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SEARCHING OF FLAVONOID COMPOUNDS AS A NEW ANTIVIRAL FOR SUDAN EBOLAVIRUS GLYCOPROTEIN USING IN SILICO METHODS

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ABSTRACT: Ebola hemorrhagic fever is a viral disease from *Ebolavirus* genus and lethal to primates, including humans. The case fatality rate is 30%-90%. Until now, no vaccines nor drugs that could effectively combat Ebola hemorrhagic fever. Sudan ebolavirus (SEBOV) is the second deadliest species after *Zaire ebolavirus*, with a fatality rate of 50-70%. In Ebola life cycle, glycoprotein (GP) is crucial for mediating *Ebolavirus* entry into the host cell. Thus, molecules that could inhibit GP activity has a potential to become an ideal therapeutic compound of Ebola hemorrhagic fever. Flavonoid compounds are potential because of its antiviral properties. In this research, the *in silico* method was utilized to investigate the potency of flavonoid compounds as an inhibitor of SEBOV GP through molecular docking and computational ADMET test. Moreover, the oral bioavailability and toxicity prediction of the flavonoid compounds were performed as well to get the best flavonoid compounds. In this research, about 1358 flavonoid compounds and 3D structure of SEBOV GP were retrieved from ChEBI database and RCSB PDB, respectively. Moreover, MOE 2014.09 software was used as the primary software. Furthermore, the Osiris DataWarrior and SwissADME were used as the software for conducting computational ADMET test. In the end, cyanidin-3-(p-coumaroyl)-rutinoside-5-glucoside, myricitrin V, and 7-O-(6-feruoylglucosy) were selected as the potential inhibitor of SEBOV GP because they have the best binding affinity and low toxicity risk.

Keywords: Sudan Ebolavirus, Glycoprotein, In Silico, Molecular Docking, ADMET Test

1. INTRODUCTION

Ebolavirus is a genus of virus that causes the deadly Ebola hemorrhagic fever (EHF). Case fatality rate of EHF is between 30-90% [1]. *Ebolavirus* is classified in group V ((-) ssRNA), *Mononegavirales* order, and *Filoviridae* family. There are five species of *Ebolavirus*: Ebola virus (*Zaire ebolavirus*, EBOV), Sudan virus (*Sudan ebolavirus*, SEBOV), Taï Forest virus (*Taï Forest ebolavirus*, once *Côte d'Ivoire ebolavirus*, CIEBOV), Bundibugyo virus (*Bundibugyo ebolavirus*, BEBOV), and Reston virus (*Reston ebolavirus*, REBOV). *Reston ebolavirus* only infects primate non-human. Until now, no medicine nor vaccine can efficiently cure EHF.

SEBOV outbreak occurred for the first time in 1976 at Nzara and Maridi, Sudan with 284 people were being infected, and 153 people lost their life. The species got its name from the country it first identified. The most significant outbreak happened in 2000-2001 at Gulu, Masindi, and Mbarara, Uganda which infected 425 people and killed 224 people. Another case of SEBOV occurred in England at 1976; Uganda at 2011, 2012, and 2013; Sudan at 1979 and 2004 [2]. Considering the long list of the outbreaks; it is important to find a therapeutic compound for treating SEBOV infection.

Glycoprotein (GP), nucleoprotein (NP), RNA-polymerase (L), VP35, VP30, VP40, and VP24 are the protein that existed in SEBOV genome. The most important protein for penetration of host cells is the GP. Glycoprotein core is GP_1-GP_2 [1]. GP mechanism to penetrate into the host cell is by attaching to the host cell surface, and then getting into the cytoplasm by endocytosis pathway. The inhibitor can disrupt conformation change [3], thus rendering the virus to get into the cell [1].

