MOLECULAR DOCKING COMPOUNDS IN METHANOL EXTRACT OF MANGO LEAVES (*Mangifera indica* L.) AS ANTI-INFLAMMATORY AGENT

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Abstract

Previous studies have shown that mango has anti-inflammatory properties. Anti-inflammatory drugs are compounds or medicines used to treat diseases caused by inflammation. The most commonly used anti-inflammatory drug is a non-steroidal anti-inflammatory drug (NSAID). The drug works by inhibiting the enzyme cyclooxygenase (COX). The purpose of this study was to determine the profile metabolites present in the methanol extract of mango leaves and their interaction with the COX enzyme. This study includes an analysis of the compounds contained in the methanol extract of mango leaves using LCMS / MS and molecular docking studies of these compounds. Compounds detected by MS include $C_{26}H_{24}O_{14}$, $C_{45}H_{84}O_{14}$, Khellol-β-D-glucoside, Mangiferin, and Nevadensin-5-β-D-glucoside. Analysis of docking result was based on ΔG and Ki and the binding interactions that occur. Compounds that are compatible with COX1 and COX2 are Khellol βD glucoside with G and Ki values of 7.49 kcal/mol and 3.23 μM and 8.32 kcal/mol and 0.7919 μM, respectively. Through the molecular docking process, it was confirmed that khellol β-D-glucoside may be activated as an anti-inflammatory agent.

Keywords: *anti-inflammatory, COX, docking, mango, Mangifera indica L.*

Introduction

A large number of plants produce various secondary metabolites with low molecular weights. These secondary metabolites are also known as specialized metabolites which collectively act as an effective supply against biotic and abiotic stresses. In addition, many identity compounds from a plant have been found to be useful and have been used as food ingredients, nutraceuticals, traditional medicines, and pharmacies (Alseekh et al., 2020).

Mango *(Mangifera indica* L.) is one of the fruits of choice in the world. One of the most widely consumed fruit crops in the tropics and subtropics. For more than 4000 years *Mangifera indica* has become an important component in Ayurvedic medicine, various parts of the plant are used as toothpaste, antiseptic, astringent, anti-inflammatory, laxative, and diuretic (Igbari et al., 2019).

Inflammation is a normal response to a noxious stimulus that threatens the body and can vary from a localized response to a generalized response. Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)) is widely used for the treatment of rheumatic diseases, such as rheumatoid arthritis and pain. NSAID Pharmacological effects are caused by the suppression of prostaglandin synthesis from arachidonic acid by inhibition of the cyclooxygenase (COX) enzyme involved in prostaglandin biosynthesis (Maseda & Ricciotti, 2020).

There are two isoforms of the cyclooxygenase enzyme with different levels of selectivity, COX-1 enzyme which is found in most cells and plays an important role in gastric mucosal protection, platelet aggregation, and renal

blood flow. COX-2 is an isoenzyme that is induced and expressed during inflammation. However, NSAID long-term use of flurbiprofen and ibuprofen can cause several side effects including gastro-ulcer, cardiovascular toxicity, renal failure, and asthma (Maseda & Ricciotti, 2020). Therefore, efforts are needed to find inflammatory effects with less risk (Igbari et al., 2019). Where these inhibitors allow new agents for long-term use and for prophylactic use in certain chronic diseases.

Research conducted by Annisa 2019, showed that mango leaves have the ability as an anti-inflammatory agent (Annisa, 2019). This study aims to determine the compounds contained in the methanol extract of mango leaves and perform molecular docking of these compounds with COX enzymes to see the potential of these compounds as inflammatory agents.

Research Methods

Tools and materials

The tools used in this research are: LC-MS instrument, a set computer with Windows 10 64 bit specifications and AutoDock 4.2 program on OS Windows, Chimera 1.10.1, and Discovery Studio. The materials used in this study were: Mango leaves, 96% ethanol, Chloroform, 2 M HCl, 10% HCl, Methanol, Methanol (PA), N-Hexan, 25% Ammonia (PA), Anhydrous MgSO4, Iodine. Potassium iodide, Aqua dest, Ethyl Acetate and protein.

