Indo. J. Chem. Sci. 14 (2) (2025)

Indonesian Journal of Chemical Science

http://journal.unnes.ac.id/sju/index.php/ijcs

In Silico Study on the Antidiabetic Activity of Shallot Plants (Allium cepa) Targeting the Dipeptidyl Peptidase-4 (DPP4) Receptor

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Info Artikel

Diterima : 20-01-2025

Disetujui : 05-05-2025

Dipublikasikan : 29-08-2025

Keywords:

Bawang merah

DPP4

Penambatan Molekuler

Abstrak

Pendahuluan: Diabetes mellitus tipe 2 merupakan suatu gangguan pada sel beta pankreas dalam mensekresikan insulin yang ditandai dengan hiperglikemia atau meningkatnya kadar gula darah melebihi kondisi normal. DPP-4 telah diidentifikasi sebagai target penting dalam pengobatan diabetes tipe 2 karena perannya dalam regulasi glukosa darah dan produksi insulin. Penelitian ini bertujuan untuk membuktikan potensi senyawa yang terkandung di dalam bawang merah *(Allium cepa)* yang berperan sebagai inhibitor DPP-4 secara in silico melalui penambatan molekul. Metode: Penelitian dilakukan dengan menggunakan perangkat lunak Chemdraw, Chem 3D, AutoDockTools 1.5.6, BIOVIA Discovery Studio 2021, dan Ligandscout. Hasil: Terdapat 20 senyawa bawang merah yang diuji melalui pengujian *lipinski rule of five*, prediksi ADMETOKS, hingga penambatan molekuler. 2,4-Dimethylthiophene adalah senyawa yang paling optimal aktivitasnya dengan DPP4. Senyawa ini memiliki konstanta inhibisi (KI) dan binding energy (ΔG) terkecil, serta ditemukan 3 ikatan yang serupa dengan senyawa alami, yaitu TYR B: 666, TYR B: 662, dan SER B: 630.

Abstract

Introduction: Type 2 diabetes mellitus is a condition characterized by hyperglycemia, or elevated blood sugar levels, resulting from the dysfunction of pancreatic beta cells in insulin secretion. DPP-4, an enzyme involved in the regulation of blood glucose and insulin production, has been identified as a significant target for the treatment of type 2 diabetes. The objective of this study aims to investigate the potential of certain specific compounds found in shallots (*Allium cepa*) to act as DPP-4 inhibitors through molecular docking simulations. Methods: This study utilizemployed various software tools, including Chemdraw, Chem 3D, AutoDockTools 1.5.6, BIOVIA Discovery Studio 2021, and Ligandscout, to conduct the research. Results: A total of 20 shallot compounds were tested using the Lipinski Rule of Five, ADMETOKS prediction, and molecular docking. 2,4-Dimethylthiophene exhibited the most optimal activity with DPP-4. This compound displayed the lowest inhibition constant (KI) and binding energy (ΔG), and three bonds similar to natural compounds (TYR B: 666, TYR B: 662, and SER B: 630) were detected

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p-ISSN 2252-6951
e-ISSN 2502-6844

Introduction

Type 2 diabetes mellitus is a chronic condition characterized by elevated blood sugar levels, known as hyperglycemia, that go beyond the normal range. Insulin, a hormone, is crucial for regulating blood sugar levels. However, individuals with type 2 diabetes mellitus experience a disruption in the secretion of insulin by the pancreatic beta cells. As a result, the body's cells become less responsive to insulin, leading to ineffective utilization of the insulin produced (Galicia-Garcia et al., 2020). According to the 2018 Basic Health Research, known as Riskesdas, the prevalence of diabetes has significantly increased compared to the 2013 Riskesdas study, rising from 6.9% to 8.5%. This increase is particularly notable in Indonesia, where the number of individuals affected by type 2 diabetes reached 41,817 people in 2022 (Kemenkes RI, 2018). As a result, Indonesia has the highest diabetes prevalence among ASEAN member countries. Additionally, according to the International Diabetes Federation (IDF), Indonesia ranks fifth globally in terms of the number of diabetes patients, following China, India, Pakistan, and the United States. It is estimated that there were 19.5 million individuals with diabetes in Indonesia in 2021, with projections suggesting that this number will reach 28.6 million by 2045 (IDF, 2021).

The pathophysiology of incretin currently serves as a promising target for the development of innovative antidiabetic agents. Patients diagnosed with type-2 diabetes mellitus exhibit a deficiency in incretin hormones, specifically GLP-1 (glucagon-like polypeptide-1) and GIP (glucose-dependent insulinotropic polypeptide), which play a critical role in regulating insulin secretion after meals (Gasbjerg et al., 2018). GLP-1 is secreted by enteroendocrine L cells in the small intestine and exerts its effects by stimulating insulin secretion, reducing glucagon levels, and delaying gastric emptying. On the other hand, GIP is a hormone secreted by neuroendocrine K cells in the stomach and proximal small intestine. The rapid degradation of incretin hormones is facilitated by the DPP-4 enzyme, a serine protease expressed in various tissues such as the intestinal endothelium, kidneys, liver, and blood vessels (Singh et al., 2021). DPP-4 enzymatically breaks down the NH2-terminal amino acids of GIP and GLP-1, resulting in the loss of their insulinotropic effects (Nauck & Meier, 2018). Drugs belonging to the DPP-4 inhibitor group inhibit the function of DPP-4. By inhibiting DPP-4, these inhibitors can elevate the levels of incretin hormones, including glucagon-like peptide-1 (GLP-1) and insulinotropic peptide (GIP), thereby increasing insulin secretion by pancreatic beta cells and consequently reducing postprandial and fasting hyperglycemia (Deacon, 2020).

