

Bioinformatics Study: Molecular Docking of Cat Whiskers Flavonoid Compounds for the Development of Antidiabetic Candidates

Saeful Amin^{1*}, Febia Citraeni Rusdaita², Riska Prasetiawati³

Universitas Bakti Tunas Husada¹, Universitas Garut^{2,3}

Correspondence Email: saefulamin@universitas-bth.ac.id*

Abstract

Diabetes mellitus is a chronic disease characterized by hyperglycemia. In silico studies have been done on flavonoid derivative compounds from the cat's whiskers plant (*Orthosiphon stamineus* Benth.) on protein tyrosine phosphatase 1B (2F70) and aldose reductase enzymes (4YVP and 4YVX) to see if they can be used as diabetes medicines. The molecular docking of the test compounds found three that were more active than the comparison ligands. One of these was the luteolin compound, which can stop the Protein Tyrosine Phosphatase 1B receptor from working with an ΔG value of -8.05 kcal/mol and a K_i value of 1.25 μM by forming amino acid residues. SER A: 216, ARG A: 221, ALA A: 217, VAL A: 490, TYR A: 46, and ASP A: 181 shows that the sinensetin compound can stop the aldose reductase enzyme from working, working with a ΔG value of -7.72 kcal/mol and a K_i value of 2.19 μM to form amino acid residues. ALA B: 25, GLU B: 224, LEU B: 54, HIS B: 222, TRP B: 227, and the 6-hydroxy-5,7,4'-trimethoxyflavone compound can stop the aldose reductase enzyme from working (ΔG value of -7.02 kcal/mol, K_i 7.14 μM), which creates amino acid residues. HIS B:117, TYR B:55, SER B:217, PHE B:306. Based on the analysis of these compounds, they exhibit good physicochemical and pharmacokinetic profiles. Additionally, the toxicity analysis confirms that none of these compounds are mutagenic, carcinogenic, or harmful to humans.

Keywords: antidiabetic, flavonoids, molecular docking.

INTRODUCTION

Diabetes mellitus (DM) ranks fourth among chronic non-communicable diseases globally, following cardiovascular, cancer, and chronic respiratory diseases (Amalina Ahmad Azam, 2017). Based on data from the Basic Health Research (RISKESDAS) in 2018, the national prevalence of DM sufferers in the population aged >15 years increased by 2% compared to data in 2013 (Indonesia, 2018).

Diabetes mellitus (DM) refers to a condition of high blood glucose levels, also called hyperglycemia (Aria Wahyuni, 2016). The rise in prevalence leads to an increase in the use of oral drugs among patients with type 2 diabetes mellitus, which in turn affects the incidence of side effects. Raden et al. (2017) found that most people experienced significant effects on the consumption of oral antidiabetic drugs, specifically nausea and hypoglycemia. Radden Jody Utama Putra (2017) suggests using drugs with mechanisms like protein tyrosine

phosphate 1B inhibitors and aldose reductase inhibitors for the treatment of type 2 diabetes mellitus.

Photophosphorylation causes insulin-stimulated kinase activity to decrease, resulting in decreased cell response. There is a link between insulin resistance and the amount of protein tyrosine phosphate 1B in muscle and fat tissue found in clinical studies (V. Sreenivasa Murthy, 2002). Researchers have identified protein tyrosine phosphate 1B inhibition as an innovative target to address sensitivity to insulin resistance (Alfred Goodman Gilman, 2012; Phi Hung Nguyen, 2019). Various parts of the body contain the aldose reductase enzyme, which reduces glucose to sorbitol. Poor sorbitol metabolism leads to excessive accumulation, which can exacerbate diabetes mellitus (Karisma Enggar Saputri, 2016; Wai Ho Tang, 2012).

