Journal Syifa Science and Clinical Research, 4(1), 1-9. [CrossRef]
30. Azzahra, A., Farhani, N., Syahfitri, W., Pasaribu, S.F. (2022). Potensi kandungan flavonoid dalam kayu bajakah sebagai antidiabetes. Jurnal Pendidik Tambusai, 6(2), 14345-14350.
31. Ghorbani, A. (2017). Mechanisms of antidiabetic effects of flavonoid rutin. Biomedicine & Pharmacotherapy, 96, 305-312. [CrossRef]
32. Ullah, A., Munir, S., Badshah, S.L., Khan, N., Ghani, L., Poulson, B.G. (2020). Important flavonoids and their role as a therapeutic agent. Molecules. 25(22), 1-39. [CrossRef]
33. Shehadeh, M.B., Suaifan, G.A.R.Y., Abu-Odeh, A.M. (2021). Plants secondary metabolites as blood glucose-lowering molecules. Molecules, 26(14), 1-46. [CrossRef]
34. Blahova, J., Martiniakova, M., Babikova, M., Kovacova, V., Mondockova, V., Omelka, R. (2021). Pharmaceutical drugs and natural therapeutic products for the treatment of type 2 diabetes mellitus. Pharmaceuticals, 14(8), 1-32. [CrossRef]
35. Syifa, P., Bertha, R. (2023). Uji *in silico* aktivitas senyawa kumarin turunannya terhadap enzim alfa-glukosidase antidiabetes. Jurnal Riset Farmasi, 3(1), 9-16. [CrossRef]
36. Chen, X., Li, H., Tian, L., Li, Q., Luo, J., Zhang, Y. (2020). Analysis of the physicochemical properties of acaricides based on Lipinski's rule of five. Journal of Computational Biology, 27(9), 1397-1406. [CrossRef]
37. Truzzi, F., Tibaldi, C., Zhang, Y., Dinelli, G., D'Amen, E. (2021). An overview on dietary polyphenols and their biopharmaceutical classification system (BCS). International Journal of Molecular Sciences, 22(5514), 1-23. [CrossRef]
38. Mishra, M., Arukha, A.P., Patel, A.K., Behera, N., Mohanta, T.K., Yadav D. (2020). Multi-drug resistant coliform: Water sanitary standards and health hazards. Journal of King Saud University-Science, 32(9), 1-8. [CrossRef]
39. Annisa, F.S., Hilda, A., Taufik, M.F. (2022). Uji aktivitas *in silico* senyawa amritoside, tinosporaside dan turunannya sebagai kanditat senyawa. Bandung Conference Series Pharmacy, 2(2), 1-10. [CrossRef]
40. Sari, I.W., Junaidin, Pratiwi, D. (2020). Studi molecular docking senyawa flavonoid herba kumis kucing (*Orthosiphon stamineus* B.) pada reseptor α-glukosidase sebagai antidiabetes tipe 2. Journal Farmagazine, 7(2), 54-60. [CrossRef]
41. Prasetiawati, R., Damayanti, A., Suwandi, D.W. (2023). Simulasi dinamika molekuler senyawa aktif akar pakis tangkur (*Polypodium feei* METT) sebagai inhibitor enzim inducible nitric oxide synthase (iNOS). Prosiding Seminar Nasional Diseminasi Penelitian, 3(1), 390-400.
42. Laily, A.N., Khoiri, A.N. (2016). Identifikasi senyawa antidiabetes secara *in silico* pada *Carica pubescens* Lenne & K. Koch. El–Hayah. 5(4), 135-142. [CrossRef]
43. Ali, M., Hassan, M., Ansari, S.A., Alkahtani, H.M., Al-Rasheed, L.S., Ansari, S.A. (2024). Quercetin and kaempferol as multi-targeting antidiabetic agents against mouse model of chemically induced type 2 diabetes. Pharmaceuticals, 17(6), 757. [CrossRef]
44. Yu, R., Zhang, Y., Wang, T., Duan, J., Li, X. (2024). Effect of tricin on cardiomyocyte damage caused by diabetic cardiomyopathy (DCM). BMC Cardiovascular Disorders, 24(1), 1-8. [CrossRef]

45. Niaz, A., Adnan, A., Bashir, R., Mumtaz, M.W., Raza, S.A., Rashid, U., Tan, C.P., Tan, T.B. (2021). The *in vitro* α-glucosidase inhibition activity of various solvent fractions of *Tamarix dioica* and 1H-NMR based metabolite identification and molecular docking analysis. Plants. 10(1128), 1-14.
