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Analysis of Molecular Docking Chemical Content of Lime (*Citrus aurantiifolia* (Christm.) Swingle) Against Diabetes Mellitus Therapy Targets and Prediction of Pharmacokinetic Profiles and Toxicity

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A B S T R A C T

Diabetes mellitus drugs currently available are sulfonylureas, biguanides, thiazolidines, and alpha glucoside inhibitors which are widely used to control hyperglycemia. These drugs cannot prevent complications of diabetes, and these drugs should not be used continuously because it causes undesirable pathological conditions. The essential oil in lime peel is rich in phenolics, especially flavonoids, which can prevent oxidative stress. This research was carried out computationally to determine the affinity of the compound mechanism in lime, pharmacokinetic profile, and toxicity of the chemical content of lime which is thought to have antihyperglycemic activity using chemoinformatics studies. The hardware used is an Asus laptop X441UB-GA502T Intel Core I5 – 8250 DDR 4 4GB HD 1TB VGA mix 110 2GB screen 14 "DVD-RW WIN 10 ORI. The software used is PLANTS (PROTEIN-LIGAND ANT SYSTEM), YASARA, Marvin sketch, Swisstargetprediction, SwissADME, and Toxtree. All compounds in lime were most active against the target protein PPARγ with an average value of -78.0092, while the positive control value of thiazolidinediones was -90.3393. The highest inhibitory affinity of the compound contained in lime was hesperidin with the target protein DPP-4 of -113.614, higher than the positive control sitagliptin with an inhibitory affinity value of -107.591. Hesperidin absorption in the digestive tract is low, the topology polar surface area (TPSA) value is 234.29Å2, and low polarity and high lipophilicity. There are unexpected heterocyclic compounds, so it becomes a warning against the potential for genotoxic carcinogenicity, namely oxygen element "o".

1. Introduction

Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin (a hormone that regulates blood glucose), or when the body cannot effectively use the insulin it produces. Around 462 million people have type 2 diabetes, 6.28% in the world (4.4% aged 15–49 years, 15% aged 50–69 years, and 22% aged over 70 years), or a prevalence rate of 6059 cases per 100,000. The prevalence of type 2 diabetes in the world is predicted to increase to 7,079 people per 100,000 by 2030. Currently, available diabetes mellitus drugs are sulfonylureas, biguanides, thiazolidines, and alpha glucoside inhibitors which are widely used to control hyperglycemia. These drugs cannot prevent complications of diabetes, and these drugs should not be used continuously because they cause unwanted pathological conditions. Traditional medicinal plants that have anti-diabetic properties can be useful sources for the development of diabetes drugs.[1-8]

The essential oil in the peel of lime (*Citrus aurantifolia* (Christm. Swingle) contains as many as 60 volatile compounds consisting of 13 monoterpenoids (80.36%), 20 sesquiterpenoids (6.51%), 3 terpene aldehydes (3, 79%), 12 alcohol terpenes (4.54%), 8

aliphatic oxygens (0.43%), 3 terpene esters (2.85%), and 1 ketone terpene (0.01%). Of phenolics, especially flavonoids included in the seeds, epidemiological evidence shows that consumption of oranges reduces the risk of chronic diseases, such as type 2 diabetes and obesity. This is due to its high fiber content and several flavonoids that can prevent oxidative stress. Several studies using citrus essential oil Lime juice showed a significant decrease in fasting blood sugar and liver glucose, while liver glycogen increased in a rat model of diabetes mellitus, indicating the potential of lime essential oil as a current modality for diabetes mellitus disorders.[9-17]

Nobiletin is one of the flavonoid compounds contained in lime essential oil. Various in vivo studies have been conducted on this compound to test its effectiveness in blood sugar regulation. Several studies have shown the potential of nobiletin compounds in lowering blood glucose levels and increasing glucose uptake to target cells.[18-21] However, there are no studies that specifically evaluate the potential of flavonoid compounds of lime essential oil on specific receptors that work in blood sugar regulation. This study aims to evaluate what proteins are predicted to be the target of chemical compounds in lime, to find out which compounds are predicted to have a high inhibitory affinity for GK, GSK-3β, PPARγ, α-amylase, DPP-4, to determine the predicted profile. Pharmacokinetics of compounds in lime that have good affinity predictions for anti-diabetic target proteins and prediction of compound toxicity in limes.