One of the plants used for antidiabetic purposes is the cat's whiskers plant (*Orthosiphon stamineus* Benth.) M. Amzad Hossain (2011), which has a chemical compound component in the form of flavonoid compounds. Various studies have proven that flavonoid compounds can reduce glucose absorption and increase glucose tolerance in people with diabetes mellitus (Avtar Chand Rana, 2019).

METHOD

Physicochemical Analysis

We conducted a physicochemical analysis of the test compound using a program on the online site Lipinski's Rule of Five (<http://scfbio-iitd.res.in>) to determine the parameters. The molecular weight of a compound must be <500 g/mol, the log P value <5, the hydrogen bond donor <5, and the hydrogen acceptor <10.

Pharmacokinetic and Toxicity Analysis

Pharmacokinetic analysis aims to see the absorption and distribution of the test compound in the body with molecular modeling. This test uses the online site PrADMET (<http://preadmet.bmdrc.org/>). We conducted an analysis of the predicted toxicity properties of the test compound using the Toxtree 3.1.0® application.

Preparation and Preparation of Macromolecules

We got the macromolecular structure I needed from the Protein Data Bank (PDB) and then used the Discovery Studio Visualizer® software to prepare it. We do this to eliminate the water molecules, creating two distinct files during the preparation process: receptors and ligands. We remove water molecules attached to the macromolecule to ensure the docking process remains uninterrupted (Riska Prasetiawati, 2018).

Validation of the Docking Method

We validate the method by redocking the natural ligand with the target receptor using the AutoDock Tools® application. The method can be said to be valid when looking at the root mean square deviation (RMSD) value <2 Å (Juni Ekowati, 2018). We carry out a grid-box (binding area) arrangement in the docking process to limit the search for receptor-ligand conformation. A file in the format (dock.gpf) contains the results of the grid box arrangement (Doni Dermawan, 2019).

Ligand Optimization

We used the reference ligand and the test ligand. They were flavonoid derivative compounds from the cat's whiskers plant (*Orthosiphon stamineus* Benth.). The process of protonation was carried out using ChemDraw Professional 15.0® software, utilizing the

minimized energy method, and the results were then saved in the format (.pdb), as reported by Riska Prasetiawati in 2018.

Docking Simulation

Using AutoDock Tools® software, we conducted a docking simulation of the test compound to determine its optimal affinity for the test receptor. We set the grid box to match the validation data and then set the docking parameters using the Genetic Algorithm method. The results of the docking simulation are ΔG , K_i , and the type of bond to the amino acid residue (Fauzan Zein Muttaqin, 2019; Susanti, N. M. P., & I. P. D. N. Parahyangan, 2018).

RESULT AND DISCUSSION

Physicochemical Analysis

Lipinski's Rule of Five is a parameter for analyzing the physicochemical properties of a compound. The purpose of conducting physicochemical property analysis in in silico research is to determine the physical and chemical properties of a compound to determine the formulation method of drug preparations.

Table 1. Physicochemical Analysis

No	Compound	BM (g/mol)	Log P	Donor H	Acceptor H	Description
1.	Sulfamic acid	429	0,83	4	10	Compliant
2.	Glibenclamide	493,5	4,7	3	8	Compliant
3.	Glimepiride	490	4,15	3	9	Compliant
4.	Metformin	129	-1,23	5	5	Compliant
5.	Chlorpropamide	276,5	2,81	2	5	Compliant
6.	Cirsimaritin	314	2,73	2	6	Compliant
7.	Eupatorin	344	2,73	2	7	Compliant
8.	Ladanein	314	2,73	2	6	Compliant
9.	Luteolin	286	2,12	4	6	Compliant
10.	Quercetin	297	-1,43	5	7	Compliant
11.	Salvigenin	313	0,711	1	6	Compliant
12.	Sinensetin	352	1,74	0	7	Compliant
13.	4'hydroxy-5,6,7-trimethoxyflavone	328	3,03	1	6	Compliant
14.	4', 5,6,7-tetra	342	3,33	0	6	Compliant
15.	methoxyflavone	358	3,04	1	7	Compliant
16.	5-hydroxy 6,7,3,4'-tetra methoxyflavone	328	3,03	1	6	Compliant

Based on the analysis of all flavonoid compounds found in cat's whiskers (*Orthosiphon stamineus* Benth.) plants, they meet the parameters based on Lipinski's Rule of Five.