Analysis of metabolites using LC-MS

Mass spectrometry consists of three main parts, namely ion source, mass analyzer and detector. Prior to identification, the sample was prepared by the method solid phase extraction (SPE) is then injected into the LC-MS. ESI method is used to convert sample molecules into ions. Ionization generates a large number of ions through the exchange of charges in solution and forms a complete molecular ion to aid in the initial identification which will then be further identified by MS (Chaleckis et al, 2019).

Molecular docking

1) Docking preparation

Protein preparation was carried out by selecting the protein in the active form on the protein data bank site. Next is native protein ligand separated from the Discovery Studio program to separate between macromolecules and native ligands (Bandgar, 2010). Preparation Receptor was obtained through the PDB website (Protein data bank), then download with GDP format. Next open the app Discovery studio visualization to clean receptors that are still dirty. The first step: click the script menu then select the next selection select water molecule and the last one press delete on the keyboard. Step second, click the script menu then select the next selection select the ligand then delete. If the receptor has overall clean, Step last press on the file menu then save as the receptor in the format GDP.

Optimization of the three-dimensional structure was carried out to examine the physicochemical properties of the test ligands and native ligands using Gaussian software. The optimization of the structure is carried out on a three-dimensional structure with the DFT computational method (Saputra, 2018).

2) Method validation

Method validation of molecular docking done by restore native ligands on target proteins that have removed their native ligands using AutoDock 4.2 software. This validation is said to be valid if the RMSD value obtained is < 2 Å (Saputra, 2018).

3) Docking compound test

Molecular docking process using AutoDock tools application and AutoDock Vina. receptor structure and ligands that have been optimized separately stored in one folder same. For molecular docking use AutoDock tools first by preparing

receptors the following stages: Open the application AutoDock tools then click read molecular on the file menu and select receptors to be docked. Receptors existing ones added with hydrogen and check the option all hydrogen, method no Bond order, yes atomic renumber includes new hydrogen, then click OK. After the receptor has added hydrogen, click grid macromolecular then click choose then click the receptor and finally select molecule. The stored receptors format is in PDBQT format. Furthermore, for the preparation of the ligand, the steps are as follows: click the ligand then click new input click open and select the ligand that is in the folder. If the ligand is out on working layer click torsion tree to set the number torsion on the ligand, then save the ligands in the format PDBQT.

The next step is to prepare the place where the ligand will be binds to the receptor by as follows: select the receptor format PDBQT then click the grid box on grid menu, then adjust point on the x (red), y (green) axis, z (blue). Open Discovery studio visualization to see the active side or the binding site for the receptor area then right click and search until the area is found. Next, Reopen the AutoDock tools application and input the found x, y, z values on receptors in the Discovery studio visualization.

The next step is to open the application Notepad and input data such as receptor, ligand, center x, center y, center z, size x, size y, size z, and accuracy.

Then save in the folder that has been made. Next enter the application AutoDock Vina consisting of vina, vina split, and vina license in that folder has been created, then open the application Command prompt/terminal and input format (e.g. C:\user\Customer>D:\cd vina..), then enter the formula for calculation in command prompt: Config conf.txt – log log.txt then it will be binding affinity result of the ligand appears researched. After that use vina split to separate the result from the ligand one by one using the formula D:\vina>vina-split .. input out. dbqt. The last stage is visualization with how to drag the receptor and out ligand 1 in Discovery studio visualization later see the results in 2D and 3D.

The optimized compounds were re-docking with proteins that had their native ligands removed using the AutoDock 4.2 program using a grid box. Where the analysis results will show binding affinity and the inhibition constant of the compound with the lowest conformation and binding energy to bind to the target protein (Saputra, 2018).

