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Molecular Docking of Active Compounds of *Syzygium myrtifolium* Walp. Leaves on Leukotriene A4 Hydrolase Receptors as Colorectal Anticancer

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Abstract

Active compounds found in *Syzygium myrtifolium* Walp. leaves such as flavonoids, phenolics, and betulinic acid are known to have pharmacological activities. This research aimed to find active compounds found in *Syzygium myrtifolium* Walp. leaves, which have anticancer activity by inhibiting the protein leukotriene A4 hydrolase. Molecular docking methods are used to predict the activity and affinity between ligand-proteins. The research was conducted in silico on the active compound in *Syzygium myrtifolium* Walp. leaves, which met the five criteria of Lipinski's rule for leukotriene A4 hydrolase with PDB code 3U9W. The software used were YASARA, MarvinSketch, and PLANTS, which can optimize ligands and bind ligand molecules to receptors. Then it was visualized using Discovery Studio Visualizer and analyzed the prediction of pharmacokinetics and toxicity. Docking results show that the four active compounds from the leaves of *Syzygium myrtifolium* Walp., namely bis (2-ethylhexyl) hexanedioate, 3-octadecyne, 1- octadecene, and (2E,6E)-farnesol have a lower docking score compared to bestatin; therefore, these four compounds have the potential to inhibit leukotriene A4 hydrolase receptors and can be candidates for colorectal anticancer compounds.

1. Introduction

Cancer is a disease characterized by uncontrolled division of abnormal cells. When a cancerous growth occurs in the colon or rectum, it is called colorectal cancer [1]. Several risk factors associated with the development of colorectal cancer are excessive alcohol consumption, smoking, being overweight or obese, a family history of colorectal cancer, excessive consumption of processed and red meat, and inflammatory bowel disease [2]. Based on Global Cancer Statistics in 2020, more than 1.9 million new cases of colorectal cancer and 935,000 deaths are expected in 2020, representing about one in 10 cancer cases and deaths. Overall, colorectal cancer ranks third in incidence but second in case of death [3].

One of the causes of colorectal cancer is the uncontrolled development of the enzyme leukotriene A4 hydrolase (LTA4H). LTA4H is an enzyme that acts as an epoxide hydrolase and aminopeptidase. LTA4H can cause carcinogenesis and form leukotriene B4 which plays a role in inflammation and cancer development. LTA4H can be used as an inflammatory marker in colorectal cancer so that inhibition of LTA4H can reduce the development of colorectal cancer [4, 5]. Colorectal cancer treatment can be done with chemotherapy, surgery, and radiation, but it is ineffective in curing cancer. Therefore, compounds derived from plants have recently attracted the attention of researchers as an alternative to cancer prevention and treatment because compounds derived from plants have low costs and side effects [6].

Syzygium myrtifolium Walp. belonging to the Myrtaceae family can be developed as medicine. In Indonesia, the plant *Syzygium myrtifolium* Walp. known as Pucuk Merah and often found as an ornamental plant [7]. Based on several studies, the active compounds contained in the plant *Syzygium myrtifolium* Walp. has various pharmacological activities, including antioxidant, antibacterial, antifungal, antiviral, antitumor, antidiarrheal, and antispasmodic [8, 9, 10]. Methanol extract of the leaves of *Syzygium myrtifolium* Walp. has anticancer activity against human colorectal carcinoma cells, namely HCT 116. This activity is due to the active compound in *Syzygium myrtifolium* Walp. leaf extracts, such as phenolic compounds, flavonoids, and betulinic acid [11]. Flavonoids found in *Syzygium myrtifolium* Walp. leaves are Cyanidine 3-galactoside, Delphinidin 3-O-β-D-glucopyranoside, peonidin, and others [12]. Besides that, there are also terpenoid compounds, such as α- thujene, (E)-Nerolidol, (2E,6E)-farnesol, and α- terpinene [13].

The process of drug discovery takes years because the process is long and complex, so as technology develops, research with computational assistance is developed. In silico research using the molecular docking method can be done computationally. Computational molecular docking is routinely performed at various stages of the drug discovery process. It is often used to predict the binding orientation of drug candidates to protein targets to predict the affinity and activity of small molecules [14].

Once the biological activity is known, it is necessary to identify the receptor targets that can be inhibited by a compound and explore the most potential active compounds and see how active compounds are contained in the extract by conducting in silico tests based on the molecular docking method. The presence of in silico test results data can strengthen the results of the in vitro tests carried out [15]. In line with docking, it is necessary to evaluate and optimize drug efficiency for bioactive compounds in the body by determining their pharmacokinetic properties and toxicity.

