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Molecular docking analysis of major active compounds of pomegranate peel extract (*Punica granatum* L.) in inhibiting cyclooxygenase enzyme

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Abstract

Background: Inflammation is the body's physiological response to an injury. Injury that affected the body can be a chemical agent, physical, or biological agent. Nowadays the inflammatory condition treated by eliminating the main etiological factor then prescribing anti-inflammatory drugs such as NSAIDs, but according the data in 2021 shown that 78,8% patients has gastritis side effect. Pomegranate peel extract (PPE) has good anti-inflammatory ability because it containing the highest concentration of flavonoid.

Objectives: To predict the molecular inhibition of major active compounds (epigallocatachin gallate, ferulic acid, chlorogenic acid, gallic acid, caffeic acid, cianidanol, epicatechin, and punicalagin) in PPE against cyclooxygenase enzyme (COX-1 & COX-2) using in silico study.

Method: Preparation of the active compounds of PPE, prediction of their activity, ADMET predicting test, physicochemical test, and molecular docking simulation.

Results and discussions: In silico test showed that all common active compound of PPE that have potential as anti-inflammatory drugs. All of MAC PPE had value of Pa > 0.5. ADMET prediction showed that all common active compounds can distribute systemically because had score of log mucosal permeability < 2.5. All of common active compound in PPE had negative prediction not to toxic or triggering dermatitis contact against oral mucosa through ADMET prediction. The result of molecular docking of chlorogenic acid and punicalagin against protein of COX-2 showed ΔG_{bind} value more than mefenamic acid and arachidonic acid against COX-1 and COX-2 in range of -3,0 to -9,1 kcal/mol which the most effective as an anti-inflammatory is punicalagin (-4,0 & -9,1 kcal/mol).

Conclusion: PPE had potential as anti-inflammatory drugs through COX-1 & COX-2 inhibition with the best anti-inflammation ability is ferrulic acid and punicalagin.

Keywords: Pomegranate peel extract; Anti-inflammation; Molecular docking; Cyclooxygenase enzyme; Drug discovery

1. Introduction

Inflammation is the body's physiological response to an injury that would be considered a foreign body [1,2]. Inflammatory conditions are also often found in the oral cavity with 5 cardinal signs which include calor, tumor, dolor,

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rubor, and functio laesa [3]. The inflammatory process that occurs is mediated by strong inflammatory mediators such as prostaglandin E2 (PGE2) as a product of arachidonic acid (AA) bioconversion by the COX-2 enzyme [4–6]. Uncontrolled production of PGE2 will continuously encode the recruitment of immunocompetent cells which causes an increase in destructive responses to tissues, causing the inflammatory process to become chronic. Reviewing the activity of PGE2 which plays an important role in the inflammatory process, the therapy that is often given is anti-inflammatory drugs that inhibit COX-2 in converting AA to PGE2 such as Non-Steroid Anti-Inflammatory Drugs (NSAIDs) [7–9].

Nevertheless, NSAIDs can have gastritis side effects in patients. Reporting from research conducted in 2021 showed that the prevalence of gastritis patients due to side effects of consuming NSAIDs was 78.8%. This incident occurs because the active compounds of NSAIDs that are consumed orally (tablets, pills, and capsules) will inhibit the COX-2 enzymes in the stomach. Decreased production of PGE2 in the stomach can eliminate the function of PGE2 as a gastroprotective, causing stomach irritation (gastritis). In addition, drug delivery using oral preparations can reduce the bioavailability of a drug thereby reducing the load absorbed by the body [10]. COX-2 is an enzyme responsible for the biosynthesis of prostaglandins, which play a key role in promoting inflammation [11]. COX-2 is a membrane-bound enzyme that is located in the endoplasmic reticulum. Inhibitors of COX-2 are used as anti-inflammatory drugs to reduce inflammation and pain. The success of COX-2 inhibitors is attributed to their ability to reduce inflammation without affecting the production of natural mucus lining that protects the inner stomach. Using in silico study, researcher could predict the compound wich have lower binding affinity (high binding ability) to gain a binding complex with COX-2 active site [12–14].

Indonesia is megabiodiversity country that has a very broad agricultural sector with an area of agricultural land reaching 10.45 million hectares per year by 2022 with the main agricultural commodities being biopharmaceutical plants. One of the biopharmaceutical plants that thrives in Indonesia is the pomegranate (*Punnica granatum* L.). Pomegranate (*Punnica granatum* L.) is a fruit plant that can grow up to 5-8 m. This plant is thought to have originated in Iran, but has long been bred in the mediterranean region. This plant is also found in South China and Southeast Asia. Pomegranate is one of the most abundant fruits in Southeast Asia, including Indonesia. Pomegranate has been widely used as herbal medicine to prevent various diseases since the days of ayurvedic medicine. Pomegranate contains several compounds that have the potential to be used as natural products in the field of dentistry. The main compounds contained in pomegranate peel include tannin acid, catechin, and gallocatechin. Pomegranate contains ellagic acid, ellagitannins, gallic acid, and punicalagin. The tannins contained in pomegranate consist of 3 compounds, namely ellagitannins, gallotannins, hydroxy benzoic acid, and hydroxy cinnamic acid. The first compound is ellagitanin. Ellagitanin is responsible for the antioxidant activity of the pomegranate. The main ellagitannin compound in pomegranate fruit is punicalagin. Pomegranate is rich in polyphenols, such as tannins, flavonoids, gallic acid, ellagic acid and punicalagin. A kind strong antioxidant which is about 92% of total antioxidant activity of pomegranate. Ellagic acid found in pomegranate seeds while punicalagin was only found in the outer peel, and is thought to have twice the antioxidant ability of red wine and green tea [15–18].

Polyphenols have a role as antioxidants that can fight free radicals dangerous free. The polyphenol content higher can be found on fruit skins such as pomegranate, grape, and apples. Pomegranate polyphenols have an important role in preventing the development of free radicals in the body as well as repairing damaged body cells, as well as being able to provide protection against disease. The edible part of the pomegranate (about 50% of the total fruit weight) consists of 80% juice and 20% seeds. Based on the results of research conducted by Tyagi (2012) on making juice by mixing the seeds and skin together, it has been proven that the skin contains a lot of polyphenolic compounds which have high potential for value addition as a potential resource of phenolics, proanthocyanidins, and flavonoids which are referred to as antioxidants. This study aims to determine the polyphenolic compounds from pomegranate fruit and seed extracts.

Reviewing the problems arising from the use of anti-inflammatory drugs NSAIDs orally and considering the potential of the pomegranate herbal plant which thrives in Indonesia. This study aim is to analyzing the potential of pomegranate peel extract as an anti-inflammatory. Researches found the gap that previous study still questionable about spesific inhibition of active compound in pomegranate peel against cyclooxygenase enzyme. Looking from that gap, the researcher conducting this research which is focusing on discovering the inhibition mechanism of pomegranate peel extract against cyclooxygenase both COX-1 and COX-2 that play key rols in inflammatory conditions [19–21]. This research is expected to be able to provide specific predictions regarding the inhibition ability of the pomegranate peel extract (PPE) against COX-1 and COX-2..

2. Material and methods

2.1. Material

2.1.1. Pomegranate peel extract compound

This study used the active compound of PPE which was downloaded and prepared in 2 dimensions from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). The active compounds used in the PPE are flavonoid compounds with the highest levels including epigallocatachin gallate (CID 65064), ferulic acid (CID 445858), chlorogenic acid (CID 1794427), gallic acid (CID 370), caffeic acid (CID 689043), cianidanol (CID 9064), epicatechin (CID 72276), and punicalagin (CID 44584733). As a comparison, the researchers used 2 comparator compounds, namely the active compounds of COX non-selective inhibitor anti-inflammatory drugs, namely mefenamic acid (CID 4044) and arachidonic acid (CID 444899) as native ligands of COX enzyme. The target of this study is active conformation of protein COX-1 (PDB ID 6Y3C) and COX-2 (PDB ID 5IKR) which was downloaded and prepared for its 3-dimensional structure from the RCSB Protein Data Bank web page (https://www.rcsb.org/). Selection of COX-1 and COX-2 is based on enzyme sources isolated from humans, has a resolution of more than 2Å, and has the most favorable region value of 90% in the Ramachandran range which is effective for protein docking targets [21,22].

2.1.2. Research tools

This research uses a bioinformatics approach (in silico) so that it utilizes database pages and supporting applications that run the Python program. Each stage of the in silico test method uses different database pages and applications, including the Biovia and PyMol applications for preparation of test materials and visualization of docking results, the Way2Drug page (http://www.way2drug.com/PASSOnline) for predicting PASS, page Lipinski Rule of Five (http://www.scfbio-iitd.res.in/) for physicochemical tests, pkCSM website (https://biosig.lab.uq.edu.au) for predicting ADME and toxicity, Uniprot website (https://www.uniprot.org/) to identify active research target sites, as well as PyRx applications for molecular docking tests [21,23].

2.2. Methods

2.2.1. Preparation of ligand molecular structure and protein structure

The preparation of the test material begins with downloading the test compound and targets through the PubChem and RCSB PDB databases. Once downloaded, the test and target compounds are named according to the compound name because when downloaded they are in the form of CID numbers. After that, determine the active site of COX-1 (PDB ID 6Y3C) and COX-2 (PDB ID 5IKR) which plays an important role in cyclooxygenase activity via the Uniprot site. After entering the PDB ID code, the peptide chain sequence is obtained which must be isolated before docking. Next, prepare the target protein using the Biovia application to remove water molecules and native ligands which are also downloaded from the database. After preparation, isolation of the peptide chain which became the active site of COX-1 at 384th peptide chain and COX-2 at 371st peptide chain, was carried out using the PyMol application [24].

2.2.2. Prediction of activity spectra for substances test

Prediction of activity spectra for subtances or PASS prediction is carried out by entering the canonical SMILES or SMILES sequences of GA, EA, and Cat test compounds obtained from the PubChem website into the Way2Drug website to predict their bioactivity. After that, the Probable to Active (Pa) and Probable to Inactive (Pi) values will be obtained. Of the many bioactivity Pa and Pi values displayed, the Pa and Pi values used as a guide in determining anti-inflammatory opportunities are the Pa and Pi values of anti-inflammatory parameters. A test compound is said to be active for an activity if it has a Pi value <0.3 and Pa variations are categorized into 3 groups. A compound is categorized as having a high chance of bioactivity if it has a Pa value >0.7. If the Pa value of a compound is 0.3<Pa<0.7 it means that the compound is still in the active group having a certain bioactivity. However, if a compound has a Pa value <0.3 then the compound is predicted to have a very low chance of being active in a bioactivity [25,26].