2. Methods

This research was carried out computationally to determine the affinity of the compounds in lime, the pharmacokinetic profile, and the toxicity of the chemical content of lime which is thought to have antihyperglycemic activity, using chemoinformatics studies. The hardware used is an Asus laptop X441UB-GA502T Intel Core I5 – 8250 DDR 4 4GB HD 1TB VGA mix 110 2GB screen 14 "DVD-RW WIN 10 ORI. The software used is PLANTS (PROTEIN-LIGAND ANT SYSTEM), YASARA, Marvin sketch, Swisstargetprediction, SwissADME, and Toxtree.

Downloaded macromolecules from the Protein Data Bank (PDB), the three-dimensional structure of the target ligand using the Marvin sketch application, and the canonical smile structure, which can be viewed at (https://pubchem.ncbi.nlm.nih.gov/). The compounds to be tested are bergapten, isopimpinellin, imperatorin, isobergapten, kaemferol, myricetin, 4',5,7- Trihydroxy-3,6-dimethoxy flavone, rutin, hesperidin, β-Sitosterol. Herniarin, isopimpinellin, citropten, oxypeucedanin, bergamottin and 5-geranyloxy-7-methoxycoumarin, rutin, apigenin, quercetin, kaempferol, nobiletin, tangeretin, hesperidin, Limonene, linalool and linalyl acetate, 6',7'-Dihydroxybergamottin. Compound searches both 2D and 3D, on the web server application https://pubchem.ncbi.nlm.nih.gov/, Canonical SMILES are entered on the web server application http://www.swisstargetprediction.ch/ and https://sea.bkslab. org/, the target protein is inserted into the diabetes mellitus pathway on the keeg webserver https://www.genome.jp/kegg-bin/show_pathway?hsa04930.

The macromolecules were prepared using YASARA, the water content in the macromolecules was removed, the macromolecules were added with hydrogen charge and stored in “yob” format, then the original protein and ligands were separated and stored in “mol2” format. Compound 3D structure creation, canonical smiles on pubchem webserver application https://pubchem.ncbi.nlm.nih.gov/ copied to marvinsketch, compound conditioned at PH 7.4 and save file with ligand2D.mrv format. Ligands were made into 10 molecular dynamics conformations and stored in the ligand.mol2 format.

The macromolecules were validated using the PLANTS application, and the original ligands were made into 10 molecular dynamics conformations. The highest score was found using the PLANTS application. The RMSD values were calculated using the YASARA application, and the conformational values with the best scores were tied to the original

ligands. RMSD (Root Mean Square Deviation) calculation, the RMSD value is used to measure the similarity of coordinates (pose) between two atoms. RMSD value must be less than 2.00 Å. The prepared ligands, along with the original protein and ligands, were put into one folder. The PLANTS application was run, the results of molecular docking were obtained from 10 conformations, and the highest score was chosen. The best ranking results tethering files are stored in the form of a "csv" file.

Discovery studio is a comprehensive software suite for analyzing and modeling molecular structures, sequences, and other data relevant to research science. Includes functionality to view and edit data and perform basic data analysis. Discovery Studio Visualizer suite of software Discovery Studio. Discovery Studio Visualizer is an interactive environment for viewing and editing molecular structures, sequences, X-ray reflection data, scripts, and other data. Visualize in the discovery studio visualizer, open the folder, select the prepared macromolecule, the ligand with the highest conformation is added, and the interaction of the ligand with the receptor is shown in the form of a 2D diagram.

The high failure rate of drug candidates in the later stages of testing is usually due to an unfavorable pharmacokinetic profile. The pharmacokinetic profile includes the nature of absorption, distribution, metabolism, excretion, and toxicity (ADMET). Therefore, it is necessary to conduct ADMET studies as early as possible to avoid the failure of the drug candidate. The ADME study uses the swiss ADME application with the web address http://www.swissadme.ch/ by opening the pubchem web https://pubchem.ncbi.nlm.nih.gov/ the compound is entered into the pubchem search field, copy of the canonical smile. Embedded in swiss ADME http://www.swissadme.ch/ select the red arrow > *run*, the ADME display for the compound will appear, if you want it to be shaped like an egg, select "show boiled egg", to predict the toxicity of the compound using the toxtree webserver application https://apps.ideaconsult.net/data/ui/toxtree.