4) Data analysis

Data analysis seen from the value of binding affinity resulting from the result re-docking with using docking procedure. Score of binding affinity indicates the bond strength between the test compound and the target work. Where the lower the binding affinity value, the more stable the bond between the compound and the receptor (Saputra, 2018).

Results and Discussion

Analysis of metabolite profile using LC-MS/MS

LC-MS/MS was used to determine the metabolite profile of the mango leaf methanol extract and to find out what compounds were contained in the sample. The results of the LC-MS/MS analysis are used to analyze the metabolite of a sample. Chromatography is used to detect molecular weights, two-dimensional signal intensity in raw data, etc. Furthermore, analysis using mass spectroscopy was carried out to identify the compounds contained in the sample. The method used to determine the method of ionization in this study was the electrospray (ESI) method, where the analyte that was already in the liquid was inserted into the capillary and then sprayed into the gas phase (fine aerosol).

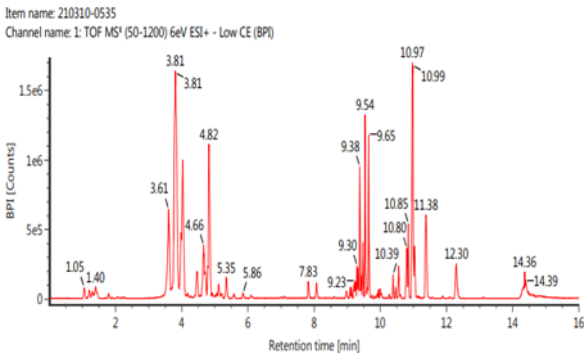


Figure 1. LC-MS chromatogram

Figure 1 is the results of chromatogram. It can be seen that there are approximately 20 peaks which indicate that there are 20 compounds identified. However, due to the limitations of MS readings available at the Indonesian Education Laboratory, only 5 compounds were identified by name and only 3 compounds were identified as structures.

The test results showed 5 compounds detected by MS, at 4.03 minutes C26H24O14 was detected, C45H84O14 was detected at 10.97 minutes, Khellol-β-D-glucoside was detected, at 3.81 minutes Mangiferin was detected and at 4.83 minutes Nevadensin-5- was detected. O-β-D- glucoside. However, only three compounds were detected by the library, namely Mangiferin, Khellol-β-D-glucoside and Nevadensin-5-O-β-D-glucoside.

Compound docking

1) Target protein preparation

COX-1 and COX-2 receptors was downloaded by PDB ID code 1EQG and 4PH9. The search for the structure and binding site of this receptor was carried out by searching the RCSB Protein Data Bank website. The code 1EQG and 4PH9 were chosen because the three-dimensional structure of these COX-1 and COX-2 receptors has already formed a complex with native ligands and affected the activity. The PDB data from 1EQG and 4PH9 suggest that the three-dimensional structure of the receptor crystal COX-1 and COX-2. This is determined by the X-Ray Diffraction method has a resolution of 2.61 and 1.81 and was published in 2001. The format used in downloading the three-dimensional structure of the receptor via PDB is *.pdb format which is suitable as input in the molecular docking stage.

This selection is based on the chemical properties of native ligands that are the same as binding to the receptor which can affect the test results. Therefore, the same receptor that binds to ibuprofen is chosen. NSAIDs can effectively suppress the hyperinflammatory response. The use of light and moderate doses can be considered to reduce inflammation in the initial treatment of cytokine storms and in certain cases (Rusdiana, 2020). This receptor selection process aims to obtain protein macromolecules or proteins without ligands and obtain native ligands from these proteins.