Flavonoid is the biggest phytonutrient group in a natural product. It is very abundant in various plants, fruits, and vegetables. It has low toxicity, high bioavailability, antioxidant activity, and free radical activity [4]. They have the potential to become therapeutical medicine for SEBOV infection by inhibiting its imperative proteins, particularly GP [5]. Thus, this research aims to find the best flavonoid-based drug candidate that can disrupt the activity of SEBOV GP through molecular docking simulation and computational ADMET test, based on their binding affinity, molecular interaction and the toxicity prediction of the selected flavonoid compounds.

2. RESEARCH METHODOLOGY

This research utilized some online and offline software such as Molecular Operating Environment (MOE) 2014.09, Osiris DataWarrior v4.5.1, NCBI BLAST (National Center for Biotechnology

Information Basic Local Alignment Search Tool), and SwissADME. Additionally, the Chemical Entities of Biological Interest (ChEBI) database and RCSB PDB (Research Collaboratory for Structural Bioinformatics Protein Data Bank) were used as the primary sources of the experimental data. The general pipeline in this research referred to the previous research that has been validated and approved [6]–[8].

First, the flavonoid compounds were retrieved from ChEBI database [9],[10]. Thus, a total of 1358 flavonoid compounds were obtained and saved in .sdf file format. RCSB PDB was the source for SEBOV GP database. Then, NCBI BLAST was used to find the appropriate SEBOV GP sequence. Finally, MOE 2014.09 [11],[12] software was used to perform the molecular docking simulation.

The protein preparation in this study was conducted by downloading the 3D structure of SEBOV GP after the sequence alignment of SEBOV GP sequences had been performed. Followed by the elimination of the unwanted amino acid chains, solvents, and ligands. 'Protonate 3D' and 'Energy Minimization' protocols were performed to add explicit hydrogen and to optimize the protein conformation, respectively. AMBER99 was set as the forcefield because of its suitable for protein and nucleic acid structure. Solvation was set in a 'Gas Phase' which means the simulation did not include solvent into the calculation. Root mean square (RMS) gradient was set at 0,05 kcal/Å.mol, meaning energy minimization will be stopped when RMS gradient reached 0,05 kcal/Å.mol [13]. The active site was chosen using 'Site Finder' feature on MOE 2014.09, which the Site 2 was picked because it has an interaction with Endosomal Receptor Niemann-Pick C1 [14].

The preparation of flavonoid database was needed to make sure there is no identical ligand to be used in the docking phase. 'Wash' protocol was performed to simulate the state of the ligand in the body by either protonating the amine functional group ($-NH_3^+$) or deprotonating the carboxylate functional group ($-COO^-$). Additionally, MMFF94 (modified) forcefield was explicitly chosen as a partial charge of the ligand for its compatibility with the small organic molecule. Finally. RMS gradient value was set at 0,001 kcal/Å.mol in 'Energy Minimization' phase.

In this study, The molecular docking simulation was performed by choosing 'Triangle Matcher' as the placement method. The function of the 'Triangle Matcher' is to adjust ligand to the active site based on charge group and spatial fit. The role of 'London dG' scoring is to calculate Gibbs energy for binding for specific conformation or pose to allow the ligand orientation and position as an inhibitor of the protein [13],[15].

A toxicology test is needed to accurately measure the ligand properties such as ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties for every compound that will be tested to human. In this research, the chosen flavonoid compounds will be tested by using two software, namely Osiris DataWarrior and SwissADME.

3. RESULT AND DISCUSSION

In this research, the ligands and the protein that involved in the docking simulation must be prepared first. A total of 1358 flavonoid ligand was obtained from the ChEBI database. In addition, the selected SEBOV glycoprotein was obtained by employing NCBI BLAST. The result itself can be seen in Fig. 1. In this step, the 3D structure of SEBOV GP with the PDB ID: 3VE0 was chosen because of the sequence similarity that obtained from the NCBI Database. The general procedure for preparing the SEBOV GP protein structure was performed by using LigX module on MOE 2014.09 software.