Pharmacokinetic and Toxicity Analysis

The pharmacokinetic analysis, conducted using the PREADMET online site on Human Intestinal Absorption (HIA) cells and human colon adenocarcinoma (Caco-2), elucidates the absorption process, while the Protein Plasma Binding (PPB) value explains the distribution process. Utilizing the Ames test method, we assess the mutagenic potential of compounds through in vitro testing on *S. typhimurium* bacteria, employing Benigni/Bossa rule base parameters and the Kroes TTC decision tree.

Table 2. Pharmacokinetic Analysis (Absorption and Distribution)

No.	Compound	Absorption		Distribution
		HIA (%)	CaCo-2 Cell (nm sec)	Plasma Protein Binding (%)
1.	Sulfamic acid	71,37 ^a	13,69 ^b	66,78 ^b
2.	Glibenclamide	95,90 ^a	5,99 ^b	100 ^a
3.	Glimepiride	95,08 ^a	6,50 ^b	100 ^a
4.	Metformin	45,66 ^b	21,08 ^b	3,95 ^b
5.	Chlorpropamide	96,48 ^a	1,40 ^c	90,56 ^a
6.	Cirsimaritin	93,34 ^a	8,34 ^b	88,06 ^b
7.	Eupatorin	93,45 ^a	7,14 ^b	85,46 ^b
8.	Ladanein	93,37 ^a	9,76 ^b	87,68 ^b
9.	Luteolin	79,43 ^a	4,54 ^b	99,71 ^a
10.	Quercetin	59,72 ^b	3,43 ^c	58,99 ^b
11.	Salvigenin	96,49 ^a	33,07 ^b	87,41 ^b
12.	Sinensetin	98,89 ^a	51,23 ^b	86,24 ^b
13.	4'-hydroxy-5,6,7-trimethoxyflavone	96,49 ^a	33,07 ^b	87,65 ^b
14.	4', 5,6,7-tetramethoxyflavone	98,44 ^a	53,77 ^b	88,11 ^b
15.	5-hydroxy-6,7,3,4'-tetramethoxyflavone	96,81 ^a	30,48 ^b	85,83 ^b
16.	6-hydroxy-5,7,4'-trimethoxyflavone	96,49 ^a	34,62 ^b	87,28 ^b

Table 3. Toxicity Analysis

No.	Compound	Mutagen (<i>amst</i> test)	Carcinogen		Kroes TTC
			Genotoxic	Non genotoxic	
1.	Sulfamic acid	(-)	(-)	(-)	No risk
2.	Glibenclamide	(-)	(-)	(+)	No risk
3.	Glimepiride	(+)	(+)	(-)	Low risk
4.	Metformin	(-)	(-)	(-)	No risk
5.	Chlorpropamide	(-)	(-)	(+)	No risk
6.	Cirsimaritin	(-)	(-)	(-)	No risk
7.	Eupatorin	(-)	(-)	(-)	No risk
8.	Ladanein	(-)	(-)	(-)	No risk
9.	Luteolin	(-)	(-)	(-)	No risk
10.	Quercetin	(-)	(-)	(-)	No risk
11.	Salvigenin	(-)	(-)	(-)	No risk
12.	Sinensetin	(-)	(-)	(-)	No risk
13.	4'-hydroxy-5,6,7-trimethoxyflavone	(-)	(-)	(-)	No risk
14.	4', 5,6,7-tetramethoxyflavone	(-)	(-)	(-)	No risk
15.	5-hydroxy-6,7,3,4'-tetramethoxyflavone	(-)	(-)	(-)	No risk
16.	6-hydroxy-5,7,4'-trimethoxyflavone	(-)	(-)	(-)	No risk