2) Docking method validation

The parameters used for the validation of the docking method are the RMSD value must be < 2 Å, and the Inhibition Constant Value must not be more than 100 μ M. RMSD is a measure of the deviation distance between the conformation of the test ligand and the X-ray ligand. RMSD is used to assess whether combination mode prediction is successful, it is very important for docking procedure verification8. Table 2 and Table 3 collect the KI with the lowest RMSD for each ligand. From the docking data, it is obtained that the grid box size values for COX-1 receptors are 40 x, 40 y, 40 z, with a grid center x = 68.328, a grid center y = -22.231, a grid center z = 189.725 and uses a grid spacing of 0.375 while for COX-2 receptors are 60 x, 60 y, 60 z, with grid center x = 19304, grid center y = 14,877, grid center z = 68,001 and use a grid spacing of 0.375 with the Lamarckian Genetic Algorithm (LGA) method.

Table 1. Target proteins and native ligands

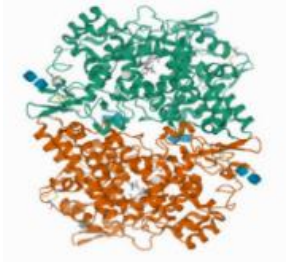
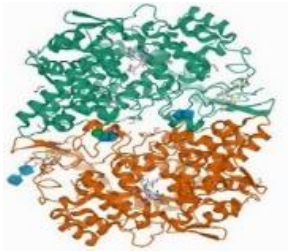
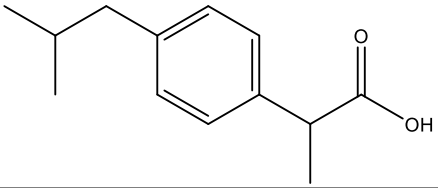
	COX-1	COX-2
PDB ID	1EQG	4PH9
Method	X-Ray Diffraction	X-Ray Diffraction
Resolution	2.61	1.81
Structure		
Native ligand (ibuprofen)		

Table 2. COX-1 receptor validation parameter

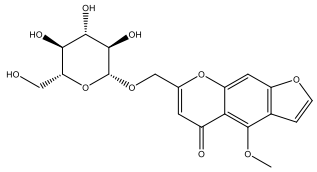
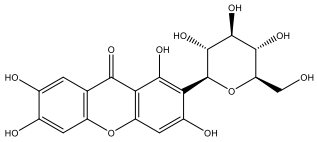
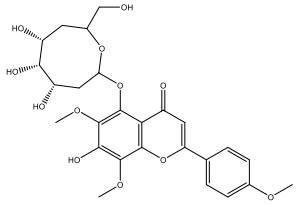
Grid Box			RMSD	Grid Spacing	Grid Center		
X	Y	Z			X	Y	Z
40	40	40	0.930 A	0.375	68.328	22.231	189.725

Table 3. Parameters of COX-2 receptor validation

Grid Box			RMSD	Grid Spacing	Grid Center		
X	Y	Z			X	Y	Z
60	60	60	0.661 A	0.375	19.304	14.877	68.001

In this study, 3 test compounds were used resulting from the characterization of mango leaf methanol extract using the LCMS/MS instrument conducted at the Indonesian Education Laboratory (LIPI) (Table 4).

Table 4. Test ligand

Compound Code	Compound Name	Two-Dimensional Chemical Structure
L1	Khellol-β-D-glucoside	
L2	Mangiferin	
L3	Nevadensin-5-O-β-D-glucoside	

3) Geometry optimization

The test compounds were made in 2D and 3D structures and geometric optimization was carried out to determine their physicochemical properties. Molecular anchoring studies aim to help develop new pharmacological agents that can interact effectively with drug targets and produce biological activity. Lipinsky's Five Rules are practical parameters for evaluating a drug or determining whether a compound with a certain pharmacological or biological activity has properties acceptable to the human body. These parameters describe the important molecular characteristics of the drug through the human body, including the processes of absorption, distribution, metabolism and excretion (Benouis et al, 2010).

