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Research Article

Virtual Screening of Flavonoid Compounds as A Main Protease Inhibitor for Anti-Sars-Cov-2 Candidates

Saeful Amin[1*], Fadhilah Utami[2], Selvira Anandia Intan Maulidya[2]

[1] Departement of Pharmacy, Bakti Tunas Husada University, Tasikmalaya, West Java, Indonesia
[2] Departement of Pharmacy, Garut University, Garut, West Java, Indonesia

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*Corresponding author: saefulamin@universitas-bth.ac.id

Abstract
In 2019, the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) was discovered in Hubei Province, China. After one year, no drug therapy has been approved, necessitating the development of SARS-CoV-2 drugs. To screen new drug candidates from natural product databases, the in-silico method, a drug discovery process based on computer simulations, can be utilized. Flavonoid is one of the most common compounds found in nature. They are secondary metabolites compounds contains phenolic functional group that can be virtually screened by predicting antiviral activity, drug likeness prediction, pharmacokinetic prediction, toxicity prediction, and molecular docking simulation Virtual screening is used in molecular docking simulations to design drugs based on activity prediction, compound similarity with oral drugs based on similar physical properties, pharmacokinetic profiles that include absorption and distribution, toxicity, and interactions of compounds with targets. The main protease used by target receptors is an enzyme that is important in determining SARS-CoV-2 survival. The structure of SARS-CoV-2 main protease code ID PDB 5RL4, 5R7Y and 7BUY is used in molecular docking simulation. The results of virtual screening of 80 flavonoid compounds showed that there are two most potential compounds, namely naringin and rutin that have lower ΔG values than the three native ligands, predictions of toxicity and good activity and a fairly good distribution profile.
Keywords: Flavonoids, Main Protease, SARS-CoV-2, Virtual Screening

Skrining Virtual Senyawa Flavonoid sebagai Inhibitor *Main Protease* untuk Kandidat Anti-Sars-Cov-2

Abstrak
Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) merupakan virus baru yang ditemukan tahun 2019 di Provinsi Hubei, Cina. Setahun setelah COVID-19 ini muncul belum ditemukan obat pasti dalam penangan terapi, sehingga perlu dilakukan pembuatan dan pengembangan obat SARS-CoV-2. Salah satu cara untuk memangkas waktu dan biaya pembuatan obat baru yaitu dengan metode in silico skrining virtual senyawa yang terdapat di alam. Flavonoid merupakan salah satu senyawa yang banyak ditemukan di alam dan dilakukan skrining secara virtual dengan memprediksi aktivitas antivirus, prediksi *druglikeness*, prediksi farmakokinetika, prediksi toksisitas, dan simulasi *molecular docking*. Reseptor target yang digunakan pada simulasi molecular docking yaitu main protease yang merupakan enzim yang menentukan kelangsungan hidup SARS-CoV-2. Struktur SARS-CoV-2 *main protease* kode ID PDB 5RL4, 5R7Y dan 7BUY digunakan dalam simulasi *molecular docking*. Senyawa *flavonoid* yang dinyatakan paling berpotensi sebagai kandidat anti SARS-CoV-2 pada penelitian ini yaitu senyawa *narigin* dan *rutin.*
Kata Kunci: *Flavonid, Main Protease*, SARS-CoV-2, Skrining Virtual

1. Introduction

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is a virus that causes coronavirus disease 2019 (COVID-19). In December 2019 the virus was first discovered in Hubei Province, China, spreading to 190 other countries.[1] World Health Organization (WHO) declared COVID-19 a global pandemic. The statement was delivered in March 2020 after cases increased to 20 million cases worldwide and the death rate reached 0.5-6%.[2] COVID-19 attacks the respiratory system causing acute pneumonia. In addition, other symptoms that are first caused in people infected with this virus are fever, dry cough, shortness of breath, sore throat, and fatigue.[3]

A year after COVID-19 emerged, there is still no definite cure for COVID-19 specific therapies. So it is necessary to make and develop new drugs for anti-SARS-CoV-2.[4] SARS-CoV-2 binds to ACE2 (Angiotensin Converting Enzyme 2) thus infecting host cells and causing immunogenicity. ACE2 is a SARS-CoV-2 receptor.[5] Part of SARS-CoV-2 that plays an important role in the replication and transcription of the virus is the main protease, so the main protease is identified as the target of the work of anti-SARS-CoV-2 drugs.[6]

Compounds contained in plants have become one of the options for becoming medicinal candidates. One of the compounds of secondary metabolites that are most produced from plants is flavonoids. Flavonoids are one of the secondary metabolite derivatives of phenolic compound groups.[7] Examples of compounds that belong to the group of flavonoid compounds are apigenin, quercetin, luteolin, naringin, rutin, isoquercetin, ginkgetin, ochnaflavones and others. These compounds have been proven to have antiviral activity.[8] So it can be possible flavonoid derivative compounds have antiviral activity in SARS CoV-2.[7]