This research aims to analyze the potential of the active compound of *Syzygium myrtifolium* Walp. leaves as an in silico colorectal anticancer using the molecular docking method and predictive analysis of the active compound's pharmacokinetic properties and toxicity, which has potential as a colorectal anticancer.

2. Methodology

The research method used was an in silico experimental research by conducting physicochemical screening through drug-likeness analysis based on Lipinski's Rule of Five, molecular docking, prediction of pharmacokinetics and toxicity of the active compounds in the leaves of *Syzygium myrtifolium* Walp., which has the potential to inhibit the leukotriene A4 hydrolase receptor.

2.1. Materials and Tools

The materials used were the target protein leukotriene A4 hydrolase (LTA4H) available in the Protein Data Bank with code 3U9W, bestatin reference ligand available in PubChem, and test ligands derived from the 2D structures of 64 active compounds found in *Syzygium myrtifolium* Walp. leaves and depicted in MarvinSketch with SMILES format of active compounds available on PubChem. The tools used in this research were an Intel Celeron N3060 dual-core 1.6 GHz TurboBoost 2.48 GHz processor specifications laptop with 2 GB RAM, Windows 10 64-bit as the operating system, and the software used were YASARA View, MarvinSketch, PLANTS 1.1, BIOVIA Discovery Studio Visualizer, PubChem, Protein Data Bank, SwissADME, and pkCSM.

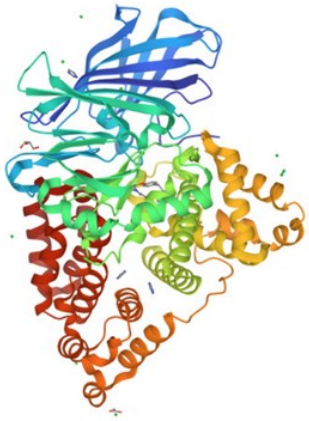

Figure 1. Structure of LTA4H protein

2.2. Analysis Drug-Likeness

Sixty-four active compounds in the leaves of *Syzygium myrtifolium* Walp. were obtained from a literature search [13, 16, 17, 18]. Each active compound was analyzed for drug-like properties according to Lipinski's Rule of Five by uploading SMILES of the active compound to the swissADME web server (http://www.swissadme.ch/index.php). Compounds that comply with Lipinski's Rule of Five were continued to the molecular docking process.

2.3. Target protein preparation

Leukotriene A4 hydrolase receptor target protein (LTA4H) (PDB code: 3U9W) was downloaded in .pdb format via the Protein Data Bank (https://www.rcsb.org/structure/3U9W). Then the target protein was prepared using YASARA software by separating native ligands and residues, such as water, from the target protein. Then the results of the target protein preparations were saved in the Mol2-Sybyl Mol2 file format.

2.4. Ligand preparation

The ligand structure was obtained from the database on the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). The structure was downloaded by copying the SMILES and then prepared on the MarvinSketch software. Compounds that did not have SMILES format in PubChem, their structures were drawn directly in MarvinSketch. The ligand was protonated at pH 7.4 and was confirmed by 20 conformers. Ligand preparation results were saved in .mol2 file format. The molecular structure of all the ligands can be seen in Figure 2.