2.2.3. Physicochemical test

Physicochemical tests were carried out by uploading the 2-dimensional conformations of the test compounds od PPE to the Lipinski Rule of Five page to determine their drug-likeness characteristics. The Lipinski test results contain 5 parameters including molecular mass, Log P, donor hydrogen bonds, acceptor hydrogen bonds, and molar refractivity. A compound is categorized as a drug-like compound if it fulfills 2 of the 5 Lipinski parameters such as molecular mass <500 dalton (Da), Log P <5, donor hydrogen bonds <5, acceptor hydrogen bonds <10, and molar refractivity in the range of 40-130 [27–29].

2.2.4. ADME prediction test

ADME or pharmacokinetic prediction is done with the help of the PkCSM website. This prediction is the same as the PASS prediction which utilizes the SMILES sequence or the canonical SMILES as a marker for the compound to be predicted. After the SMILES sequence is inputted into the column, the ADME prediction will take place. The prediction results displayed contain each parameter, namely administration, distribution, metabolism, and excretion. Because the research being conducted wanted to know the ability of the active compounds when distributed systemically, the ADME parameters used must also be specific. Some of them are for the administration aspect the water solubility parameters (in numeric) and skin permeability (in numerical) are taken, the distribution aspect uses the human fraction unbound parameter (in percentage), the metabolism aspect uses the CYP2D6 parameter of substrates and inhibitors, and the excretion aspect uses the total clearance parameter (in numeric). Each parameter has limitations so that it is said to be optimal. Reviewing the administration aspect, a compound is said to be water soluble/polar and able to penetrate the surface of the skin/mucosa if it has a yield of less than -2. In the distribution aspect, a compound is said to be able to be circulated if it has a human fraction unbound value >0%. In terms of metabolism, it is said that it does not interfere with the metabolism of other drugs and does not interact with other drugs if it has a negative value for CYP2D6, both substrates and inhibitors. In the aspect of excretion if it has an excretion value >0 per minute [30,31].

2.2.5. Toxicity prediction test

Toxicity tests are carried out with the help of the pkCSM website and or ToxTree. This prediction is the same as the PASS and ADME predictions which use the SMILES sequence or the canonical SMILES as a marker for the compound to be predicted. After the SMILES sequence is inputted into the column, the prediction of toxicity will take place. The prediction results displayed include each parameter, namely AMES toxicity, human maximum tolerated dose, lethal dose of 50, hepatotoxicity, and skin sensitization. Each parameter has its own requirements to be categorized as non-toxic. A compound is categorized as non-toxic if it is negative for AMES toxicity, hepatotoxicity and skin sensitization. The human maximum tolerated dose and lethal dose 50 parameters aim to determine the maximum dose that the body can accept in units of mg/KgBB. This value can be used as a database for determining dose in in vitro and in vivo tests [32,33].

2.2.6. Molecular docking test

The molecular docking test was carried out by uploading the test compound, comparator compound, and target protein to the PyRx application. After uploading, run the PyRx program to find out the results of binding affinity in units of kcal/mol, mode, RMSD lower bound and upper bound. A compound is said to have a tendency to form bonds with the target protein if it has a low binding affinity value. The lower the binding affinity value of a compound, the lower the energy needed to form bonds. As a result, a compound will have a high tendency to form bonds with the target protein. The mode parameter reflects the variation of the bonds formed. The RMSD parameter shows the level of accuracy and precision of the resulting predictions. In general, compounds will have a low binding affinity value and are optimum in the RMSD lower bound and zero upper bound modes [19,22,34,35]. After finishing the docking predicting test, the next step is visualization to confirm the enzymatic reaction position against the active site. Visualization of the docking results was carried out to profile the location, type, and number of bonds formed between the test compound and the target protein. The visualization process utilizes the PyMol and Biovia applications. Visualization is done by uploading the docked conformation that is inserted into the target protein. After that it can be known the location, type, and number of bonds formed [36].

3. Results

3.1. Prediction of activity spectra for substances test result

The PASS test results shown that the epigallocatechin gallate compound had a chance to be active as an anti-inflammatory is 0.623 with a chance of being inactive to become an anti-inflammatory is 0.027. The next data regarding the ferulic acid compound has a chance to be active as an anti-inflammatory is 0.604 with a chance of being inactive to become an anti-inflammatory is 0.031. The next data regarding the chlorogenic acid compound has a chance to be active as an anti-inflammatory is 0.598 with a chance of being inactive to become an anti-inflammatory is 0.032. The next data regarding the gallic acid compound has a chance to be active as an anti-inflammatory is 0.548 with a chance of being inactive to become an anti-inflammatory is 0.044. The next data regarding the caffeic acid compound has a chance to be active as an anti-inflammatory is 0.651 with a chance of being inactive to become an anti-inflammatory is 0.023. The next data regarding the cianidanol compound has a chance to be active as an anti-inflammatory is 0.548 with a chance of being inactive to become an anti-inflammatory is 0.044. The next data regarding the epicatechin compound has a chance to be active as an anti-inflammatory is 0.548 with a chance of being inactive to become an anti-inflammatory is

0.044. The next data regarding the punicalagin compound has a chance to be active as an anti-inflammatory is 0.983 with a chance of being inactive to become an anti-inflammatory is 0.004. The latest data regarding the mefenamic acid compound has a chance to be active as an anti-inflammatory is 0.644 with a chance of being inactive to become an anti-inflammatory is 0.024.

Table 1 Predicition of activity spectra for subtances test result of PPE active compound with control drug mefenamic acid.

Compound	PASS score anti-inflammation aspect	
	Pa Score	Pi Score
Epigallocatechin gallate	0.623	0.027
Ferulic acid	0.604	0.031
Chlorogenic acid	0.598	0.032
Gallic acid	0.548	0.044
Caffeic acid	0.651	0.023
Cianidanol	0.548	0.044
Epicatechin	0.548	0.044
Punicalagin	0.893	0.004
Mefenamic acid	0.644	0.024

3.2. Physicochemical test result

Table 2 Physicochemical test result of PPE active compound using Lipinski rule of five (RO5).

Compound	Molecular Mass	Hydrogen Bond Donor	Hydrogen Bond Acceptor	Log P	Molar Refractivity	Druglike-ness
Standard	**≤500 Da**	**≤5**	**≤10**	**≤5**	**40-130**	**+**
Epigallocatechin gallate	332	5	10	0.837	67.392	+
Ferulic acid	194	2	4	0.556	45.351	+
Chlorogenic acid	354	6*	9	1.286	78.631	+
Gallic acid	170	4	5	-0.189	32.578	+
Caffeic acid	180	3	4	0.031	40.737	+
Cianidanol	290	5	6	1.093	68.131	+
Epicatechin	290	5	6	1.093	68.131	+
Punicalagin	312	5	6	-0.053	77.146	+

*Value not inquire lipinski rule of five (RO5)

The physicochemical test results showed that all compounds fulfill at least 2 parameters Lipinski's requirements because they had a molecular mass less or equal 500 Da, hydrogen bond donor less or equal to 5, hydrogen bond acceptor less or equal to 10, log P less or equal to 5, and molar refractivity between 40-130.

3.3. ADME prediction result

Table 3 ADME prediction test result of PPE active compound.

Compound	Water Solubility	Log MW	Human Fraction Unbound (%)	CYP2D6 Substrate & Inhibitor	Total Clearance (ml/min/kg)
Epigallocatechin gallate	-2.193	2.521	83.2	Negative	67.392
Ferulic acid	-2.817	2.288	34.3	Negative	45.351
Chlorogenic acid	-2.449	2.549	65.8	Negative	78.631
Gallic acid	-2.560	2.230	61.7	Negative	32.578
Caffeic acid	-2.330	2.255	52.9	Negative	40.737
Cianidanol	-3.117	2.462	23.5	Negative	68.131
Epicatechin	-3.117	2.462	23.5	Negative	68.131
Punicalagin	-2.998	2.494	37.4	Negative	77.146

The results of ADME predictions show the results of the parameters of each aspect consisting of administration, distribution, metabolism, and excretion. Each compound predicited can soluble into water that construct the major composition of body because have water solubility less than -2 and have a small structure of molecules because has value of molecular weight logaritmic less than 3. As a drug that consumed by patients and will distribute inside the body, all of pomegranate active compound has potential to distribute actively inside of blood stream and confirmed bu score of human fraction unbound more than 0. 8 of 8 active compounds also predicted can't alteration the enzyme of CYP2D6 that play main role in xenobiotics metabolism. All of drugs that insert over the body must have potential and ability to excreted out from the body. All of active compound in pomegranate peel predicted can be excreted by urinary tract because has more than 0 value of renal secretion in milimeter.

3.4. Toxicity test result

Table 4 Toxicity test result of PPE active compound.

Compound	AMES Toxicity	Hepatotoxicity	Human Maximum Tolerated Dose (mg/Kg BW/day)	Skin Sensitization	LOAEL Chronic Toxicity (mol/Kg MW/day)
Epigallocatechin gallate	Negative	Negative	5.105	Negative	3.987
Ferulic acid	Negative	Negative	12.078	Negative	2.065
Chlorogenic acid	Negative	Negative	0.735	Negative	2.982
Gallic acid	Negative	Negative	5.012	Negative	3.060
Caffeic acid	Negative	Negative	13.964	Negative	2.092
Cianidanol	Negative	Negative	2.742	Negative	2.500
Epicatechin	Negative	Negative	2.742	Negative	2.500
Punicalagin	Negative	Negative	2.742	Negative	9.877

The toxicity test results show parameter data that will determine whether pomegranate peel extract compounds have toxic abilities when distributed systemically and whether they are systemic toxic. All of compounds are negative for AMES toxicity, so they are considered not to cause genetic mutations, have a maximum dose threshold of more than 0, lethal dose, and do not cause mucosal / skin irritation because they are negative for skin sensitization.