Multiple studies have provided evidence that shallots possess the ability to lower blood sugar levels. Flavonoid compounds in shallots that are associated with their hypoglycemic effects (Amzad et al., 2021). Therefore, the purpose of this study was to examine the in silico influence of DPP-4 inhibitors on the activity of shallot compounds, which exhibit antidiabetic properties. Shallots are known to contain flavonoids, polyphenols, and organosulfur compounds, all of which contribute to powerful antioxidant benefits that can effectively combat free radicals and inflammation. We conducted an investigation into shallot-derived herbal plants exhibiting DPP-4 inhibition mechanisms by analyzing their physicochemical properties in accordance with Lipinski's Rules of Five to determine their physicochemical viability. We also performed an ADME prediction analysis to evaluate the absorption potential of these compounds, focusing on Caco2 cell permeability, which estimates their absorption by human intestinal cells.

Method

2.1 Tools and Materials

The study utilized Autodock as a supporting software tool, along with various other software programs. Specifically, PubChem was used for two-dimensional test compound modeling, Swiss Drug Design for measuring physicochemical parameters, Preadmet and Dude Docking as databases for the active side of the test compound, LigandScout for pharmacophore modeling and validation, RCSB Protein Data Bank (RCSB PDB) as a database, Biovia for distinguishing between ligands and receptors, and Autodock for ligand and receptor design.

2.2 Procedure

2.2.1 Analysis of Physicochemical Properties of Alpha-Glucosidase

In the search for herbal plants with DPP-4 inhibitory properties, the red onion was selected as it contains an active compound known as alpha-glucosidase. The physicochemical properties of alpha-glucosidase were analyzed using the Lipinski rule, specifically the Rules of Five (RO5), with the assistance of Swiss Drug Design tools. A comprehensive analysis was conducted on the permeability, molecular weight, and number of hydrogen bonds of 20 active compounds.

2.2.2 Prediction Analysis of Absorption, Distribution, Metabolism, Excretion, and Toxicology on Alpha-Glucosidase

The prediction of Absorption, Distribution, Metabolism, Excretion, and Toxicology (ADMET) on alpha-glucosidase utilizes tools provided by the https://preadmet.webservice.bmdrc.org/ platform. The independent variables employed in ADMET prediction include Absorption in Human Intestinal Absorption (HIA) 0 and Caco-2 cells, Distribution to Plasma Protein Binding (PPB) and Blood Brain Barrier (BBB), as well as toxicity assessments such as mutagenicity or Ames tests, and carcinogenicity studies in mouse and rabbit models.

2.2.3 Pharmacophore Model

The process of pharmacophore modeling starts with searching for an active compound database, consisting of both active sites and decoys. This database is obtained from the https://dude.docking.org/targets tool and is stored in a file-based format, specifically .ldb. The active site database is used in pharmacophore modeling using LigandScout. To ensure validity, the resulting pharmacophore is subjected to validation by creating an AUC-ROC curve plot and selecting an AUC value higher than 70% with the highest hits. Following this step, a screening of the test compounds is conducted.

2.2.4 Molecular Docking Simulation

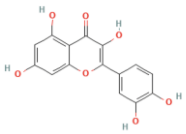
The molecular docking simulation commences with the separation of ligands and receptors utilizing the Biovia tool. The receptors were sourced from https://www.rcsb.org/ and subsequently isolated in pdb format using Biovia. The natural ligands of the receptors were then prepared by adding hydrogen atoms, rendering the compounds non-polar, assigning gasteiger charges, and applying torque in the Autodock tool. The preparation results were subsequently verified by establishing docking parameters and conducting an RMSD search. The accepted RMSD requirement is <2%. Compounds that meet the docking requirements are visualized in both 2D and 3D. The docking simulation can be further validated through redocking.

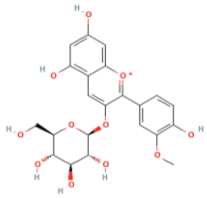
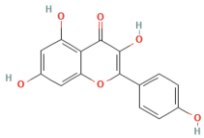
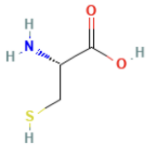
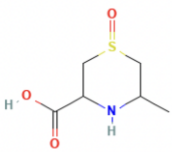
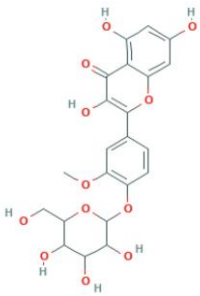
Results and Discussion

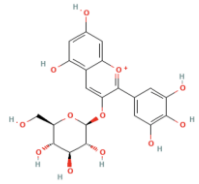
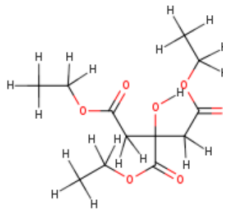
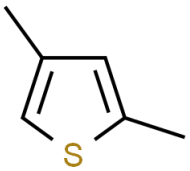
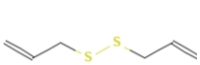
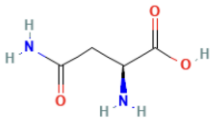
Analysis of Physicochemical Properties of Alpha-Glucosidase

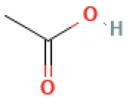
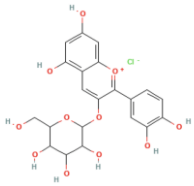
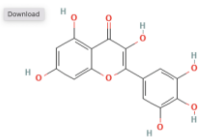
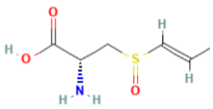
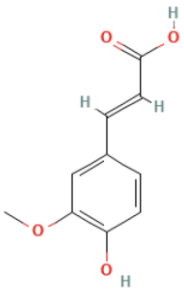
Physicochemical property analysis, according to Lipinski's Rules of Five (RO5) established in 1997, is used to evaluate a compound's similarity to a drug or drug-like substance. This assessment is made based on several criteria, including permeability, molecular weight, and the number of hydrogen bonds. A compound can be considered a potential orally administered drug if it violates no more than one of Lipinski's Rules of Five (Ivanovic et al., 2020). Lipinski's guidelines, known as the Rules of Five (RO5), state that a compound must have an optimal molecular weight below 500 g/mol, fewer than five hydrogen bond donors, less than ten acceptors, and a partition coefficient below five (Lipinski, 2000). If a compound violates no more than one of Lipinski's Rules of Five, it may have the potential to be administered orally as a drug (Ivanović et al., 2020).