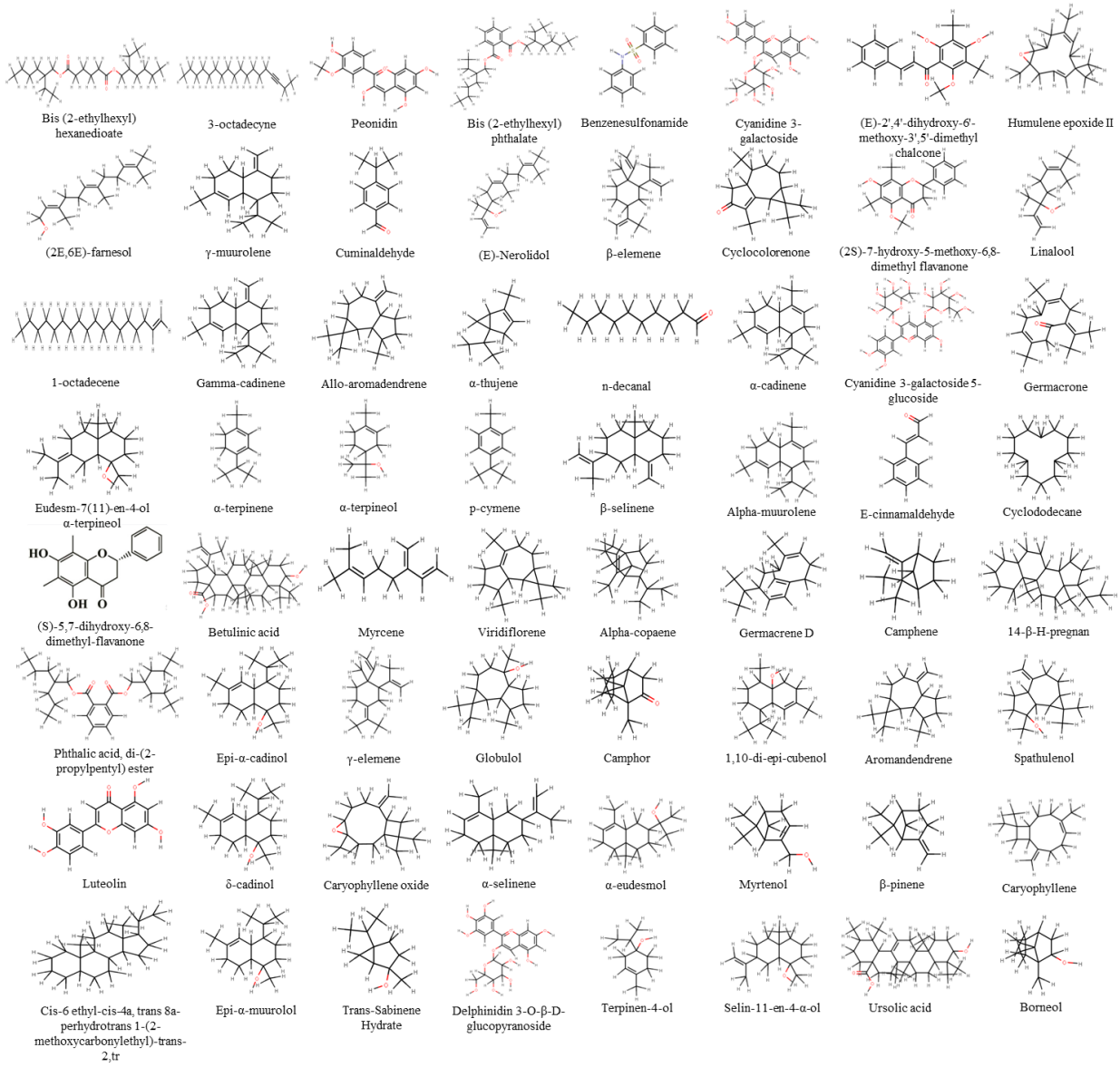

Figure 2. Molecular structure of active compounds in the leaves of *Syzygium myrtifolium* Walp. used as a test ligand

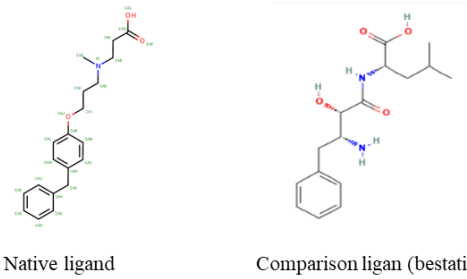

Figure 3. Molecular structure of native ligand and comparison ligan (bestatin)

2.5. Molecular Docking Method Validation

Prior to method validation, a search for the active binding site between the protein and native ligand was carried out in the PLANTS software. After the active binding site was known, the protein and native ligand were docked to obtain the best conformation of the ligand. Validation was done using YASARA software to determine the RSMD (Root Mean Square Deviation) value by combining the native ligand file steps with the best conformation file from the docking results.

2.6. Molecular Docking Analysis with PLANTS

Molecular docking was done using PLANTS software on a Windows operating system. The results of preparing the target protein file, the native ligand, and the tested ligands were prepared in one folder with the PLANTS application and its supporting files, and then searched for the active binding sites between proteins and ligands. The molecular docking process was executed with the command: "plants --mode screen pc_3u9w.txt". Molecular docking results could be viewed in the terminal with the command: "cd results" followed by "more bestranking.csv".

2.7. Docking Result Visualization

Docking visualization was performed using Discovery Studio Visualizer software. Before visualization, the best docking results of the ligands were combined with the target protein file on YASARA and then visualized in 2D in Discovery Studio Visualizer.

2.8. Predictive Analysis of Pharmacokinetics and Toxicity

Pharmacokinetic and toxicity predictions were conducted using the pkCSM web server. SMILES of the active compound with the best docking score was uploaded on the web server (https://biosig.lab.uq.edu.au/pkcsm/prediction).

