WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

In Silico Screening of the Potential of Flavonoid Compounds (Rutin and Catechin) in Gardenia (*Gardenia augusta, Merr.) Leaves* as Antidyslipidemia

I Made Wahyu Yogatama [a], ***Desak Ketut Ernawati*** [b], ***I Made Jawi.*** [a,b,*]

**Department of Pharmacology and Therapy, Faculty of Medicine, Udayana University, Jalan PB Sudirman, Denpasar, Bali. Postal Code 80232, Indonesia*

DOI : https://doi.org/10.55248/gengpi.5.0724.1816

A B S T R A C T

Dyslipidemia is a risk factor for various diseases which are still a big problem in Indonesia, such as cardiovascular disease (PERKI, 2022). Manifestations of dyslipidemia are an increase in the concentration of total cholesterol, LDL (low density lipoprotein) and triglycerides as well as a decrease in HDL (high density lipoprotein) in the blood. The statin group is the first line to reduce LDL levels whose mechanism of action is inhibition of the mevalonate pathway in HMG-CoA reductase in cholesterol biosynthesis. The statin drug that is often prescribed is simvastatin. Side effects of myopathy and increased liver enzymes can occur in patients using statin drugs. These side effects can worsen the condition of dyslipidemia patients, therefore it is necessary to develop new drugs that can be used as alternative therapy for dyslipidemia. One way to develop new drugs can be done through in silico methods . Flavonoid compounds in gardenia leaves are responsible for antioxidant activity. The flavonoid compounds that are mostly contained in the ethanol extract of gardenia leaves are rutin and catechin. The antioxidant mechanism works as an antihyperlipidemic by inhibiting LDL oxidation. In addition, flavonoids play a role in reducing blood cholesterol levels by inhibiting HMG-CoA reductase. This study aims to determine the pharmacokinetic profile and affinity of flavonoid compounds (rutin and catechin) of gardenia leaves towards the target protein (HMG-Coa reductase) in silico. Based on Lipinski's rules, catechins fulfill the characteristics of a drug-like compound. Meanwhile, the rutin compound only meets the log P value requirements. In terms of pharmacokinetics (ADMET), the rutin compound has a poor absorption value, namely 28.301%, while the catechin compound has a good absorption value, namely 72.619%. Data on binding energy values show that the compoundsrut and catechin have an affinity for HMG-Coa reductase. The binding energies of the rutin and catechin compounds are -2.92 and -5.32 (kcal/mol), respectively. This shows that the test compound has the potential to be developed as an antidyslipidemic agent. The bond that occurs between the test compound and the HMG-Coa reductase protein is able to inhibit the formation of endogenous cholesterol through the mevalonate pathway in the liver.

Keywords: dyslipidemia, rutin, catechin, In Silico, molecular docking

1. Introduction

Dyslipidemia is a risk factor for various diseases which are still a big problem in Indonesia, such as cardiovascular disease. Manifestations of dyslipidemia are an increase in the concentration of total cholesterol, LDL (*low density lipoprotein)* and triglycerides as well as a decrease in HDL (*high density lipoprotein)* in the blood (PERKI, 2022). The principles of dyslipidemia therapy are a strict diet low in fat or cholesterol, regular physical activity or exercise, losing weight and managing your lifestyle. After all the non-pharmacological therapy that has been carried out is inadequate, it is necessary to carry out therapy with drugs /pharmacological (PERKI, 2022). The statin group can be used as a first line to reduce serum LDL levels whose mechanism of action is to reduce cholesterol synthesis in the liver by inhibiting the mevalonate pathway in 3-hydroxy-3-methyl-glutarly-coenzyme A (HMG-CoA) reductase which plays a role in cholesterol biosynthesis. One of the statin drugs that is often prescribed is simvastatin. Side effects of myopathy and increased liver enzymes can occur in patients using statin drugs. Myopathy can take the form of myalgia, myositis, or rhabdomyolysis (Pedersen and Tobert, 2004). These side effects can worsen the condition of dyslipidemia patients, therefore it is necessary to develop new drugs that can be used as alternative therapy for dyslipidemia. One way to develop new drugs can be done through *in silico methods*.