Research in silico with virtual screening methods is one way to the discovery of new drugs for COVID-19. Virtual screening conducted was molecular docking simulation, antiviral activity prediction, drug likeness prediction, pharmacokinetic prediction and toxicity prediction of the compound. This computational method certainly strongly supports the discovery and development of drugs, especially in terms of saving time and costs incurred.[9]

2. Methods

2.1. Instrument

The research instruments were AMD A9-9425 RADEON R5 processor, 5 COMPUTE CORES 2C+3G, (2 CPUs), ~3.1GHz, RAM (Random Access Memory) 4.00GB, Windows 10 Pro 64-bit. The first stage begins by collecting compounds based on libraries and encrypting them virtually using the Pass (Prediction of Activity Spectra for Substances) Online (*http://pharmaexpert.ru/passonline/*). The next screening is drug likeness prediction based on the parameters of Lipinski's Rule of Five using an online site (http://scfbio-iitd.res.in). The second prediction is the prediction using Pre-ADMET server (*http://preadmet.bmdrc.org/*). Further predict toxicity using the Toxtree®. Several applications are used at this level, the first of which is the Discovery Studio Visualizer® to prepare macromolecules. Then, for flavonoid compounds, use the AutoDock Tools® software, which has two stages: validation and molecular docking simulation.

2.2. Materials

The research material used was macromolecules as receptors obtained through the Protein Data Bank (PDB) accessed on *http://www.rcsb.org/* with PDB ID 5RL4, 5R7Y and 7BUY

The structure of test compounds was downloaded from the PubChem web site (http://pubchem.ncbi.nlm.nih.gov) for optimization using ChemDraw®, and then developed using Marvin Sketch 17.2,13® prior to molecular docking simulation of flavonoid derivative compounds. Using the web portal SwissDock (*http://.swissdock.ch*), the most recent ligand docking optimization test was conducted for receptors that had been isolated in preparation. The value of ΔG, the relationship of chemical bonds and amino

acid residues visualized with Discovery Studio Visualizer®, is the end product of this analysis.

2.3. Procedures

The first stage begins by collecting compounds based on libraries and encrypting them virtually using the PASS (Prediction of Activity Spectra for Substances) Online (*http://pharmaexpert.ru/passonline/*).[10] Furthermore, compound predictions include drug likeness prediction, pharmacokinetic prediction (absorbance and distribution) as well as toxicity prediction of flavonoid compounds. Flavonoids conducted testing are chrysin, baicalein, apigenin, acacetin, scutellarein, hispidulin, luteolin, chryseoriol, diosmetin, tricetin, tricin, tangeretin, wogonin, rhoifolin, galangin, fisetin, kaemferol, kaemferide, robinetin, herbacetin, quercetin, rhamnetin, isorhamnetin, myricetin, querstagenin, gossypetin, isoquercetin, rhamnazin, rutin, morin, pachypodol, apigenidin, luteolinidin, pelargonidin, sciadopitysin, peonidin, delphinidin, malvinidin, rosinidin, daidzein, formononetin, genistein, biochanin A, orobol, tectorigenin, baptigenin, liquiritigenin, pinocembrin, narigenin, sakuranetin, eriodictyol, hesperetin, homeriodictyol, parietin, epigallocatechin, catechin, naringin, pinobanksin, aromadendrin, fustin, taxifolin, aghatisflavone, cupressuflavone, cyanidin, amentoflavone, ginkgetin, robustaflavone, hinokiflavone, ochna flavone, phlorizin, phloretin, isoliquiritigenin, chalconarigenin, butein, okanin, sulfuretin, aureusidin, maritimetin and leptosidin.

The drug likeness prediction based on the parameters of Lipinski's Rule of Five using an online site (*http://scfbio-iitd.res.in*).[11] The second prediction is the prediction using Pre-ADMET server (*http://preadmet.bmdrc.org/*).[12] Further predict toxicity using the Toxtree.[13] The main stage in this study is molecular docking using the Discovery Studio Visualizer to prepare macromolecules.[14] Then using the application AutoDock Tools to namely validation and molecular docking simulation for flavonoid compounds.[15] The structure of flavonoid derivative compounds was downloaded on PubChem online site (*http://pubchem.ncbi.nlm.nih.gov*) and optimization using Marvin Sketch.[12] The last performed ligand docking optimization results test with receptors that have been separated in preparation using the online site SwissDock (*http://.swissdock.ch*). The final result of this study is the value of ΔG, the interaction of chemical bonds and amino acid residues visualized using Discovery Studio Visualizer.[16]

3. Results

3.1. Compounds Antiviral Activity Prediction

The results showed from 80 flavonoid compounds there are 71 compounds that have a value of Pa>Pi with the activities shown are antiviral influenza, herpes, hepatitis, HIV, picornavirus, adenovirus, rhinovirus, trachoma, CMV and poxvirus.