3. Results and Discussion

3.1. Results of Analysis of Drug-likeness

The drug-likeness analysis aims to determine the similarity of the tested compounds with drugs so that if these compounds are administered orally as drugs, they may have good absorption and permeability in the body. Analysis of drug-like properties can be used as a guide in developing new targets with promising biological activity. This analysis is based on Lipinski's rule, which states that a compound has good absorption or permeability properties if its molecular weight is less than 500 Dalton; LogP values less than 5; the number of hydrogen bond acceptors is less than 10; the number of hydrogen bond donors is less than 5, and the molar refraction is between 40-130 [19]. The results of drug-like properties of the 64 active compounds of *Syzygium myrtifolium* Walp. leaves can be seen in Table 1.

Table 1. Results of drug-likeness of the active compound of the leaves of *Syzygium myrtifolium* Walp. based on the Lipinski rule

Compound	MW	LogP	HBA	HBD	MR	Lipinski violations
Luteolin	286.24	1.86	6	4	76.01	0
(E)-2',4'-dihydroxy-6'-methoxy-3',5'-dimethyl chalcone	298.33	2.64	4	2	86.72	0
(2S)-7-hydroxy-5-methoxy-6,8-dimethyl flavanone	298.33	2.78	4	1	83.95	0
(S)-5,7-dihydroxy-6,8-dimethyl-flavanone	284.31	2.65	4	2	79.48	0
Cyanidine 3-galactoside	449.38	-3.22	11	8	108.29	2
Cyanidine 3-galactoside 5-glucoside	611.53	-5.96	16	11	140.42	3
Delphinidin 3-O-β-D-glucopyranoside	465.38	-2.99	12	9	110.32	2
Peonidin	301.27	-1.94	6	4	80.64	0
Betulinic acid	456.70	3.79	3	2	136.91	1
Ursolic acid	456.70	3.71	3	2	136.91	1
Bis (2-ethylhexyl) hexanedioate	370.57	5.46	4	0	110.44	1
1-octadecene	252.48	5.05	0	0	88.17	1
Bis (2-ethylhexyl) phthalate	390.56	4.77	4	0	116.30	0
Alpha-copaene	204.35	3.40	0	0	67.14	0
Caryophyllene	204.35	3.29	0	0	68.78	0
Aromandendrene	204.35	3.26	0	0	67.14	0
Alpha-muurolene	204.35	3.38	0	0	69.04	0
Gamma-cadinene	204.35	3.39	0	0	69.04	0
Caryophyllene oxide	220.35	3.15	1	0	68.27	0
Cyclododecane	168.32	3.01	0	0	57.68	0
3-octadecyne	250.46	4.99	0	0	86.80	0
Linalool	154.25	2.70	1	1	50.44	0
α-thujene	136.23	2.67	1	0	45.22	0
Camphene	136.23	2.58	0	0	45.22	0
β-pinene	136.23	2.59	0	0	45.22	0
Myrcene	136.23	2.89	0	0	48.76	0
α-terpinene	136.23	2.70	0	0	47.12	0
p-cymene	134.22	2.51	0	0	45.99	0
Trans-Sabinene Hydrate	154.25	2.47	1	1	46.90	0
Camphor	152.23	2.12	1	0	45.64	0
Borneol	154.25	2.29	1	1	46.60	0
Terpinen-4-ol	154.25	2.51	1	1	48.80	0
α-terpineol	154.25	2.51	1	1	48.80	0
Myrtenol	154.23	2.34	1	1	46.38	0
n-decanal	156.27	2.72	1	0	50.38	0
Cuminaldehyde	148.20	2.03	1	0	46.41	0
E-cinnamaldehyde	132.16	1.65	1	0	41.54	0
β-elemene	204.35	3.37	0	0	70.42	0
γ-elemene	204.35	3.40	0	0	70.42	0
Allo-aromadendrene	204.35	3.26	0	0	67.14	0

Compound	MW	LogP	HBA	HBD	MR	Lipinski violations
γ-muurolene	204.35	3.39	0	0	69.04	0
Germacrene D	204.35	3.32	0	0	70.68	0
β-selinene	204.35	3.28	0	0	68.78	0
Viridiflorene	204.35	3.30	0	0	67.14	0
α-selinene	204.35	3.31	0	0	68.78	0
α-cadinene	204.35	3.38	0	0	69.04	0
(E)-Nerolidol	222.37	3.64	1	1	74.00	0
Spathulenol	220.35	2.88	1	1	68.34	0
Globulol	222.37	3.08	1	1	68.82	0
Humulene epoxide II	220.35	3.11	1	0	69.91	0
1,10-di-epi-cubenol	222.37	3.24	1	1	70.72	0
Epi-α-cadinol	222.37	3.15	1	1	70.72	0
Epi-α-muurolol	222.37	3.15	1	1	70.72	0
δ-cadinol	222.37	3.15	1	1	70.72	0
α-eudesmol	222.37	3.18	1	1	70.46	0
Selin-11-en-4-α-ol	222.37	3.10	1	1	70.46	0
Germacrone	218.33	2.96	1	0	70.88	0
Eudesm-7(11)-en-4-ol	222.37	3.10	1	1	70.46	0
(2E,6E)-farnesol	222.37	3.71	1	1	73.96	0
Cyclocolorenone	218.33	2.82	1	0	67.34	0
Benzensulfonanilide	233.29	1.86	2	1	63.56	0
Phthalic acid, di-(2-propylpentyl) ester	390.56	4.68	4	0	116.30	0
14-β-H-pregnan	288.51	4.08	0	0	94.08	0
Cis-6 ethyl-cis-4a, trans 8a-perhydrotrans 1-(2-methoxycarbonyl ethyl)-trans-2,tr	380.52	2.96	5	2	105.63	0