Indonesia has quite high biodiversity when compared to areas with subtropical and polar climates. One plant that has the potential to have an antidyslipidemic effect is the gardenia plant (*Gardenia augusta*, Merr.) because the bioactive compounds contained in gardenia leaves include flavonoids, saponins, iridoid glycosides and essential oils (Norhabibah*et al* ., 2022). Research conducted by Uddin R., *et al*., 2014, shows that *Gardenia augusta*Merr. *leaf extract,* has antioxidant activity comparable to the standard antioxidant vitamin C. The phenolic compounds and flavonoid content in gardenia leaves are responsible for antioxidant activity. The flavonoid compounds that are mostly contained in the ethanol extract of gardenia leaves are rutin and catechin (Uddin, *et al.* 2014). The antioxidant mechanism works as an antihyperlipidemic by inhibiting LDL oxidation. Apart from that, flavonoids play a role in reducing blood cholesterol levels by inhibiting HMG-CoA reductase (Octavelia W., and Kusuma S., 2022).

Based on the description above, gardenia leaves have the potential to be developed as a therapeutic agent that can prevent an increase in LDL levels through the mevalonate pathway, so it is necessary to carry out *in silico research* to determine the pharmacokinetic profile and affinity of gardenia leaf flavonoid compounds for the HMG Coa-reductase enzyme.

2. Research Materials and Methods

2.1 Material

3-dimensional structure of HMG-Coa reductase (pdb id: 1HW9), rutin, catechin downloaded from the website https://www.rcsb.org/pdb/home/home.do.

2.2 Tool

2.2.1 Hardware

The hardware used is an HP laptop with an AMD A8-7410APU Radeon R5 Graphics processor and a Microsoft Windows 10 64 bit operating system.

2.2.2 Software

a. Autodock 4.2 software

b. ArgusLab Software

c. Chimera Software 1.17.3

d. SWISS ADME

e. pkCSM

2.3 Work procedures

a. Preparation of HMG-Coa Reductase Enzyme Structure (Target Protein)

This stage is carried out by separating the HMG-Coa Reductase protein from *the native ligand* that is still attached. The Chimera 1.17.3 program is used to separate the target protein so that space (*pocket/cavity*) is available and the size and coordinates *of the pocket are known* as a *docking site* for the test compound on the target protein.

b. *Molecular Docking* Methods

Validation of the method was carried out by *docking* the target protein without *ligand* (obtained from target protein preparation) with *the native ligand* (simvastatin) using the Autodock 4.2 program. The method is said to be valid if it meets the RMSD value ≤ 2Å and the coordinates and size of the gridbox obtained are recorded (Allen and Rizzo, 2014).

c. Optimization of 3-Dimensional Structure of Test Compounds

The three-dimensional structures of the rutin and catechin compounds were downloaded from the website https://pubchem.ncbi.nlm.nih.gov/ . The 3-dimensional structures of the rutin and catechin compounds were optimized using ArgusLab software. The aim of optimization was to obtain a more stable structural conformation with a lower total energy (Puspitasari et al, 2022). Structural geometry optimization was carried out using the AM1 semi-empirical computing method and energy calculations were carried out on the structure before and after the optimization process.

d. *Docking of* Test Compounds with Target Proteins

After the optimization stage, the test compounds (rutin and ketekin) were docked with the target protein, namely HMG-Coa reductase, which had its *native ligand removed* using the Autodock 4.2 application. The coordinates and size of the grid box used are based on the results of method validation. The results obtained are the conformation and binding energy of the test compound to the target protein.

e. Analysis of Drug-Like Properties and Pharmacokinetic Profile

The SwissADME program was used to analyze the drug-like properties of flavonoid compounds contained in gardenia leaves. This program can be accessed via the website (http://www.swissadme.ch/). Analysis of drug-like properties was carried out based on Lipinski's basic rules (log P partition coefficient value, molecular weight, number of hydrogen bond donors and acceptors).

