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Potential of Indonesian Medicinal Plant Biodiversity as CHK1 Inhibitor Agent for Cancer Treatment by Bioinformatics and Computational Chemistry

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Indonesia is rich in flora diversity, which has the potential as compounds that can be validated as candidate CHK1 inhibitor agents. The research was conducted in *silico* by performing molecular docking using Autodock tools. The reference ligand was found from the 4FT3 receptor with the H1K code on the PDB. The result of docking in the reference ligand obtained a Binding Free Energy value of -6.48 kcal/mol. The next process was carried out molecular docking to 24 selected test compounds. The processes carried out are molecular docking, molecular visualization, "Lipinski's Rule of Five" testing and Pre-ADMET testing. Based on Binding Free Energy, 18 molecules met the criteria and the best 3 were selected for analysis, namely stigmasterol, laurifolin, and quercetin with Binding Free Energy values of -10.40 kcal/mol, -8.70 kcal/mol, and -8.04 kcal/mol respectively. Based on the Pre-ADMET test, quercetin belongs to GSH toxicity class 4 which means the compound has a mild toxicity effect compared to other compounds. The molecular dynamics results showed that quercetin is the most stable compound and binds the longest compared to the others. The results can be continued for further research through laboratory testing stages in vitro and in vivo.

Keywords: CHK1 | in silico | quercetin | reference ligand |

According to the Basic Health Research (Riskesdas)[1], cancer is one of the diseases that cause high mortality rates in Indonesia. There are several factors that cause individuals to develop cancer, such as family history, age, and lifestyle and environment. Mutations that cause cancer can occur in various genes. One well-known mutation is a mutation to TP53 that causes the production of P53 to be disrupted. When P53 production is disrupted, cell growth becomes uncontrolled, leading to cancer[2]. To date, no drugs have been found that can target the P53 protein directly. However, an alternative protein has a synthetic lethality relationship with the P53 protein, CHK1. This protein controls the cell cycle so inhibition of this protein can stop protein growth[3]. Inhibition of the CHK1 protein is an interesting cancer treatment pathway as it is able to reactivate p53 pathway and promote cell death. The CHK1 protein contains a specific amino acid residue called THR68 (Threonine 68) that plays a crucial role as it can restore the function of the p53 tumour pathway in cancer cells that have lost p53 activity. When it is inhibited, it will respond by disrupting the DNA Damage response pathway and leading to replication stress, causing DNA damage and triggerings apoptosis in cancer cells that have defective p53. Indonesia is an archipelago that has 35,000 islands and contains various types of flora and fauna. This is supported by more than 40,000 plant species found and 180 species identified as medicinal plants. The potential biodiversity in Indonesia is very high, making the discovery of cancer drugs more feasible[4].

Methods

The research was conducted in silico to test the potential of candidate secondary metabolites as ligands for CHK1 protein. CHK1 crystal structure data was taken from the RCSB Protein Data Bank (PDB). Data on candidate secondary metabolites were obtained through PubChem. Binding of the reference ligand will be carried out using the assistance of AutoDock Tools. The validation of the results of the reference ligand follows the condition of RMSD value below 2Å. AutoDock Tools also provides a record of the binding energy between the candidate compound and the receptor at certain positions. Next, molecular visualisation was carried out with the Discovery Studio Visualizer to view the interaction between the ligand and the receptor. Molecular dynamics was then conducted to confirm the docking results of the best candidate compounds. The testing of chemical compounds against the five Lipinski rules and Pre-Absorption Distribution Metabolism Excretion Toxicity (Pre-ADMET) was conducted to predict the pharmacokinetic properties and toxicity of drug compounds which determine safety for human consumption. Various secondary metabolite compounds were tested through the same stages to find the most optimal compound.

Tool

The tools used in this study were Windows computers with Intel(R) Core(™) i5 specifications, 16GB RAM, and Windows 10 OS; OpenBabel software, AutoDock Tools, PyMOL, and Discovery Studio Visualizer 2017.

Materials

The materials used in this study were obtained from RCSB PDB and PubChem. Materials include the crystal structure of CHK1 (PDB code: 4FT3), the 3D structures of 24 compounds[5] (Table 1).

Detailed Procedure

The crystal structures of CHK-1 are collected from RCSB PDB website. The unique ligand is seen on the website and noted to be the reference ligand. By AutoDock Tools, the CHK-1 are separated into the receptor and the reference ligand. Docking of receptor and reference ligands is done to observe the RMSD value and binding energy. The dimension of the grid box (40, 40, 40) and the coordinates X, Y, Z (7.302, -4.298, 10.05) are recorded to be the control variable. Molecular docking is done between the receptor of CHK-1 and the secondary metabolites. Each of the docking

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conducted has a total of 10 runs with 10 poses. The best pose of the ligand is picked by validating the binding energy. The best compounds are then chosen by validating the binding energy of the compounds with the reference ligand. The interaction of the receptor and the best compounds are observed using Discovery Studio Visualizer.