3. Results and Discussion

Validation was carried out on macromolecules to be used as protein targets with RMSD values < 2Å to 2.5Å. The RMSD value is calculated using the YASARA app. The smaller the RMSD value, the more similar the positions of the two overlapping compound structures. The RMSD value in table 3 is not more than 2.5 Å, so it can be used to calculate the bond energy of the ligands.

Table 1. Macromolecular validation value.

No.	Macromolecules	Protein	RMSD value
1.	4GQR	α-amylase	2,0205 Å
2.	2OGZ	DPP-4	1,6783 Å
3.	3VF6	GK	0,6846 Å
4.	4AFJ	GSK-3β	0,5208 Å
5.	2F4B	PPARγ	1,2573 Å

Ligands used with each conformation 10 conformational ligands, molecular docking using PLANTS application, ligand preparation using Marvin sketch, the values of 10 conformations were sorted, and the lowest energy was taken from each ligand on 5 protein targets, namely α-amylase, DPP-4, GK, GSK3β, and PPARγ. The molecular docking results of the 26 ligands can be seen in Table 2.

The lowest native ligand binding energy value is in the DPP-4 target protein. The ligands with the lowest

binding energy values for the DPP-4 target protein were hesperidin and rutin, both of which were lower than the original ligand value. The native ligand of the target protein α-amylase, has a higher binding value when compared to the binding energy values of herperidine, rutin, and bergamottin. The molecular docking of the native ligand to the target protein GSK-3β was higher than that of hesperidin, rutin, and β-sitosterol, while the binding value of the target protein with the native GK and PPARγ ligands was lower than that of all lime ligands.

The average value of the lowest tethering energy on the target protein PPARγ, meaning that compounds from lime have the ability to increase insulin sensitivity, but when compared to the positive control of thiazolidinediones compounds that act on the target protein PPARγ, the total of all compounds contained in lime has a higher tethering energy value greater than the thiazolidinediones. The lowest binding energy value of the hesperidin compound on the DPP-4 target protein was lower in binding energy than the positive control DPP-4 sitagliptin. The value of the binding of positive control is in Table 3.

Table 2. Molecular docking of lime ligand.

No.	**Compound**	**Target protein**				
		α-amylase (4GQR)	DPP-4 (2OGZ)	GK (3VF6)	GSK3-β (4AFJ)	PPAR-γ (2F4B)
1.	Original ligand	-81,6808	-100.835	-114,401	-80,4033	-127,884
2.	Kaempferol	-75,8882	-74,9459	-75,3639	-76,8403	-78,2587
3.	Myricetin	-81,5452	-78,8692	-75,1793	-78,2631	-81,6573
4.	4',5,7- Trihydroxy-3,6-dimethoxy flavone	-75,6926	-67,3863	-67,9296	-72,2276	-80,8061
5.	Rutin	-92,7556	-103,474	-94,7604	-99,5822	- 108.293
6.	Hesperidin	-96.2378	-113.614	-102.863	-102.18	-109.566
7.	β-Sitosterol	-85,9502	-84,5591	-90,1018	-85,3586	-94,4781
8.	Apigenin	-77.9771	-74,6851	-76,0892	-75,9988	-77,7393
9.	Quercetine	-80,3594	-78,229	-75,6734	-76,6689	-79,5999
10.	Nobiletin	-58,6881	-64,2824	-56,644	-56.8102	-69.58
11.	Tangeritin	-59.4608	-60.2368	-56.7977	-55.0432	-67.838
12.	Bergapten	-63.5632	-64.9699	-72.0945	-65.272	-70.4613
13.	Isopimpinellin	-67.3118	-67.8114	-72.853	-68.4008	-74.9096
14.	Imperatorin	-74.8328	-76.0135	-83,7131	-74.0827	-83.9595
15.	Isobergapten	-62, 4047	-63.6614	-71.2084	-64.2708	-72, 1212
16.	7-Methoxycoumarin/Herniarin	-57.839	-63.6791	-69.3056	-62.2814	-65.9686
17.	Citropten/5,7-Dimethoxycoumarin	0	0	0	0	0
18.	Oxypeucedanin	-77.0879	-75, 2972	-79,345	-71,9025	-80,5497
19.	Bergamottin	-84,5534	-94,9497	-94,0496	-79,6341	-97,1304
20.	5-geranyloxy-7-methoxycoumarin	-82,3692	-89,8726	-96,6513	-83,9513	-94,9641
21.	limonene	-57.8961	-60.6716	-68.2346	-57.194	-64.2344
22.	linalool	-60.5807	-68.564	-69.4874	-59, 1357	-65,8844
23.	6',7'-Dihydroxybergamottin	-89,7358	-93,7335	-101,875	-88,2777	-102,567
24.	linalyl acetate	-64,6153	-72,9306	-79,2421	-64,5345	-73,6472
25.	limonin	-75,2794	-85,2873	-72,9395	-67,8521	-76.0099
Mean		-70,7541	-73,5841	-75,1939	-70,3439	-78,0092
SD (Standard deviation)		19.0238	21.0081	20.3733	19.4701	21.2355