The docking simulation between flavonoid ligands and SEBOV GP protein was performed using MOE 2014.09 software. This simulation determines the binding affinity of the selected ligands in the binding site of the protein/enzyme by knowing the Gibbs free binding energy ($\Delta G_{binding}$) value, inhibition constants, molecular interaction in the respective binding site. Moreover, the interaction between the ligand and the key residues that play a crucial role in the protein/enzyme is also observed. If the selected ligand has lower $\Delta G_{binding}$ value and higher inhibition constants than the standard ligand and may bind with the key residues at the binding site of the protein/enzyme, then the selected ligand has better chance to be a drug candidate than the standard ligand. The docking simulations were conducted three times; The first two 'Rigid Docking' protocol was performed to eliminate the low affinity and bad pose ligand that reflected by its RMSD value. While the 'Induced-Fit Docking' protocol was carried out for the best ligands to observe the molecular interactions and binding affinity of the selected compounds. In this research, About four ligands, namely neplanocin A, 3-deazaneplanocin A, toremifene, and clomiphene were chosen as the standard ligands [16].

In this study, about 1358 flavonoid compounds underwent docking simulation against SEBOV GP, with only 553 out of 1358 flavonoid ligands passed the first docking simulation due to having a lower $\Delta G_{binding}$ value than the standard ligands. Afterward, the second 'Rigid Docking' simulation yielded best 100 flavonoid ligands that have higher binding affinity than the standard ligands. Finally, the 'Induced-Fit Docking' was conducted subsequently, resulting seven ligands, namely monarda in, cyanidin-3-(p-coumaroyl)-rutinoside-5-glucoside, cyanidin 3-glucoside 5-caffeoylglucoside, myricitrin

V, prurient 6''-O-gallate, 7-O-(6-feruoylglucosyl)isoorientin, and petunidin 3-O-(6-O-(E)-4-coumaroyl-beta-D-glucoside), as the best ligands in the docking simulation which have lower $\Delta G_{binding}$ value than the four standards. Moreover, cyanidin-3-(p-coumaroyl)-rutinoside-5-glucoside was observed to have the lowest $\Delta G_{binding}$ value among all with -9,6941 kcal/mol. Followed by myricetin V and 7-O-(6-feruoylglucosyl)isoorientin, with a $\Delta G_{binding}$ value of -9,1728 kcal/mol and -9.0058 kcal/mol, respectively. These are much lower than the lowest $\Delta G_{binding}$ value among all of the standard ligands (clomiphene), which has a $\Delta G_{binding}$ value of -7.5146 kcal/mol. The results of molecular docking simulation in this study are shown in Table 1.

