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THE VIRTUAL SCREENING OF SECONDARY METABOLITES OF MIANA LEAVES (*Plectranthus scutellarioides* (L) R. Br) AGAINST PLASMODIUM FALCIPARUM LACTATE DEHYDROGENASE ENZYME (PfLDH)

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ABSTRACT

Malaria is a deadly disease that still occurs in some tropical countries. Medicines derived from plant parts have long been used as herbal medicines to treat malaria as part of Indonesian traditional medicine practice. Miana has been used as one of the antimalarial herbs by the South Sulawesi community. This study describes the in-silico study by tethering the secondary metabolite compound molecule of miana leaf (*Plectranthus scutellarioides* (L.) R.Br.) To the *Plasmodium falciparum* lactate dehydrogenase (PDB ID code: 1CET) as the first-line test to determine the antimalarial activity of Miana leaves. The results showed that the free energy binding value of 4 secondary metabolites of miana leaves, namely Scutellarioidone A (-6.32 kcal/mol), Scutellarioidone D (-6.06 kcal/mol), Coleon O (-6.07 kcal/mol), Fredericone B (-6.13 kcal/mol), was better than its natural ligands, Chloroquine (-5.89 kcal/mol). From the visualization of the docking results, all compounds have hydrogen bonds with different amino acid residues. Based on the results of predictions of pharmacokinetics, toxicity, and Lipinski's rule of five, all secondary metabolites from Miana leaves meet the criteria as a promising drug. However, some compounds can pose a safety risk. From the overall computational analysis, it can be predicted that the Scutellarioidone D can be used as a candidate for antimalarial drugs.

Keywords: Miana Leaf, In Silico, *Plasmodium falciparum* Lactate Dehydrogenase.

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INTRODUCTION

Malaria is a disease caused by the parasite *Plasmodium sp*. through the female Anopheles mosquito vector. The prevalence of malaria in the world is relatively high. According to WHO data, in 2018, around 228 million malaria cases occurred worldwide, with the number of deaths reaching approximately 405,000 people.[1] Malaria signs include a high fever, headaches, chills, myalgia, malaise, and gastrointestinal (GI) issues. *P. falciparum* species can invade erythrocytes of all ages, isolate themselves in blood vessels, and develop vasoactive products, causing parasitemia, hypoglycemia, and multiorgan failure.[2,3] The malaria parasite *P. falciparum* enzyme lactate dehydrogenase (PfLDH) is studied as a possible molecular drug target. Chloroquine, as a widely used malaria drug, can interact directly with PfLDH in the NADH-binding pocket, creating the same bond position as the adenyl cofactor ring, resulting in chloroquine acting as a PfLDH inhibitor.[4,5] In the final step of glycolysis, the LDH enzyme catalyzes the conversion of pyruvate to lactate, which is needed for energy production in living cells.[6] Several compounds may compete with NADH to control PfLDH activity and inhibit hematin-to-hemozoin conversion. Hematin that accumulates and stays present in the parasite's food vacuole can eradicate the parasite. Several quinoline-derived

compounds, such as chloroquine, are thought to form complexes with hematin dimers to inhibit the development of hemozoin. NADH analogs have been identified as new possible PfLDH inhibitors.[7]
Various types of plant are used for traditional medicines to treat malaria; one of these plants is miana (*Plectranthus scutellarioides* (L) R.Br).[8] The people of South Sulawesi have local antimalarial ingredients consisting of miana leaves, betel fruit, and honey. The secondary metabolites isolated from miana leaves include scutellarioidone A; scutellarioidone B; scutellarioidone C; 6-acetylfredericone B; scutellarioidone D; scutellarioidolide A; coleon O; coleon G; lanugone K; fredericone B[9] quercetin[10], caffeic acid, and rosmarinic acid, apigenin and luteolin glycoside.[11,12] The activities of miana leaves that have been studied include its anti-inflammatory, anti-proliferative[8,9], and anti-diabetic properties[13], cyclooxygenases (COXs), and xanthine oxidase (XO) enzyme inhibition[14], and its use as an antihelmintic[15], anticestode[16], and antipyretic.[17] Miana is a Lamiaceae plant belonging to the genus Plectranthus. The Lamiaceae family's Plectranthus genus includes a variety of plants with varied organic activity and usage in traditional recuperation methods. Over 300 species in the genus are found in tropical and subtropical zones of Africa, Asia, and Australia.[18] In other studies, it has been stated that *Plectranthus Amboinicus* (Lour) Spreng, either the extract or some of the active compounds contained therein, has antimalarial activity.[19-21] The in-silico study was conducted first to streamline time and cost. In this study, compounds that have antimalarial activity will be identified by looking at the value of free energy. Docking is often used to predict the bond orientation of minor molecular drug candidates to their protein targets to predict the affinity and activity of small molecules.[22] Based on the description above, the antimalarial activity of the secondary metabolite compounds of miana leaves (*Plectranthus scutellarioides* (L) R.Br) was tested *in silico* against the *Plasmodium falciparum* lactate dehydrogenase (PfLDH) enzyme to see any interaction of the compounds with enzymes by looking at the value of the free binding energy (FEB) and the inhibition constant (Ki).