46. Arthur, D.E., Uzairu, A. (2019). Molecular docking studies on the interaction of NCI anticancer analogues with human phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit. Journal King Saud University-Science, 31(4), 1151-1166. [CrossRef]
47. Alandijany, T.A., El-Daly, M.M., Tolah, A.M., Bajrai, L.H., Khateb, A.M., Alsaady, I.M. (2023). Investigating the mechanism of action of anti-dengue compounds as potential binders of Zika virus RNA-dependent RNA polymerase. Viruses, 15(7), 1-22. [CrossRef]
48. Febrianda, L.H., Jawi, I.M., Widiarthini, I.A.A., Aman, I.G.M. (2024). Uji penghambatan aktivitas enzim alfa-glukosidase terhadap dua produk jamu kapsul ekstrak kayu manıs (*Cinnamomum burmannii*). Jurnal Medika Udayana, 13(02), 53-58.
49. Poongunran, J., Perera, H.K.I., Jayasinghe, L., Fernando, I.T., Sivakanesan, R., Araya, H., Fujimoto, Y. (2017). Bioassay-guided fractionation and identification of α-amylase inhibitors from *Syzygium cumini* leaves. Pharmaceutical Biology, 55(1), 206-211. [CrossRef]
50. Hilma, R., Gustina, N., Syahri, J. (2020). Pengukuran total fenolik, flavonoid, aktivitas antioksidan dan antidiabetes ekstrak etil asetat daun katemas (*Euphorbia heterophylla* L.) secara *in vitro* dan *in silico* melalui inhibisi enzim α-glukosidase. ALCHEMY Jurnal Penelitian Kimia,16(2), 240-249. [CrossRef]
51. Mechchate, H., Es-Safi, I., Louba, A., Alqahtani, A.S., Nasr, F.A., Noman, O.M. (2021). *In vitro* alpha-amylase and alpha-glucosidase inhibitory activity and *in vivo* antidiabetic activity of *Withania frutescens* L. foliar extract. Molecules, 26(2), 1-10. [CrossRef]
52. Sinulingga, S., Subandrate, S., Safyudin, S. (2020). Uji fitokimia dan potensi antidiabetes fraksi etanol air benalu kersen (*Dendrophtoe petandra* (L) Miq). Jurnal Kedokteran dan Kesehatan, 16(1), 76-83. [CrossRef]
53. Abdelmonsef, M., Shawky, E., Ghareeb, D.A., El Naggar, E.M.B., El Newehy, N.M. (2024). Comprehensive metabolomics and chemometrics unravel potential anti-diabetic metabolites of pumpkin (*Cucurbita pepo* L.) fruits through UPLC-QqQ-MS and GC–MS analyses. Food Research International, 192(114771), 1-16. [CrossRef]
54. Yuniarto, A., Selifiana, N. (2018). Aktivitas inhibisi enzim alfa-glukosidase dari ekstrak rimpang bangle (*Zingiber cassumunar* Roxb.) secara *in vitro*. Media Pharma Indonesia, 2(1), 22-25. [CrossRef]
55. Fadhli, H., Hendri, S.N., Nurain, N.A. (2021). Aktivitas inhibisi enzim alfa-glukosidase dari ekstrak kulit batang bunga kupu-kupu (*Bauhinia semibifida* Roxb) secara *in vitro*. Jurnal Ilmiah Ibnu Sina: Ilmu Farmasi dan Kesehatan, 6(2), 223-231. [CrossRef]
56. Zabidi, N.A., Ishak, N.A., Hamid, M., Ashari, S.E., Mohammad, L.M.A. (2021). Inhibitory evaluation of *Curculigo latifolia* on α-glucosidase, DPP (IV) and *in vitro* studies in antidiabetic with molecular docking relevance to type 2 diabetes mellitus. Journal of Enzyme Inhibition and Medicinal Chemistry, 36(1), 109-121. [CrossRef]
57. Souhoka, F.A., Hattu, N., Huliselan, M. (2019). Uji aktivitas antioksidan ekstrak metanol biji kesumba keling (*Bixa orellana* L). Indonesian Journal of Chemical Research, 7(1), 25-31. [CrossRef]
58. Kashtoh, H., Baek, K.H. (2022). Recent updates on phytoconstituent alpha-glucosidase inhibitors: An approach towards the treatment of type two diabetes. Plants, 11(20), 1-25. [CrossRef]
59. Seboletswe, P., Kumar, G., Kubone, L., Olofinsan, K., Idris, A., Islam, M.S., Singh, P. (2024). Ultrasound-assisted synthesis of 4-thiazolidinone Schiff bases and their antioxidant, α-glucosidase, α-amylase inhibition, mode of inhibition and computational studies. Medicinal Chemistry Research. [CrossRef]