Table 5. Geometry optimization

Compound	Total Energy (au)	HOMO (au)	LOMO (au)	Energy Gap (au)
L1	-1487.755	-0.062	-0.220	-0.158
L2	-1561.791	-0.062	-0.022	-0.040
L3	-1910.836	-0.063	-0.214	-0.151

Table 6. Parameters of physicochemical properties based on Lipinski's Rules

Compound	MW (g/mol)	LogP	H donor	H acceptor	Eligibility
Native ligand	214.2	3.78	2	2	Eligible
L1	408.1	-2.11	4	10	Eligible
L2	422.1	-1.43	8	11	Not eligible
L3	534.2	0.45	5	12	Not eligible

Based on this rule, three compounds were analyzed to determine whether they met Lipinski's requirements. Judging from the results of Lipinski's five-choice rule, the native ligands and the 3 test ligands in the table show that only the Khelol-β-D-glucoside compound meets Lipinski's criteria, so it is possible that these compounds have interactions with receptor proteins. Table 5 shows that L1 has the highest HOMO value of -2.11 au, so it can be concluded that compared to other ligands, this compound has a greater ability to donate electrons. Compared to other ligands, L1 also has a higher gapp value, this indicates that the compound has a high level of stability.

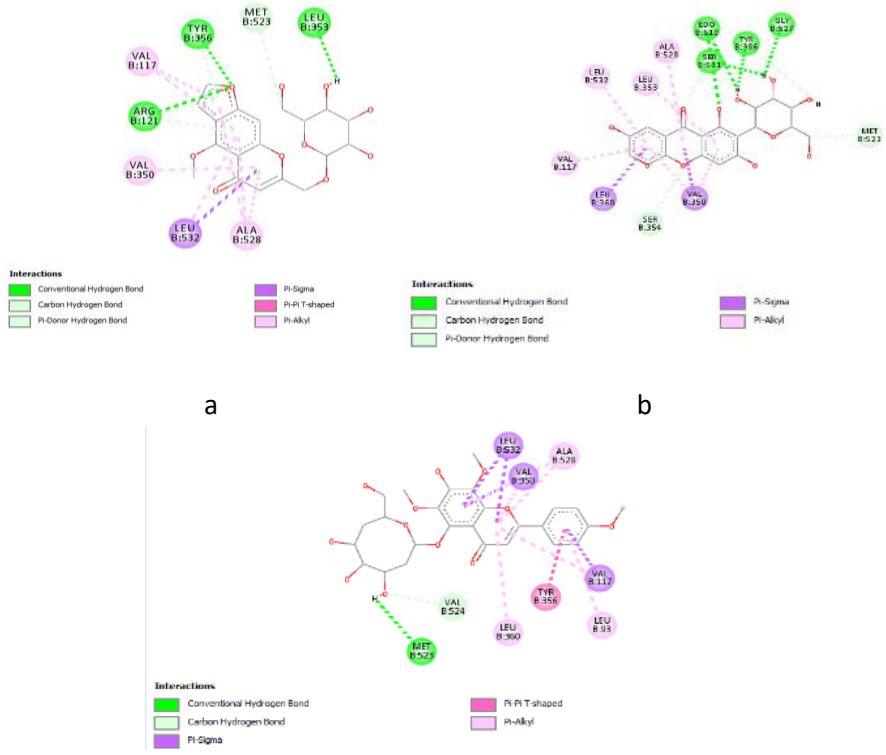


Figure 2. Visualization of COX-1.ligand interactions, a. Khellol-β-D-glucoside; b. Mangiferin; c. Nevadensin-5-O-β-D-glucoside

4) Docking compound test

The docking carried out in this study is oriented docking because it determines the grid box as the docking target. In oriented docking, the ligand is flexible and the target protein is rigid. The requirements for docking with AutoDock are ligands and protein targets that have previously been validated by the Lamarckian Genetic Algorithm (LGA) method.

The results of molecular anchoring in this study include the value of the inhibition constant (KI) and Root Mean Square Deviation (RMSD), as well as the interaction of ligands with amino acid residues in protein macromolecules. The conformation of each docking ligand is ordered from the smallest to the largest based on the value of the inhibition constant (KI). The inhibitory constant (KI) is the energy required for the ligand to interact (bind) with the receptor at the binding site. The smaller the value of the inhibitory constant (KI), the more stable the ligand binding to the receptor.