Table 3. Positive control docking value.

No.	Positive control	Highest docking value
1.	4-Benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione (GSK-3β)	-63,399
2.	Sitagliptin (DPP-4)	-107,591
3.	Acarbose (α-amylase)	-99, 8547
4.	Thiazolidinediones (PPARγ)	-90.3393

Visualization of hesperidin and sitagliptin with the target protein DPP-4 can be seen in Figure 7. The aromatic compound hesperidin forms conventional hydrogen bonds with amino acids, and hesperidin binds to amino acids CYS A:551; GLN A:553; TRP A:629. The aromatic compound hesperidin forms a pi anion bond with the amino acid GLU A:205. A hydrogen carbon bond is formed on hesperidin and sitagliptin with the amino acid TYR A:547. The overlapping pi bonds were seen in the aromatic compound hesperidin with amino acids TYR A: 666, sitagliptin with amino acids PHE A: 357, and TYR A: 662. An unfavorable donor bond is formed between hesperidin and the amino acid ARG A:358.

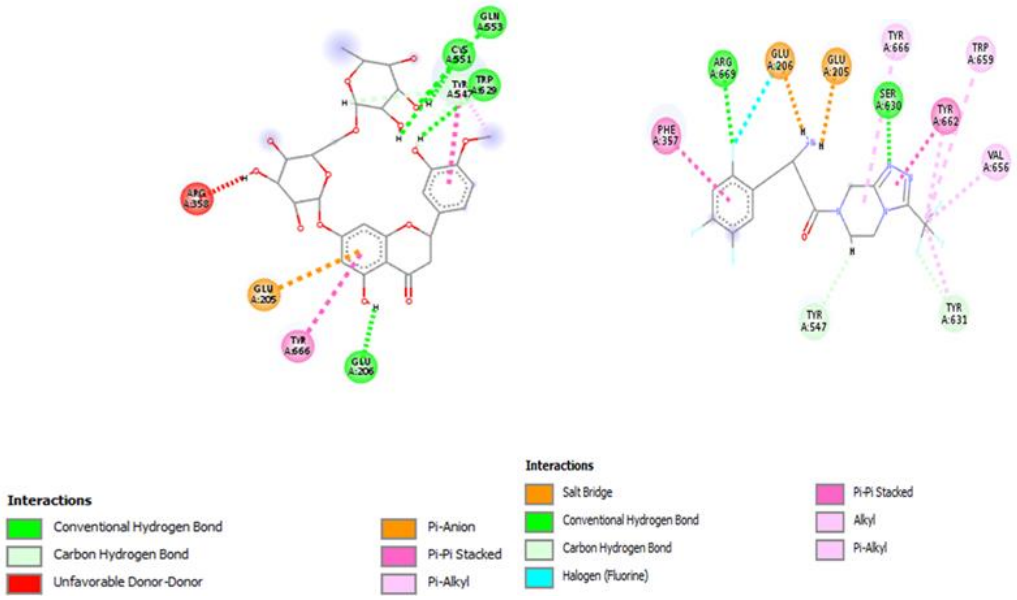

Figure 1. Interaction between hesperidin and DPP-4 target protein (right), sitagliptin interaction, and DPP-4 target protein (left).