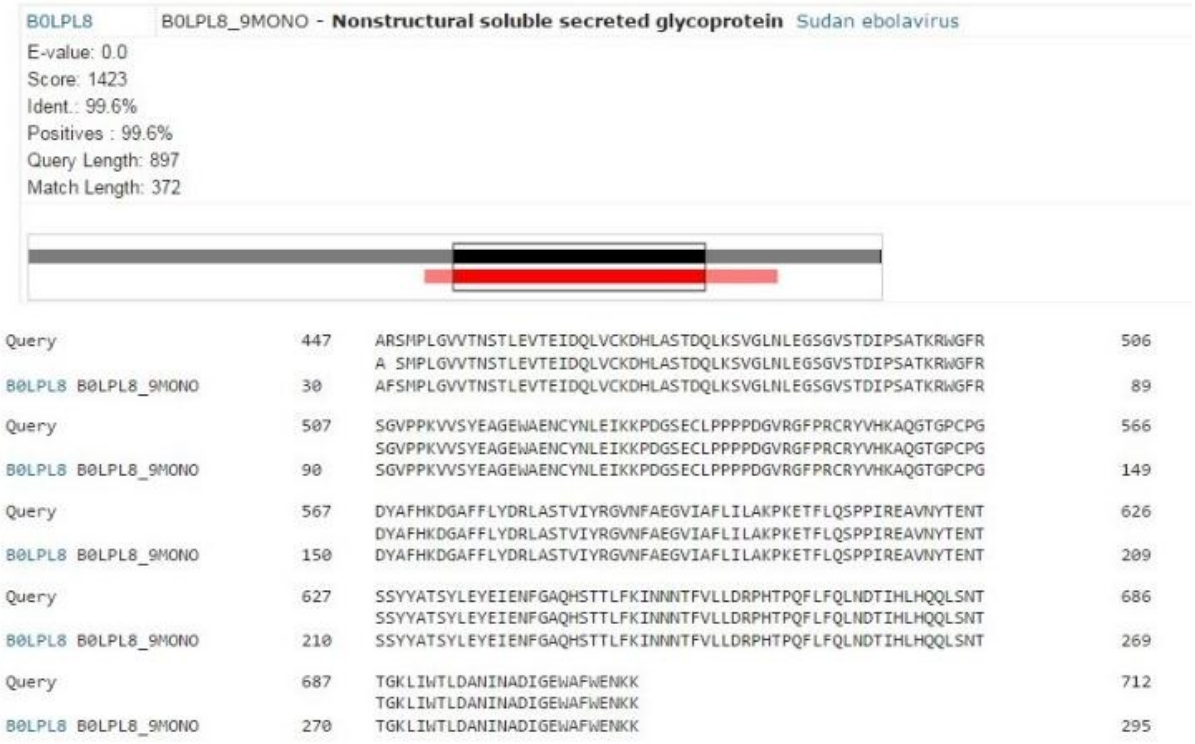

Fig. 1 Similarity of SEBOV GP with GP from the other *Ebolavirus* (99,6%)

Table 1. Molecular docking simulation result

No	Molecule Name (with Molecular Structure)	$\Delta G_{binding}$ (kcal/mol)	RMSD
1	Monardaein	-8,5716	1,6116
2	Cyanidin-3-(p-coumaroyl)-rutinoside-5-glucoside	-9,6941	3,1720
3	Cyanidin 3-glucoside 5-caffeoylglucoside	-8,1407	2,3497
4	Myrciatrin V	-9,1728	1,8359
5	Prurin 6''-O-gallate	-8,4739	1,8922
6	7-O-(6-feruoylglucosyl)isoorientin	-9,0058	1,8618
7	Petunidin 3-O-(6-O-(E)-4-coumaroyl-beta-D-glucoside)	-8,6197	2,1168
S1	3-deazaneplanocin A	-6.0057	1,2494
S2	Clomiphene	-7.5146	1.6039
S3	Neplanocin A	-6.1226	1.7294
S4	Toremifene	-7.3515	1.6344

In addition, the molecular interaction between the ligands and the protein was taken into account in determining the best ligand. Cyanidin-3-(p-coumaroyl)-rutinoside-5-glucoside forms three hydrogen bond interactions, two with Ile285 and one with Arg136. Also, the positively charged oxygen from the ligand forms an ion contact with the side chain of Glu71. About 19 residues from the protein interact with the ligands through van der Waals interaction. On the other hand, myrciacitrin V, the ligand with the second lowest $\Delta G_{binding}$, interact with Glu71 and Gly72 through side-chain hydrogen bond and Trp104 through backbone hydrogen bond. Moreover, the ligand also interacts with 18 protein

residues by van der Walls interaction. 7-O-(6-feruoylglucosyl)isoorientin forms three hydrogen interactions with a backbone of Trp104 and Ala283, also side chain of Asn107. In term of van der Waals interaction, this ligand interacts with 24 residues. From the molecular interaction, 7-O-(6-feruoylglucosyl)isoorientin ligand was selected to have the best molecular interaction. Both molecular interaction of the selected flavonoid ligands with SEBOV GP, as well as their 2D structure, can be seen in Fig. 2.