In the pharmacokinetic analysis, all test ligands, with the exception of quercetin, exhibit a favorable absorption profile and a poor distribution profile. However, luteolin, due to its strong binding to plasma proteins, exhibits a favorable distribution. In terms of the toxicity profile, the in vitro method (AMES test) confirms that all test ligands are non-mutagens and not carcinogenic. Their structural form, as determined by the Benigni/Bossa rule base parameters, indicates that they are either genotoxic or non-genotoxic. We found that all test compounds have an exposure threshold of no. 1, which is no more than 0.15 µg/day. We attribute this to the dose-response analysis we conducted on carcinogenic compounds. This means that the test compound is neither dangerous nor safe.

Molecular Docking Simulation

Method Validation

The docking validation process involves reattaching the natural ligand to the target receptor. We check the method's correctness by looking at the RMSD value, which should be less than 2 (June Ekowati, 2018). The RMSD validation results for the ID 2F70 method are 1.307; for ID 4YVP, it is 1.952; and for ID 4YVX, it is 0.510.

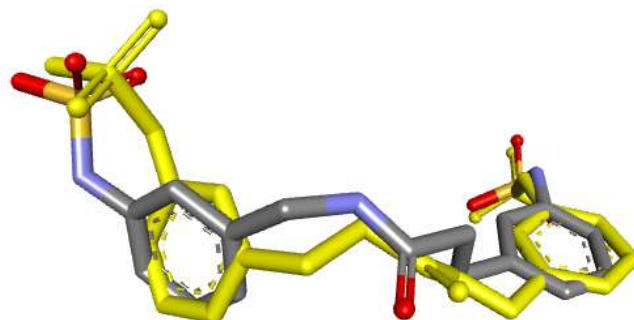


Figure 1. Overlapping Visualization of Validation Results of Sulfamic Acid Method Against Protein Receptor Tyrosine Phosphatase 1B (PTP1B) ID 2F70

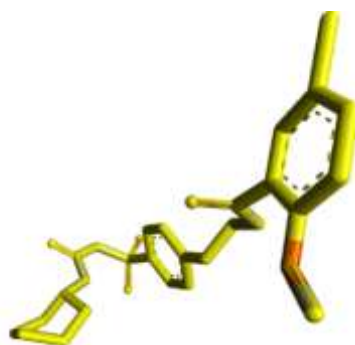


Figure 2. Overlapping Visualization of Validation Results of Glibenclamide Method Against Aldose Reductase Receptor ID 4YVP



Figure 3. Overlapping Visualization of Glimepiride Method Validation Results Against Aldose Reductase Receptor ID 4YVX

Table 4. Method Validation Results

No.	Receptor	Native Ligand	RMSD (Å)	ΔG (kcal/mol)	Ki (μM)
1.	Protein Tyrosine Phosphatase 1B (PTP1B) ID 2F70	sulfamic acid	1,037	-13,43	143,32x10 ⁻⁶
2.	Aldose Reductase ID 4YVP	Glibenclamide	1,952	-11,27	5,5x10 ⁻³
3.	Aldose Reductase ID 4YVX	Glimepiride	0,510	-9,19	182,99x10 ⁻³

Table 5. Molecular Docking Results of ID 2F70

No.	Test Ligand	ΔG (kcal/mol)	Ki (μM)
1.	Native Ligand sulfamic acid	-13,43	143,32x10 ⁻⁶
2.	Metformin comparator ligand	-2,76	9490
3.	Cirsimaritin	-7,13	5,98
4.	Eupatorin	-6,52	16,62
5.	Ladanein	-7,41	3,72
6.	Luteolin	-8,05	1,25
7.	Quercetin	-7,94	1,51
8.	Salvigenin	-6,46	18,40
9.	Sinensetin	-6,72	11,78
10.	4'-hydroxy-5,6,7-trimethoxyflavone	-7,26	4,73
11.	4', 5,6,7-tetramethoxyflavone	-7,02	7,13
12.	5-hydroxy-6,7,3,4'-tetramethoxyflavone	-6,62	14,05
13.	6-hydroxy-5,7,4'-trimethoxyflavone	-7,47	3,33