EXPERIMENTAL

Hardware and Software

The hardware used in this research was a set of personal computers with the following specifications: Intel® Core ™ i5-6400 CPU @ 3.90 GHz (4CPUs), GPU Nvidia GeForce GTX, 970 Gigabyte OC Edition, 8GB DDR4 RAM. Software programs used for this research were ChemDraw Ultra 12.0, MarvinSketch 5.2.5.1, Molegro Molecular Viewer 2.5, Autodock 4.2.6, and AutoDockTools 1.5.6, BIOVIA Discovery Studio Visualizer v21.1.0.20298, Toxtree 3.1.0, AMBER 16, and several web server's programs such as PdbSum, PreADMET, and RCSB PDB.

Preparation of Ligands and Receptor

The ligands used for this study were scutellarioidone A; scutellarioidone B; scutellarioidone C; 6-acetylfredericone B; scutellarioidone D; scutellarioidolide A; coleon O; coleon G; lanugone K; fredericone B; quercetin, and rosmarinic acid. The receptor was *Plasmodium falciparum* Lactate dehydrogenase (PfLDH) which can be downloaded via the Protein Data Bank (http://www.rcsb.org/) with the code PDB 1CET. The ligands were built in two dimensions using Marvin Sketch software and then protonated to produce a pH of 7.4. Then the structural conformation was determined by conducting a conformational exploration.[24,25] The 1CET receptor was separated from the ligands and water molecules using Molegro Molecular Viewer software so that only the protein structure of *P. falciparum* L-Lactate dehydrogenase (PfLDH) was used as the target protein. Then polar hydrogen was added so it could bind to the ligand using the AutoDockTools 1.5.6 program.

Molecular Docking and Visualization

The docking method is valid if the RMSD value is ≤ 2 Å [26,27], because of the similarity between the change of distance and the best conformation of the simulation Ligand with the distance and conformation of the initial ligand. The genetic algorithm was adjusted for 100 runs to see the best position for the bond between the ligand and the receptor. Molecular docking to the ligand-binding site of the PfLDH receptor was carried out for all tested ligands using the AutoDockTools program. The binding affinity of the 1CET-ligand complexes was expressed in docking scores. The results obtained from this docking process were the lowest Free Energy Binding (FEB).[28,29] Visualization was presented to determine the ligand-receptor interactions

in 2D and 3D forms. The best dock results were used to create complex files for visualization using BIOVIA Discovery Studio 2016 software.

Pharmacokinetics and Toxicity Predictions

The PreADMET program is a web-based application for predicting absorption, distribution, metabolism, and excretion data of test compounds using the in-silico method. The program can be accessed online through http://preadmet.bmdrc.org/.[25] The Toxtree application is used to predict the toxicity of test compounds with the parameters of Cramer Rules, the Kroes TTC decision tree, and the Benigni/Bossa Rulebase.[29,30]

Screening Ligand-Based Drugs Likeness (Drug Scan)

Lipinski's Rule of Five parameters could be determined using Marvin Sketch software. These parameters include log P <5, molecular weight <500 g/mol, molar refractory 40-130, hydrogen bond donor <5, and hydrogen bond acceptor <10.[31]