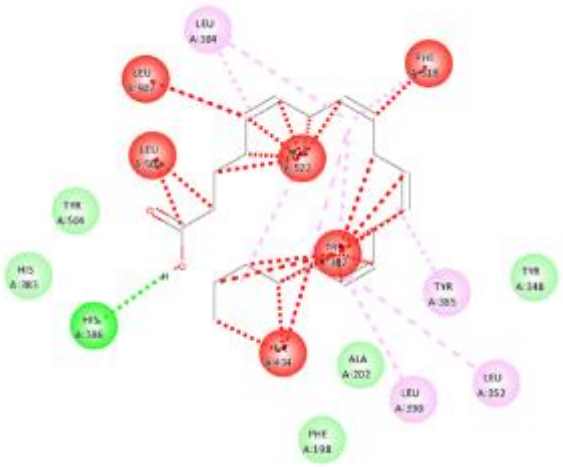
3.5. Molecular docking test result

Table 5 Molecular docking test result of PPE active compound with comparative compound of arachidonic acid and mefenamic acid.

Compound	Binding Affinity (kcal/mol)		Mode
	COX-1 PDB ID 6Y3C	COX-2 PDB ID 5IKR	
Arachidonic acid	-2.6	-3.9	0
Mefenamic acid	-3.0	-4.9	0
Epigallocatechin gallate	-2.9	-4.0	0
Ferulic acid	-3.0	-4.9	0
Chlorogenic acid	-3.2	-4.5	0
Gallic acid	-3.3	-4.5	0
Caffeic acid	-1.7	-5.0	0
Cianidanol	-2.7	-3.8	0
Epicatechin	-2.8	-3.4	0
Punicalagin	-4,0	-9.1	0

The results of the molecular docking test determine the ability and anti-inflammatory potential of pomegranate peel extract compounds reviewed through their ability to inhibit COX-2 compared to comparison compounds. GA compounds in mode and RMSD 0 have binding affinity values of -3.4 kcal / mol, this value is not lower than celecoxib but lower than arachidonic acid. Cat compounds in mode and RMSD 0 have binding affinity values of -4.9 kcal/mol, this value is lower than celecoxib and arachidonic acid. EA compounds in mode and RMSD 0 have binding affinity values of -4.6 kcal / mol, this value is not lower than celecoxib but lower than arachidonic acid.

3.6. Visualization result



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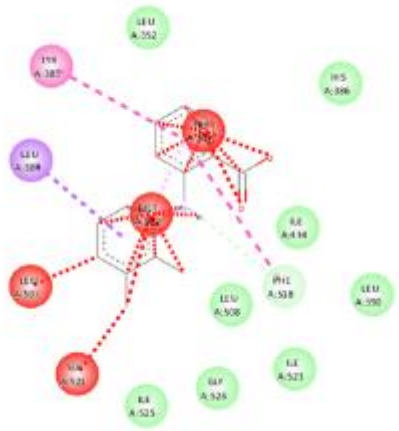

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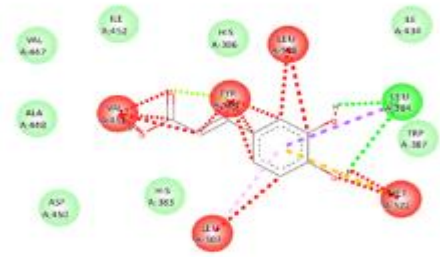

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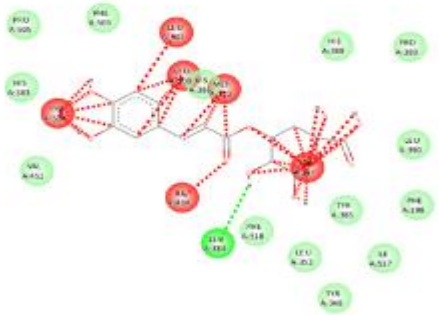

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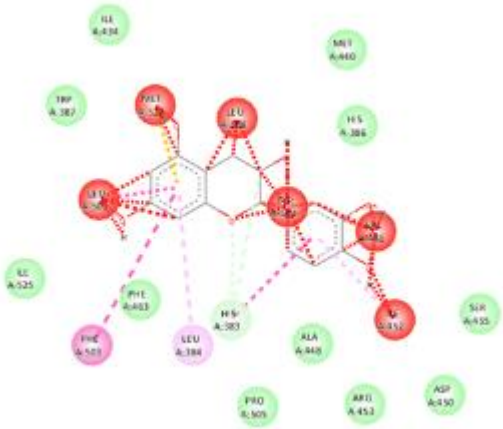

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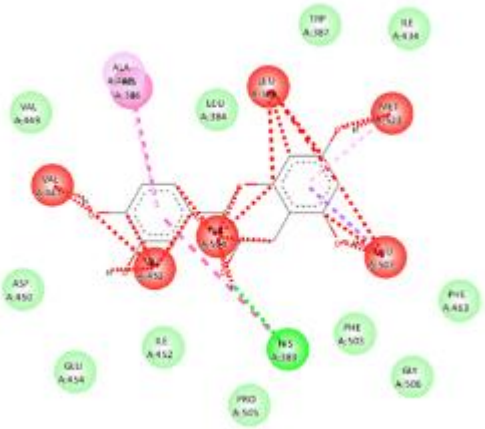

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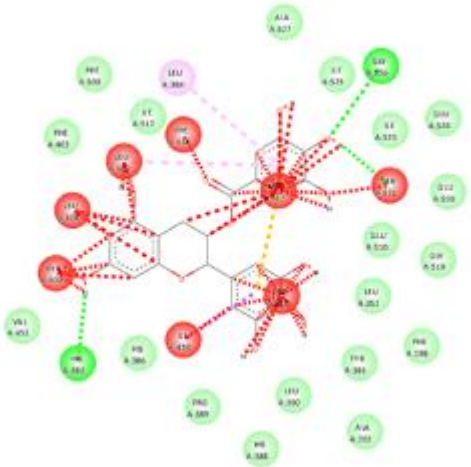

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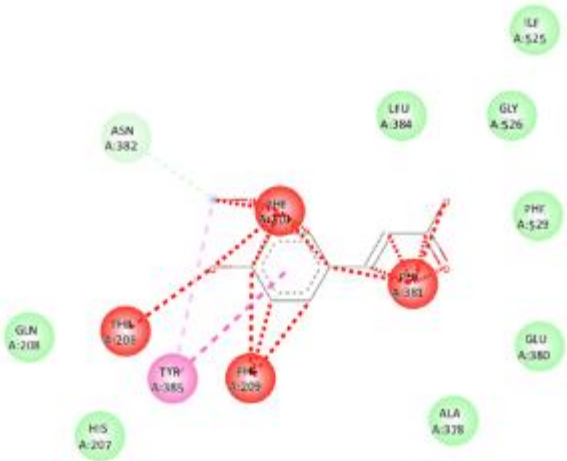

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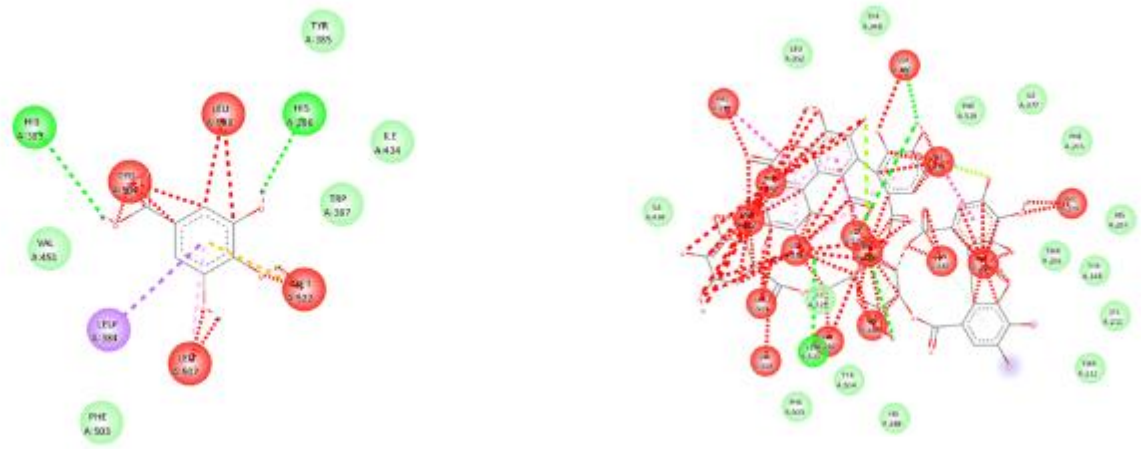

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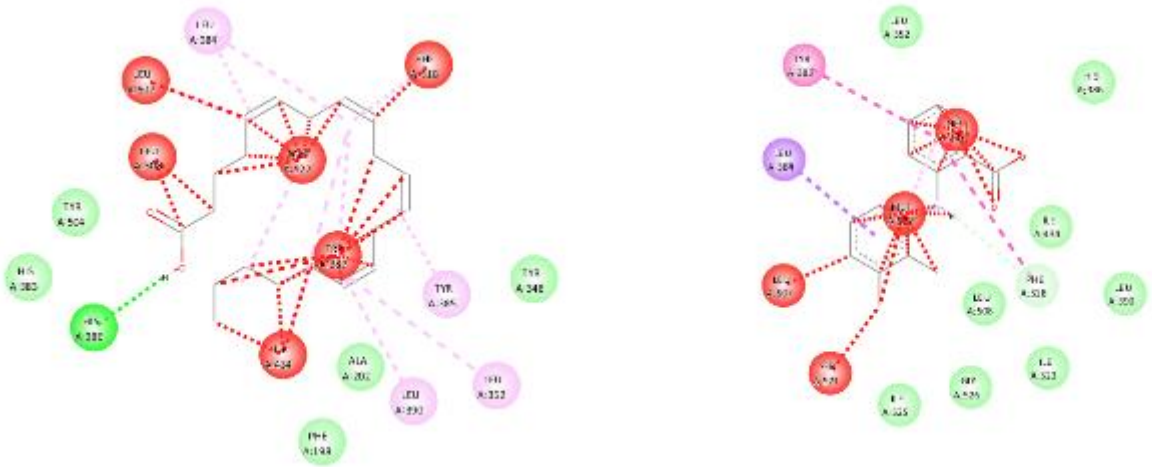
Figure 1 Visualization of arachidonic acid (a), and mefenamic acid (b), caffeic acid (c), chlorogenic acid (d), cianidanol (e), epicatechin (f), epigallocatechin gallate (g), ferulic acid (h), gallic acid (i), and punicalagin (j) against COX-1 enzyme (PDB ID 6Y3C). Different colors and lines indicate the formation of the type of bond between the test compound and the enzyme peptide. Light green indicates the formation of a pi-donor hydrogen bond, dark green indicates the formation of a van der Waals bond, red indicates the formation of an unfavorable bump bond, dark purple indicates the formation of a pi-sigma bond, light purple indicates the formation of a pi-pi bond, yellow indicates the formation of a pi-donor hydrogen bond. formation of pi-sulfur bonds, and pink indicates the formation of pi-alkyl bonds.