The data obtained from molecular anchoring is prediction of bond conformation based on bond energy in the form of position and type of bond as well as bond affinity. Compared with other tested ligands, Khelol-β-D-glucoside has the best binding affinity and inhibition constant G, namely mangiferin and Nevadensin-5-O-β-D-glucoside.

However, the value of this binding affinity has not been able to determine the pharmacological activity that occurs. Therefore, it is necessary to carry out further experimental verification, either through in vitro or in vivo tests. Nevertheless, docking plays an important role as the first step in the development and design of new drugs because it has its own advantages over in vivo and in vitro methods (Brooijmans et al, 2010).

5) Compound interaction

The interaction analysis was carried out to determine the contribution of the affinity of the ligand to the receptor due to the electrostatic interaction between the ligand and the receptor. From the determination of the interaction, the bond energy values for the three test compounds sequentially in Figure 2 are -5.10 kcal/mol, -2.96 kcal/mol, -2.99 kcal/mol and in Figure 3 are -6.83 kcal/mol, - 2.96 kcal/mol, -0.34 kcal/mol. The carboxylate inhibitors take part in a pole that includes two hydrogen bonds between the inhibitor and ARG-120, a salt bridge between ARG-120 and a hydrogen bond between the phenolic hydroxyl inhibitors of TYR-355.