Finally, the root means square deviant (RMSD) score is also considered for determining whether the ligand pose that created during the docking simulation is acceptable and can be replicated during the real interaction when the ligand acts as the drug or inhibitor for the respective protein. The acceptable value for each docking pose is below 2.0 Å [17]. In this research, about four out of the seven best ligands have RMSD score below 2.0 Å, namely monarda in (1,6116 Å), myricetin V (1.8359 Å), Prurin 6’’-O-gallate (1.8922 Å), 7-O-(6-feruoylglucosyl)isoorientin (1.8618 Å). It means that these ligands pose which were generated during the docking process is acceptable and can be considered as a model for the real interaction that may happen during the inhibition process of the respective ligands into the SEBOV GP.

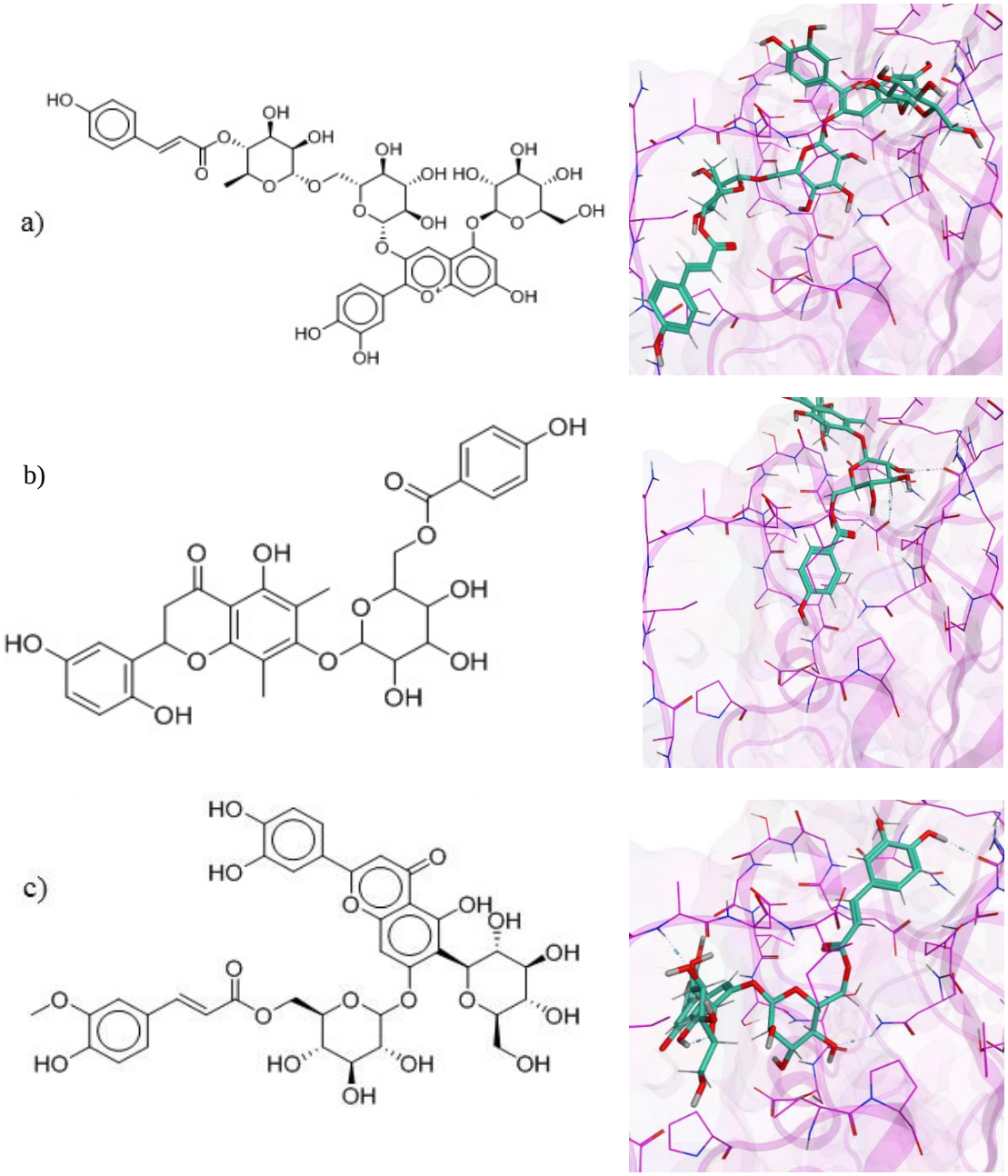

Fig. 2 The two-dimensional structure of the best ligands (left) and binding pattern of SEBOV GP (right) with (a) cyanidin-3-(p-coumaroyl)-rutinoside-5-glucoside, (b) myrciatrin V, and (c) 7-O-(6-feruoylglucosyl)isoorientin

Table 2. Molecular properties of the selected ligands by Osiris DataWarrior

Ligand	Druglikeness						
	MW	clogP	clogS	H-acceptor	H-donor	TPSA (Å^2)	Druglikeness
1	903,813	-4,9096	-5,286	23	8	357,92	-16,4980
2	598,555	-1,3391	-4,887	22	13	344,67	-11,8510
3	786,690	-0,7744	-4,384	19	12	305,98	-9,7867
4	625,557	2,0912	-4,202	13	7	212,67	0,7934
5	911,749	1,0577	-3,218	14	8	232,90	0,7608
6	586,500	-0,5711	-3,531	19	11	312,05	-3,3256
7	773,671	1,4906	-4,812	14	8	215,83	-5,5318
S1	262,268	-0,8991	-0,433	7	4	219,00	1,7618
S2	406,975	3,7701	-4,147	2	1	353,00	-2,5935
S3	263,256	-1,1785	-1,069	8	4	215,00	1,7618
S4	406,975	3,5829	-4,798	2	1	356,00	-3,4787