Note: MW: Molecular Weight; LogP: High lipophilicity; HBA: Hydrogen Bond Acceptors; HBD: Hydrogen Bond Donors; MR: Molar Refractivity

Excellent absorption and permeability are achieved when a compound fulfills all the parameters of the Lipinski rule, and the permissible rule violation limit is one rule [20]. Based on this, cyanidin 3-galactoside, cyanidin 3-galactoside 5-glucoside, and delphinidin 3-O-β-D-glucopyranoside have poor absorption in the human body because they have two or more parameters that are out of range; thus, the docking procedure is discontinued for these three compounds. The active compounds in *Syzygium myrtifolium* Walp. leaves which are stimulated against leukotriene A4 hydrolase receptors in this research are 61 active compounds with good absorption in the body.

3.2. Validation of Molecular Docking

The molecular docking was validated by measuring the value of the ligand's Root Mean Square Deviation (RMSD) from its reference position to the appropriate receptor after optimal superimposition of the receptor. The docking process has high accuracy if the RSMD value is below 2 Å. The lower the RMSD score, the more accurate the docking process is [21]. The RMSD value obtained after re-docking between the best conformation of the native ligand and the receptor is 1.5714 Å, so the method used has validity with high accuracy, and the pose level is similar to the native ligand. The results of the best conformation pose similarity with the native ligand can be seen in Figure 4.

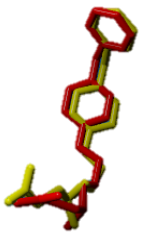

Figure 4. The best conformational (yellow) pose similarity of the native ligand (red)

3.3. Molecular Docking

In the research that has been done, the ligands used are the native ligand, the reference ligand, and the test ligand. The test ligands were obtained from 61 active compounds in *Syzygium myrtifolium Walp*. leaves, which have good absorption in the body. The docking score describes the binding affinity directly related to the Gibbs energy of ligand-protein binding [22]. The more negative the docking score, the more stable and stronger the binding affinity is between the ligand and protein.

Table 2. Docking results of native ligands and comparison ligands

Compound	Docking Score (kcal/mol)
Native ligands: 28P	-120.759
Comparison ligands: Bestatin	-104.601

Table 3. Docking results of active compounds of *Syzygium myrtifolium* Walp.

Compound	Docking score (kcal/mol)
Bis (2-ethylhexyl) hexanedioate	-115.502
3-octadecyne	-113.790
1-octadecene	-112.531
(2E,6E)-farnesol	-106.599
Bis (2-ethylhexyl) phthalate	-102.883
(E)-Nerolidol	-101.921
Phthalic acid, di-(2-propylpentyl) ester	-100.541
Luteolin	-94.727
Cis-6 ethyl-cis-4a, trans 8a-perhydrotrans 1-(2-methoxycarbonyl ethyl)-trans-2,tr	-92.219
Benzensulfonanilide	-90.241
(S)-5,7-dihydroxy-6,8-dimethyl-flavanone	-84.128
α-selinene	-82.391
β-elemene	-82.027
n-decanal	-81.963
β-selinene	-81.000
Alpha-copaene	-80.300
Linalool	-80.234
14-β-H-pregnan	-79.164
α-eudesmol	-79.084
Terpinen-4-ol	-77.557
Spathulenol	-76.999
α-terpineol	-76.987
α-terpinene	-76.962
Gamma-cadinene	-76.340
γ-muurolene	-76.337
p-cymene	-76.208
Myrcene	-76.068
Epi-α-cadinol	-75.767
δ-cadinol	-75.753
Epi-α-muurolol	-75.737
Viridiflorene	-75.663
Cyclocolorenone	-74.501
α-cadinene	-74.054
Alpha-muurolene	-74.031
Germacrene D	-74.027
Germacrone	-73.128
1,10-di-epi-cubenol	-72.867
Myrtenol	-72.777
Selin-11-en-4-å-ol	-72.729
Caryophyllene	-72.526
Peonidin	-72.437
Eudesm-7(11)-en-4-ol	-72.378
Allo-aromadendrene	-72.297
Cuminaldehyde	-72.083
α-thujene	-72.072
Betulinic acid	-72.040
γ-elemene	-71.657
Caryophyllene oxide	-70.589
Trans-Sabinene Hydrate	-70.567
(E)-2',4'-dihydroxy-6'-methoxy-3',5'-dimethyl chalcone	-70.194
Globulol	-69.366
Humulene epoxide II	-69.338
(2S)-7-hydroxy-5-methoxy-6,8-dimethyl flavanone	-69.187
E-cinnamaldehyde	-68.568