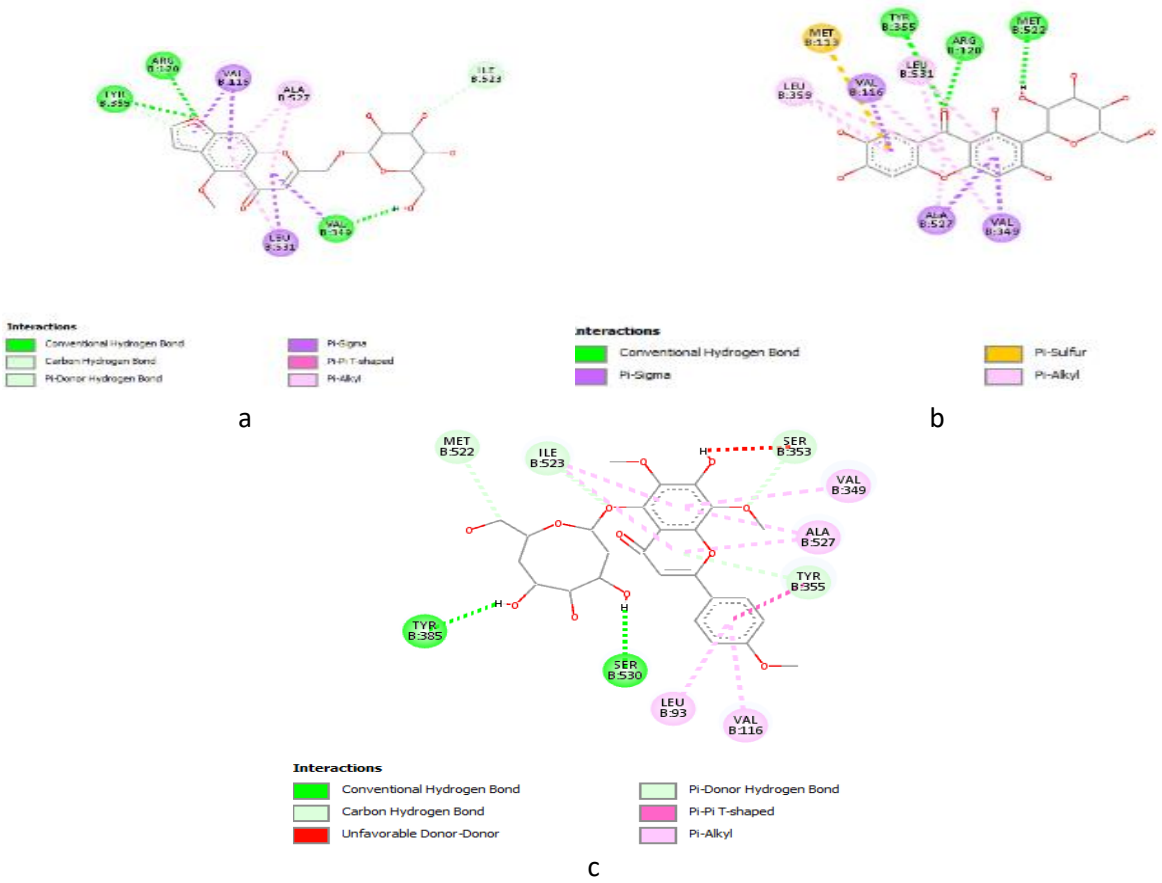


Figure 3. Visualization of COX-2.ligand interactions, a. Khellol-β-D-glucoside; b. Mangiferin; c. Nevadensin-5-O-β-D-glucoside

Conclusion

Based on the research that has been done, there are 5 metabolite compounds detected by MS from group flavonoids including $C_{26}H_{24}O_{14}$, $C_{45}H_{84}O_{14}$, Khellol-β-D-glucoside, Mangiferin, Nevadensin-5-O-β-D-glucoside. Through the molecular docking process, the compounds obtained Khellol-β-D-glucoside is predicted to be active as an anti-inflammatory agent.

Aknowledgment

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References

Alseekh, S., Perez de Souza, L., Benina, M., & Fernie, A. R., 2020, The style and substance of plant flavonoid decoration; towards defining both structure and function. Phytochemistry, 174(March).

Anisa, N., Amaliah, N.A., Haq, P.M.A., dan Arifin, A.N., 2019, Efektifitas Anti-Inflamasi Daun Mangga (Mangifera indica) Terhadap Luka Bakar Derajat Dua. Jurnal Sainsmat, 8(1): 1-7.

Bandgar, B. P., & Gawande, S. S., 2010, Synthesis and biological screening of a combinatorial library of β-chlorovinyl chalcones as anticancer, anti-inflammatory and antimicrobial agents. Bioorganic and Medicinal Chemistry. 2010; 18 (5): 2060-2065.

Benouis, S., Ferkous, F., Kraim, K., Allali, A., & Saihi, Y., 2010, Molecular docking studies on gingerol analogues toward mushroom tyrosinase. Journal of the Chemical Society of Pakistan, 42: 6.

Brooijmans, N., Mobilio, D., Walker, G., Nilakantan, R., Denny, R. A., Feyfant, E., Diller, D., Bikker, J., & Humblet, C., 2010, A structural informatics approach to mine kinase knowledge bases, Drug Discovery Today, 15, 5-6 , 203-209

Chaleckis, R., Meister, I., Zhang, P., & Wheelock, C. E., 2019, Challenges, progress and promises of metabolite annotation for LC–MS-based metabolomics. Current Opinion in Biotechnology, 55, 44–50.

Igbari, A. D., Nodza, G. I., Adeusi, A. D., & Ogundipe, O. T., 2019, Morphological characterization of mango (Mangifera indica L.) cultivars from south-west Nigeria. Ife Journal of Science, 21(1), 155.

Maseda, D., & Ricciotti, E., 2020, NSAID–Gut Microbiota Interactions. In Frontiers in Pharmacology.

Rusdiana, T., & Akbar, R., 2020, Perkembangan Terkini Terapi Antikoagulan Pada Pasien Covid-19 Bergejala Berat. Jurnal Sains Farmasi & Klinis. 7 (3): 248-253.

Saputra, D. P. D., 2018, Molecular Docking Sianidin dan Peonidin sebagai Antiinflamasi pada Aterosklerosis Secara In Silico. Jurnal Farmasi Udayana, 7(1), 28.