Table 1. The compounds used in molecular docking

No.	Secondary Metabolites	Ligand	Pubchem CID
1.		Flavone	10680
2.		Flavanol	25201487
3.		Isoflavone	72304
4.		Flavanone	10251
5.	Flavonoid	Anthocyanin	101115386
6.		Laurifolin	44257868
7.		Quercetin	5280343
8.		Myricetin	528167
9.		Elatin	44257938
10.		Chalcone	637760
11.		Phenol	996
12.	Phenolic	Catechin	1203
13.		Chrysin	5281607
14.		Kaempferol	5280867
15.		Stigmasterol	5280794
16.		Campesterol	173183
17.	Steroid	Ergosterol	444679
18.		Lanosterol	246983
19.		Brassinosteroid	13039058
20.		Isoquinoline	8405
21.		Quinolizidine	119036
22.	Alkaloid	Indole	798
23.		Imidazole	795
24.		Acridine	9215

This is conducted to view and validate further the 2D structure of the interaction. After validation, molecular dynamic simulation is conducted towards the best compounds with receptors..The molecular dynamic simulation is done with a period of 100 nanoseconds (ns). The best compounds are then tested with Lipinski's rule of five through SCFBio Lipinski website. It's done to determine the compatibility of the compounds with pharmacological activity and physical suitability to be used as active drugs. The procedure was then followed by testing Pre-Absorption Distribution Metabolism Excretion Toxicity through pkCSM website. It's done as a parameter of pharmacokinetic properties and drug toxicity to ensure the drug's safety for human consumption.

Result

Molecular Docking

The study uses the crystal structure of CHK1 (4FT3) as the receptor and reference ligand (Table 2) that sets in the binding energy of -6.48 kcal/mol and RMSD of 0.84Å. The results were evaluated by the parameter of RMSD below 2Å for best docking results. RMSD values measure the similarity of the structure of the real ligand

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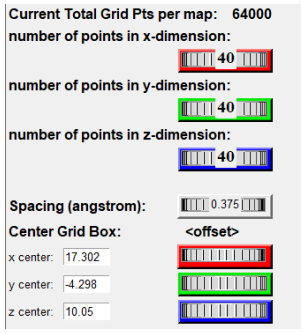
position in the receptor with the computed position of the reference ligand; this suggests that a ligand-receptor docking with a low RMSD value is considered to be accurate. As the RMSD value of the reference ligand is 0.84Å, the structure of the ligand with the initial real ligand has a high similarity. The binding energy of the docking is preferably to be at a low negative value as it shows the lowest energy of binding in the receptor's active site.

Table 2. The molecular docking result of the reference ligand

Root Mean Square Deviation (RMSD) dan Binding Free Energy	Dimension and Coordinates of Grid Box	2D Visualization

Binding Energy	Cluster RMSD	Reference RMSD
-6.48	0.00	0.84
-6.48	0.35	0.73
-6.48	0.35	0.74
-6.48	0.36	0.74
-6.48	0.29	0.74
-6.48	0.29	0.75
-6.48	0.31	0.75
-6.46	0.40	0.72
-6.45	0.41	0.76
-6.44	0.23	0.76



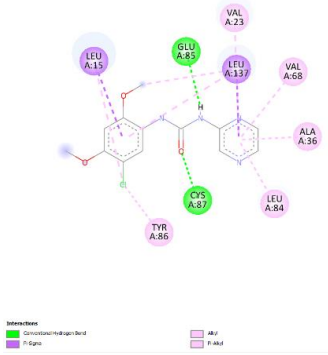


Table 3. The molecular docking results of 24 secondary metabolites compounds

No.	Secondary Metabolites	Ligand	Binding Free Energy ΔG
		Reference Ligand	RMSD: 0,76Å Binding Free Energy: -6.48 kcal/mol
1.	Flavonoid	Flavone	Binding Free Energy: -7.11 kcal/mol
2.		Flavanol	Binding Free Energy: -7.19 kcal/mol
3.		Isoflavone	Binding Free Energy: -7.11 kcal/mol
4.		Flavanone	Binding Free Energy: -7.02 kcal/mol
5.		Anthocyanin	Binding Free Energy: -6.58 kcal/mol
6.		**Laurifolin**	**Binding Free Energy: -8.70 kcal/mol**
7.		**Quercetin**	**Binding Free Energy: -8.04 kcal/mol**
8.		Myricetin	Binding Free Energy: -7.76 kcal/mol
9.		Elatin	Binding Free Energy: -8.01 kcal/mol
10.	Phenolic	Chalcone	Binding Free Energy: -7.16 kcal/mol
11.		Phenol	Binding Free Energy: -3.85 kcal/mol
12.		Catechin	Binding Free Energy: -7.89 kcal/mol
13.		Chrysin	Binding Free Energy: -7.39 kcal/mol
14.		Kaempferol	Binding Free Energy: -7.44 kcal/mol
15.		**Stigmasterol**	**Binding Free Energy: -10.40 kcal/mol**
16.		Campesterol	Binding Free Energy: -10.30 kcal/mol