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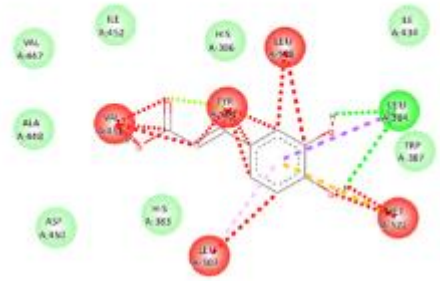

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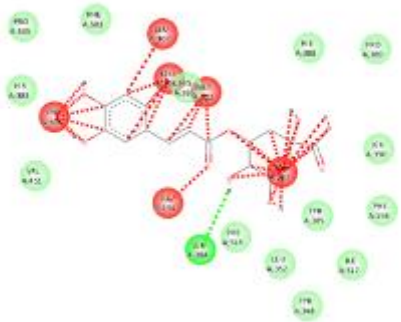

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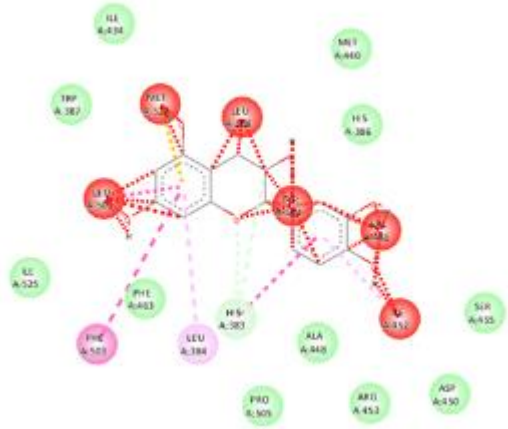

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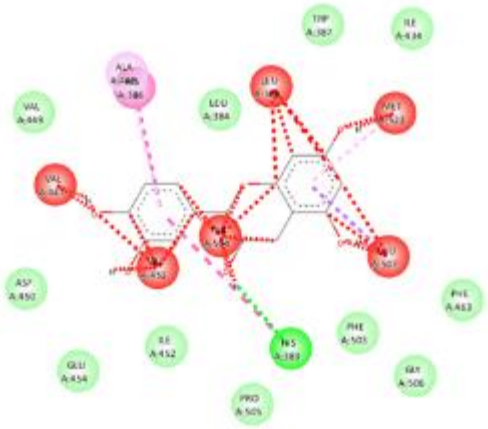

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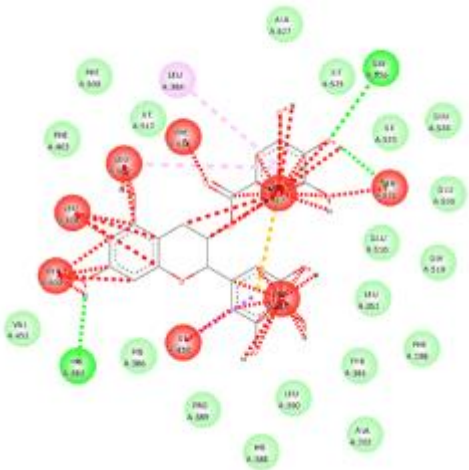

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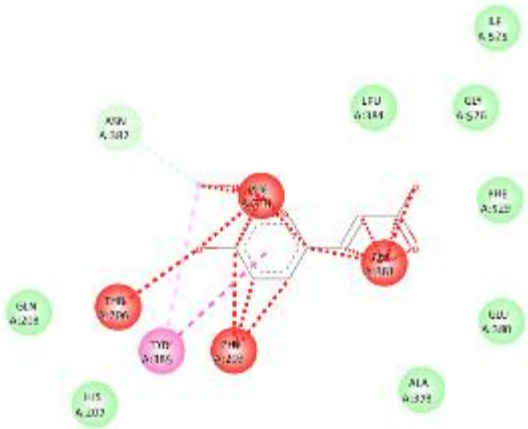

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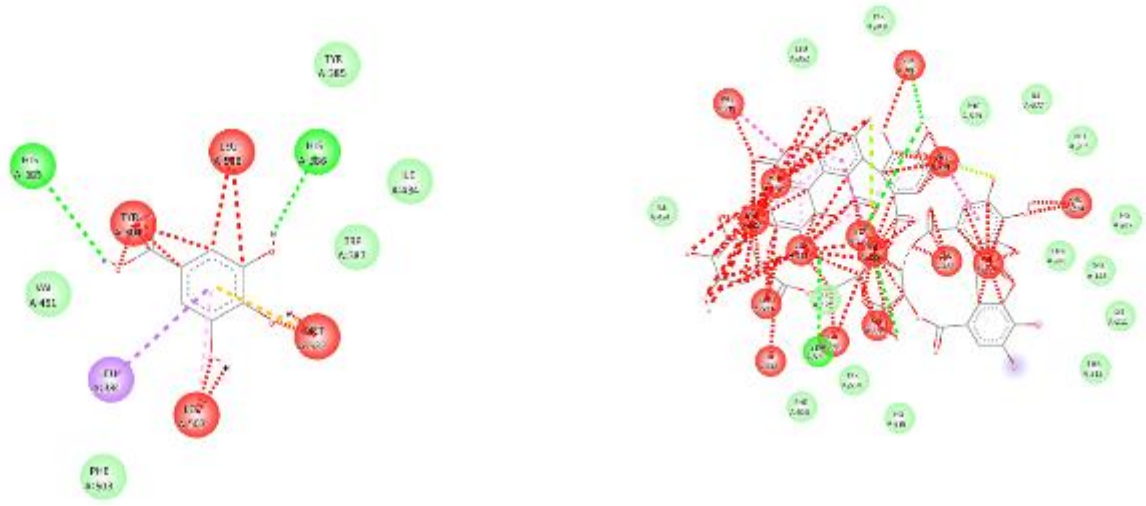

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Figure 2 Visualization of arachidonic acid (a), and mefenamic acid (b), caffeic acid (c), chlorogenic acid (d), cianidanol (e), epicatechin (f), epigallocatechin gallate (g), ferulic acid (h), gallic acid (i), and punicalagin (j) against COX-2 enzyme (PDB ID 5IKR). Different colors and lines indicate the formation of the type of bond between the test compound and the enzyme peptide. Light green indicates the formation of a pi-donor hydrogen bond, dark green indicates the formation of a van der Waals bond, red indicates the formation of an unfavorable bump bond, dark purple indicates the formation of a pi-sigma bond, light purple indicates the formation of a pi-pi bond, yellow indicates the formation of a pi-donor hydrogen bond. formation of pi-sulfur bonds, and pink indicates the formation of pi-alkyl bonds.

The visualization results of molecular docking tests between the test compound and the comparison compound against the target protein showed identical bond locations. This is indicated by the location of the compound binding to peptides in the COX-1 (Figure 1) and COX-2 (Figure 2) enzyme. This result will listed in next table of peptide binding. Table 6 will confirm the result of binding location of each compound in pomegranate peel extract and comparation compound in figure 2.

Table 6 Molecular docking test result of pomegranate active compound with comparative compound of arachidonic acid and mefenamic acid.

Protein Target	Compound	Binding Location (Peptide)
COX-1 (PDB ID 6Y3C)	Arachidonic acid	HIS 386, HIS 383, TYR 504, LEU 508, LEU 507, **LEU 384**, MET 522, PHE 518, TRP 387, TYR 385, TYR 384, ILE 434, ALA 202, LEU 390, LEU 352, PHE 198
	Mefenamic acid	TYR 385, LEU 352, HIS 386, ILE 434, LEU 390, PHE 518, LEU 508, ILE 523, GLY 526, ILE 525, SER 521, LEU 507, **LEU 384**, MET 522, THP 387
	Epigallocatechin gallate	VAL 447, ILE 452, HIS 386, LEU 508, ILE 434, **LEU 384**, TRP 387, MET 522, LEU 507, HIS 383, ASP 450, ALA 448, TYR 504, VAL 451
	Ferulic acid	ILE 525, GLY 526, **LEU 384**, PHE 529, PHE 381, PHE 210, GLU 380, ALA 378, PHE 209, TYR 385, ASN 382, THR 206, GLN 208, HIS 207
	Chlorogenic acid	PRO 505, PHE 503, HIS 383, LEU 507, LEU 508, TYR 504, VAL 451, HIS 386, MET 522, ILE 434, **LEU 384**, PHE 518, TRP 387, HIS 388, TYR 385, LEU 352, TYR 348, ILE 517, PHE 198, LEU 390, PRO 389
	Gallic acid	TYR 385, ILE 434, HIS 386, LEU 508, HIS 383, TYR 504, TRP 387, MET 522, VAL 451, **LEU 384**, LEU 507, PHE 503
	Caffeic acid	VAL 447, ILE 452, HIS 386, LEU 508, ILE 434, **LEU 384**, TYR 504, VAL 451, ALA 448, ASP 450, HIS 383, LEU 507, MET 522, TRP 387