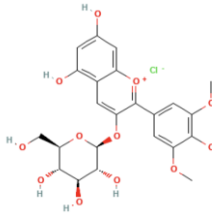
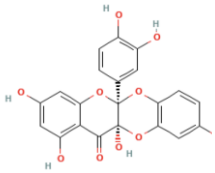
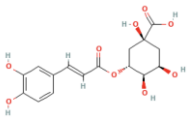
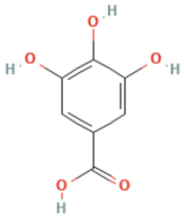
Table 1. Lipinski's Rule of Five (RO5) Prediction Results

No.	Compound Name	Molecular weigh (< 500 Da)	Log P (<5)	hydrogen bond		Description Qualified/Unqualified)
				donor (<5)	acceptor(< 10)	
1.	Quercetin 	302.24 g/mol	1.23	5	7	Qualified
2.	Peonidin 3'-glucoside	463.41 g/mol	-0.69	7	11	Unqualified

No.	Compound Name	Molecular weigh (< 500 Da)	Log P (<5)	hydrogen bond		Description Qualified/Unqualified)
				donor (<5)	acceptor(< 10)	
						
	Kaempferol					
3.		286.24 g/mol	1.58	4	6	Qualified
	Cysteine					
4.		121.16 g/mol	-1.31	2	3	Qualified
	Cycloalliin					
5.		177.2 g/mol	-1.44	2	4	Qualified
	Isorhamnetin-4'-glucoside					
6.		478.40 g/mol	0.00	7	12	Unqualified

No.	Compound Name	Molecular weigh (< 500 Da)	Log P (<5)	hydrogen bond		Description Qualified/Unqualified)
				donor (<5)	acceptor(< 10)	
7.	Delphinidin 3'-glucoside 	465.38 g/mol	-1.55	9	12	Unqualified
8.	Triethyl citrate 	276.28 g/mol	0.93	1	7	Qualified
9.	2,4-Dimethylthiophene 	140.20 g/mol	2.09	0	1	Qualified
10.	Diallyl disulphide 	146.27 g/mol	2.39	0	0	Qualified
11.	Asparagine 	113.14 g/mol	-1.38	4	4	Qualified

No.	Compound Name	Molecular weigh (< 500 Da)	Log P (<5)	hydrogen bond		Description Qualified/Unqualified)
				donor (<5)	acceptor(< 10)	
12.	Acetic-acid 	60.05 g/mol	-0.09	1	2	Qualified
13.	Cyanidin-3-glucoside 	484.84 g/mol	-1.99	8	11	Unqualified
14.	Myricetin 	318.24 g/mol	0.79	6	8	Qualified
15.	Isoaliin 	177.22 g/mol	-1.21	2	4	Qualified
16.	Ferulic acid 	194.18 g/mol	1.36	2	4	Qualified
17.	Malvidine 3' glucoside	528.89 g/mol	-1.90	7	12	Unqualified

No.	Compound Name	Molecular weigh (< 500 Da)	Log P (<5)	hydrogen bond		Description Qualified/Unqualified)
				donor (<5)	acceptor(< 10)	
						
	Alliuocide					
18.		426.33 g/mol	1.34	6	10	Unqualified
	Chlorogenic acid					
19.		354.31 g/mol	-0.39	6	9	Qualified
	Gallic acid					
20.		170.12 g/mol	0.21	4	5	Qualified

The analysis of physicochemical properties was conducted on twenty compounds present in alpha-glucosidase. Out of these, only fourteen compounds met the requirements. These compounds include Quercetin, Kaempferol, Cysteine, Cycloalliin, Triethyl citrate, 2,4-dimethylthiophene, diallyl disulphide, Asparagine, Acetic acid, Myricetin, Isoaliin, Ferulic acid, Chlorogenic acid, and Gallic acid (Table 1.). On the other hand, the following compounds did not meet the requirements due to inappropriate hydrogen bonds: peonidin 3'-glucoside, isorhamnetin-4'-glucoside, delphinidin 3'-glucoside, Cyanidin-3-glucoside, and Alliuocide. Additionally, Malvidine 3' glucoside did not meet the requirements due to its large molecular weight and inappropriate hydrogen bonds. Compounds with a molecular weight exceeding 500 g/mol, like Malvidine 3' glucoside, face challenges in drug distribution because they struggle to penetrate the lipid bilayer membrane. The partition coefficient plays a role in determining the compound's solubility in the body by comparing its solubility ratio in two different solutions. Permeability is expressed as the Log P value, which is directly proportional to lipid solubility (Preeti et al., 2023). A Log P value below 5 indicates good solubility in the body, as the body's barrier consists of a lipid bilayer. Compounds with negative Log P values, such as Peonidin 3'-glucoside, Cysteine, Cycloalliin, Delphinidin 3'-glucoside, Asparagine, Acetic acid, Cyanidin-3-glucoside, Isoaliin, Malvidine 3' glucoside, and Chlorogenic acid, face difficulties in penetrating

the lipid bilayer. The number of hydrogen bond acceptors and donors influences a compound's ability to form hydrogen bonds(Coimbra et al., 2020). Failure to meet the Lipinski Rule of Five criteria indicates poor bioavailability and makes oral administration not recommended. Based on these findings, fourteen out of the twenty compounds found in shallots can be considered potential drug compounds suitable for oral administration.