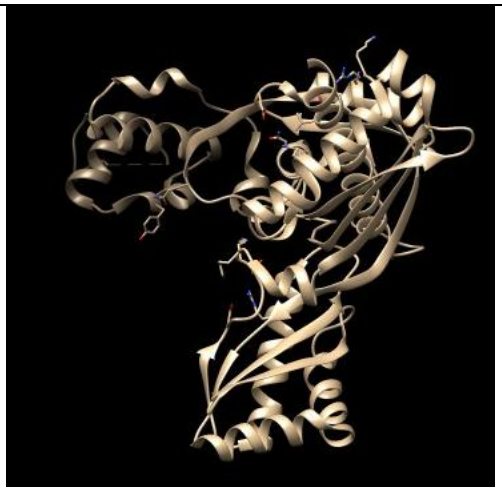
The pharmacokinetic profile was predicted using the pkCSM program which can be accessed via the website (http://biosig.unimelb.edu.au/pkcsm/). SMILES (*Simplified Molecular Input Line Entry Specification*) data from the test compound is required when using this application. The pharmacokinetic profile is analyzed based on the parameters of absorption, distribution, metabolism, excretion and toxicity of the test compound.

3. Results and Discussion

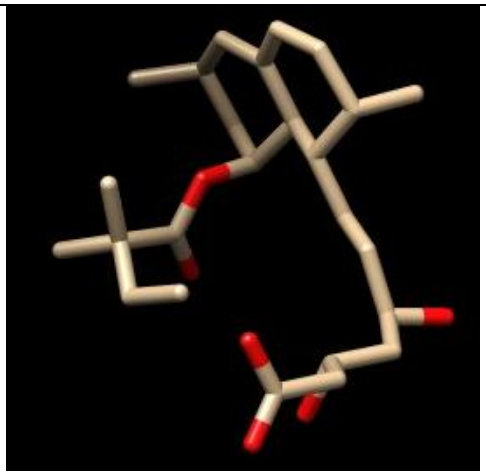
3.1 Preparation of Target Protein Structures

The target protein, namely HMG-Coa reductase, is first separated from its *native ligand* so that there is space to be used during the *docking process* with the test compound. The structure with pdb id 1HW9 is the structure of HMG-Coa reductase used in this research. There are four chains in the HMG-Coa reductase structure used, with each chain binding to *the native ligand* (simvastatin). This research uses chain A of HMG-Coa reductase.

The results obtained from this protein preparation process are protein structures without *native ligands* and *native ligands* which are then saved in the form of a pdb file. Structure of target protein (HMG-Coa reductase) and *native ligand* (simvastatin)shown in figure 1.



(a) HMG-Coareduktase



(b) Simvastatin

Figure 1. Structure of the Target Protein and *Native Ligand*

3.2 Validation of the Molecular Docking Method

This process is carried out by *docking* the target protein without *ligand* (obtained from target protein preparation) with *the native ligand* (simvastatin) using the Autodock 4.2 program. The RSMD (*Root Mean Square Deviation*) value is a validation parameter for the *molecular docking method.* RMSD is a measurement of two poses by comparing the atomic positions between the experimental structure and the structure docked *back* to the target protein (Lestari, 2015). If the RMSD value obtained is ≤ 2Å, it can be said that the method is valid. (Allen and Rizzo, 2014). The results in table 3 show that the *molecular docking method* used is valid because it has an RMSD value ≤ 2Å.

Table 3. Molecular Docking Method Validation Results

Target Proteins	***Native ligands***	**Bond Energy (kcal/mol**	**RMSD (Å)**	**Amino Acid Residues**	**Atomic Clusters**
HMG-Coa Reductase	Simvastatin (5)	-5.74	1.39	LYS735:HZ1 ASN755:HD22	O1A O5

3.3 Optimization of the 3-Dimensional Structure of the Test Compound

The 3-dimensional structures of the rutin and catechin compounds were optimized using ArgusLab software. The aim of optimization was to obtain a more stable structural conformation with a lower total energy (Puspitasari et al, 2022). The results of the structural optimization of the rutin and catechin compounds are shown in table 4. Judging from the data in table 4, the structure optimization went well as indicated by the results of the final energy being lower than the initial energy.