	Cianidanol	ILE 434, MET 440, HIS 386, LEU 508, MET 522, TRP 387, LEU 507, TYR 504, VAL 451, SER 455, ILE 452, ALA 448, HIS 383, **LEU 384**, PHE 463, PHE 503, ILE 525, PRO 505, ARG 453, ASP 450
	Epicatechin	ILE 434, TRP 387, LEU 508, LEU 384, **LEU 384**, HIS 386, ALA 448, VAL 449, MET 522, VAL 447, VAL 451, TYR 504, LEU 507, PHE 463, PHE 503, HIS 383, ILE 452, GLU 454, ASP 450, PRO 505, GLY 506
	Punicalagin	TYR 348, LEU 352, SER 530, PHE 529, ILE 377, PHE 519, TRP 387, PHE 331, PHE 205, ILE 434, MET 522, **LEU 384**, GLY 525, TYR 385, ASN 382, PHE 210, THR 206, PHE 209, HIS 207, TYR 148, LYS 211, THR 212, HIS 388, TYR 504, HIS 385, HIS 383, ILE 525, HIS 383, SER 521, PHE 503, LEU 508, LEU 507
COX-2 (PDB ID 5IKR)	Arachidonic acid	LEU 531, LEU 534, CYS 540, **PHE 371**, ASN 537, MET 535, GLY 533, TYR 373, GLY 536, LEU 386, ASN 375, LYS 532, GLN 374, THR 118, SER 114, LEU 117, LYS 369, LEU 365, TYR 115 GLN 372, PRO 363
	Mefenamic acid	PRO 127, SER 121, **PHE 371**, ASP 125, SER 126, GLN 372, GLN 374, ILE 124, LYS 532, GLY 536, TYR 373, PRO 528, PHE 529, GLY 533, ASN 375, MET 535, ASN 537, LEU 531, LEU 534
	Epigallocatechin gallate	PRO 528, ASN 375, MET 535 TYR 373, GLY 533, GLY 536, GLN 372, GLN 374, PRO 128, ARG 376, SER 121, THR 118, ASP 125, GLN 370, LYS 369, PRO 127, LYS 532, SER 126, HIS 122, ILE 124, **PHE 371**
	Ferulic acid	PHE 367, LEU 366, MET 535, ASN 368, LYS 369, GLN 370, TYR 373, GLY 536, LEU 534, ASN 537, LEU 531, **PHE 371**, CYS 540, LEU 117
	Chlorogenic acid	LEU 366, MET 535, CYS 540, ASN 537, GLY 536, LYS 532, **PHE 371**, LEU 117, GLN 374, LYS 369, THR 118, LEU 534, TYR 373, GLN 372, LEU 531, ASN 375, GLY 533, PHE 361, PHE 367, ILE 341, SER 541, VAL 538, PRO 127, ILE 539
	Gallic acid	SER 114, LEU 365, LYS 369, THR 118, ASN 368, PHE 367, GLN 370, LEU 366, TYR 373, **PHE 371**, MET 535, LEU 117, CYS 540
	Caffeic acid	TYR 373, HIS 122, GLY 536, GLN 370, GLN 372, SER 126, GLN 374, LYS 532, **PHE 371**, SER 121, ILE 124, ASN 375
	Cianidanol	GLN 370, **PHE 371**, SER 121, ILE 124, ASP 125, HIS 122, LYS 532, ASN 375, GLN 372, SER 126, PRO 128, PRO 542, GLN 374, PRO 127, ARG 376
	Epicatechin	ARG 376, GL 374, PRO 127, GLN 372, PRO 542, PRO 128, SER 126, HIS 122, ASN 375, LYS 532, SER 121, GLN 370, ASP 125, ILE 124, **PHE 371**
	Punicalagin	PHE 367, ARG 61, LYS 546, TRP 545, TYR 373, CYS 540, GLN 370, LYS 137, THR 129, PRO 542, ALA 543, SER 541, TYR 544, VAL 538, GLN 372, **PHE 371**, SER 126, PRO 127, THR 118, SER 121, GLN 374, SER 119, LEU 117, LYYS 512, ASP 125, PRO 128, PHE 142, VAL 116, ARG 120, PRO 528, LEU 531, ASN 375, GLY 536, GLY 533, HIS 122, ILE 124, LEU 123, THR 149, ARG 376,

4. Discussion

To knowing the potential of pomegranate peel extract as anti-inflammatory agent, it is necessary to conduct two main analyses of correlated potential. The potential to be analyzed includes the ability of the active compounds of pomegranate peel extract to be applied systemically and the anti-inflammatory ability of the active compounds of pomegranate peel extract in inhibiting COX-1 and COX-2. The ability of a drug to be distributed systemically must meet several aspects including being able to be absorbed by the surface that will deliver the drug, drug compounds must be designed so that the size of the molecule is able to penetrate and diffuse passively into the layers of the body. Reviewed in silico, this can utilize several tests including physicochemical tests, specific ADME predictions to map pomegranate peel extract compounds so that they can be absorbed, and toxicity tests that also specifically predict the sensitization of pomegranate peel extract compounds when applied systemically [37,38].

The ability of the active compounds of pomegranate peel extract to be applied systemically can be reviewed from the results of physicochemical test prediction, ADME prediction, and toxicity tests. Physicochemical tests (Table 2) can classify an active compound as drug-like or non-drug-like. The active compound of pomegranate peel extract has a molecular mass of less than or equal to 500 dalton. This shows that all eight active compounds pass the mass parameter of the Lipinski molecule. This can predict that all compounds are able to penetrate the somatic cell membrane because the molecular size is not too complex / large comparing to membrane cell of somatic cell. Reviewing the next parameters, the active compound of pomegranate peel extract has donor and acceptor hydrogen bonds that are respectively less than equal to 5 and 10. The value indicates that the active compound of pomegranate peel extract has a good ability to be distributed by passive diffusion to the target [27–29]. The fourth parameter is log P. This parameter reflects that compounds less than 5 have good polarity properties so that they can be flowed into blood plasma and classified as polar drug/ soluble compound in water solution. This parameter is urgent to detected at in silico test because the main component of whole body is water especially in blood plasm. The active compound of pomegranate peel extract has Log P values of less than 5. This value reflects that the active compound of pomegranate peel extract has good polar properties so that it can dissolve and be circulated throughout the body through blood vessels and delivered inside the somatic cell. The last physicochemical parameter is molar refractivity. This parameter reflects that compounds that have values between 40-130 are able to maintain the position and location of the bond with the target protein. The active compound of pomegranate peel extract is able to maintain the position and location of the bond with the target protein because it has a molar refractivity value between 40-130. This may indicate that all compounds are able to maintain the strength and binding position of the target protein. Of the five physicochemical test parameters, it can be categorized that all active compounds of pomegranate peel extract have drug-like characteristics because they meet at least 2 predetermined parameters [27,35,39].

After predicted the drug-likeness characteristic of the active compound of pomegranate peel extract, we should know the ADME aspect of it. ADME prediction plays an important role in determining whether the active compounds of pomegranate peel extract can be applied systemically (Table 3). From this predicting test can be analyzed 4 main aspects containing aspects of administration, distribution, metabolism, and excretion. Looking from the administrative aspect, the active compound of pomegranate peel extract has water solubility values of less than -2. A compound will be able to dissolve in water if it has a water solubility value lower than -2. From the results of the administrative aspect, the active compound of pomegranate peel extract is able to dissolve in water solvents so that it can be circulated throughout the body where the majority is composed of water and is easy to distribute. The next parameter is skin permeability. This parameter can indicate that a compound can be distributed topically through the skin. However, the mucosal structure in the oral cavity has a structure that is not the same as the histological structure of adnexal skin in general and more complex than the skin structure. In addition to its different histological structure, the density of the diameter of the pores of the oral mucosa (Log K_{ow} <0.5) has the narrowest pore diameter value compared to mucosal pores (Log K_{ow} ~0.5) or skin in general (Log K_{ow} ~0.7-0.8). Therefore, a compound that can be predicted to diffuse passively to enter through the oral mucosa is a compound with a value of MW <300 Da. Compounds that have MW <300 Da (Log MW <3) have excellent hydrophilic properties and have an optimal Log P value of less than 5. The pomegranate peel extract compound has MW log values less than 3. This value is categorized as still included in the threshold range of MW log values, a compound that is able to penetrate the mucous membrane of the oral cavity or through digestive membrane [30,40].

The next parameter is human fraction unbound or known as HFU. These parameters reflect compounds that can circulate in blood plasma freely and can be distributed towards destination tissues and produce therapeutics. Generally, all drug compounds consumed by humans will be bound by plasma proteins in the blood which will later form an inactive formation called drug plasma binding protein. This HFU value is the mathematical result of the ratio between the active/unbound fraction of plasma proteins with the total number of doses consumed in the range of 0 to 1. The greater the HFU value or the closer to 1, the more drug fractions that are active and can be distributed to the target so that they can cause pharmacological and therapeutic effects. From the results of pharmacokinetic analysis of HFU known that all of pomegranate peel extract has active formation more than 23% in range 23.5%-83.2% partitions are predicted to be active compounds and able to cause pharmacological responses [41–44]. The next parameter is CYP2D6. Normally, the body will metabolize drugs that enter the body through xenobiotic metabolic mechanisms. Xenobiotic metabolism is played by cytochrome P450 enzymes. In bioinformatic database, this cytochrome enzyme is replaced by CYP2D6. Ensuring that a test active compound has a negative value for the substrate or inhibitor against CYP2D6 is to avoid interactions between drugs and reduce the possibility that the drug will not be converted to inactive and excreted by the body. All of pomegranate peel extract compounds have negative values against CYP2D6 which means that these active compounds are predicted not to interfere with xenobiotic metabolism and interact with other drugs. The last parameter is excretion. In addition, through prediction pkCSM is also able to predict the rate of excretion of active compounds absorbed by the body. This parameter aims to determine the rate of excretion while ensuring that the active compounds consumed are not trapped in the body. Based on data obtained from the database, gallic acid, catechin, and

ellagic acid compounds will be excreted from the body through urine with successive excretion rates more than 0 ml/minute [41,45,46].