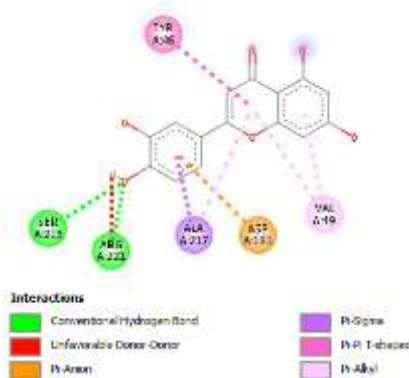


Figure 4. Visualization of Amino Acid Residues of Luteolin Compound Against ID 2F70

Molecular docking simulation of the test compound against the Protein Tyrosine Phosphatase 1B (PTP1B) receptor ID 2F70 showed a negative free energy value; luteolin has the lowest free energy value with a value of -8.05 kcal/mol and an inhibition constant of 1.25 μ M, which forms amino acid residues with a hydrogen bond model (SER A: 216, ARG A: 221), hydrophobic interactions, namely Pi-sigma (ALA A: 217), pi-alkyl (VAL A: 490), pi-pi T-shaped (TYR A: 46), and electrostatic interactions (ASP A: 181). Luteolin has a better affinity than the reference ligand metformin, which has a free energy value of -2.76 kcal/mol.

Table 6. Molecular Docking Results ID: 4YVP

No.	Test Ligand	ΔG (kcal/mol)	Ki (μ M)
1.	Native Ligand Glibenclamide	-11,27	5,5 x10 ⁻³
2.	Comparative ligand chlorpropamide	-7,24	4,67
3.	Cirsimaritin	-6,72	11,78
4.	Eupatorin	-7,22	5,12
5.	Ladanein	-6,53	16,37
6.	Luteolin	-6,61	14,40
7.	Quercetin	-6,88	9,03
8.	Salvigenin	-6,88	9,07
9.	Sinensetin	-7,72	2,19
10.	4'-hydroxy-5,6,7-trimethoxyflavone	-7,16	5,61
11.	4', 5,6,7-tetramethoxyflavone	-7,46	3,39
12.	5-hydroxy-6,7,3,4'-tetramethoxyflavone	-7,18	5,46
13.	6-hydroxy-5,7,4'-trimethoxyflavone	-7,14	5,84

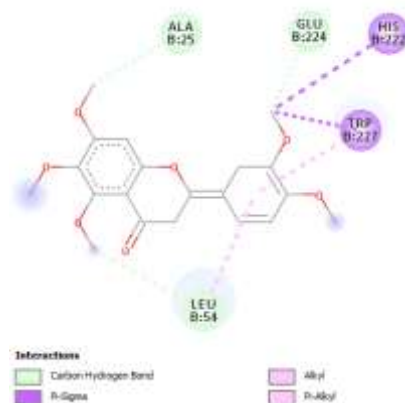


Figure 5. Visualization of Sinensetin Compound Amino Acid Residues Against ID 4YVP

In the molecular docking simulation against the aldose reductase receptor ID 4YVP, sinensetin has the lowest free energy value of 7.72 kcal/mol and 2.19 μM , which forms amino acid residues with a hydrogen bond model (ALA B: 25, GLU B: 224, LEU B: 54), hydrophobic bonds, namely Pi-sigma (HIS B: 222, TRP B: 227), alkyl (LEU B: 54), and pi-alkyl (TRP B: 227). With a free energy of -7.24 kcal/mol and a K_i value of 4.67 μM , sinensetin binds better to chlorpropamide, which is used as a standard.