Molecular Dynamics Simulation

The compounds with the lowest FEB were subjected to Molecular Dynamics (MD) simulations. The AMBER ff14SB force field for proteins was used in the MD simulations. The ligand was exposed to the General AMBER force field (GAFF), and TIP3P water was added to the 10x10x10 box. After neutralizing ligand charges with restrained electrostatic potential, topology files were created (RESP). Amber16's Sander module was used for minimization, heating, and equilibration. In a dynamic simulation, before entering the molecular production stage, a heating system is used to allow the ligands and receptors to interact with the previously given force fields. Heating is done in three stages, from 0 to 310 K, at regular intervals, to approximate the physiological temperature of the body. This is followed by equilibration to bring the system back to a constant state before producing molecular dynamics simulations. The MD results can be used to calculate the Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF).[32-34]

RESULTS AND DISCUSSION

Molecular Docking and Visualization

Based on data from several journals, the compounds contained in miana leaves include scutellarioidone A, scutellarioidone B, scutellarioidone C, 6-acetylfredericone B, scutellarioidone D, scutellarioidolide A, coleon O, coleon G, lanugone K, fredericone B[9], quercetin[10], and rosmarinic acid.[12] The receptor in the simulation of molecular docking is the *Plasmodium falciparum* lactate dehydrogenase (PfLDH) enzyme with the code PDB ID 1CET, which contains the natural ligand chloroquine (CLQ).[5] Before docking, the test, and natural ligands passed the protonation stage at pH 7.4 to adjust the pH of the blood in the human body. This was the confirmation stage to get the most stable molecular position to interact with the receptor's active site. Receptor preparation ensures water removal because it can block the ligand-receptor interaction.

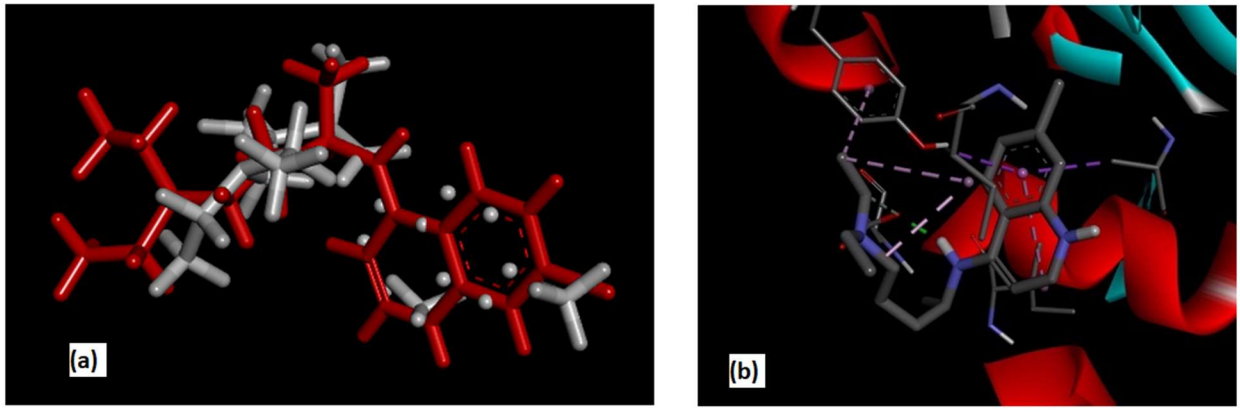


Fig.-1: (a) Overlay Conformation of CLQ Validated Results (white) and CLQ Crystallographic Results (Red); (b) Natural Ligand Complex (CLQ) against the 1CET Receptor