Table 4. Test Compound Geometry Optimization Results

Compound	**Initial energy (kcal/mol)**	**Final energy (kcal/mol)**
Rutin	-283.31	-330.62

Catechin	-128.44	-148.29

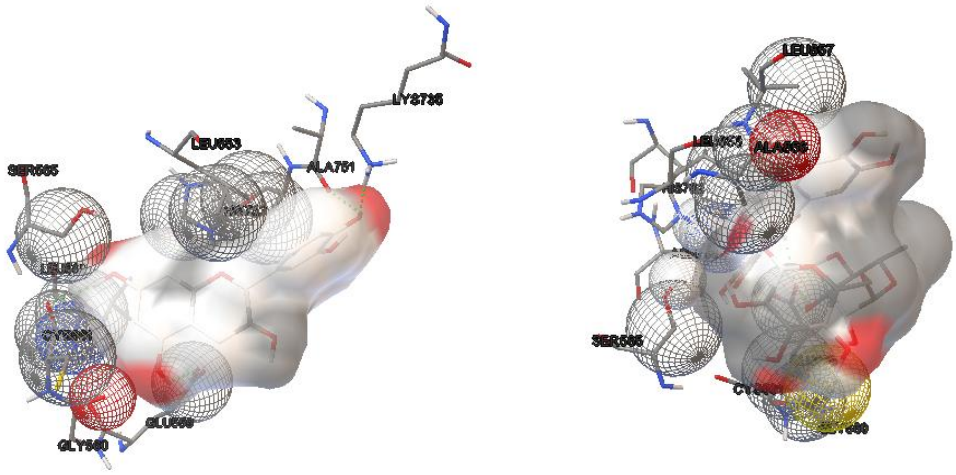
3.4 Docking of the Test Compound to the Target Protein

The Autodock 4.2 program was used to *dock* flavonoid compounds (rutin and catechin) to the active site of the target protein (HMG-Coa reductase) with the same size and coordinate settings as the coordinates of the interaction site of *the native ligand* (simvastatin). The data obtained from the *docking process* of the test compound with the target protein is in the form of bond energy data and the hydrogen bonds formed. Binding energy indicates the affinity of the test compound for the target protein. A negative bond energy indicates an affinity between the test compound and the target protein. The smaller the bond energy obtained, the more stable the bond formed (Laksmiani et al., 2016). The binding energy and list of amino acid residues that bind compounds (rutin and catechin) to the target protein (HMG-Coa reductase) can be seen in table 5. A visualization of the interactions that occur between the test compound and the target protein is shown in figure 2.

Table 5. Results of *Docking* of Flavonoid Compounds on Target Proteins

Target Proteins	Compound	Bond Energy (kcal/mol)	Amino Acid Residues	Atomic Clusters
HMG-Coa Reductase	Rutin	-2.92	GLU559 : OE2	H20
			GLU559 : OE2	H28
			GLU559 : OE2	H21
			GLY560 : O	H19
			ASN755: O14	O14
	Catechin	-5.32	LYS735:HZ1	O6
			CYS561 : O GLU559 : OE2	H12
			ALA751 : O	H11
				H14

Data obtained shows that the rutin and catechin compounds have an affinity and form hydrogen bonds with the HMG-Coa reductase protein. One of the test compounds, namely catechin, has a greater affinity for the HMG-Coa reductase protein compared to simvastatin, which is indicated by a smaller binding energy value. This shows that the test compound has the potential to be developed as an antidyslipidemic agent. The formation of a bond between the test compound and the HMG-Coa reductase protein is able to inhibit the formation of endogenous cholesterol through the mevalonate pathway in the liver (Pawlak *et al.*, 2015).