Prediction Analysis of Absorption, Distribution, Metabolism, Excretion, and Toxicology (ADMET) for Alpha-Glucosidase

ADMET prediction involves the evaluation of the ADMET process within the human body. It utilizes in silico and computational methods to predict the pharmacokinetic behavior and potential toxicity of chemical compounds or drugs. This allows for effective screening of candidates with undesirable ADME profiles and minimizes toxicity during the early stages of drug development. By identifying drug candidates with optimal ADME characteristics and minimal potential toxicity, this approach accelerates and streamlines the drug development process in a cost-effective manner. Consequently, it reduces the need for extensive preclinical and clinical trials.

Table 2. ADMET Prediction Results

No.	Compound Name	Absorption		Distribution		Toxicology		
		HIA (%)	Caco-2 (nm/sec)	PPB (%)	BBB	Mutagens	Carcinogen	
							Rat	*Mouse*
1.	Quercetin	63.485215	3.4129	93.236103	0.172765	+	+	-
2.	Peonidin 3'-glucoside	35.220925	6.84935	65.409551	0.035777 8	-	-	-
3.	Kaempferol	79.439289	9.57744	89.608221	0.286076	+	+	-
4.	Cysteine	56.878627	19.7083	32.855927	0.106899	+	-	+
5.	Cycloalliin	73.806639	0.808763	0.000000	0.12069	+	-	+
6.	Isorhamnetin-4'-glucoside	18.547050	7.17989	33.795103	0.029637 4	+	-	-
7.	Delphinidin 3'-glucoside	7.927141	2.77355	80.024430	0.032700 2	-	-	+
8.	Triethyl citrate	65.414520	4.13632	51.002460	0.248146	+	+	-
9.	2,4-Dimethylthiophene	100.000000	40.0553	97.195467	1.37316	+	+	-

10.	Diallyl disulphide	98.196039	21.7358	88.641588	1.47765	+	+	+
11.	Asparagine	41.517363	7.20784	4.543152	0.070763 9	+	+	+
12.	Acetic-acid	78.718707	20.7623	64.815653	0.621308	-	+	-
13.	Cyanidin-3-glucoside	31.594108	20.2882	62.867107**	0.029179 3**	-	-	-
14.	Myricetin	40.964049	0.991395	96.784810	0.110308	+	+	-
15.	Isoaliin	73.040567	0.426392	1.416188	0.090837 4	+	-	-
16.	Ferulic acid	90.603297	21.1177	50.414225	0.758419	+	+	-
17.	Malvidine 3’ glucoside	47.352909	20.7839	44.571546**	0.028398 8**	-	-	+
18.	Alliuocide	56.063127	1.55101	100.000000	0.147226	-	+	-
19.	Chlorogenic acid	13.008462	18.7845	31.477494	0.028924 2	+	-	+
20.	Gallic acid	53.696852	13.8492	65.384676	0.348084	+	+	-

Absorption prediction in the human body is determined by the HIA (Human Intestinal Absorption) and Caco-2 values. HIA predicts the absorption in the intestine. Capacityof absorption in the intestine is categorized into three levels based on their HIA values: poor absorption capacity (0-20%), moderate absorption capacity (20-70%), and high absorption capacity (70-100%) (BMDRC, 2017). Among the compounds tested, 2,4-Dimethylthiophene exhibited the highest HIA value of 100%, while Delphinidin 3'-glucoside showed the lowest HIA value of 7.927% (Table 2.). These results suggest that 2,4-Dimethylthiophene is highly absorbed in the intestine, whereas Delphinidin 3'-glucoside is poorly absorbed. The Caco-2 value prediction assesses compound absorption by Caco-2 cells, derived from human adenocarcinoma colon cells. It measures the compound's ability to be absorbed by these cells. Compound absorption in Caco-2 cells is categorized into three levels: low absorption (<4), medium absorption (4-70), and high absorption (>70) (BMDRC, 2017). Among the compounds tested, 2,4-Dimethylthiophene exhibited the highest value of 40.055 nm/sec, while Isoaliin showed the lowest value of 0.426 nm/sec.

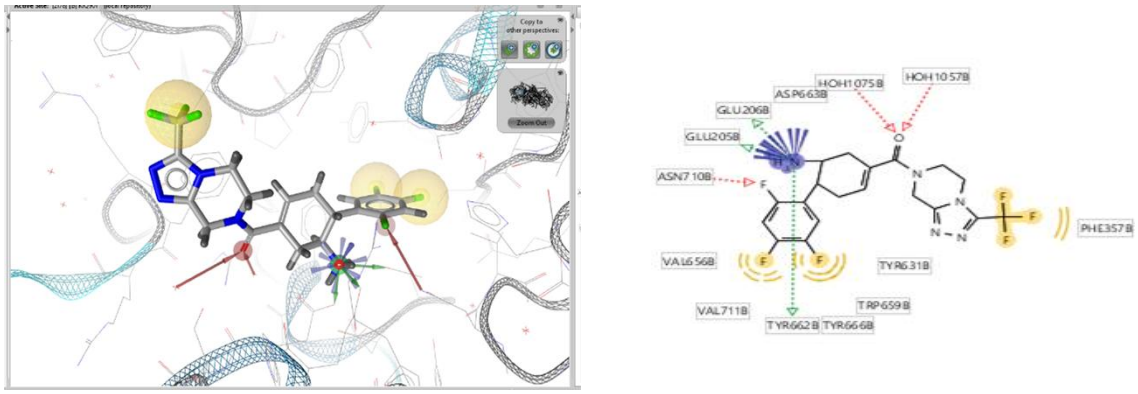
The distribution of drug compounds in the body is described by PBB (Plasma Protein Binding) and BBB (Blood-Brain Barrier). The PPB parameter reflects compounds that bind to proteins in the blood (Di, 2021). Binding affinity is categorized as strongly bound (>90%) or weakly bound (<90%) (BMDRC, 2017). Alliuocide exhibited the highest value of 100%, indicating a very strong protein binding affinity. On the other hand, Cycloalliin showed the lowest value of 0%, suggesting no binding affinity. Strong protein binding affinity can lead to high toxicity and affect the compound's half-life in the body. Compounds that are loosely bound at low levels may not be effective in their use (Afzal et al., 2023). The BBB parameter indicates the