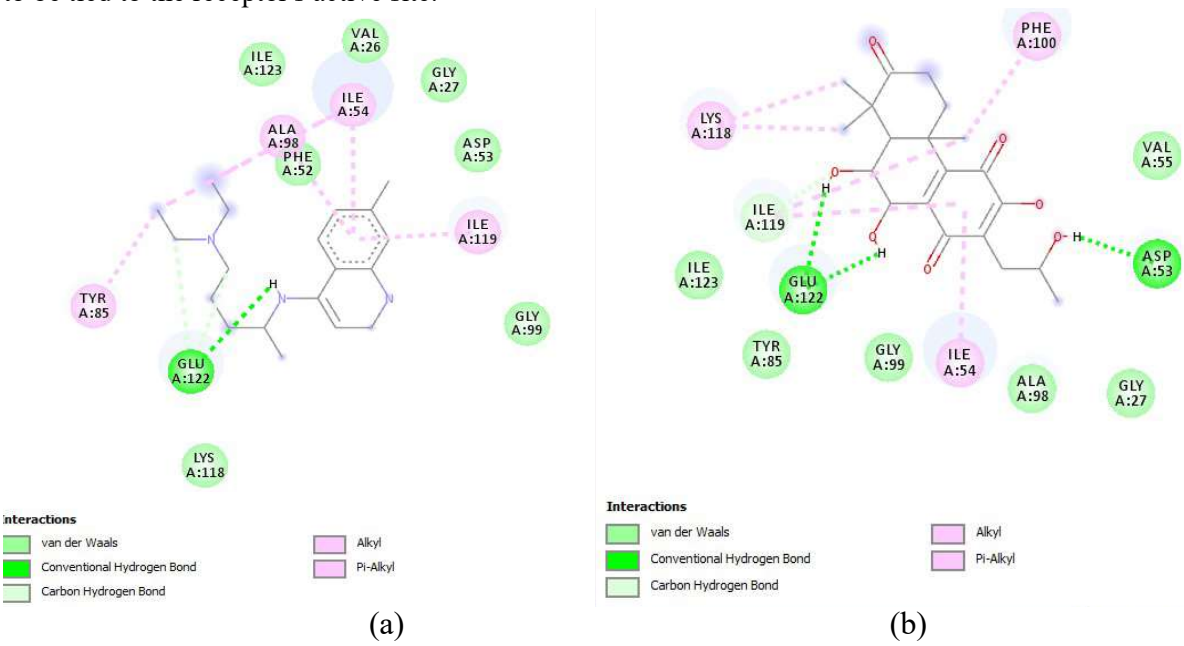
Figure-1 shows the redocking validation of the 1CET receptor with a grid box size of 30 x 34 x 30 and x, y, and z coordinates (36.211; 10.539; and 19.336) for the chloroquine ligand yielding an RMSD value of 1.13 Å. The valid receptor parameters are seen from the RMSD parameter ≤ 2 Å, which shows similarities based on comparing the change in distance and the best ligand conformation in the simulation with the distance and initial ligand conformation

Table-1: Docking Results of the Compounds of Miana Leaves in LfLDH Enzyme Receptor

No.	Compounds	FEB (Kcal/mol)	Ki (μM)	Amino Acid Interaction	
				Hydrogen Binding	Hydrophobic Binding
1.	Scutellarioidone A	-6.32	23.36	Ile:119, Glu:122, Asp:53	Ile:123, Tyr:85, Gly:99, Ala:98, Gly:27, Val:55
2.	Scutellarioidone B	-5.58	81.10	Ile:119, Glu:122, Tyr:85, Gly:99	Val:55, Gly:27
3.	Scutellarioidone C	-5.06	194.37	Glu:122, Gly:27, Ala:98,	Lys:118, Leu:115, Gly:99, Ser:28, Phe:52, Ile:123, Tyr:85
4.	6-Acetylfredericone B	-5.37	115.51	Tyr:85, Glu:122, Ile:199, Asp:53, Gly:27,	Leu:115, Val:26, Ile:123, Phe:52, Ala:98, Gly:99, Phe:100
5.	Scutellarioidone D	-6.06	36.41	Glu:122, Tyr:85, Gly:99, Ile:119, Phe:100	Ile:123, Ala:98, Asp:53
6.	Scutellarioidolide A	-4.35	645.83	Glu:122	Ala:98, Gly:99, Phe:100, Leu:115, Lys:118, Ile:119
7.	Coleon O	-6.07	35.29	Leu:115, Glu:122, Ile:119	Asn:116, Ala:98, Tyr:85, Ile:54
8.	Coleon G	-4.57	449.18	Glu:122, Ile:119	Tyr:85, Ile:54, Ile:123, Phe:100
9.	Lanugone K	-5.48	95.61	Glu:122, Ile:119	Gly:99, Gly:27, Asp:53, Tyr:85, Ile:123, Ile:54
10.	Fredericone B	-6.13	32.26	Glu:122, Tyr:85, Gly:99, Asp:53, Ile:119	Ala:98, Phe:100, Leu:115, Ile:123
11.	Quercetin	-5.63	74.09	Asp:53, Lys:118,	Gly:27, Val:55, Phe:100, Ile:121, Glu:122, Ile:123, Tyr:85, Phe:52, Val:26
12.	Rosmarinic Acid	-4.39	604.41	Glu:122, Tyr:85,	Ile:123, Phe:52, Val:26, Gly:99
13.	Chloroquine	-5.89	48.31	Glu:122	Lys:118, Gly:99, Asp:53, Gly:27, Val:26, Ile:123, Phe:52