(a) HMG-Coa Reductase-Quercitrin (b) HMG-Coa Reductase-Rutin

Figure 2. Visualization of HMG-Coa Reductase Bonds with Flavonoid Compounds in Gardenia Leaves

3.7 Analysis of Characteristics of Drug-Like Compounds and Pharmacokinetic Profiles

The characteristics of drug-like compounds from flavonoid compounds in gardenia leaves were analyzed based on the Lipinski *Rule of Five* . Lipinski's *Rule of Five* or Lipinski's 5 basic rules states that a compound has drug-like properties if it has a molecular weight of less than 500 Daltons, a log P partition coefficient value of <5, the number of hydrogen bond donors is not more than 5, and the number of hydrogen bond acceptors is no more than of 10 (Lipinski, 2000). The data in table 1 shows that the catechin compounds in gardenia leaves meet all the requirements for molecular weight parameters, log P partition coefficient values and the number of hydrogen bond acceptors and donors. Meanwhile, rutin compounds only meet the partition coefficient parameter log P. Based on these results, it can be concluded that catechin compounds have properties similar to drugs, so they have the potential to be developed as drugs.

Table 6. Results of analysis of characteristics of drug-like compounds based on Lipinski's rules

Compound	Molecular Mass (Da)	Log P	Number of Hydrogen Bond Acceptors	Number of Hydrogen Bond Donors
Rutin	610,521	-1.68	16	10
Catechin	290,271	1.33	6	5

The pharmacokinetic properties of the flavonoid compounds rutin and catechin contained in gardenia leaves were predicted using the pkCSM program. Prediction of pharmacokinetic properties includes ADMET (absorption, distribution, metabolism, excretion, and toxicity). The predicted pharmacokinetic properties of gardenia leaf flavonoid compounds can be seen in table 2.

The absorption profile is seen from absorption parameters in the gastrointestinal tract and interaction with P-glycoprotein. The distribution profile is seen from the parameters of distribution volume and binding to plasma proteins. The metabolic profile is seen from the parameters of compounds as CYP enzyme substrates and compounds as CYP enzyme inhibitors. The excretion profile is seen from the clearance value and the toxicity profile is seen from the hepatotoxicity parameters.

Absorption in the gastrointestinal tract is categorized as poor if it has an absorption percentage of <30%, medium category between 30% to 80%, and high category if more than 80% (Perez *et al.*, 2004). Catechin compounds have moderate levels of gastrointestinal absorption, while rutin compounds have low levels of gastrointestinal absorption as shown in Table 2. P-glycoprotein (Pgp) is a *transporter* that can influence the absorption and release of a drug compound so that the interaction of a compound with P-glycoprotein (Pgp) can reduce the levels of drug compounds in cells thereby reducing the effect of the compound (Ahmed *et al.*, 2022). Catechin is a test compound which is a substrate of Pgp, so the absorption of the compound is affected by Pgp activity.

Table 7. Prediction Results of Pharmacokinetic Profiles of Flavonoid Compounds in Gardenia Leaves

Compound	Absorption		Distribution		Metabolism		Excretion	Toxicity
	Gastrointestinal absorption	**Pgp Substrate**	**Distribution volume (log L/kg)**	**Free Drug Fraction**	**CYP substrates**	**CYP inhibitors**	**Clearance (mL/min/kg)**	**Hepatotoxic**
Rutin	28.301%	No	-0.065	0.303	-	-	-0.27	No
Catechin	72.619%	Yes	1,559	0.139	-	-	0.29	No

Volume of distribution (Vd) is a pharmacokinetic parameter that describes whether a drug remains in plasma or is redistributed to other tissue compartments. If the Vd value of a drug is high, the drug tends to leave the plasma and enter the extravascular compartment of the body so that a higher dose is required to reach a certain plasma concentration (Shargel and Yu, 2016). The distribution volume is categorized as low if the VD value is < 0.71 L / kg (log VDss< -0.15) and high if the VD value is > 2.81 L / kg (log VDss> 0.45) (Nursanti, 2023). Based on the table above, it is known that rutin compounds have a low volume of distribution so they require a low dose to reach a certain plasma concentration. From the free drug fraction value, plasma protein binding can be calculated. Plasma protein binding is the degree of distribution of protein binding in the blood, so that the body can distribute drugs bound to plasma proteins in the blood. The greater the ability to bind plasma proteins, the better the distribution of drug compounds in the blood (Nusantoro and Fadlan, 2020). Rutin and catechin compounds have plasma protein binding percentages of 69.7% and 86.1%, respectively.