ability of a compound to penetrate the blood-brain barrier (Zhang, 2024). BBB criteria are divided into three scale levels: low penetration (<1), medium penetration (1-2), and high penetration (>2) (BMDRC, 2017). Among the compounds tested, Diallyl disulphide exhibited the highest BBB value of 1.477, while Chlorogenic acid showed the lowest value of 0.0289.

Toxicity can be predicted using the Ames Test and Rodent Carcinogenic parameters. The Ames Test parameters involve using bacteria to determine the mutagenic potential of a compound (Vijay et al., 2018). The Ames test results are classified as positive or negative. Apositive prediction result indicatesing mutagenicity and a negative prediction result indicating non-mutagenicity (BMDRC, 2017). Based on these findings, it was determined that 14 out of 20 compounds possess mutagenic properties. These compounds including Quercetin, Kaempferol, Cysteine, Cycloalliin, Isorhamnetin-4'-glucoside, Triethyl citrate, 2,4-Dimethylthiophene, Diallyl disulphide, Asparagine, Myricetin, Isoaliin, Ferulic acid, Chlorogenic acid, and Gallic acid. Rodent carcinogenic parameters are used to predict whether a test compound can induce tumor formation or preneoplastic lesions in rodents, specifically mice (Suarez-Torres et al., 2020). This assessment is based on two criteria: a positive result indicates evidence of carcinogenic potential, while a negative result indicates no evidence of carcinogenic potential (BMDRC, 2017).

Model Farmakopor

Pharmacophore model creation requires the utilization of active and decoy databases containing test compounds. These databases are processed through logandscout to generate pharmacophores, which are visualized in both two-dimensional and three-dimensional formats (refer to Figure 1). As a result of this process, a total of 10 pharmacophore models were successfully created. The selection of these pharmacophores was based on evaluation of their ROC curve plots.



(a) (b)

Figure 1. Visualization Results of Pharmacophore Modeling (a) 2 Dimensions (b) 3 Dimensions

Model 1 demonstrates the highest AUC value at 90%, yielding a notable number of 210 hit compounds amongst a pool of 100 active compounds and 400 decoy compounds (Figure 2.).

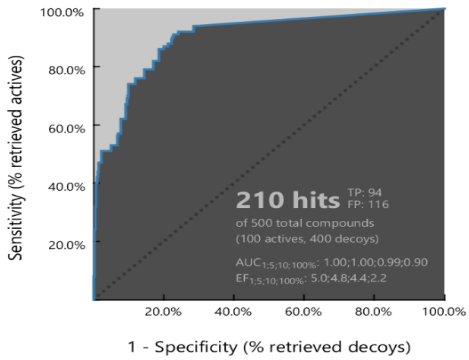


Figure 2. Pharmacopore Validation Curve

In model 1, we screened test compounds that specifically target the DPP4 receptor. Four compounds, Asparagine, Cysteine, Isoalliin, and Cycloalliin, are known to have activity against this receptor. Among these compounds, Asparagine showed the highest Pharmacophore Fit Score value of 45.19 (Figure 3.).

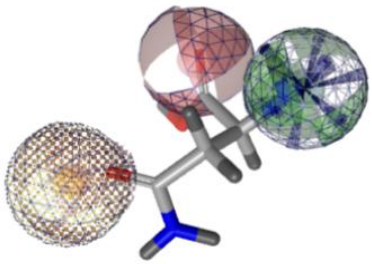


Figure 3. Compound Screening Results

Molecular Docking Simulation

During this phase, the process of molecular docking is conducted. Molecular docking is a molecular modeling technique that is utilized to predict the interaction between proteins and small molecules (ligands). These interactions can have an impact the biological functionality of proteins. To perform molecular docking, the receptor macromolecule must be prepared, and the computational chemistry method used must be validated to closely approximate the physiological conditions observed in the human body. In this specific process, molecular docking is performed on secondary metabolite compounds found in shallots (Allium cepa), with a target on the Dipeptidyl peptidase-4 (DPP4) enzyme with PDB ID 2i78. The Dipeptidyl peptidase-4 (DPP4) enzyme is a 110 kDa glycoprotein expressed on the surface of various cells. This exopeptidase selectively cleaves the N-terminal dipeptide from various substrates, including cytokines, growth factors, neuropeptides, and incretin hormones. DPP4 expression is significantly dysregulated in several disease conditions, such as inflammation, cancer, obesity, and diabetes, as incretin hormones, particularly glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide (GIP) are the primary regulators of post-prandial insulin secretion (Kalhotra et al., 2020).

Table 3. Molecular docking results of red onion compounds (Allium cepa) with natural ligands and reference compounds.