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17.	Steroid	Ergosterol	Binding Free Energy: -10.33 kcal/mol
18.		Lanosterol	Binding Free Energy: -9.81 kcal/mol
19.		Brassinosteroid	Binding Free Energy: -9.28 kcal/mol
20.	Alkaloid	Isoquinoline	Binding Free Energy: -4.95 kcal/mol
21.		Quinolizidine	Binding Free Energy: -4.83 kcal/mol
22.		Indole	Binding Free Energy: -4.41 kcal/mol
23.		Imidazole	Binding Free Energy: -2.57 kcal/mol
24.		Acridine	Binding Free Energy: -6.21 kcal/mol

▲Legend : Bolded Compound represents the best compounds based on the parameter

Twenty-four secondary Indonesia natural compound were tested for their potency, and three of them were chosen as the most suitable compounds. The binding energy of the reference ligand was set as the parameter, indicating that compounds with binding energy lower than the reference ligand were considered valid. The three selected compounds, namely quercetin, laurifolin, and stigmasterol, had the lowest binding energy values of -8.04 kcal/mol, -8.70 kcal/mol, and -10.4 kcal/mol, respectively (Table 3).

Molecular Visualization

Molecular visualization was conducted towards the three best compounds to confirm their interaction with receptor[6]. Hydrogen bonds are an important component used in the analysis because they indicate the level of intermolecular stability. Thus, obtaining more hydrogen bonds results in a greater bond-free energy value between the kinase enzyme and the substrate. Within the hydrophobic interactions, there are two types of bonds - pi-alkyl and alkyl bonds - that help confirm the ligand and structural interactions with the receptor binding pocket.

Molecular Dynamics Simulations

The three compounds were subjected to molecular dynamics simulations to confirm their efficacy. The results showed that quercetin exhibited the highest stability among the three compounds. In contrast, laurifolin initially showed smooth binding with the receptor, but later detached from it. On the other hand, stigmasterol performed poorly in the simulation, showing immediate detachment from the receptor. These findings suggest that quercetin may be a promising candidate for further studies on its interaction with the receptor. However, the poor performance of stigmasterol warrants further investigation into its binding mechanism[7].

Lipinski's Rule of Five

To assess the compounds' suitability as drug candidates, Lipinski's rule of five was employed to test their solubility and permeability[8]. The examination was conducted on the three best compounds and the reference ligands. The results revealed that both the reference ligand and the best compounds, quercetin and laurifolin, passed the examination, indicating their potential as drug candidates. However, stigmasterol failed the examination with a Log-P value of 7.800803, suggesting that it may not be a suitable drug candidate due to its poor solubility and permeability. These findings highlight the importance of considering the physicochemical properties of compounds when selecting potential drug candidates.

Pre-ADMET

The Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) tests were performed on the reference ligands and the three selected compounds. Results showed that none of the compounds exhibited skin permeability. Only stigmasterol was found to have the ability to pass through the central nervous system (BBB) and exhibited a relatively good LD50 value. In contrast, the co-crystallized ligands displayed satisfactory values across all test indicators.

Discussion

Molecular Docking

The Binding Free Energy value is a parameter used to determine if a reaction occurs spontaneously or not. The more negative the binding energy results, the stronger the intermolecular interactions [9]. Based on the data obtained, the reference ligand has a free energy value of -6.48 kcal/mol when the grid box coordinates are 17.302, -4.298, and 10.050 with an RMSD value of 0.84 Å. Among all the secondary metabolites, stigmasterol is the compound that will produce the strongest bond at -10.40 kcal/mol. However, steroid secondary metabolites are reactive compounds and can interact with various active sites (Table 4). This is because steroids contain several functional groups like carbonyl, hydroxyl, and double bonds capable of forming strong covalent bonds with other molecules. Steroids also have complex 3D structures and are highly lipophilic, allowing them to have a strong affinity for lipid membranes and other hydrophobic membranes. The combination of high reactive nature, complex 3D structure, and lipophilic properties makes steroids are at high risk in producing false positive values. Thus, data from other compound groups was needed for data reliability. The Flavonoid group is one of the best alternatives where there are Laurifolin compounds with -8.70 kcal/mol and Quercetin with -8.04 kcal/mol. Therefore, the compounds above are compounds that can potentially be drug candidates for CHK1 kinase inhibitor agents.

In the in silico method, the best compounds determined from the Binding Free Energy analysis are continued for visualisation in two dimensions. Indicate the level of intermolecular stability. Thus, the more hydrogen bonds are obtained, the bond-free energy value generated between the kinase enzyme and the substrate will also be greater. Within the hydrophobic interactions, there are types of pi-alkyl and alkyl bonds that help confirm the ligand and structural interactions.