Cytochrome P450 enzymes have a role in phase I drug metabolism. Several drug compounds act as cytochrome P450 substrates which can induce expression or inhibit the action of cytochrome P450. If the cytochrome P450 enzyme is induced, drug metabolism will increase so that the pharmacological effect of the drug decreases. If the compound is in the form of a prodrug, enzyme induction will increase the pharmacological effect and increase the possibility of achieving toxic effects (Katzung*et al.*, 2015). Inhibition of cytochrome P450 enzymes causes a decrease in enzyme activity in metabolizing drugs so that it can increase drug levels in the blood which causes toxicity (Almazroo*et al.*, 2017). Rutin and catechin compounds are not substrate compounds for cytochrome P450 enzymes so these compounds do not affect the metabolism that takes place in the liver.

The excretion of the rutin compounds and catechins contained in gardenia leaves was assessed by the clearance parameters of these compounds from the body. The clearance values for rutin and catechin compounds were -0.27 and 0.29 mL/minute/kg, respectively. The toxicity of a compound is assessed by its potential to cause liver damage (hepatotoxic). Rutin and catechin compounds do not have hepatotoxic properties.

4. Conclusion

Catechin compounds fulfill drug-like properties based on Lipinski's basic rules. Analysis of the pharmacokinetic profile and *molecular docking* of the test compound against the target protein shows that the rutin and catechin compounds have an affinity and form bonds on the active site of the target protein, so they are able to inhibit the action of the HMG-Coa reductase enzyme *in silico*.

REFERENCES

Ahmed, J, A. Hamid AA, A. Halim KB, and C. Has AT. 2022. P-glycoprotein: new insights into structure, physiological function, regulation and alterations in disease. *Heliyon*. Vol. 8(*6*):e09777.

Allen, WJ and RC Rizzo. 2014. Implementation of the Hungarian algorithm to account for ligand symmetry and similarity in structure-based design. *J. Chem. Inf. Model.* Vol. 54(*2*): 518–529.

Almazroo, OA, Miah, MK, Venkataramanan, R. 2017. Drug Metabolism in the Liver. Clin Liver Dis. Vol. 21(1):1-20.

Cai, H., David G. Harrison. 2000. *Endothelial Dysfunction in Cardiovascular Diseases The Role of Oxidant Stress.* Department of Medicine, Emory University School of Medicine, Atlanta.

Dewijanti, ID, W. Mangunwardoyo, A. Dwiranti, M. Hanafi, and N. Artanti. 2020. Short communication: Effects of the various source areas of Indonesian bay leaves (*Syzygiumpolyanthum*) on chemical content and antidiabetic activity. *Biodiversity* Vol. 21(*3*): 1190-1195.

Ezeh, KJ and O. Ezeudemba. 2021. Hyperlipidemia: A Review of the Novel Methods for the Management of Lipids. *Cureus.* Vol. 13(*7*)

Hartanti, L, SMK Yonas, JJ Mustamu, S. Wijaya, HK Setiawan, and L. Soegianto. 2019. Influence of extraction methods of bay leaves (*Syzygiumpolyanthum)* on antioxidant and HMG-CoA Reductase inhibitory activity. *Heliyon.* Vol. 5(*4*):e01485.

Katzung, BG, & Trevor, AJ 2015. Basic & Clinical Pharmacology. Edition XIII. San Francisco, USA: McGraw-Hill.

Laksmiani, NPL, NLPV Paramita, and IMAG Wirasuta. 2016. In Vitro and In Silico Antioxidant Activity of Purified Fractions from Purple Sweet Potato Ethanolic Extract. *International Journal of Pharmacy and Pharmaceutical Sciences.* Vol. 8:177-181

Lestari, T. 2015. StudiInteraksiSenyawaTurunan 1,3-Dibenzoiltiourea sebagaiRibonukleotidaReduktase Inhibitor. *JurnalFarmasi Indonesia.* Vol.7:163-169

Lipinski, CA 2000. Drug-like properties and the causes of poor solubility and poor permeability. *Journal of Pharmacological and Toxicological Methods* . Vol.44(*1*): 235-249.