Compound	Docking score (kcal/mol)
Camphene	-67.658
Cyclododecane	-67.469
Aromandendrene	-66.222
β-pinene	-65.402
Ursolic acid	-63.290
Borneol	-58.833
Camphor	-54.662

Based on Tables 2 and 3, both the active compound from the leaves of *Syzygium myrtifolium* Walp. and the reference compound bestatin has a higher docking score than the native ligand against the leukotriene hydrolase A4 receptor. However, several active compounds exist in *Syzygium myrtifolium* Walp. leaves, which had a lower docking score than the bestatin comparator compounds, namely bis(2-ethylhexyl)hexanedioate, 3-octadecyne, 1-octadecene, and (2E,6E)-farnesol. This shows that these four compounds interact better with target proteins than the comparator compounds in inhibiting leukotriene A4 hydrolase and can be potential drug candidates for colorectal cancer therapy.

3.4. Docking Visualization

Docking visualization was performed to see the interactions between the active compound ligands and the leukotriene A4 hydrolase (LTA4H) protein.

The interactions between proteins and ligands can affect the increase in bond affinity, where the strength of the affinity bonds between proteins and ligands will be stronger with the many interactions due to chemical bonds that occur [23]. The binding affinity can be affected by the hydrogen bonds, hydrophobic bonds, and Van der Waals bonds between the ligand and the receptor. Besides being influenced by chemical bonds, bond affinity can be affected by free bond energy and the number of bound amino acid residues. Hydrogen bonds play a role in increasing the binding affinity of compounds for receptors [24, 25]. The test ligand from the active compound with the same chemical interaction as the comparator protein compound has the potential to inhibit receptor action and has a strong interaction [26].

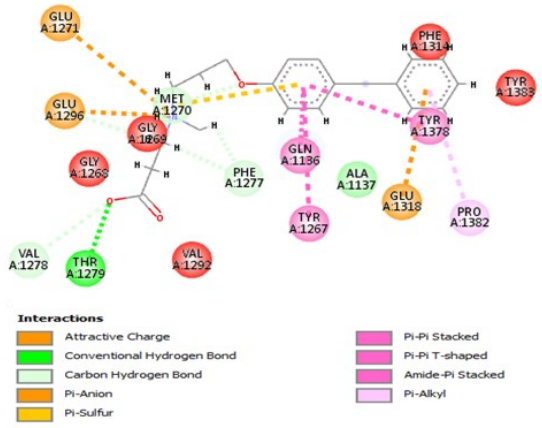

Figure 5. Visualization of native ligand interactions with protein

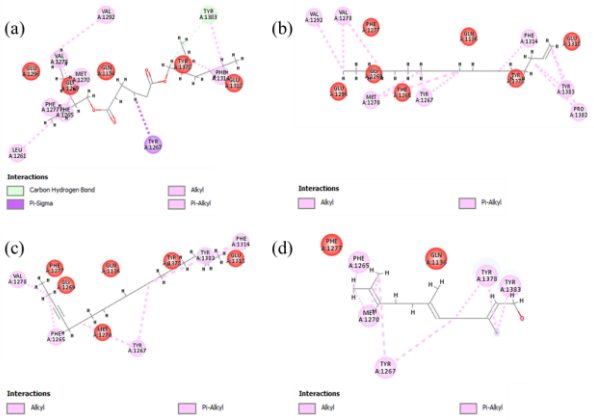

Figure 6. Visualization of ligand interaction best docking score with protein, (a) Bis (2-ethylhexylhexanedioate–protein; (b) 1-octadecene–protein; (c) 3-octadecyne–protein; and (d) (2E,6E)-farnesol–protein

Bond interactions formed on amino acid residues can play a role in knowing the chemical structure of a compound in inhibiting proteins. The bonds formed based on Figures 5 and Figure 6 are carbon-hydrogen bonds and hydrophobic interactions. Hydrogen bonds are strong bonds compared to hydrophobic bonds. If a compound has hydrogen bonds, then the compound has the potential to inhibit a receptor. The carbon-hydrogen bonds are only present in bis(2-ethylhexyl) hexanadioate at the TYR1383 residue, while the other three compounds do not have carbon-hydrogen bonds.