Stigmasterol does not form a hydrogen bond. However, stigmasterol has a hydrophobic interaction: alkyl residues LEU^{137}, VAL^{23}, CYS^{87}, LEU^{15}, ALA^{36}, LEU^{84}, and LYS^{38}.
Laurifolin forms hydrogen bonds on residues CYS^{87}, GLU^{85}, SER^{147}, and GLU^{134}. This compound also has hydrophobic bonds, namely pi-alkyl on ALA^{36}, LEU^{15}, dan VAL^{23} and pi-sigma on LEU^{137}. Quercetin can also make hydrogen bonds on residues GLU85, CYS87, GLU91, and ASP94. This compound also has hydrophobic bonds, namely pi-alkyl on ALA36 and LEU15 and pi-

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sigma on VAL23 and LEU137. Based on the compounds obtained, it can be analysed that stigmasterol has a high bond-free energy but cannot make hydrogen bonds. Thus, it can be assumed that stigmasterol gives a false positive result as it is also a part of the sterol group and requires more testing. For the flavonoid group, laurifolin and quercetin have more hydrogen bonds than the reference ligands. Thus, it can be assumed that laurifolin and quercetin can replace the reference ligand in binding to the receptor because the two compounds can form more hydrogen bonds, so the bond between the receptor and laurifolin and quercetin is stronger. The three compounds above also have the ability to form hydrophobic bonds between alkyl, pi-alkyl, and pi-sigma.

Table 4. The Molecular Interaction of Tested Compounds

Tested Compounds	Hydrogen Bonds	Hydrophobic Interaction	
		Type of Bonds	Amino Acid Residue
Reference Ligand	GLU85 CYS87	Pi-Sigma	LEU^{15} LEU^{137}
		Pi-Alkyl	VAL^{23} VAL^{68} LEU^{84} ALA^{36} TYR^{86}
Stigmasterol	-	Alkyl	LEU^{137} VAL^{23} CYS^{87} LEU^{15} ALA^{36} LEU^{84} LYS^{38}
Laurifolin	CYS^{87} GLU^{85} SER^{147} GLU^{134}	Pi-Alkyl	ALA^{36} LEU^{15} VAL^{23}
		Pi-Sigma	LEU^{137}
Quercetin	GLU^{85} CYS^{87} GLU^{91} ASP^{94}	Pi-Alkyl	ALA^{36} LEU^{15}
		Pi-Sigma	VAL^{23} LEU^{137}

Table 5. Specification of Indonesian plants

Plant's name	Scientific name	Origin	Part that is used	Metabolite Secondary Composition
Ranti Leaves or Bobosa	*Solanum nigrum L.*	Java and Maluku	Leaves	Flavonoid, Alkaloid, Saponin, and Tannin
Marungga (Kelor) Leaves	*Moringa oleifera*	East Nusa Tenggara	Leaves	Flavonoid, Alkaloid, Phenolate, and Triterpenoid
Cemara Sumatera	*Flemingia macrophylla*	West Sumatera	Leaves	Flavonoid, Alkaloid, Phenolic, and Triterpenoid

Based on the compounds obtained, it can be analyzed that stigmasterol has a high bond-free energy but cannot make hydrogen bonds. Thus, it can be assumed that stigmasterol gives a false positive result as it is also a part of the sterol group and requires more testing. For the flavonoid group, laurifolin and quercetin have more hydrogen bonds than the reference ligands. Thus, it can be assumed that laurifolin and quercetin can replace the reference ligand in binding to the receptor because the two compounds can form more hydrogen bonds, so the bond between the receptor and laurifolin and quercetin is stronger. The three compounds above also have the ability to form hydrophobic bonds between alkyl, pi-alkyl, and pi-sigma.

The result of molecular docking enables the discovery of the potential for best compounds such as stigmasterol, laurifolin, and quercetin. As these compounds can be found in natural resources, it also may exist in plant species originated in Indonesia. This is because Indonesia is the second largest biodiversity country and rich in natural resources.

In Indonesia's plant biodiversity, three plants were found and chosen as a representative that could be sources of the above compounds [10]. First, ranti or bobosa leaves are plants that can be found in Java and Maluku. This plant was identified to contain flavonoids, alkaloids, saponins, and tannins. Second, Sumatran cypress from West Sumatra. The leaves of the Sumatran cypress contain flavonoids, alkaloids, phenolic and triterpenoid compounds. Third, marungga or moringa leaves from East Nusa Tenggara. In the leaves, flavonoids, triterpenoids/steroids, alkaloids, phenolics, and tannins were found[11].

Molecular Dynamics Simulation

Molecular dynamics is utilized to confirm the docking results obtained. This is necessary because docking only reveals a limited number of poses, whereas molecular dynamics can provide animated poses at a nanosecond scale, enabling us to observe the interactions between molecules.

The confirmation of the obtained docking results was carried out through molecular dynamics to visualize the interactions between the ligands at a nanosecond scale. The molecular dynamics results demonstrated that the best ligand based on the initial docking test, stigmasterol, was unstable with RMSD values ≥2 and was eventually released from the CHK1 protein. This finding indicates that stigmasterol is a false positive, which could be attributed to its reactive properties and its tendency to bind to other protein receptors with mostly van der Waals interactions. In contrast, the compounds laurifolin and quercetin demonstrated RMSD values <2, indicating greater stability and longer binding times. Quercetin exhibited a more stable graph than laurifolin and persisted in binding with receptors for up to 100 nanoseconds. While laurifolin displayed a tendency to detach and rebind, further testing on other receptors is needed to determine its potential as an inhibitory agent.