Based on the docking results in Table-1, it was shown that compounds 1, 5, 7, and 10 have a better FEB value than the natural ligands. The lesser FEB value indicates that the conformation of the ligand-receptor is more stable. In contrast, a higher value of FEB suggests that the conformation of the complex is less stable. The more negative FEB values indicate better affinity of ligand-protein complexes and more stable interaction between the compounds and receptors. These will signify the activity of the compound. FEB

results will be directly proportional to the value of KI, whereas the value of KI gives an overview of the ability of compounds to inhibit an enzyme.[29] Figure-2 shows the interaction between ligands (chloroquine and Scutellarioidone A) and receptors (amino acid residues), both ligands have hydrogen bonds with different amino acid residues. The more the ligand binds with the receptor amino acids, the closer the distance between these bonds and the more stable. The presence of hydrogen bonds can affect a drug's chemical-physical properties so that it plays an essential role in the drug's biological activity. In comparison, this hydrophobic bond indicates the level of solubility of the drug in the cell membrane, which is predicted to be tied to the receptor's active site.



(a) (b)

Fig.-2: The 2D Visualization of Docking Results (a) Chloroquine; (b) Scutellarioidone A

Pharmacokinetics and Toxicity Predictions

Pharmacokinetic predictions of compounds were carried out using the PreADMET program with parameters of Caco-2, human intestinal absorption (HIA), and plasma protein binding (PPB), as shown in Table-2. Caco-2 cells are derived from the human adenocarcinoma colon, and they have a role as a route for drug transport through the epithelium of the intestine. Based on the table above, it can be seen that all secondary metabolite compounds of Miana leaves have the ability to penetrate moderate cell membranes because the values of the Caco-2 parameter are in the range of 4-70.2.[9] A compound must be able to penetrate the cell membrane to reach the target tissue so that it can provide activity.[35] The results of pharmacokinetic predictions can be seen in Table-2.

The HIA parameter was used to predict the percentage of drug absorption in the human intestine. Drug absorption was the number of drugs taken from the place of application to the systemic circulation. Compounds 1, 11, and 12 have a moderate ability to be absorbed in the body because they have HIA parameter values in the range of 20-70%, while other compounds can be well absorbed in the body with values range of 70-100%.[36] The efficiency of drugs can be affected by the degree of binding of the drug with proteins in the blood plasma (Plasma Protein Binding). The weaker the bond between the drug and plasma protein, the more efficient the drug crosses cell membranes. With Plasma Protein Binding (PBB) values <90%, all test compounds, except compound 11, have better results than chloroquine as a natural ligand. This means that they are weakly bonded to the plasma protein and have an excellent ability to be distributed in the body. Most of the secondary metabolite compounds tested from miana leaves are weakly bound to plasma protein and can be absorbed into the human body. The interaction between the ligand and the receptor shows how the ligands interact. The drugs with weak bonds have good stability, and they will easily detach so that they can be excreted. Meanwhile, the strong bonds will accumulate in the body and become free radicals because they cannot be separated from the receptors.[37]

Table-2. Pharmacokinetics Predictions of Miana Leaf Compounds

No	Compounds	Parameters		
		Caco-2 (nm/sec)	HIA (%)	PPB (%)
1.	Scutellarioidone A	20.99	63.15	50.65
2.	Scutellarioidone B	20.83	84.90	78.30
3.	Scutellarioidone C	20.11	80.41	70.87
4.	6-Acetylfredericone B	20.94	84.82	86.58
5.	Scutellarioidone D	20.76	92.51	86.14
6.	Scutellarioidolide A	29.61	97.98	88.21
7.	Coleon O	20.68	91.59	74.52
8.	Coleon G	20.68	91.59	79.80
9.	Lanugone K	21.07	90.19	80.99
10.	Fredericone B	20.76	92.52	81.95
11.	Quercetin	3.41	63.48	93.24
12.	Rosmarinic Acid	20.72	62.49	86.24
13.	Chloroquine	56.61	98.06	92.53