A salt bridge is formed between the amide compound and the amino acid GLU A:205; GLU A:206. Nitrogen and fluorine in aromatic compounds form conventional hydrogen bonds, nitrogen with amino acids SER A:630 and fluorine with amino acids ARG A:669; GLU A:206. Fluorine forms hydrogen carbon bonds with amino acids TYR A:631, pi alkyl, and alkyl bonds are formed between aromatic compounds and amino acids TYR A: 666 ; TRP A:659 ; VAL A:656 ; TYR A:662 ; TYR A:631.

The visualization results between lime compounds and positive controls both work on the active site of the target protein DPP-4 (2OGZ), so lime compounds have better binding affinity energy than positive controls sitagliptin. The amino acid equation that acts on the active site of DDP-4 can be seen in Table 4.

Table 4. The active side of the compound bond in lime and positive control of DPP-4.

No.	Amino acids	Compounds and bond types			
		Sitagliptin (positive control)	Hesperidin	Rutin	Bergamottin
1.	GLU A206 and GLU A205	Salt bridges are formed between amides and amino acids	Formed conventional hydrogen bonds	Formed conventional hydrogen bonds	-
2.	TYR A547	Hydrogen carbon bonds	Hydrogen carbon bonds	Hydrogen carbon bonds	Alkyl and pi alkyl bonds
3.	TYR A666	Alkyl and pi alkyl bonds	Overlapping pi and pi bonds	-	Alkyl and pi alkyl bonds
4.	SER A630	Conventional hydrogen bonds formed between nitrogen and amino acids	-	Conventional hydrogen bonds	Hydrogen carbon bonds
5.	PHE A357	Overlapping pi and pi bonds	-	Overlapping pi and pi bonds	-
6.	ARG A669	Conventional hydrogen bonds formed between fluorine and amino acids	Unfavorable donor bond	Conventional hydrogen bond	-
7.	TRP A659	Alkyl and pi alkyl bonds	-	-	Alkyl and pi alkyl bonds
8.	TYR A662	Overlapping pi and pi bonds	-	-	Overlapping pi and pi bonds
9.	TYR A631 and VAL A656	Alkyl and pi alkyl bonds	-	-	Alkyl and pi alkyl bonds

The compounds that will be predicted for pharmacokinetic tests are compounds with the highest binding energy affinity values can be seen in Table 7. The bioavailability radar of compounds is based on 6 physicochemical parameters of the compounds, namely Lipophilicity (XLOGP3 between 0.7 to +5.0), Size (molecular weight between 150 to 500 g/mol), polarity (total polar area between 20 to 130 $Å^2$), Solubility (log S not higher than 6), Saturation (Csp3 fraction not less than 0.25), Flexibility (number of ties that can be twisted no more than 9). On the bioavailability radar, sitagliptin has good lipid solubility, while bergamottin is very fat-soluble or has high lipophilicity. The molecular weight of bergamottin is still included in the bioavailability parameter, while rutin and hesperidin are not included in the parameter. Polarity has been seen from the TPSA value on the bioavailability radar. Bergamottin has a good polarity compared to rutin and hesperidin. The solubility in the water of bergamottin is moderate, as seen at the log S value. The higher the saturation affects the solubility of a compound, the saturation of bergamottin is relatively low. Therefore the solubility of bergamottin is moderate.[22] Flexibility affects the properties of a compound to become a drug, and if the compound has flexibility of more than 9, then the compound will be difficult to synthesize into a drug.

Table 5. Physicochemical values of compounds based on bioavailability radar.

No.	Compound	Parameters bioavailability					
		Log p (XLOGP3) (0.7 -5)	BM (150-500) g/mol	TPSA (20-130) Å²	Log S (ESOL) (<6)	Fraction Csp3 (> 0.25)	Flexibility ≤9
1.	Sitagliptin	0.7	407.3	77.04	soluble	0.44	6
2.	Hesperidin	-0.14	610	234.29	soluble	0.54	7
3.	Rutin	-0.33	610	269.43	soluble	0.44	6
4.	Bergamottin	5.29	338 ,40	52.58	Moderate solubility	0.29	6