Compound	Cluster	Binding Energy (kkal/mol)	Ki (uM)	Interaction with amino acids		
				Hydrogen Bonds	Van Der Waals Bond	Others
Natural Ligands						
KIQ (1S,6R)-3-{[3-(TRIFLUOROMETHYL)-5,6-DIHYDRO[1,2,4]TRIAZOLO[4,3-A]PYRAZIN-7(8H)-YL]CARBONYL}-6-(2,4,5-TRIFLUOROPHENYL)CYCLOHEX-3-EN-1-AMINE	1	-11.00	8,67	ARG B : 669 TYR B : 662 ARG B : 125 ASN B : 710	-	PHE B : 357 TYR B : 666 SER B : 630 GLU B : 205 GLU B : 206 ASP B : 663

Active Compounds of Red Onion (Allium cepa)						
Quercetin	1	-6.9	8.77	ARG B : 358 GLU B : 206 ARG B : 669 TYR B : 547	-	PHE B : 357
Peonidin 3'-glucoside	1	-6.27	25.38	HIS B : 126 SER B : 209 ARG B : 358 TYR B : 666	-	PHE B : 357
Kaempferol	1	-6.64	13.69	ARG B : 125 GLU B : 205 TYR B : 662	-	-
Cysteine	1	-4.11	975.65	VAL B : 546 TYR B : 631	-	TYR B : 547 TYR B : 666 SER B : 630
Cycloalliin	1	-5.63	74.64	HIS B : 126 GLU B : 206 ARG B : 669	-	TYR B : 666 GLU B : 205
Isorhamnetin-4'-glucoside	1	-7.56	2.87	ARG B : 358 GLU B : 206 ARG B : 669 TYR B : 547 TYR B : 662	-	PRO B : 550 TYR B : 666 PHE B : 357
Delphinidin 3'-glucoside	1	-6.53	16.22	HIS B : 740 ASN B : 710	-	TYR B : 662

				GLU B : 205 HIS B : 126 GLU B : 206		TYR B : 666 ARG B : 125
Triethyl citrate	1	-4.11	964.45	TYR B : 662 TYR B : 631 TYR B : 666 TYR B : 547 HIS B : 740 SER B : 630	-	PHE B : 357 TRP B : 659 VAL B : 656
2,4-Dimethylthiophene	1	-4.02	1.13	-	-	VAL B : 656 TYR B : 631 TYR B : 666 TRP B : 659 TYR B : 662 VAL B : 711 HIS B : 740 SER B : 630
Diallyl disulphide	1	-3.26	4.10	HIS B : 740	-	TYR B : 662 TYR B : 631 VAL B : 656 TYR B : 666 VAL B : 711 TRP B : 659 TYR B : 547
Asparagine	1	-4.72	348.71	ARG B : 669 GLU B : 205 GLU B : 205	-	TYR B : 547
Acetic-acid	1	-1.81	46.79	SER B : 630 HIS B : 740 ARG B : 125	-	-

Cyanidin-3-glucoside	1	-6.49	17.57	ARG B : 125 ARG B: 669 GLU B : 206 TYR B : 631 HIS B : 740 ASN B : 710	-	SER B : 630 TYR B : 662 PHE B : 357
Myricetin	1	-6.59	14.72	ARG B : 358 ARG B : 669 GLU B : 206 TYR B : 547	-	PHI B : 357 VAL B : 207
Isoaliin	1	-6.02	38.44	TYR B : 547 TYR B : 662 TYR B : 666 SER B : 630 GLU B : 205 GLU B : 206	-	TYR B : 631 VAL B : 656 TRP B : 659 ASN B :710
Ferulic acid	1	-4.38	615.70	HIS B : 740 SER B : 630 ARG B : 125 LYS B : 554	TRP B : 629 GLY B : 632 GLY B : 628 VAL B : 546	TYR B : 547
Malvidine 3'-glucoside	1	6.79	10.57	SER B : 630 GLU B : 205 ARG B : 669 GLU B : 206	-	TYR B : 547 CYS B : 551 PHE B : 357
Alliuocide	1	-7.51	3.1	HIS B : 740 ARG B : 125 TYR B : 631 TYR B : 547 TRP B : 629	-	GLY B : 632

Chlorogenic acid	1	-4.78	311.27	GLU B : 205 TYR B : 631 TYR B : 666 TYR B : 547	ASN B : 710 ARG B : 125 TYR B : 662 HIS B : 740 SER B : 630 GLY B : 549 SER B : 209 ARG B : 358 PHE B : 208 ARG B : 669 GLU B : 206	PHE B : 357
Gallic acid	1	4.15	915.49	ARG B : 358 ARG B : 669 GLU B : 206	-	-

In this study, the inhibitory activity of the DPP4 enzyme in shallots was assessed using various test compounds. The test compounds utilized in this study included Quercetin, Penonidin 3'-glucoside, Kaempferol, Cysteine, Cycloalliin, Isorhamnetin-4'-glucoside, Delphinidin 3'-glucoside, Triethyl citrate, 2,4-Dimethylthiophene, Diallyl disulphide, Asparagine, Acetic acid, Cyanidin-3-glucoside, Myricetin, Isoaliin, Ferulic acid, Malvidine 3' glucoside, Alliuocide, Chlorogenic acid, and Gallic acid. KIQ was utilized as the comparative compound, serving as a natural ligand. The molecular docking process begins by establishing the size and positioning of the grid box, which is then validated. This grid box is used to map the receptor in three-dimensional coordinates (x, y, z), enabling the identification of the ligand's conformation with the lowest energy. The re-docking results are considered valid if they exhibit a low RMSD (Root Mean Square Deviation) value (≤ 2) and if the grid box generates an appropriate binding energy. The RMSD assessment involves comparing the position of the natural ligand with the re-docking results, thereby evaluating the computational method's ability to replicate the experimental procedure.

The molecular docking results include several parameters, such as the number of hydrogen bonds, the inhibition constant value (Ki), and the free energy value of the bond (ΔG). These parameters elucidate the ligand's ability to bind to the receptor. A higher similarity in hydrogen bonds between the test ligand and the natural ligand indicates that the test ligand has potential in inhibiting the activity of the target protein or receptor by displacing the natural ligand. A low or negative ΔG value indicates the possibility of bond formation between the ligand and the macromolecule. The Ki value represents the concentration required to achieve half of the maximum inhibition value, with a smaller Ki value indicating a stronger affinity between the ligand and the macromolecule.