As similar as another drug, the existing herbal medicine candidates must confirmed that their ability is not giving a toxic effect against body. These concern can confirmed by do the toxicity predicting at in silico test. From the results of pkCSM analysis regarding toxicity aspects (Table 4), it was found that the three active compounds of pomegranate peel extract gave negative results for AMES toxicity. AMES toxicity is a state of cellular mutation caused by the activity of chemicals in the body. If a compound is said to be positive for AMES toxicity, then the compound when consumed and in the body will be a mutation factor and trigger mutations [47]. Similar with another drugs, the drug that has herbal medicine-based must been converted into a inactive form before it excreted from the body. Liver is one of several organs that play main role as xenobiotic metabolism against the drugs. We expected that the herbal medicine drugs that entered the body is not give a toxic activity against hepatic cell, and it will confirmed through hepatotoxicity parameter. All of pomegranate peel extract compound have a negative value of hepatotoxicity. From these result it shown that all compound doesn't give a toxic activity against the hepatic cell when metabolism at the liver. The next parameter is the human maximum tolerated dose. Dose is the quantity of a compound that researchers use to become a drug and will eventually be consumed by humans. The benefit of knowing the dosage threshold is to determine the therapeutic dose of a drug compound so that it does not become a toxic dose for the body. All of pomegranate peel extract compounds showed maximum tolerated dose values of more than 0 mg/Kg BW per day. From this information it will be able to be the basis for determining therapeutic doses, drug use doses, and lethal doses before in vitro and in vivo analysis [48]. As linear with our research purposes to developing and know the potential of pomegranate peel extract as anti-inflammatory drug candidate, we also analyze the predicting of skin sensitization of each compound. This parameter is urgent because we don't want when pomegranate peel extract applied on the oral mucosa will gave the effect of irritation like dermatitis contact allergic. All of pomegranate peel extract have negative result of skin sensitization. It shown that all compound not irritating oral mucosa when applied on it [49].

After we predicting all of part in distribution aspect, we will discussing about the ability of pomegranate peel extract as anti-inflammatory agent. First of all we need to predicting the probability of each compound active as anti-inflammatory drugs biocomputationally by PASS predicting. PASS software allows estimating the probability profile of biological activity based on the structural formula of drug-like organic compounds. Estimates are based on analysis of structure-activity relationships for training sequences of compounds with known biological activity. Biological activity is qualitatively considered as active or inactive in the PASS program. The PASS algorithm models the classification of structure-activity relationships based on training sets with known biological structures and activities of known pharmaceutical agents. The results of the PASS prediction are presented as a ranking list of various biological activities with calculated probabilities Pa ("becoming active") and Pi ("becoming inactive") [25,26].

The values of Pa and Pi vary between 0.000 and 1.000. A compound is considered experimentally active with Pa>Pi. A Pa>0.7 value reflects that a test compound has a very high chance of being active against a biological activity. A value of 0.5<Pa<0.7 reflects a good probability of experimental pharmacological action. If the value of Pa<0.3, the chance of finding activity experimentally is smaller, but may indicate the chance of finding a new compound. Based on PASS prediction result (Table 1), from that result shown that 1 from 8 compound has very high chance active as anti-inflammatory activity by punicalagin because it has Pa value more than 0.7 (0.893). 7 of 8 compound of pomegranate peel extract predicted has good probability active as anti-inflammatory agent because it has Pa score more than 0.5 but still less than 0.7 and has Pi value less than 0.3. This prediction is confirmed by our comparation drugs (mefenamic acid). We know that mefenamic acid is one of anti-inflammatory drugs non steroid classes (NSAIDs). The ability of mefenamic acid actively as anti-inflammatory drug is confirmed by Pa value more than 0.5 and categorized as good probability active as anti-inflammatory drugs. This shows that major active compounds in pomegranate peel extract have the potential to be active as anti-inflammatory compounds with the greatest chance of active compounds equal or better than mefenamic acid [26,50].

The result of PASS prediction is confirming by molecular docking test. Based on the results of molecular docking tests results by inhibiting COX-1 and COX-2 enzyme (Table 5). One of cardinal symptomp of inflammatory conditions is pain. Pain is one of discomfort sensation that regulated by the body to shown there is a tissue injury and tissue destruction caused of pathological condition. Pathogenesis of pain played role by one of inducible enzyme namely COX-1. Several drug can used for inhibit the COX-1 activity to convert arachidonic acid to prostaglandin E2 (PGE2). This study shown that main active compound of pomegranate peel extract has ability to inhibit COX-1 activity. This statement is approved by the result of several main compound has binding affinity equal or lower than the comparation compound (arachidonic acid and mefenamic acid). That several compound is ferulic acid has binding affinity -3.0 kcal/mol, chlorogenic acid -3.2 kcal/mol, gallic acid -3.3 kcal/mol, and punicalagin -4.0 kcal/mol. 4 of 8 compounds mentioned before has binding affinity equal and also lower than arachidonic acid and mefenamic acid that indicates that compound

are able to precede arachidonic acid in forming bonds with COX-1 than mefenamic acid and arachidonic acid. The other 4 compounds has higher binding affinity than arachidonic acid and/or mefenamic acid. Epigallocatechin gallate has binding affinity of -2.9 kcal/mol, cianidanol -2.7 kcal/mol, epicatechin -2.8 kcal/mol. This value is shown that 3 compounds mentioned before is has binding ability to inhibit COX-1 better than arachidonic acid but not good ass mefenamic acid. The term "better" have meaning of that compounds will make a binding formation as soon as possible when it compared to arachidonic acid. So when the active site of COX-1 is inhibited by that compound, arachidonic acid can't to bind and can't to convert to PGE2 formation. The lowest activity to inhibit COX-1 is caffeic acid. Caffeic acid has binding affinity value of -1.7 kcal/mol. This value shown that caffeic acid can't preceded to make an inhibition form at active site of COX-1 than arachidonic acid and mefenamic acid. This phenomenon is causing by higher number of binding affinity value. The binding affinity value is predicting value of energy that used when the protein make a binding formation with the micro molecule/ligands. The body will choose the reaction way with the lower energy needed to prevent over-used ATP.

In addition to the COX-1 enzyme, there is another cyclooxygenase enzyme that plays a role in the inflammatory process, namely the COX-2 enzyme. COX-2 enzyme is one of the enzymes that plays an important role in the production of PGE2 in inflammatory processes that trigger inflammatory cascades such as immunocompetent cell recruitment, oedema, and trigger the production of pro-inflammatory cytokines. Similar to the COX-1 enzyme, this enzyme works by converting arachidonic acid compounds into PGE2. The inhibitory ability possessed by pomegranate peel extract is also shown by the binding affinity value of each active compound against the COX-2 enzyme which will be compared with arachidonic acid and mefenamic acid compounds. The results showed that there were 3 out of 8 dominant compounds of pomegranate peel extract that had the ability to inhibit the active site of COX-2 better than the two comparison compounds. These compounds are ferulic acid compounds with binding affinity values of -4.9 kcal/mol, caffeic acid -5.0 kcal/mol, and punicalagin -9.1 kcal/mol. This value reflects that the energy required to form a bond between the ligand and the active site of the COX-2 protein is likely to be very low compared to the energy required by the COX-2 enzyme to form a bond with arachidonic acid and mefenamic acid compounds. So that the three compounds will be able to form an inhibitory conformation with the active site of COX-2. However, other compounds turned out to have lower anti-inflammatory abilities compared to the anti-inflammatory abilities of mefenamic acid but still have anti-inflammatory abilities because they are better than arachidonic acid control. The compound is epigallocatechin gallate with binding affinity value of -4.0 kcal/mol, chlorogenic acid -4.5 kcal/mol, and gallic acid -4.5 kcal/mol. Based on their binding affinity value, the three compounds showed that they still have anti-inflammatory ability through COX-2 active site inhibition because they have the ability to form barriers ahead of the native ligand compound arachidonic acid, but their anti-inflammatory ability is not better than the gold standard compound of mefenamic acid. However, there are 2 compounds out of 8 predominant compounds of pomegranate peel extract have poor inhibitory ability compared to the two comparison compounds. The two compounds are cianidanol with binding affinity -3.8 kcal/mol and epicatechin -3.4 kcal/mol. The higher binding affinity value compared to the comparison compounds arachidonic acid and mefenamic acid makes the two compounds mentioned earlier unable to precede mefenamic acid and arachidonic acid in inhibiting the active site of COX-2. So that makes both compounds have a low tendency to inhibit and are predicted to be unable to provide anti-inflammatory or weak effects. In addition to reviewing affinity values, RMSD values also need to be considered. The lower bound and upper bound RMSD values reflect that the test compounds that are predicted in silico are close to the data to be obtained in the laboratory and the accuracy is higher if these two RMSD values are close to 0. Both mefenamic acid, arachidonic acid, and all pomegranate peel extract compounds have lower and upper bound RMSD values of 0 indicating that their accuracy of docking prediction is high and similar with laboratory result [19,22,34,51–53].

Based on the results of molecular docking test visualization, all of pomegranate peel extract have similar molecular activity with comparison compounds because of the location of the identical binding to peptides in the COX-1 and COX-2 enzyme so that it has good anti-inflammatory abilities (Table 6). Regarding in COX-1 enzyme active site, the test and comparison compounds/ligand have the same bond location in LEU 384 (Leucine 384). This prediction is accuracy because regarding to the biological databases of active site of COX-1 protein is on peptide 384th. All of binding predicting in COX-1 enzyme on point on their active site. In line with the prediction results for COX-1, molecular docking results from the active compound pomegranate peel extract and comparison compounds against the COX-2 enzyme also showed identical binding locations in peptide PHE 371 (Phenylalanine 371). This is in line with a biological database that maps that the active site location of the COX-2 PDB ID 5IKR enzyme is at peptide 371. This also indicates that all compounds above previously have the correct binding location at the active site. Inhibition of the active sites of both enzymes that play an important role in the inflammatory process will lead to a decrease in the bioconversion process of arachidonic acid into PGE2. As mentioned earlier, PGE2 is one of the most powerful pro-inflammatory mediators in the process of metabolizing immunocompetent cells. With a decrease in PGE2 levels in the inflamed tissue, the prolonged (chronic) inflammatory process will be stopped immediately to prevent unexpected excess damage to normal tissues. Through this research, it was able to show that herbal medicine made from pomegranate peel extract

has a good prediction of its ability to trigger anti-inflammatory activity as well as synthetic chemical drug compounds that have been widely used. The results of this research can be a predictive reference for further research on the potential of herbal medicine, especially in the field of dentistry. [21,36,54–57].