Table 7. Molecular Docking Results for ID 4YVX

No.	Test Ligand	ΔG (kcal/mol)	K_i (μM)
1.	Native Ligand Glimepiride	-9,19	182,99 $\times 10^{-3}$
2.	Comparative ligand chlorpropamide	-6,03	37,93
3.	Cirsimaritin	-6,38	20,93
4.	Eupatorin	-7,01	7,32
5.	Ladanein	-6,56	15,66
6.	Luteolin	-6,05	36,94
7.	Quercetin	-5,95	43,48
8.	Salvigenin	-6,45	18,87
9.	Sinensetin	-6,70	12,36
10.	4'hydroxy-5,6,7-trimethoxyflavone	-6,37	21,39
11.	4', 5,6,7-tetramethoxyflavone	-6,88	9,02
12.	5-hydroxy-6,7,3,4'-tetramethoxyflavone	-6,48	17,90
13.	6-hydroxy-5,7,4'-trimethoxyflavone	-7,02	7,14

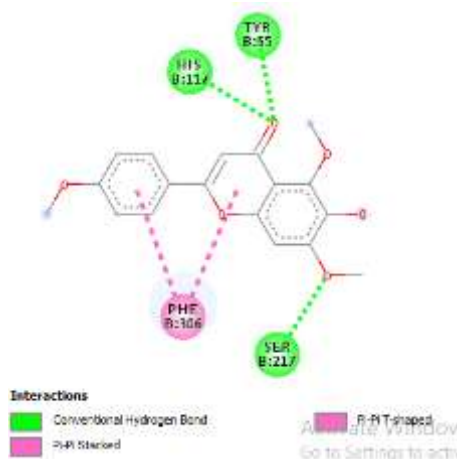


Figure 6. Visualization of Amino Acid Residues of Compound 6-Hydroxy-5,7,4'-trimethoxyflavone Against ID 4YVP

In the molecular docking simulation against the aldose reductase receptor ID 4YVP, the compound 6-hydroxy-5,7,4'-trimethoxyflavone has the lowest free energy value of -7.02 kcal/mol and K_i 7.14 μ M on the compound 6-hydroxy-5,7,4'-trimethoxyflavone by forming amino acid residues with a hydrogen bond model (HIS B: 117, TYR B: 55, SER B: 217) and hydrophobic interactions, namely Pi-Pi (PHE B: 306). The 6-hydroxy-5,7,4'-trimethoxyflavone ligand binds better than chlorpropamide, which has a free energy of -6.03 kcal/mol and a K_i value of 37.93 μ M.

CONCLUSION

Researchers conducted an in-silico study on 11 flavonoid derivative compounds from the cat's whiskers plant (*Orthosiphon stamineus* Benth.) against the Protein Tyrosine Phosphatase 1B receptor and aldose reductase. The results of physicochemical analysis showed that all test compounds had physicochemical properties in accordance with the parameters. Pharmacokinetic analysis of absorption and distribution profiles showed that all test compounds had good absorption profiles except for quercetin and luteolin compounds, which had good distribution profiles, while all other test compounds had poor distribution profiles. Toxicity analysis showed that all compounds were non-mutagenic and non-carcinogenic and safe for humans. The results of the molecular docking of the test compounds showed that the affinity formed was better than that of the comparison ligand. The luteolin compound can inhibit the activity of the protein tyrosine phosphatase 1B receptor; the sinensetin and 6-hydroxy-5,7,4'-trimethoxyflavone compounds can inhibit the activity of the aldose reductase enzyme.