Based on the results of molecular docking analysis, it has been determined that the compound 2,4-Dimethylthiophene has exhibited the smallest inhibition constant (KI) and binding energy (ΔG) values. This compound demonstrates three bonds similar to natural compounds, namely TYR B: 666, TYR B: 662, and SER B: 630 (Table 3). The selection of 2,4-Dimethylthiophene as the most optimal compound among the 20 test ligands analyzed using the molecular docking method, Lipinski prediction, and ADMETox highlights its superior performance in terms of physicochemical properties, stability, and ability to interact with the protein target. These interactions are also present in other natural compounds such as Isoaliin and Kaempferol, suggesting that 2,4-Dimethylthiophene mimics the binding characteristics of known DPP-4 inhibitors. Consequently, this compound demonstrates significant potential as an inhibitor due to its strong interactions and bonding patterns analogous to those of other natural compounds. When compared to other compounds that satisfy Lipinski's criteria, including Quercetin, Kaempferol, Cysteine, Cycloalliin, Triethyl citrate, diallyl disulfide, Asparagine, Acetic acid, Myricetin, Isoaliin, Ferulic acid, Chlorogenic acid, and Gallic acid—2,4-Dimethylthiophene stands out in terms of absorption (Caco2), Ki value, and interaction

with active residues. Therefore, it has been selected as the primary candidate based on its superior performance across all key evaluation parameters.

Conclusion

An in silico study was conducted to investigate the effects of shallots (Allium cepa) on the DPP4 receptor. Various software programs were utilized in the study, including ChemOffice 2010, BIOVIA Discovery Studio 2020, LigandsScout, and AutoDock Tools 1.5.6. The results of the molecular docking study showed that 2,4-Dimethylthiophene had the most favorable activity with the DPP4 receptor compared to the other 20 test ligands. Lipinski's prediction results indicated that 2,4-Dimethylthiophene met the necessary requirements, with a molecular weight of 140.20 g/mol, a Log P value of 2.09, and a hydrogen bond acceptor value of 0 and donor value of 1. The ADMETox prediction results showed that 2,4-Dimethylthiophene exhibited a high %HIA value of 100% and a moderate CaCo-2 permeability value of 40.055 nm/sec. The distribution test revealed a %PPB value of 97.195%, indicating strong binding of the drug to plasma proteins and good distribution ability. The compound also had a moderate BBB value of 1.373%. However, it is important to note that the study identified potential shortcomings, such as positive toxicity predictions for 2,4-Dimethylthiophene in the Ames test and positive carcinogenicity results in rats, while yielding negative results in mice. Therefore, further research is necessary to determine the potential of 2,4-Dimethylthiophene as an antidiabetic agent.

Discussion

Shallots (Allium cepa) contain various bioactive compounds, including flavonoids (quercetin, rutin, isoquercitrin), phenolics (gallic acid, catechin, epicatechin), and organosulfur compounds (allicin, alliin, diallyl sulfide, and ajoene) (Shang et al., 2019). Several certain compounds, particularly rutin, quercetin, and gallic acid, have demonstrated, both in silico and in vitro, the capacity to inhibit the DPP-IV enzyme, which plays an important role in regulating blood glucose levels. In addition, other natural DPP-4 inhibitors include anthocyanins, aspalathin, chrysin, eriodictyol, hispidulin, kaempferol, lepidoside, mangiferin, naringenin, naringin, procyanidin, rhamnoside, and terpenoids (Ansari et al., 2021).

Based on research conducted by Elvira and Nathalia in 2020 involved administering shallots to ten respondents with elevated blood sugar levels. The findings revealed that seven out of ten respondents achieved normal blood sugar levels, as evidenced by a dependent t-test yielding a p-value of 0.001 (<0.05), indicating a significant effect on blood sugar levels in individuals with Diabetes Mellitus (Elvira and Nathalia, 2020). Additionally, a study by Hidayat and Zahroh in 2017 involving fourteen respondents with diabetes mellitus demonstrated a reduction in blood sugar levels following the administration of red onion powder. The analysis, utilizing the Wilcoxon Signed Ranks Test, produced a probability value of 0.001, confirming the impact of red onion supplementation on lowering blood sugar levels in this patient population.

Until now, there have been no studies explicitly addressing the effects of red onion compounds on the DPP-4 enzyme. However, the in silico assessment conducted in this study indicated that the compound 2,4-Dimethylthiophene, found in red onions, exhibits superior binding affinity for the DPP-4 enzyme compared to other compounds. This discovery suggests a promising avenue for further research into the potential of red onions as natural DPP-4 inhibitors, contributing valuable insights to the scientific community.

This study was conducted in silico. Therefore, it cannot directly characterize the compound's activity within the human biological system. Consequently, further research is necessary through in vitro and in vivo investigations to confirm the inhibitory activity of DPP-4 and to evaluate the compound's safety, toxicological potential, and efficacy under actual biological conditions.

Acknowledgements

The author would like to express gratitude to Diah Lia Aulifa for her invaluable contributions to the data collection process in this study.

Reference

Afzal, M., Kazmi, I., Kaur, R., Hosawi, S. B. I., Kaleem, M., Alzarea, S. I., & Ahmad, M. M. 2023. Introduction to molecular pharmacology: basic concepts. *How Synthetic Drugs Work: Insights into Molecular Pharmacology of Classic and New Pharmaceuticals*, 1–25. https://doi.org/10.1016/B978-0-323-99855-0.00001-4.

BMDRC. 2017. Toxicity Prediction. Tersedia online di *https://preadmet.webservice.bmdrc.org/toxicity-prediction/*. [Diakses pada 08 Juni 2023].