5. Conclusion

The main active compounds from pomegranate peel extract are predicted to have good anti-inflammatory abilities against COX-1 and COX-2 enzymes with the best compounds in anti-inflammatory abilities are ferulic acid and punicalagin compounds. In addition, through these studies it can be predicted that the compound can be distributed systemically. In the future, researchers hope that this research can be developed comprehensively in vitro and in vivo.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Pahwa R, Goyal A, Jialal I. Chronic Inflammation. Pathobiology of Human Disease: A Dynamic Encyclopedia of Disease Mechanisms [Internet]. 2022 Aug 8 [cited 2023 Feb 15];300–14. Available from: https://www.ncbi.nlm.nih.gov/books/NBK493173/

[2] Hannoodee S, Nasuruddin DN. Acute Inflammatory Response. Nature [Internet]. 2022 Nov 14 [cited 2023 Feb 14];206(4979):20. Available from: https://www.ncbi.nlm.nih.gov/books/NBK556083/

[3] Cavaillon JM. Once upon a time, inflammation. J Venom Anim Toxins Incl Trop Dis [Internet]. 2021 [cited 2023 Apr 18];27. Available from: https://pubmed.ncbi.nlm.nih.gov/33889184/

[4] Mahesh G, Anil Kumar K, Reddanna P. Overview on the Discovery and Development of Anti-Inflammatory Drugs: Should the Focus Be on Synthesis or Degradation of PGE2? J Inflamm Res [Internet]. 2021 Feb [cited 2023 Feb 15];14:253–63. Available from: https://pubmed.ncbi.nlm.nih.gov/33568930/

[5] Das UN. Essential Fatty Acids and Their Metabolites in the Pathobiology of Inflammation and Its Resolution. Biomolecules [Internet]. 2021 Dec 1 [cited 2023 Jul 29];11(12). Available from: https://pubmed.ncbi.nlm.nih.gov/34944517/

[6] Davis FM, Tsoi LC, Wasikowski R, denDekker A, Joshi A, Wilke C, Deng H, Wolf S, Obi A, Huang S, Billi AC, Robinson S, Lipinski J, Melvin WJ, Audu CO, Weidinger S, Kunkel SL, Smith A, Gudjonsson JE, Moore BB, Gallagher KA. Epigenetic regulation of the PGE2 pathway modulates macrophage phenotype in normal and pathologic wound repair. JCI Insight [Internet]. 2020 Sep 3 [cited 2023 Jul 29];5(17). Available from: https://pubmed.ncbi.nlm.nih.gov/32879137/

[7] Ghlichloo I, Gerriets V. Nonsteroidal Anti-inflammatory Drugs (NSAIDs). Treatment of Chronic Pain Conditions: A Comprehensive Handbook [Internet]. 2022 May 19 [cited 2023 May 2];77–9. Available from: https://www.ncbi.nlm.nih.gov/books/NBK547742/

[8] Rajat S, Kumar MM, Kumar PA, Abhishek S, Krishna K, Rajat S, Kumar MM, Kumar PA, Abhishek S, Krishna K. An insight of non-steroidal anti-inflammatory drug mefenamic acid: A review. https://gsconlinepress.com/journals/gscbps/sites/default/files/GSCBPS-2019-0066.pdf [Internet]. 2019 May 30 [cited 2023 May 2];7(2):052–9. Available from: https://gsconlinepress.com/journals/gscbps/content/insight-non-steroidal-anti-inflammatory-drug-mefenamic-acid-review

[9] Badan Penelitian dan Pengembangan Kesehatan Kementerian RI. Riset Kesehatan Dasar Republik Indonesia 2018 [Internet]. Jakarta, Indonesia; 2019 [cited 2023 Jan 19]. Available from: http://repository.bkpk.kemkes.go.id/3514/1/Laporan%20Riskesdas%202018%20Nasional.pdf

[10] Feyisa ZT, Woldeamanuel BT. Prevalence and associated risk factors of gastritis among patients visiting Saint Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia. PLoS One [Internet]. 2021 Feb 1 [cited 2023 Feb 15];16(2):e0246619. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0246619

[11] Chaushu L, Gavrielov MR, Chaushu G, Zar K, Vered M. Orabase Promotes Oral Epithelization in a Wound Healing Rat Model: An Immunohistochemical Study. Appl Immunohistochem Mol Morphol [Internet]. 2021 May 1 [cited 2023 Jul 29];29(5):E39–45. Available from: https://pubmed.ncbi.nlm.nih.gov/33208673/

[12] Gao J, Karp JM, Langer R, Joshi N. The Future of Drug Delivery. Chemistry of Materials [Internet]. 2023 Jan 24 [cited 2023 Jul 29];35(2):359–63. Available from: https://pubs.acs.org/doi/full/10.1021/acs.chemmater.2c03003

[13] Vargason AM, Anselmo AC, Mitragotri S. The evolution of commercial drug delivery technologies. Nature Biomedical Engineering 2021 5:9 [Internet]. 2021 Apr 1 [cited 2023 Jul 29];5(9):951–67. Available from: https://www.nature.com/articles/s41551-021-00698-w

[14] Serafini G, De Biase A, Lamazza L, Mazzucchi G, Lollobrigida M. Efficacy of Topical Treatments for the Management of Symptomatic Oral Lichen Planus: A Systematic Review. Int J Environ Res Public Health [Internet]. 2023 Jan 1 [cited 2023 Jul 29];20(2):1202. Available from: https://www.mdpi.com/1660-4601/20/2/1202/htm

[15] Imanu SF, Leginis SN, Iqbal M, Surboyo MDC. Pomegranate extract mechanism in inhibiting the development of oral cancer: A review. Indonesian Journal of Dental Medicine. 2023 May 15;6(1):37–42.

[16] Vučić V, Grabež M, Trchounian A, Arsić A. Composition and Potential Health Benefits of Pomegranate: A Review. Curr Pharm Des [Internet]. 2019 Aug 27 [cited 2023 Nov 3];25(16):1817–27. Available from: https://pubmed.ncbi.nlm.nih.gov/31298147/

[17] Xiang Q, Li M, Wen J, Ren F, Yang Z, Jiang X, Chen Y. The bioactivity and applications of pomegranate peel extract: A review. J Food Biochem [Internet]. 2022 Jul 1 [cited 2023 Nov 3];46(7). Available from: https://pubmed.ncbi.nlm.nih.gov/35128669/

[18] Ban Z, Fan L, Song J, Fillmore S, Guan J. Antibacterial Effect of Pomegranate Juice on Listeria innocua and E. coli in Different Media. Foods [Internet]. 2023 Sep 1 [cited 2023 Nov 3];12(17). Available from: https://pubmed.ncbi.nlm.nih.gov/37685180/

[19] Saikia S, Bordoloi M. Molecular Docking: Challenges, Advances and its Use in Drug Discovery Perspective. Curr Drug Targets [Internet]. 2019 Oct 25 [cited 2023 Apr 30];20(5):501–21. Available from: https://pubmed.ncbi.nlm.nih.gov/30360733/

[20] Stanzione F, Giangreco I, Cole JC. Use of molecular docking computational tools in drug discovery. Prog Med Chem [Internet]. 2021 Jan 1 [cited 2023 Apr 30];60:273–343. Available from: https://pubmed.ncbi.nlm.nih.gov/34147204/

[21] Khoswanto C, Siswandono. Molecular Docking Analysis of Quercetin and Diclofenac as Cox-2 Potential Inhibitors [Internet]. Research Gate. 2022 [cited 2023 May 13]. p. 552–5. Available from: https://www.researchgate.net/publication/363652337_Molecular_Docking_Analysis_of_Quercetin_and_Diclofenac_as_Cox-2_Potential_Inhibitors

[22] Pinzi L, Rastelli G. Molecular docking: shifting paradigms in drug discovery. Int J Mol Sci. 2019;20(18):4331.

[23] Kusuma SMW, Utomo DH, Susanti R. Molecular Mechanism of Inhibition of Cell Proliferation: An In Silico Study of the Active Compounds in Curcuma longa as an Anticancer. J Trop Biodivers Biotechnol. 2022 Nov 14;7(3):74905.

[24] Utomo DH, Kita M. Binding Mode of Actin–Aplyronine A–Tubulin Heterotrimeric Complex Revealed by Molecular Dynamics Simulation. Bull Chem Soc Jpn [Internet]. 2023 Jan 13 [cited 2023 Jul 30];96(2):120–6. Available from: https://www.journal.csj.jp/doi/10.1246/bcsj.20220299

[25] Rudik A V., Dmitriev A V., Lagunin AA, Filimonov DA, Poroikov V V. PASS-based prediction of metabolites detection in biological systems. SAR QSAR Environ Res [Internet]. 2019 Oct 3 [cited 2023 Jun 12];30(10):751–8. Available from: https://pubmed.ncbi.nlm.nih.gov/31542944/

[26] Dmitriev A V., Filimonov DA, Rudik A V., Pogodin P V., Karasev DA, Lagunin AA, Poroikov V V. Drug-drug interaction prediction using PASS. SAR QSAR Environ Res [Internet]. 2019 Sep 2 [cited 2023 Jun 17];30(9):655–64. Available from: https://pubmed.ncbi.nlm.nih.gov/31482727/

[27] Chen X, Li H, Tian L, Li Q, Luo J, Zhang Y. Analysis of the Physicochemical Properties of Acaricides Based on Lipinski's Rule of Five. J Comput Biol [Internet]. 2020 Sep 1 [cited 2023 May 1];27(9):1397–406. Available from: https://pubmed.ncbi.nlm.nih.gov/32031890/

[28] Chagas CM, Moss S, Alisaraie L. Drug metabolites and their effects on the development of adverse reactions: Revisiting Lipinski's Rule of Five. Int J Pharm [Internet]. 2018 Oct 5 [cited 2023 May 1];549(1–2):133–49. Available from: https://pubmed.ncbi.nlm.nih.gov/30040971/

[29] Ivanović V, Rančić M, Arsić B, Pavlović A. Lipinski's rule of five, famous extensions and famous exceptions. Popular Scientific Article. 2020;3(1):171–7.