Toxtree software is a program designed to help investigators determine what toxicological properties research substances have. The toxicological properties examined mainly are carcinogenicity and mutagenicity, both of which are major concerns for human health. The Toxtree program deals specifically with test compounds, and determining toxicity is a cornerstone of drug development. The results of toxicity prediction can be seen in Supplementary Table-S1. The toxicity examination was carried out to predict the level of toxicity of the secondary metabolites of miana leaves in the human body. The results are shown in Table-S1. In this test, three toxicity parameters were used, i.e., Cramer Rules to see the level of toxicity from its functional groups, the Kroes TTC decision tree to estimate the exposure threshold for drug compounds in humans, and the Benigni/Bossa release to determine if the compound can cause carcinogenicity and mutagenicity.[30] High toxicity (Class III) means that high concentrations of the compounds are not guaranteed to be safe in use. From a safety point of view, Class III states that the substance's chemical structure gives an initial effect that is not too strong. However, a substance with this chemical structure may have significant toxicity. For example, heterocyclic substances, heteroaromatics, and other cyclic substances have side chains with reactive functional groups. The secondary metabolite compounds of miana leaves have functional groups, namely heteroaromatic groups, that contribute to increased toxicity.[38] Based on the Kroes TTC decision three parameter results, it was estimated that all compounds, except compounds 6, 12, and 13, have a negligible risk of toxicity. In contrast, the three compounds (6, 12, and13) are not expected to pose a safety risk. As for their Benigni/Bossa rulebase parameters, almost all test compounds can cause genotoxic carcinogenicity, but the results are negative for nongenotoxic, carcinogenicity, and mutagenicity. So, the results indicate that the compounds will not cause cancer.

Screening Ligand-Based Drugs Likeness (Drug Scan)

Lipinski's Rule of Five predictions was carried out on all test compounds as this is a requirement for drug candidates to be used orally.[38,39] The results are shown in Table-3. Molecular weight parameters related to the drug distribution process are fulfilled so these compounds can penetrate the cell membrane by passive diffusion.

Table-3: Screening of Ligand-Based Drug Likeness (Drug Scan of Miana Leaf Compounds)

No	Compounds	Parameters				
		Molecular Weight (MW)	Donor Proton	Acceptor Proton	Log P	Refractory Molar
		< 500 g/mol	< 5	< 10	< 5	40 - 130
1.	Scutellarioidone A	378.17	4	10	0.53	97.90
2.	Scutellarioidone B	448.21	2	10	2.03	115.57
3.	Scutellarioidone C	460.17	3	10	1.99	117.13
4.	6-Acetylfredericone B	432.19	2	10	3.04	112.00
5.	Scutellarioidone D	388.19	2	8	2.23	105.19

6.	Scutellarioidolide A	474.22	0	8	2.83	127.15
7.	Coleon O	388.19	2	8	1.73	101.84
8.	Coleon G	388.19	2	8	1.83	101.03
9.	Lanugone K	390.20	2	8	2.19	101.18
10.	Fredericone B	388.19	2	8	2.13	105.99
11.	Quercetin	302.04	5	8	2.16	76.86
12.	Rosmarinic Acid	360.08	5	9	3.00	90.95
13.	Chloroquine	319.18	1	4	3.93	96.42

Log P values for all test compounds were not greater than five (< 5). A significant Log P value indicates that the molecule is increasingly hydrophobic. The more hydrophobic the nature of a molecule, the higher the toxicity level of the molecule. Hydrophobic molecules will be held longer in the lipid bilayer because the lipid bilayer has hydrophobic properties.[31,39] Besides that, the molecules will also be distributed more widely in the body, reducing the selectivity of the target enzyme. The hydrogen bond donor values were <5, and the hydrogen bond acceptor values were <10. These values are related to the biological activity of a drug molecule. The presence of hydrogen bonds can affect the physical-chemical properties of a compound. The modification of these properties can affect the biological activity of a compound. The refractory molar values for all test compounds were between 40-130, which is the total polarizability value of drug molecules. It is very dependent on temperature, refractive index, and pressure.[39]