As it was found, quercetin is known to be the most stable compound and lasts the longest in binding to the receptor. RMSD Receptor graphic data (Figure 2), quercetin actually tends to be at 5Å compared to stigmasterol and laurifolin (Figure 1) which has a lower value. Thus, the higher RMSD of the receptor can make the compound more stable and bind longer.

Furthermore, the Root-Mean-Square Fluctuation (RMSF) graph also shows quercetin starting at the N-Terminal with a lower value up to a higher value at the C-Terminal. The graph that tends to go up shows that the protein becomes more reactive and moves. Quercetin, which is the compound that is proven to be the most stable, has an upward trend so it can be said that quercetin prevents the CHK1 protein from working. CHK1 itself is a signalling protein that can give signals to P53 which can give orders to produce cells abnormally. If quercetin prevents the CHK1 protein from working, then P53 cannot receive the signal given by CHK1. Thus, cells will not have abnormal growth and cause cancer.

Comparing the three images, stigmasterol (Figure 4) and laurifolin (Figure 6) are disconnected from the bonds, while quercetin remains stable (Figure 3), validating the previous data. In Figure 3, high

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fluctuations of quercetin confirm its stable binding, unlike stigmasterol which has a lower fluctuation due to detachment.

Molecular Mass Analysis

In seeing the potential of compounds as drugs, molecular mass has an important role. For a drug to form a bond with a cell receptor, it needs to penetrate the biological membrane by the process of diffusion. Drug compounds with a mass of more than 500 Daltons have a relatively larger size, making it difficult to be absorbed and penetrate biological membranes. So, it would be better if the compound has a molecular mass below 500 Daltons.

Stigmasterol has the largest molecular mass, which is 412 Daltons. Laurifolin took second place, with 356 Daltons. Then, followed by a reference ligand with a molecular mass of 308.5 Daltons, and quercetin with a molecular mass of 302 Daltons. From the data obtained, these four compounds do not have a molecular mass of >500, so they do not violate any of the “Lipinski’s Rule of Five” requirements.

H-Donor and H-Acceptor Analysis

There are two things to note: the number of hydrogen bond donors and acceptors. The number of hydrogen bond donors is the total number of bonds between hydrogen and nitrogen and hydrogen and oxygen. The number of hydrogen bond acceptors is the sum of the nitrogen and oxygen atoms. Hydrogen bond analysis can reveal a molecule's physical-chemical properties such as melting point, acidity, water solubility, and boiling point. According to Lipinski's Rule of Five, a molecule is said to be good if it has < 5 donors and < 10 hydrogen acceptors.

Quercetin has the highest number of hydrogen donors, namely 5. Followed by laurifolin which has 3 hydrogen donors, a reference ligand with 2 hydrogen donors, and stigmasterol with 1 hydrogen donor. Of the four compounds, none violates the requirements of " Lipinski's Rule of Five "; the number of hydrogen bond donors cannot be more than 5. The reference ligands and quercetin have 7 acceptors, laurifolin has 6 acceptors, and stigmasterol only has 1.

Log-P Analysis

The value of the octanol partition coefficient of water (log-P) is an important measurement in determining a compound's ability as a drug to penetrate biological membranes. The greater the log-P value, the molecule has a higher level of hydrophobicity. However, if the log-P value is too high or too low, the drug molecule cannot penetrate the biological membrane and therefore cannot work properly. According to the terms of "Lipinski's Rule of Five", the log-P value cannot exceed 5 and it is better if the result is positive.

All four compounds have positive log-P values. However, it can be seen that stigmasterol has a log-P value exceeding 5, namely 7.800803. This means that stigmasterol does not meet the requirements of "Lipinski's Rule of Five". Whereas the other three compounds have fulfilled “Lipinski’s Rule of Five” requirements. Laurifolin has a log-P value of 2.878698, comparator ligand has a log-P value of 2.791199, and quercetin has a log-P value of 2.0109.

Refraction Molar Analysis

Molar refraction is a measure of the polarizability of a drug molecule. Polarisability is the ability of a molecule to make dipole relationships momentarily with other molecules. According to "Lipinski's Rule of Five", the molar refraction value needs to be between 40 to 130 m3/mol.

Stigmasterol has the largest molar refractive value, namely 128.122742 m3/mol. Laurifolin occupies the second position with the largest molar refraction value of 92.947861 m3/mol. The reference ligand and quercetin had slightly different molar refractive values, namely 79.229385 m3/mol and 74.050476 m3/mol. Thus, the four compounds have fulfilled the requirements of "Lipinski's Rule of Five".

The comparator ligand (H1K), quercetin, and laurifolin complied with all five requirements of "Lipinski's Rule of Five" while the secondary metabolite compound stigmasterol violated one of the requirements, namely regarding the value of the octanol partition coefficient of water (log-P). Thus, according to "Lipinski's Rule of Five", the comparator ligands, quercetin, and laurifolin have the best potential to become oral drugs because they have fulfilled all the requirements needed.