REFERENCES

- Alfred Goodman Gilman, M. P. (2012). *Dasar Farmakologi Terapi Edisi 10*. Jakarta: Penerbit EGC, 4.
- Amalina Ahmad Azam, R. P. (2017). Urinary metabolomics study on the protective role of *Orthosiphon stamineus* in Streptozotocin induced diabetes mellitus in rats via 1H NMR spectroscopy. *BMC Complementary and Alternative Medicine*, 278.
- Aria Wahyuni, N. (2016). Senam Kaki Diabetik Efektif Meningkatkan Ankle Brachial Index Pasien Diabetes Melitus Tipe 2. *Jurnal Ipteks Terapan Research of Applied Science and Education*, 155-164.

- Avtar Chand Rana, B. (2019). Chemistry and Pharmacology of Flavonoids- A Review. *Indian Journal of Pharmaceutical Education and Research*, 53(1).
- Doni Dermawan, R. S. (2019). Molecular Dynamic Simulation of Estrogen Receptor Alpha Against Andrografolid as Anti Breast Cancer. *Indonesian Journal of Pharmaceutical Science and Technology*.
- Fauzan Zein Muttaqin, H. I. (2019). Studi Molecular Docking, Molecular Dynamic, Dan Prediksi Toksisitas Senyawa Turunan Alkaloid Naftiridin Sebagai Inhibitor Protein Kasein Kinase 2-A Pada Kanker Leukemia. *Pharmaoscript*, 46-64.
- I Ketut Adyana, F. S. (2013). From Ethnopharmacology to Clinical Study of Orthosiphon Stamineus Benth. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(3), 66-73.
- Indonesia, K. R. (2018). *Riset Kesehatan Dasar*.
- Juni Ekowati, N. W. (2018). Molecular Docking of Ferulic Acid Derivatives on P2Y12. *J. Math. Fund. Sci*, 203-2019.
- Karisma Enggar Saputri, N. F. (2016). Docking Molekular Potensi Anti Diabetes Melitus Tipe 2 Turunan Zerumbon Sebagai Inhibitor Aldosa Reduktase Dengan AutoDock-Vina. Fakultas Farmasi, Universitas Muhammadiyah Surakarta, 16-20.
- M. Amzad Hossain, S. (2011). Isolation And Characterisation of Flavonoids from The Leaves of Medicinal Plant Orthosiphon Stamineus. *Arabian Journal of Chemistry*.
- Phi Hung Nguyen, H. N. (2019). Glucose Uptake Stimulatory and PTP1B Inhibitory Activities of Pimarane Diterpenes from Orthosiphon stamineus Benth. *Biomolecules*.
- Priyanka Tolambiya, S. (2017). Identifiation of cost-effective drug through in-silico approach of Catharanthus roseus plant ligand-receptor docking for Type 2 Diabetes NIDDM. *Chemistry & Biology Interface*, 64-78.
- Radden Jody Utama Putra, A. H. (2017). Kejadian Efek Samping Potensial Terapi Obat Antidiabetes Pasien Diabetes Melitus Berdasarkan Algoritma Naranjo. 2(2), 45-50.
- Riska Prasetiawati, B. P. (2018). MOLECULAR DOCKING STUDY OF XANTHONE DERIVATIVE COMPOUNDS OF MANGOSTEEN RIND (*Garcinia mangostana* L.) TO ER- α (ESTROGEN RECEPTOR ALFA) AND ER- β (ESTROGEN RECEPTOR BETA) AS ANTI-BREASTCANCER. *Jurnal Ilmiah Farmako Bahari*.
- Susanti, N. M. P., D. P., & I. P. D. N. Parahyangan, I. (2018). Molecular Docking Sianidin dan Peonidin Sebagai Antiinflamasi pada Aterosklerosis Secara in Silico. *Jurnal Farmasi Udayana*, 7(1), 28-33.
- V. Sreenivasa Murthy, V. (2002). Molecular Modeling of Protein Tyrosine Phosphatase 1B (PTP 1B) Inhibitors. (10).
- Wai Ho Tang, K. J. (2012). Aldose Reductase, Oxidative Stress, and Diabetic Mellitus . 3(87).