Molecular Dynamics Simulations

A molecular dynamics (MD) simulation was conducted for the selected compounds, namely Scutellarioidone A and chloroquine, using the AMBER16 program, and the results can be described using RMSD and RMSF values. Ligand and receptor parameterization was carried out using ff14SB, GAFF force file, and AM1-BCC.[34] RMSD analysis was performed to show that the protein remains stable and is not denatured. The increasing fluctuation shows that the protein structure begins to open, and the stability of the fluctuation indicates that the ligand has reached a stable conformation to bind to the protein. It can be seen from Fig.-3 that the scutellarioidone A tends to be stable starting from 7.400 ps with a lower RMSD value than scutellarioidone D, coleon O, fredericone B, and chloroquine.

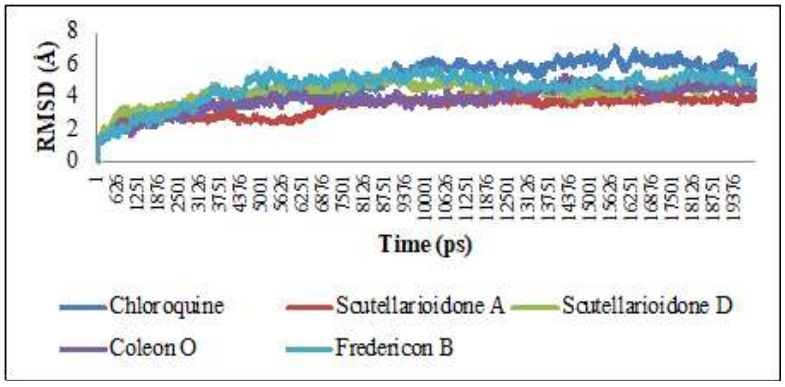


Fig.-3: The RMSD Value of the Compounds-1CET Complex during 20 ns MD.

The Root means square fluctuation (RMSF) is the difference in flexibility between residues. It identifies local changes in protein chain fluctuations. The RMSF is calculated for each residue that makes up the protein to see how much fluctuation in each residue's movement occurs during the simulation. Figure-4 shows that all residues fluctuate in the same region. Still, the most significant changes occur from the LEU39 to LEU64 residues, and the most considerable fluctuation occurs in the ILE54 amino acid residues, which occurs in the chloroquine-1CET complex. Aside from the RMSD and RMSD study, the results of dynamic molecular simulations can also be used to visualize the movement of the compound and the receptor interactions by looking at the visualization results of the trajectories from the start to the end. It can be shown in the dynamic molecular simulation of all the compound-1CET complexes that the scutellarioidone D, coleon O, fredericone B, and chloroquine are still in the binding site but the scutellarioidone A is slightly outside of the binding site; this can be seen in Fig.-5.