In this research, the computational ADMET predictions to determine the molecular properties of the best ligands and predict their toxicity potency were also conducted. Two software was deployed to calculate their properties, namely Osiris DataWarrior and SwissADME. This software were used to calculate the toxicity potency based on the structure and the fragments that contained in the respective ligands. All of these results are displayed in Table 2.

The molecular properties, druglikeness, and toxicity of the ligands and the standards were predicted using Osiris DataWarrior. Following the Lipinski's Rule of Five, all the ligands have molecular weight more than 500 g/mol, H-acceptor more than 10, H-donor more than 5, and TPSA greater than 140 Å^2 [18]. This rule must be strictly applied on the synthetic compound. However, all the best ligands in this study were natural product compounds, which naturally have good bioavailability although violating the Lipinski's RO5 [19]. Thus, the compounds still acceptable to be a drug candidate. On the other hand, the standards have the molecular properties which mostly acceptable according to Lipinski's Rule of Five. The result of drug-likeness score showed that only ligand 4 (myrciatrin V), ligand 5 (prurin 6''-O-gallate), standard 1 (3-deazaneplanocin A), and standard 3 (neplanocin A**)** that have the score higher than zero (0,7934, 0,7608, 1,7618, and 1,7618, respectively). It means that myrciatrin V and prurin 6''-O-gallate ligands have a better characteristic as a drug compound judging from their molecular fragments. Furthermore, in the toxicity test, as shown in Table 3, all of the seven flavonoid ligands have passed all of these parameters, i.e., mutagenic, tumorigenic, reproductive effective, and irritant. These results indicate that all of these proposed ligands have no demonstrated any toxicity properties based on their molecular fragments. Moreover, two standard ligands, toremifene and clomiphene, showed a high probability to have tumorigenic and reproductive effective properties. Exclusively to clomiphene, this compound is also shown to have a high probability of being mutagenic.