In the bis(2-ethylhexyl) hexanadioate compound, Pi-Sigma bonds are formed at the TYR1267 residue and alkyl bonds at the PHE1277, MET1270, and VAL1278 residues. Pi-Sigma and pi-alkyl bonds are the strongest in increasing free energy and can stabilize water molecules. The bond formed on 1-octadecene, 3- 3- octadecyne, and (2E,6E)-fernesol is a hydrophobic bond by forming many residues through the interaction of alkyl and Pi-alkyl bonds.

Based on the visualization results between the protein-native ligand complex, it was obtained that amino acid residues were formed through various interactions, namely GLU1271, GLU1296, VAL1278, THR1279, MET1270, PHE1277, GLN1136, TYR1267, ALA1137, GLU1318, TYR1378, and PRO1382. Based on the results of protein visualization with the active compound of *Syzygium myrtifolium* Walp. leaves which have the best docking score indicate that some of the amino acid residues formed are the same as the amino acid residues of the protein-native ligand complex, namely

bis(2- ethylhexyl) hexanedioate with the same four amino acid residues (VAL1278, MET1270, PHE1277, and TYR1267), 1-octadecene with the same four amino acid residues (VAL1278, MET1270, PRO1382, and TYR1267), 3- octadecyne with the same two amino acid residues (VAL1278 and TYR1267), and (2E,6E)- farnesol with the same three amino acid residues (MET1270, TYR1378, and TYR1267).

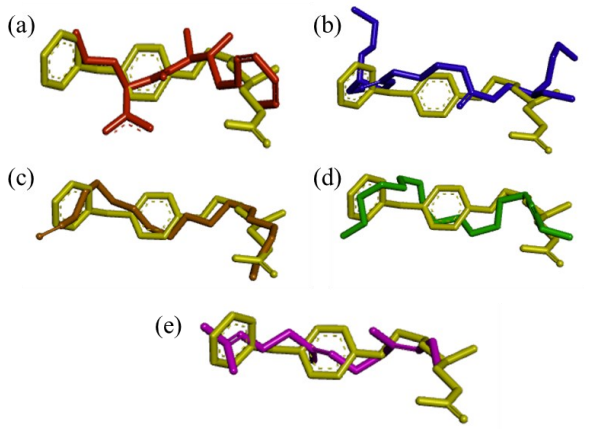

Figure 7. Superimposition of (a) comparison ligands (red), (b) bis(2-ethylhexyl) hexanedioate (blue), (c) 1- octadecene (brown), (d) 3-octadecyne (green), (e) (2E,6E)-farnesol (purple) over native ligands (yellow)

The similarity of the amino acid residues formed and also the bond interactions that occur can affect the Gibs energy value due to the docking score; the lower the Gibbs energy value, the better the ability to bind a compound to the receptor and has the potential to have inhibitory activity against the receptor [27]. Based on the visualization results of the superimposition of bis(2- ethylhexyl) hexanedioate, 1-octadecene, 3- octadecyne, (2E,6E)- farnesol, and the comparison ligands showed that bis(2-ethylhexyl) hexanedioate, 1- octadecene, 3- octadecyne, and (2E,6E)-farnesol have a slightly similar binding mode to the reference ligand. This indicates that these compounds have potency in inhibiting protein leukotriene A4 hydrolase.

3.5. Pharmacokinetic and Toxicity Prediction Results

Pharmacokinetic prediction aims to predict the absorption, distribution, metabolism, and excretion properties of a compound. Predictions have been made of the four compounds from the leaves of *Syzygium myrtifolium* Walp. which have the best docking scores, namely bis(2-ethylhexyl) hexanadioate, 1-octadecene, 3-octadecyne, and (2E,6E)-farnesol. Virtual screening was carried out in this research to obtain compounds with the best pharmacokinetic properties of the four potential compounds. Predictive parameters that have numerical values with certain limitations are Caco-2 permeability, intestinal absorption (human), skin permeability, a steady volume of distribution (VDss), blood-brain barrier (BBB) permeability, central nervous system permeability (CNS), and clearance total is used in virtual screening to determine compounds with the best pharmacokinetic properties [28].