The compound in Figure 2 of the boiled-egg model, high absorption of sitagliptin in the digestive tract is indicated by a positive p-glycoprotein blue dot, which means that sitagliptin is actively predicted to penetrate the peripheral brain barrier and is excreted from the central nervous system by p-glycoprotein. Bergamottin is marked with a red dot minus p-glycoprotein in boiled eggs, meaning that it is highly soluble in the gastrointestinal tract and can penetrate the peripheral brain barrier, but cannot be removed from the central nervous system by p-glycoprotein.[23] Hesperidin and rutin do not appear in the boiled-eggs diagram because the topological values for the polar surface area (TPSA) are 234.29Å² and 269.43Å², and their solubility in the digestive tract is low. The pharmacokinetic profile of bergamottin is an inhibitor of CYP1A2, CYP2C19, and CYP2C9, which are metabolized in the liver by cytochrome p450 enzymes.

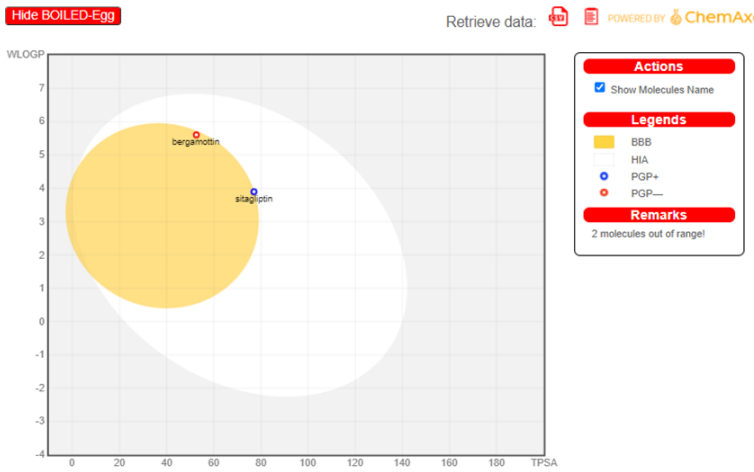

Figure 2. Pharmacokinetic profile of boiled eggs.

Compounds in lime with the lowest bond energy values were analyzed using Lipinski's "rule 5" to determine the absorption of compounds in the body can be seen in table 5. Lipinski's rule 5 was fulfilled if the molecular weight was less than 500 g/mol, High lipophilicity is expressed by log P less than 5, the hydrogen bond donor less than 5, the hydrogen bond acceptor less than 10, molar refraction must be between 40 – 130. Lipinski tests for hesperidin and rutin compounds do not meet Lipinski criteria. Compounds that meet Lipinski's rule 5 are bergamottin.

Table 6. Drug similarity is based on Lipinski's law.

No.	Compound	Molecular Weight (g/mol)	Lipophilicity (log P)	Hydrogen bond donor	Hydrogen acceptor	Molar refraction
1	Hesperidin	610,56	2,6	8	15	141,41
2.	Rutin	610,52	2,43	10	16	141,38
3.	Bergamottin	338,40	4,02	0	4	101,06
4.	Sitagliptin	407,31	2,35	1	10	87,25

Hesperidin compound and rutin both have a heterocyclic atomic structure. In the Cramer rules parameter, they are classified as class 3, which can be seen in Figure 3.

Figure 3. Cramer rules hesperidin (above), rutin (bottom).

Hesperidins and rutin compounds are not electrophilic or non-electrophilic and have halogen compounds and do not contain narcotic structural fragments, so they are not classified according to Verhaar, as can be seen in Figure 4.