In SwissADME test, all flavonoid ligands have been predicted to have low gastrointestinal (GI) absorption, mainly due to their enormous molecular weight, as well as high hydrogen bond donors and acceptors that these ligands possessed. Hence, lowering their intestinal permeability and absorption. On the other hand, three out of four standards have high GI absorption, except neplanocin A. Moreover, the Pan-assay interference compounds (PAINS) test were also predicted throughout this software. In this study, only two flavonoid ligands (Cyanidin-3-(p-coumaroyl)-rutinoside-5-glucoside and prurin 6''-O-gallate) that have zero PAINS alert, while the rest of them possessed PAINS alert due to catechol fragments that these flavonoids owned. Furthermore, the Brenk prediction was also conducted in this study using the SwissADME software as well; this software showed that only ligand 2 (Cyanidin-3-(p-coumaroyl)-rutinoside-5-glucoside) and ligand 6 (7-O-(6-feruoylglucosyl)isoorientin) produced one alert, due to hydroquinone and catechol fragments, respectively.

According to these results, all seven flavonoid ligands have low toxicity risks, judging from their mutagenic, tumorigenic, irritant, and reproductive effective potency. However, these ligands were also predicted to have low absorption rate in the gastrointestinal system. Thus, another administration route of the potential flavonoid compounds should be considered especially when the in-vitro test has validated the low-absorption rate of these ligands.

Table 3. Toxicity and absorption prediction by SwissADME and OSIRIS DataWarrior

Ligand	Toxicity			Pharmacokinetics		Medicinal Chemistry	
	Mut.	Tum.	Rep. Effect	Irr.	GI Abs.	PAINS	Brenk
1	No	No	No	No	Low	1 alert: catechol_A	3 alerts: catechol, charged_oxygen_sulfur, michael_acceptor_1
2	No	No	No	No	Low	0 alert	1 alert: hydroquinone
3	No	No	No	No	Low	1 alert: catechol_A	2 alerts: cathecol, Michael_acceptor_1
4	No	No	No	No	Low	1 alert: catechol_A	3 alerts: catechol, charged_oxygen_sulfur, michael_acceptor_1
5	No	No	No	No	Low	0 alert	4 alerts: beta_keto_anhydride, charged_oxygen_sulfur, michael_acceptor_1, more_than_2_esters
6	No	No	No	No	Low	1 alert: catechol_A	1 alert: catechol
7	No	No	No	No	Low	1 alert: catechol_A	3 alerts: catechol, charged_oxygen_sulfur, michael_acceptor_1
S1	No	No	No	No	High	0 alert	1 alert: isolated_alkene
S2	High	High	High	No	High	0 alert	1 alert: stilbene
S3	No	No	No	No	Low	0 alert	1 alert: isolated_alkene
S4	Low	High	High	No	High	0 alert	2 alert: alkyl_halide, stilbene

Note: Mut.: Mutagenic, Tum.: Tumorigenic, Rep. Effect: Reproductive Effective, Irr.: Irritant, and GI Abs.: Gastrointestinal Absorption

4. CONCLUSIONS

The result from the current study shows that flavonoid group is potential for combating SEBOV by inhibiting its glycoprotein. In this study, about 1358 flavonoid ligands were screened through molecular docking simulation, with cyanidin-3-(p-coumaroyl)-rutinoside-5-glucoside, myrciatrin V, and 7-O-(6-feruoylglucosy) were selected as the best flavonoid ligands to inhibit SEBOV GP by having the lowest Gibbs energy binding ($\Delta G_{binding}$), and modest binding interactions. Moreover, these compounds were also predicted as safe drug candidates, judging from their mutagenic, tumorigenic, reproductive effective, and irritant potency. Thus, the molecular dynamics simulations of these compounds and SEBOV GP should be taken to determine their complex stability in the human body.

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