Mirza, AZ, Althagafi II, and H. Shamshad. 2019. Role of PPAR receptors in different diseases and their ligands: Physiological importance and clinical implications. *Eur J Med Chem* Vol. 166:502–13.

Nirosha, K., M. Divya, S. Vamsi, and M. Sadiq. 2014. A review on hyperlipidemia. *Int. J Novel Trends Pharm Sci.* Vol 4(*5*): 81-92.

Norhabibah, Ali Rakhman Hakim, DariniKurniawati. 2022. Uji Kuantitatif Flavonoid Total dan AktivitasAntioksidanEkstrakDaunKacapiring (*Gardenia jasminoides Ellis*). Banjarmasin: Indonesian Journal of Pharmacy and Natural Product.Nursanti, O. 2023. PrediksiToksisitas dan FarmakokinetikaUntukMendapatkanKandidatObatAntidiabetes. *Journal of Pharmaceutical Care and Sciences* Vol. 3 (2): 1-9.

Nusantoro, Y. R., A. Fadlan. 2020. AnalisisSifatMiripObat, Prediksi ADMET, dan PenambatanMolekular Isatinil-2- Aminobenzoilhidrazon dan komplekslogamtransisiCo(II), Ni(II), Cu(II), Zn(II) Terhadap BCL2-XL. *AktaKimindo* Vol. 5(2): 114-126

Oktavelia W., and Kusuma S., 2022. Therapy for Dyslipidemia: Plant Inhibitors of HMG-CoA Reductase. *Indonesian Journal of Biological Pharmacy*. Vol 2: 159-170

Pawlak, M., P. Lefebvre, and B. Staels. 2015. Molecular mechanism of PPARα action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. *Journal of Hepatology* . Vol. 62(*3*): 720-733.

Pedersen, T and Tobert, J., 2004. Simvastatin: A Review. Drug Evaluation. Ashley Publications Ltd ISSN 1465-6566.

Pérez, MA, MB Sanz, LR Torres, RG Avalos, MP González, and HG Díaz. 2004. A topological sub-structural approach for predicting human intestinal absorption of drugs. *Eur J Med Chem* . Vol. 39(*11*):905-16.

PERKI (PerhimpunanDokterSpesialisKardiovaskular Indonesia). 2022. PanduanTatalaksanaDislipidemia. *PenerbitPerhimpunanDokterSpesialisKardiovaskularIndonesi.*

Sakaganta, A.R.I. dan A. Sukohar. 2021. Daun Salam (SyzygiumPolyanthum) SebagaiPenurun Kadar KolesterolDalamDarah. *Medula* Vol. 10(4): 618-622.

Shargel, L. and AB Yu. 2016. *Applied Biopharmaceutics and Pharmacokinetics.* New York: McGraw & Hill.

Shi, Q., J. Chen, X. Zou, and X. Tang. 2022. Intracellular Cholesterol Synthesis and Transport. *Front Cell Dev Biol*. 2022 Vol.10:819281.

Stancu, C. and A. Sima. 2001. Statins: mechanisms of action and effects. *J Cell Mol Med* . Vol. 5(*4*):378-87.

Susanti, N.M.P, N.K. Warditiani, K.A.S. Dewi, dan M. Oka. 2016. AktivitasAntihiperlipidemiaAndrografoliddariSambiloto (Andrographis paniculata (Burm. f.) Ness) secara In Silico. *JurnalFarmasiUdayana* Vol. 5(2): 58-62.

Tian, S., R. Wang, S. Chen, J. He, W. Zheng, and Y. Li. 2021. Structural Basis for PPARs Activation by The Dual PPARα/γ Agonist Sanguinarine: A Unique Mode of Ligand Recognition. *Molecules* Vol. 26(*19*):6012.

Uddin R., Moni Rani Saha, Nusrat Subhan, Hemayet Hossain, Ismet Ara Jahan, RaushanaraAkter, Ashraful Alam. 2014. HPLC-Analysis of Polyphenolic Compounds in Gardenia jasminoides and Determination of Antioxidant Activity by Using Free Radical Scavenging Assays: *Advanced Pharmaceutical Bulletin.* 4(3), 273-281.