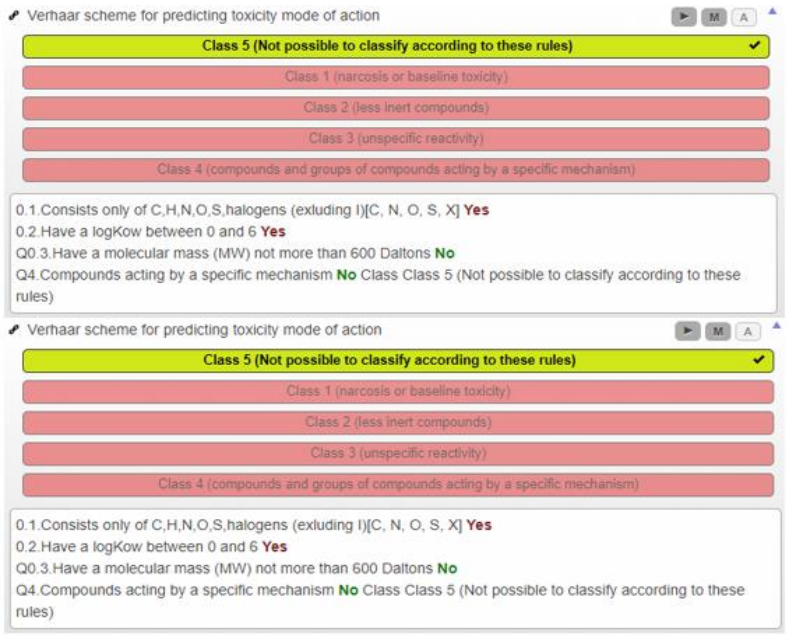

Figure 4. Verhaar sceme hesperidin(top), rutin (bottom).

Non-genotoxic carcinogens act by a variety of unclear binding mechanisms. These mechanisms can be caused by receptors, impaired homeostatic control, DNA damage, induction of cytotoxicity in lieu of cell proliferation, loss of immunity, and loss of communication between cells.[24] The results of the analysis showed that hesperidin and rutin did not contain carcinogenic genotoxic and non-genotoxic structures. There were no errors when setting the decision tree. The picture can be seen in Figure 5.

Figure 5. Benigni /Bossa hesperidin (top) and rutin (bottom).

Hesperidin and rutin compounds have an unexpected substance, thus becoming a safety issue warning structure for potential genotoxic carcinogenicity, Figure 6.

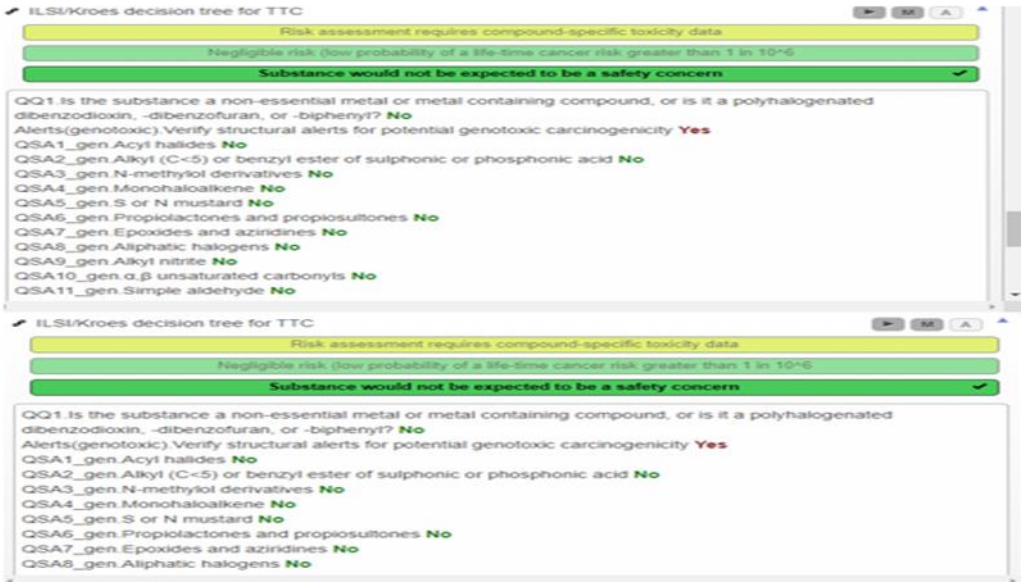

Figure 6. ILS1 hesperidin(top) and rutin (bottom).

4. Conclusion

All compounds in lime were most active against the target protein PPARγ with an average value the average was -78.0092, while the positive control value for thiazolidinediones was -90.3393. The highest inhibitory affinity of the compound contained in lime was hesperidin with the target protein DPP-4 of -113.614, higher than the positive control sitagliptin with an inhibitory affinity value of -107.591. Hesperidin absorption in the digestive tract is low, the polar surface area (TPSA) topology value is 234.29Å^2, and low polarity and high lipophilicity. There are unexpected heterocyclic compounds, so it becomes a warning against the potential for genotoxic carcinogenicity, namely oxygen element "o".

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