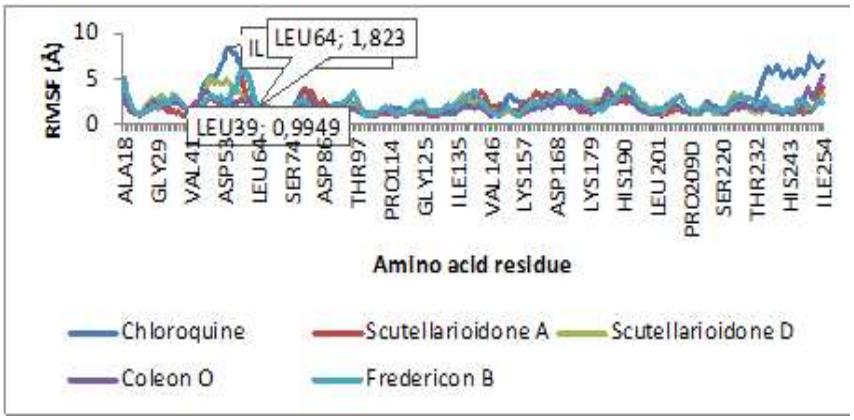


Fig.-4: The RMSF Curve during 20 ns MD Simulation for the Compounds-1CET Complex

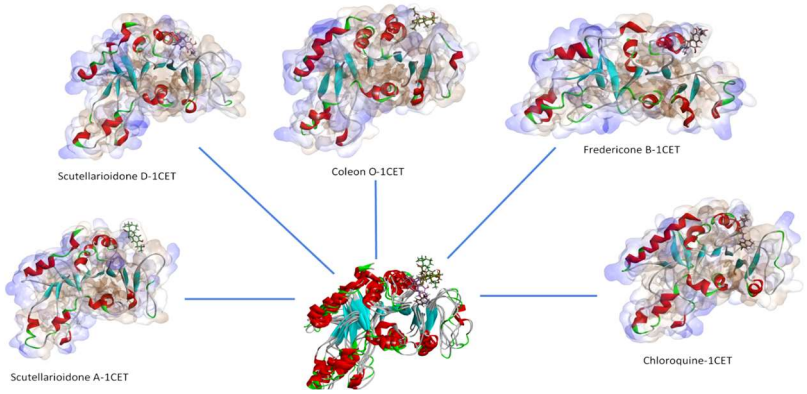


Fig.-5: The Trajectory of All the Compounds in 1CET after 20ns Molecular Dynamic Simulation

Meanwhile, the trajectory change during the scutellarioidone A simulation can be seen in Supplementary Fig.-S1. This visualization's results differ from the results of the changes in a trajectory that occurred in the other complexes. During the dynamic molecular simulations at 1ns and 5ns, the scutellarioidone A was still at the binding site and still interacted stably with the receptor. Still, starting at 5ns to 20ns, the scutellarioidone A began to move away from the receptor binding site even though there were still amino acid residues that interacted with the receptors. As a result, the interaction between the scutellarioidone A and the receptor begins to weaken, and this visualization change can be seen in Supplementary Fig.-S1. The results of the Ramachandran plot in Supplementary Fig.-S2 show the stability of the receptor due to its interaction with the compounds after a molecular dynamic simulation for 20ns. The amino acid residue in the disallow region was only in the scutellarioidone A-1CET complex (2.6%). From the results of the Ramachandran plot**,** Fig.-S2, it can be explained that at the end of the molecular dynamic simulation, 20ns, the scutellarioidone D-1CET complex was more stable than the others. This is also supported by the number of amino acid residues that contribute to the stability of the interaction, as can be seen in Fig.-6.

CONCLUSION

Based on the results of the study, it can be concluded that there are four compounds, namely scutellarioidone A (-6.32 kcal/mol), scutellarioidone D (-6.06 kcal/mol), Coleon O (-6.07 kcal/mol), and fredericone B (-6.13 kcal/mol), in silico that have better activity than the natural ligand chloroquine (-5.89 kcal/mol,) and these can be used as candidates for new antimalarial drugs. From the analysis of the dynamic molecular results for 20ns, it can be predicted that scutellarioidone D has a more stable interaction than others, and it is almost the same as chloroquine, so scutellarioidone D can be predicted to be useful as an antimalarial candidate.

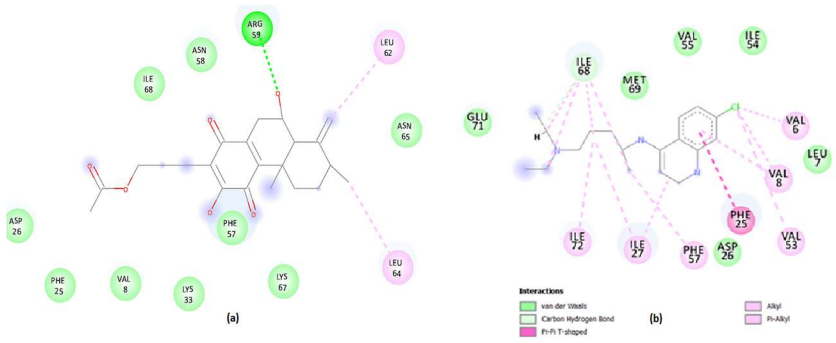


Fig.-6: The 2D visualization of Scutellarioidone D (a) and Chloroquine (b) in 1CET after 20ns Molecular Dynamic Simulation

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