Table 4. Pharmacokinetic parameters of potential compounds

Parameter	Bis (2-ethylhexyl) hexanedioate	1-octadecene	3-octadecyne	(2E,6E)-farnesol	requirement value
Caco2 permeability	1.349	1.373	1.393	1.495	>0.9
Intestinal absorption	92.853	90.834	92.171	91.531	>30%
Skin Permeability	-2.622	-2.639	-2.466	-1.514	≥-2.5
CYP2D6 substrate	No	No	No	No	-
CYP3A4 substrate	Yes	Yes	Yes	No	-
CYP1A2 inhibitor	No	Yes	Yes	No	-
CYP2C19 inhibitor	No	No	No	No	-
CYP2C9 inhibitor	Yes	No	No	No	-
CYP2D6 inhibitor	No	No	No	No	-
CYP3A4 inhibitor	No	No	No	No	-
VDss (human)	0.032	0.656	0.581	0.36	≥-0.15
BBB permeability	-0.425	0.987	0.953	0.66	>-1
CNS permeability	-2.611	-1.308	-1.148	-1.933	≥-3
Total Clearance	1.997	1.998	1.913	1.754	The higher, the better

Caco-2 cells are a type of colorectal cancer cell. The Caco-2 model was used to predict the gastrointestinal permeability of drugs. Absorption in the intestine aims to predict the proportion of compounds absorbed in the human small intestine [29]. The results of the absorption prediction analysis showed that the compounds 3- octadecyne and (2E-6E)-farnesol had better absorption values than other compounds.

VDSs (volume of distribution at steady state) is the theoretical volume at which the total dose of a drug is evenly distributed to provide the same concentration as in blood plasma. The higher the value of the volume of distribution, the more the drug will be distributed in the tissues compared to the plasma. The ability of a drug to penetrate the blood-brain barrier is an important parameter to help reduce side effects and drug toxicity or to increase the efficacy of drugs whose pharmacological activity resides in the brain [29]. The compounds that have the best distribution are 1-octadecene, 3- octadecyne, and (2E-6E)-farnesol.

Metabolism prediction provides information about possible compounds metabolized in the liver. Three compounds metabolized in the liver are bis (2- ethylhexyl) hexanadioate (CYP3A4 substrate and CYP2C9 inhibitor), 1-octadecene (CYP3A4 substrate and

CYP1A2 inhibitor), and 3-octadecyne (CYP3A4 substrate and CYP1A2 inhibitor).

Total clearance is the combination of hepatic clearance (metabolism in the liver and biliary clearance) and renal clearance (excretion via the kidneys) that is related to the bioavailability of a drug and to determine the dose to achieve steady-state concentrations [29]. The higher the total clearance value, the easier the compound is excreted. The four compounds are easily excreted. After the results of the pharmacokinetic prediction analysis are known, proceed with the prediction of toxicity based on AMES toxicity, *T. Pyriformis* toxicity, and Minnow toxicity. Data can be seen in Table 5.

Table 5. Toxicity parameters of potential compounds

Parameter	Bis (2-ethylhexyl) hexanedioate	1-octadecene	3-octadecyne	(2E,6E)-farnesol
AMES toxicity	No	No	No	No
T. Pyriformis toxicity	0.865	1.386	1.583	2.328
Minnow toxicity	-1.793	-1.811	-1.518	0.1

AMES toxicity aims to see whether a compound has mutagenic potential. The prediction results show that the four test compounds are not mutagenic and have no potential as carcinogenic compounds. Toxicity prediction of *T. Pyriformis* is used as a toxic endpoint of a compound. A value of > -0.5 log µg/L is considered toxic; the four test compounds are highly toxic from the predicted results. Toxicity prediction based on Minnow's toxicity showed that the compounds bis(2-ethylhexyl) hexanadioate, 1- octadecene, and 3-octadecyne had high acute toxicity because the log LC_{50} value < -0.30 while the compound (2E,6E)-farnesol had a low acute toxicity.

4. Conclusion

From the results of the molecular docking that has been carried out, it is known that there are four active compounds in the leaves of *Syzygium myrtifolium* Walp. which have activity and have the potential to inhibit leukotriene A4 hydrolase in colorectal cancer namely bis(2-ethylhexyl) hexanadioate, 3-octadecyne, 1- octadecene, and (2E),6E)-farnesol. It is necessary to isolate the compounds bis(2-ethylhexyl)hexanedioate, 3-octadecyne, 1-octadecene, and (2E,6E)-farnesol found in the leaves of *Syzygium myrtifolium* Walp. and further research was carried out regarding dosage and toxicity to determine the activity and safety of these four compounds as candidates for colorectal anticancer compounds with the mechanism of inhibiting leukotriene A4 hydrolase receptors.

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