Coimbra, J. T. S., Feghali, R., Ribeiro, R. P., Ramos, M. J., & Fernandes, P. A. 2020. The importance of intramolecular hydrogen bonds on the translocation of the small drug piracetam through a lipid bilayer.*RSC Advances*, *11*(2), 899. https://doi.org/10.1039/D0RA09995C.

Deacon, C. F. 2020. Dipeptidyl peptidase 4 inhibitors in the treatment of type 2 diabetes mellitus. *Nature Reviews. Endocrinology*, *16*(11), 642–653. https://doi.org/10.1038/S41574-020-0399-8.

Di, L. 2021. An update on the importance of plasma protein binding in drug discovery and development. *Expert Opinion on Drug Discovery*, *16*(12), 1453–1465. https://doi.org/10.1080/17460441.2021.1961741.

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., Ostolaza, H., & Martín, C. 2020. Pathophysiology of Type 2 Diabetes Mellitus. *International Journal of Molecular Sciences*, *21*(17), 1–34. https://doi.org/10.3390/IJMS21176275.

Gasbjerg, L. S., Gabe, M. B. N., Hartmann, B., Christensen, M. B., Knop, F. K., Holst, J. J., & Rosenkilde, M. M. 2018. Glucose-dependent insulinotropic polypeptide (GIP) receptor antagonists as anti-diabetic agents. *Peptides*, *100*, 173–181. https://doi.org/10.1016/J.PEPTIDES.2017.11.021.

International Diabetes Federation. 2021. International Diabetes Federation (IDF) Atlas 10th Edition. In Diabetes Research and Clinical Practice, 102,(2).

Ivanović, V., Rančić, M., Arsić, B., & Pavlović, A. 2020. Lipinski's rule of five, famous extensions and famous exceptions. *Chemia Naissensis*, *3*(1), 171–181. https://doi.org/10.46793/chemn3.1.171i.

Kalhotra, P., Chittepu, V. C. S. R., Osorio-Revilla, G., & Gallardo-Velazquez, T. 2020. Phytochemicals in Garlic Extract Inhibit Therapeutic Enzyme DPP-4 and Induce Skeletal Muscle Cell Proliferation: A Possible Mechanism of Action to Benefit the Treatment of Diabetes Mellitus. *Biomolecules*, *10*(2).

Kemenkes RI. 2018. Hasil Riset Kesehatan Dasar Tahun 2018. Kementrian Kesehatan RI, 53(9), 1689–1699.https://doi.org/10.3390/BIOM10020305.

Lipinski, C. A. 2000. Drug-like properties and the causes of poor solubility and poor permeability. *Journal of Pharmacological and Toxicological Methods*, *44*(1), 235–249. https://doi.org/10.1016/S1056-8719(00)00107-6.

Nauck, M. A., & Meier, J. J. 2018. Incretin hormones: Their role in health and disease. *Diabetes, Obesity & Metabolism*, *20 Suppl 1*, 5–21. https://doi.org/10.1111/DOM.13129.

Preeti, Sambhakar, S., Saharan, R., Narwal, S., Malik, R., Gahlot, V., Khalid, A., Najmi, A., Zoghebi, K., Halawi, M. A., Albratty, M., & Mohan, S. 2023. Exploring LIPIDs for their potential to improves bioavailability of lipophilic drugs candidates: A review. *Saudi Pharmaceutical Journal : SPJ*, *31*(12). https://doi.org/10.1016/J.JSPS.2023.101870.

Singh, A. K., Yadav, D., Sharma, N., & Jin, J. O. 2021. Dipeptidyl Peptidase (DPP)-IV Inhibitors with Antioxidant Potential Isolated from Natural Sources: A Novel Approach for the Management of Diabetes.*Pharmaceuticals 2021, Vol. 14, Page 586*, *14*(6), 586. https://doi.org/10.3390/PH14060586.

Suarez-Torres, J. D., Jimenez-Orozco, F. A., & Ciangherotti, C. E. 2020. The 2-year rodent bioassay in drug and chemical carcinogenesis testing: Sensitivity, according to the framework of carcinogenic action. *Toxicology Mechanisms and Methods*, *30*(6), 462–475. https://doi.org/10.1080/15376516.2020.1760986.

Vijay, U., Gupta, S., Mathur, P., Suravajhala, P., & Bhatnagar, P. 2018. Microbial Mutagenicity Assay: Ames Test. *Bio-Protocol*, *8*(6). https://doi.org/10.21769/BIOPROTOC.2763.

Zhang, W. 2024. Blood-Brain Barrier (BBB)-Crossing Strategies for Improved Treatment of CNS Disorders. *Handbook of Experimental Pharmacology*, *284*, 213–230. https://doi.org/10.1007/164_2023_689.

Elvira, M., dan Nathalia, V. 2020. Bawang merah menurunkan kadar gula darah penderita diabetes mellitus. *Jurnal Kesehatan Perintis*, *7*(2), 21–27.

Hidayat, S.A., dan Zahroh, C. 2017. Pengaruh bawang merah terhadap penurunan kadar gula darah pada penderita diabetes mellitus di Desa Sidoraharjo Kecamatan Kedamean Kabupaten Gresik. *Jurnal Ilmiah Kesehatan*, *10*(2), 263–269.

Shang A, Cao SY, Xu XY, Gan RY, Tang GY, Corke H, Mavumengwana V, Li HB. 2019. Bioactive compounds and biological functions of garlic (*Allium sativum* L.). *Foods*, *8*(7), 246. https://doi.org/10.3390/foods8070246.

Ansari P, Hannon-Fletcher MP, Flatt PR, Abdel-Wahab YHA. 2021. Effects of 22 traditional anti-diabetic medicinal plants on DPP-IV enzyme activity and glucose homeostasis in high-fat fed obese diabetic rats. *Bioscience Reports*, *41*(1). https://doi.org/10.1042/BSR20203889.