[30] Ferreira LLG, Andricopulo AD. ADMET modeling approaches in drug discovery. Drug Discov Today. 2019;24(5):1157–65.

[31] Shivanika C, Deepak Kumar S, Ragunathan V, Tiwari P, Sumitha A, Brindha Devi P. Molecular docking, validation, dynamics simulations, and pharmacokinetic prediction of natural compounds against the SARS-CoV-2 main-protease. J Biomol Struct Dyn [Internet]. 2020 [cited 2023 May 14];40(2):1. Available from: /pmc/articles/PMC7573242/

[32] Sripriya N, Ranjith Kumar M, Ashwin Karthick N, Bhuvaneswari S, Udaya Prakash NK. In silico evaluation of multispecies toxicity of natural compounds. Drug Chem Toxicol [Internet]. 2021 [cited 2023 Jul 30];44(5):480–6. Available from: https://pubmed.ncbi.nlm.nih.gov/31111731/

[33] Rim KT. In silico prediction of toxicity and its applications for chemicals at work. Toxicol Environ Health Sci [Internet]. 2020 Sep 1 [cited 2023 Jul 30];12(3):191–202. Available from: https://pubmed.ncbi.nlm.nih.gov/32421081/

[34] Pantsar T, Poso A. Binding Affinity via Docking: Fact and Fiction. Molecules [Internet]. 2018 [cited 2023 Jun 12];23(8):1DUMMY. Available from: https://pubmed.ncbi.nlm.nih.gov/30061498/

[35] Ji D, Xu M, Udenigwe CC, Agyei D. Physicochemical characterisation, molecular docking, and drug-likeness evaluation of hypotensive peptides encrypted in flaxseed proteome. Curr Res Food Sci [Internet]. 2020 Nov 1 [cited 2023 May 1];3:41–50. Available from: https://pubmed.ncbi.nlm.nih.gov/32914119/

[36] Burley SK, Bhikadiya C, Bi C, Bittrich S, Chao H, Chen L, Craig PA, Crichlow G V., Dalenberg K, Duarte JM, Dutta S, Fayazi M, Feng Z, Flatt JW, Ganesan SJ, Ghosh S, Goodsell DS, Green RK, Guranovic V, Henry J, Hudson BP, Khokhriakov I, Lawson CL, Liang Y, Lowe R, Peisach E, Persikova I, Piehl DW, Rose Y, Sali A, Segura J, Sekharan M, Shao C, Vallat B, Voigt M, Webb B, Westbrook JD, Whetstone S, Young JY, Zalevsky A, Zardecki C. RCSB Protein Data bank: Tools for visualizing and understanding biological macromolecules in 3D. Protein Sci [Internet]. 2022 Dec 1 [cited 2023 Jun 17];31(12):e4482. Available from: https://pubmed.ncbi.nlm.nih.gov/36281733/

[37] Song Y, Day CM, Afinjuomo F, Tan JQE, Page SW, Garg S. Advanced Strategies of Drug Delivery via Oral, Topical, and Parenteral Administration Routes: Where Do Equine Medications Stand? Pharmaceutics [Internet]. 2023 Jan 1 [cited 2023 Jul 29];15(1). Available from: /pmc/articles/PMC9861747/

[38] Matos C, Lobão P. Non-Steroidal Anti-Inflammatory Drugs Loaded Liposomes for Topical Treatment of Inflammatory and Degenerative Conditions. Curr Med Chem [Internet]. 2020 Mar 4 [cited 2023 Jul 29];27(23):3809–29. Available from: https://pubmed.ncbi.nlm.nih.gov/30827226/

[39] Sanjaya RE, Putri KDA, Kurniati A, Rohman A, Puspaningsih NNT. In silico characterization of the GH5-cellulase family from uncultured microorganisms: physicochemical and structural studies. J Genet Eng Biotechnol [Internet]. 2021 Dec 1 [cited 2023 Feb 15];19(1). Available from: https://pubmed.ncbi.nlm.nih.gov/34591195/

[40] Wanasathop A, Patel PB, Choi HA, Li SK. Permeability of Buccal Mucosa. Pharmaceutics 2021, Vol 13, Page 1814 [Internet]. 2021 Oct 31 [cited 2023 Aug 4];13(11):1814. Available from: https://www.mdpi.com/1999-4923/13/11/1814/htm

[41] Mulpuru V, Mishra N. In Silico Prediction of Fraction Unbound in Human Plasma from Chemical Fingerprint Using Automated Machine Learning. ACS Omega [Internet]. 2021 Mar 16 [cited 2023 Feb 15];6(10):6791–7. Available from: https://pubmed.ncbi.nlm.nih.gov/33748592/

[42] Shaker B, Ahmad S, Lee J, Jung C, Na D. In silico methods and tools for drug discovery. Comput Biol Med. 2021;137:104851.

[43] Watanabe R, Esaki T, Kawashima H, Natsume-Kitatani Y, Nagao C, Ohashi R, Mizuguchi K. Predicting Fraction Unbound in Human Plasma from Chemical Structure: Improved Accuracy in the Low Value Ranges. Mol Pharm [Internet]. 2018 Nov 5 [cited 2023 Feb 15];15(11):5302–11. Available from: https://pubmed.ncbi.nlm.nih.gov/30259749/

[44] Fagerholm U, Spjuth O, Hellberg S. Comparison between lab variability and in silico prediction errors for the unbound fraction of drugs in human plasma. Xenobiotica [Internet]. 2021 [cited 2023 Feb 15];51(10):1095–100. Available from: https://pubmed.ncbi.nlm.nih.gov/34346291/

[45] Bruno A, Costantino G, Sartori L, Radi M. The in silico drug discovery toolbox: applications in lead discovery and optimization. Curr Med Chem. 2019;26(21):3838–73.

[46] Rudik A V., Dmitriev A V., Lagunin AA, Filimonov DA, Poroikov V V. Metabolism site prediction based on xenobiotic structural formulas and PASS prediction algorithm. J Chem Inf Model [Internet]. 2014 Feb 24 [cited 2023 Jun 12];54(2):498–507. Available from: https://pubmed.ncbi.nlm.nih.gov/24417355/

[47] Marnett LJ. Adventures with Bruce Ames and the Ames test. Mutat Res Genet Toxicol Environ Mutagen [Internet]. 2019 Oct 1 [cited 2023 Feb 15];846. Available from: https://pubmed.ncbi.nlm.nih.gov/31585632/

[48] Stampfer HG, Gabb GM, Dimmitt SB. Why maximum tolerated dose? Br J Clin Pharmacol [Internet]. 2019 Oct 1 [cited 2023 Feb 15];85(10):2213–7. Available from: https://pubmed.ncbi.nlm.nih.gov/31219196/

[49] Selvestrel G, Robino F, Russo MZ. In Silico Models for Skin Sensitization and Irritation. Methods Mol Biol [Internet]. 2022 [cited 2023 Feb 15];2425:291–354. Available from: https://pubmed.ncbi.nlm.nih.gov/35188638/

[50] Chy MNU, Chakrabarty N, Roy A, Paul A, Emu KA, Dutta T, Dutta E, Ferdous I, Das R, Hasan MJ, Tasnim SM. Antibacterial, anthelmintic, and analgesic activities of Piper sylvaticum (Roxb.) leaves and in silico molecular docking and PASS prediction studies of its isolated compounds. J Complement Integr Med [Internet]. 2019 Dec 1 [cited 2023 Jun 12];16(4). Available from: https://pubmed.ncbi.nlm.nih.gov/31437123/

[51] Dar AM, Mir S. Molecular Docking: Approaches, Types, Applications and Basic Challenges. Journal of Analytical & Bioanalytical Techniques 2017 8:2 [Internet]. 2017 Mar 27 [cited 2023 May 1];8(2):1–3. Available from: https://www.omicsonline.org/open-access/molecular-docking-approaches-types-applications-and-basic-challenges-2155-9872-1000356.php?aid=88070

[52] Velázquez-Libera JL, Durán-Verdugo F, Valdés-Jiménez A, Núñez-Vivanco G, Caballero J. LigRMSD: A web server for automatic structure matching and RMSD calculations among identical and similar compounds in protein-ligand docking. Bioinformatics. 2020;36(9):2912–4.

[53] Nugraha AP, Sibero MT, Nugraha AP, Puspitaningrum MS, Rizqianti Y, Rahmadhani D, Kharisma VD, Ramadhani NF, Ridwan RD, Binti Tengku Ahmad Noor TNE, Ernawati DS. Anti-Periodontopathogenic Ability of Mangrove Leaves (Aegiceras corniculatum) Ethanol Extract: In silico and in vitro study. Eur J Dent [Internet]. 2023 Feb 23 [cited 2023 May 4];17(1):46–56. Available from: https://pubmed.ncbi.nlm.nih.gov/35453169/

[54] Fan C, Wong PP, Zhao H. DStruBTarget: Integrating Binding Affinity with Structure Similarity for Ligand-Binding Protein Prediction. J Chem Inf Model [Internet]. 2020 Jan 27 [cited 2023 Feb 15];60(1):400–9. Available from: https://pubmed.ncbi.nlm.nih.gov/31833767/

[55] Gromiha MM, Yugandhar K, Jemimah S. Protein-protein interactions: scoring schemes and binding affinity. Curr Opin Struct Biol [Internet]. 2017 Jun 1 [cited 2023 Jun 12];44:31–8. Available from: https://pubmed.ncbi.nlm.nih.gov/27866112/

[56] Linkuvienė V, Zubrienė A, Matulis D. Intrinsic affinity of protein - ligand binding by differential scanning calorimetry. Biochim Biophys Acta Proteins Proteom [Internet]. 2022 Sep 1 [cited 2023 Feb 15];1870(9). Available from: https://pubmed.ncbi.nlm.nih.gov/35934299/

[57] Seo S, Choi J, Park S, Ahn J. Binding affinity prediction for protein-ligand complex using deep attention mechanism based on intermolecular interactions. BMC Bioinformatics [Internet]. 2021 Dec 1 [cited 2023 Feb 15];22(1). Available from: https://pubmed.ncbi.nlm.nih.gov/34749664/