Absorption Test Analysis

In the absorption parameter, two categories are the main assessment: the value of absorption and skin permeability. Meanwhile, poor absorption has a value of less than 30%. The data retrieved shows that stigmasterol has an absorption rate of 94.97%, and laurifolin at 74%. And quercetin at 77%. Stigmasterol is superior to the reference ligands, with a higher absorption rate of 3.97%. This shows that stigmasterol and the reference ligand have good absorption values. Meanwhile, laurifolin and quercetin have sufficient absorption ability. Meanwhile, according to Pires et al. (2015), a compound has good skin permeability if the Log Kp value is less than -2.5 and relatively low if the Log Kp value is more than -2.5. Quercetin has a Log Kp value closest to -2.5 at -2.735, and laurifolin at -2.743 and stigmasterol in all compounds with low skin permeability where the Log Kp value is above -2.5.

Distribution Test Analysis

In testing the distribution, the two main assessment categories are the classification of the volume of distribution and the permeability of the blood-brain barrier (BBB). The volume of distribution of a drug is the volume over which the total dose of the drug needs to be uniformly distributed to achieve theoretically the same concentration as blood plasma. If the log-VD value is less than -0.15, the volume of distribution is low, so less drug is distributed in the blood tissue compared to plasma. Meanwhile, a VD log value that exceeds 0.45 is considered high. Laurifolin and quercetin have a high volume of distribution of 0.891 and 1.559 respectively, so they can be distributed evenly to reach blood plasma concentrations. The permeability of the blood-brain barrier is one part of the criteria assessed in distribution testing. This parameter examines the ability of the compound to penetrate the blood-brain barrier. The BBB parameter also shows the potential blood vessels' ability to conduct the nervous system's vasculature, which strictly regulates the movement of ions. If the compound is above LogBB <0.3, then the compound can penetrate the brain barrier properly. Meanwhile, if the compounds are under LogBB <-1, then the compounds cannot be distributed properly. Based on the data obtained, it can be concluded that stigmasterol is the most superior compound followed by laurifolin which is in the middle of the parameters, and quercetin which cannot be distributed properly.

Metabolism Test Analysis

In the involved assay, parameters involving the CYP2D6 substrate–inhibitor classification. Most metabolic reactions involve oxidation processes. One of the most common detoxification enzymes found in the liver is called cytochrome P450. This enzyme has a way of working by oxidising compounds that are considered foreign. By inhibiting cytochrome P450 enzymes, drug metabolism is also contraindicated against P450 enzymes. So this test is important to assess the ability of a compound to inhibit cytochrome P2D6 (CYP2D6). Through the data obtained, it is known that the secondary metabolites of stigmasterol, laurifolin, and quercetin, as well as the reference ligand, do not inhibit the CYP2D6 enzyme. Then, it can be concluded that the compounds tend to be metabolised by the P450 enzyme.

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Exertion Test Analysis

To predict the excretion process of a compound, it can be done by measuring the Total clearance clarification (CLTOT) and the Renal Organic Cation Transporter 2 (OCT2) substrate. Total clearance is a combination of metabolism in the liver and bile (hepatic clearance) with excretion through the kidneys (renal clearance). This is related to bioavailability, and it is important to determine the dose level in achieving steady-state concentrations. The CLTOT values of the tested compounds ranged from 0.197 to 0.618. Based on the values obtained, the speed of the compound excretion process can be predicted. Furthermore, the parameter of Renal Organic Cation Transporter 2 (OCT2) substrate is a transporter in the kidney which has an important role in the clearance of drugs and endogenous compounds. If the compound is an OCT2 substrate, the compound can cause side effects. All secondary metabolite compounds in the ADME table showed that none of them affected the OCT2 substrate.

Toxicity Test Analysis

Toxicity tests can be divided into three classifications: the Ames Toxicity classification, LD50 (Lethal Dose50), and the maximum dose tolerated by humans[12]. Toxicity tests carried out in the laboratory can take a lot of time and effort, so they are more efficient to do through software. First, the Ames Toxicity classification is a method for assessing the mutagenic potential of a compound using bacteria. Genetic damage can occur if a compound has mutagenic potential, leading to gene mutations. The reference ligand compounds, stigmasterol, quercetin, and laurifolin did not show positive results after going through the Amex Toxicity test. This means that the four compounds are predicted not to be mutagenic.

The next toxicity prediction that was carried out was the oral in *silico* toxicity test on rodents (LD50). LD50 is the amount of compound given that can cause the death of 50% of the experimental animal group. The reference ligands, quercetin, laurinol, and stigmasterol belong to the toxicity class ($2000 < LD50 \leq 5000$ mg/kg). This shows that the selected secondary metabolites have the potential to cause side effects. Thus, further testing is needed to prove the side effects of these compounds. Furthermore, all secondary metabolites above also belong to the 4 GSH toxicity class, meaning the compounds are mildly toxic. On the other hand, when viewed from the toxicity class tabulation from Hodge and Sterner (1949), all compounds in toxicity class 4 have relatively low toxicity. The maximum dose that can be tolerated is the limit where the treatment does not cause side effects or toxicity within a certain time. The maximum tolerated dose is determined in clinical trials by testing dose increases in different groups of people until the highest dose with acceptable side effects is found.

Based on the data gotten, the reference ligand can be consumed the most compared to other test compounds, with a value of 0.615 log mg/kg/day. On the other hand, quercetin as the best test compound can be consumed as much as 0.449 log mg/kg/day. Therefore, the reference ligand can be consumed in higher doses than quercetin with a dose difference of 0.166 log mg/kg/day.

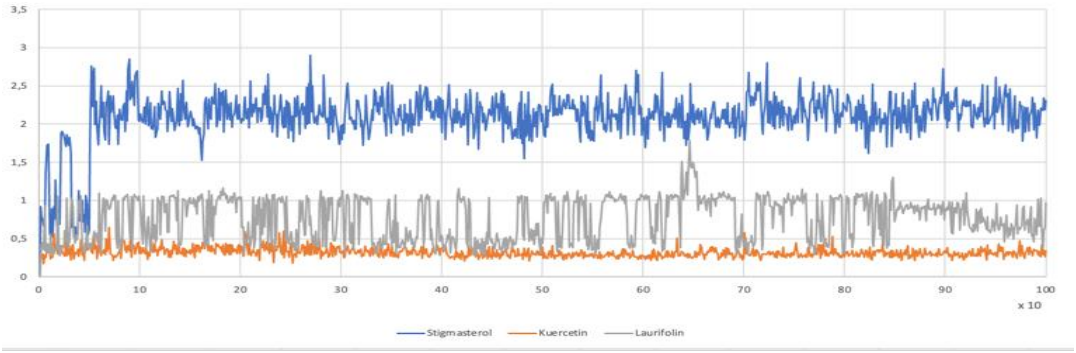

Figure 1 Molecular Dynamics of the Ligand (RMSD)

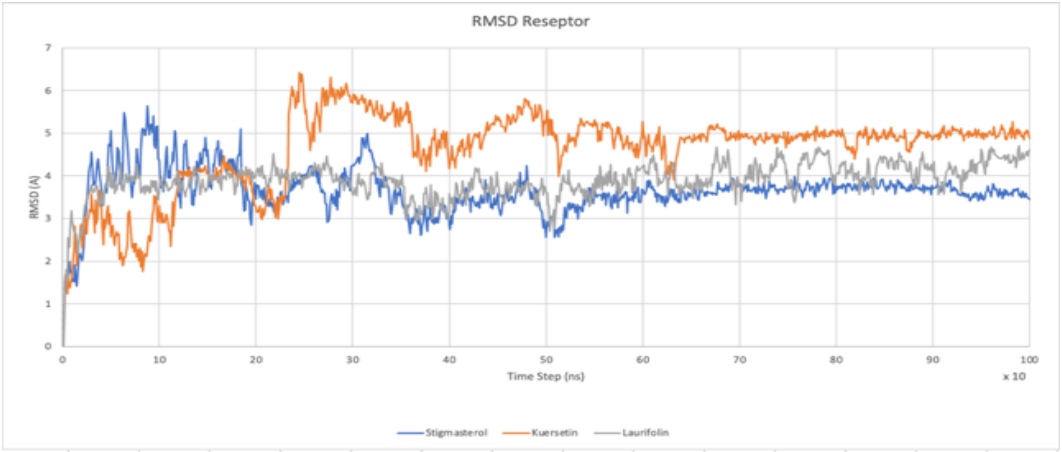

Figure 2 Molecular Dynamics of Receptor (RMSD)

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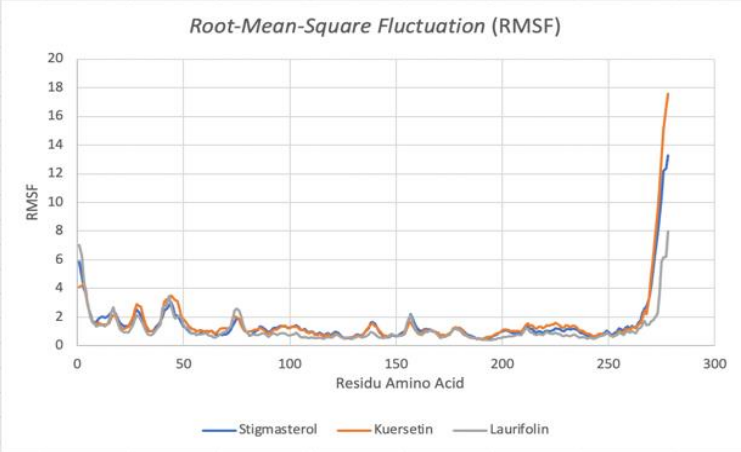

Figure 3 RMSF of stigmasterol, quercetin, and laurifolin

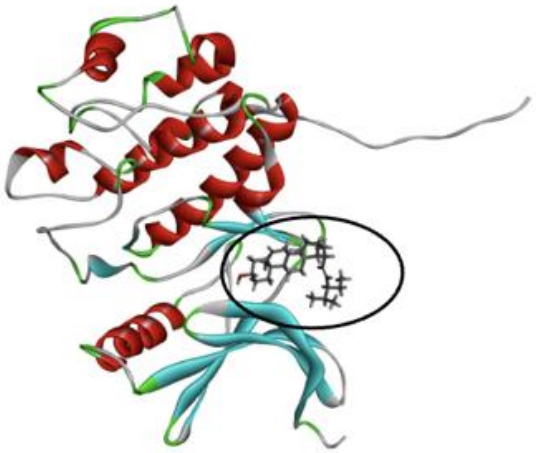

Figure 4. Stigmasterol (circled) at the last position during Molecular Dynamics simulation

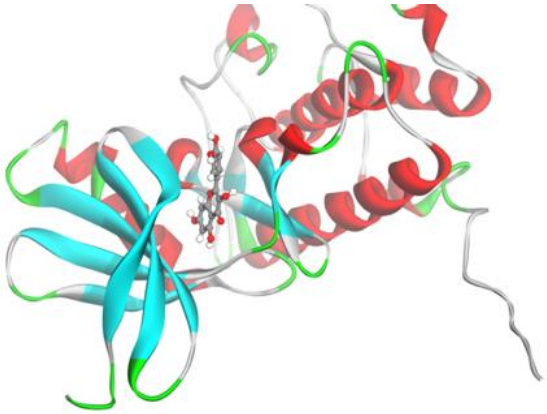

Figure 5. Quercetin at the last position in the Molecular Dynamics simulation

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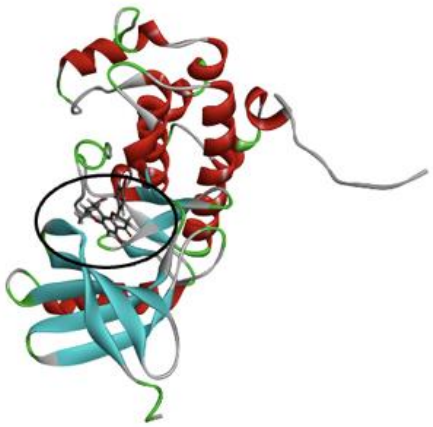

Figure 6. Laurifolin (circled) at the last position in the Molecular Dynamics simulation

Result of Lipinski's Result Rule-of-Five

Table 6. The result of Lipinski's Rule-of-Five test

Tested Compound	Result of Lipinski Rule of Five Examination				
	Molecular Mass (Dalton)	Total H-Donor	Total H-Acceptor	Log-P	Total Violation
Reference Ligand	308.5	2	7	2.791199	0
Flavone	222	0	2	3.302799	0
Flavanol	238	1	3	3.188499	0
Isoflavone	222	0	2	3.302799	0
Flavanone	224	0	2	3.393099	0
Anthocyanin	207	0	1	4.100889	0
Quercetin	302	5	7	2.010900	0
Myricetin	318	6	8	1.716500	1
Laurifolin	356	3	6	2.878698	0
Elatin	594	11	15	-1.817399	3
Chalcone	208	0	1	3.582699	0
Phenol	94	1	1	1.392200	0
Catechin	290	5	6	1.546100	0
Chrysin	254	2	4	2.713999	0
Kaempferol	286	4	6	2.305299	0
Stigmasterol	412	1	1	7.800803	1
Campesterol	400	1	1	7.634703	1
Ergosterol	396	1	1	7.330802	1
Lanosterol	426	1	1	8.479104	1
Brassinosteroid	480	4	6	3.389999	0
Isoquinoline	129	0	1	2.234800	0
Quinolizidine	139	0	1	2.024800	0
Indole	117	1	0	2.167900	0
Imidazole	68	1	1	0.409700	0
Acridine	179	0	1	3.397999	0

▲Legend : Yellow Colour represents the best compounds from the previous tests
Red Colour represents violation of a parameter

Conclusion

Based on the data and analysis obtained from this study, the conclusions that can be drawn are as follows:

1. The molecular docking process was performed on 24 secondary metabolite compounds, resulting in 18 compounds with potential to bind to protein targets and become cancer drug candidates based on their Binding Free Energy values. Among them, stigmasterol (-10.40 kcal/mol), laurifolin (-8.70 kcal/mol), and quercetin (8.04 kcal/mol) were found to have the highest potential. These three compounds also meet the criteria of "Lipinski's Rule of Five" for oral drug usage.
2. The ADMET test revealed that quercetin, the best compound based on free energy value parameters, passed 9 out of 11 tests, but did not meet the criteria for skin permeability and blood-brain barrier permeability.
3. Molecular dynamics simulation proved that the quercetin compound was the most stable compound as it can't be separated easily from the receptor in the 100ns period. Meanwhile, stigmasterol which appeared to have the lowest binding energy proved to be a false positive where the compound was unstable and released from the receptor the fastest.
4. The conducted tests have shown that quercetin is the most promising compound out of the 24 tested. Therefore, it has the potential to serve as a CHK1 inhibitor, offering an alternative cancer treatment option.

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