3.2. Drug likeness Prediction

Based on the results of research that has been conducted from 80 flavonoid compounds tested produced 64 compounds meet and 16 compounds do not meet the requirements of Lipinski's rule of five parameters.

3.3. Pharmacokinetic Predictions

Based on the results of 80 flavonoid compounds tested, there are 15 compounds that are predicted to have good absorption and distribution capabilities namely tangeretin, rhoifolin, fisetin, kaemferol, kaemferid, ramnetin, isoramnetin, ramnazin, pachypodol, daidzein, formononetin, genistein, orobol, homeriodictyol, fustin and butein. Data shown in Table 1.

3.4. Toxicity Prediction

There are 13 flavonoid compounds that are predicted to have mutagen properties based on ames toxicity test against *S. thypimurium* bacteria namely ramnetin, isoramnetin, myricetin, querstagenin, gossypetin, isoquercetin, ramnazin, pachypodol, parietin, isoliquiritigenin, chalconarigenin, butein and okanin. Data shown in Table 2.

Table 1. Pharmacokinetic prediction results using Pre-ADMET

No.	Ligand	Absorbs		Distribution
		HIA (%)	CaCo-2 Cell (nm/sec)	Plasma Protein Binding (%)
1.	Tangeretin	98,88*	53,60***	87,17 **
2.	Rhoifolin	20,17**	9,92**	54,72**
3.	Fisetin	79,43*	9,57**	88,73 **
4.	Kaemferol	79,61*	9,58**	89,61 **
5.	Kaemferid	88,192*	9,33**	83,99 **
6.	Rhamnetin	78,34*	4,93**	85,35 **
7.	Isorhamnetin	78,35*	4,94**	83,55 **
8.	Ramnazin	87,21*	5,09**	81,21 **
9.	Pachypodol	93,45*	4,38**	79,88 **
10.	Daidzein	92,65*	7,72**	88,70 **
11.	Formononetin	93,02*	21,21**	86,72 **
12.	Genistein	88,12*	5,75**	89,74 **
13.	Tectorigenin	93,04*	7,06**	89,44 **
14.	Fustin	77,82*	9,56**	88,03 **
15	Butein	97,80*	56,04**	88,98 **
16.	Naringin	15,46***	16,35**	69,33**
17.	Rutin	2,86***	7,91**	43,89**
18.	Isoquercetin	16.55***	11,96**	65,76**
19.	Ginkgetin	91,11*	11,98**	95,06*
20.	Ochnaflavone	91,62*	8,94**	100*

Description:
HIA (%) :
* : 70-100 well absorbed
** :20-70 absorbed enough
*** :<20 less absorbed
CaCo-2 (nm sec) :
* :>70 High permeability
** :4-70 medium permeability
*** : <4 low permeability
PPB (%) :
* : >90 bonded strong
** : <90 bounded weak

3.5. Molecular Docking

The docking of comparing ligands and flavonoid test compounds with SARS CoV-2 receptors main protease with different PDB ID codes (5RL4, 5R7Y, 7BUY) shows negative values so that it means that docking simulations run spontaneously and stable. Data shown in Table 3.

4. Discussion

Prediction of compounds antiviral activity used the online PASS website. The PASS Online site uses the Pa and Pi parameters. Pa is the probability value or probability of an active compound, while Pi is the probability value for an inactive compound. Compound activity is predicted to be better when the value of Pa>Pi. Compounds are most likely

to exhibit activity and have similarities to existing drugs when the value of Pa >0.7. When the value of Pa between 0.5 and 0.7, the compound has a probability of showing activity but the probability is small. Whereas if the value of Pa<0.5 then the compound does not show activity but indicates a new chemical entity.[17] There are five compounds that have a value of Pa >0.7 namely rhoifolin, isoquercetin, rutin, epigallocatechin and naringin. The five compounds show the same activity as influenza antiviral activity.

Compounds that are candidates for a drug can be said to have an ideal molecular form when it has similar physicochemical properties to oral drugs. The parameters used in determining a compound have an ideal molecular shape determined based on Lipinski's rule of five.[10] The physicochemical properties of a drug certainly affect the pharmacokinetic process of the drug. Lipinski's rule of five parameters indicate the physical properties of a compound that is the first molecular weight (BM)<500 Da, this parameter affects the distribution of drugs where the lower the BM the more able to penetrate the cell membrane. If it exceeds this value, the compound cannot diffuse through the cell membrane. The second property is the partition coefficient (Log P) <5 is a physicochemical trait that affects lipophilicity when a compound is lipophilic compounds can be held in lipids bilayer consequently increase toxicity in the body and the selectivity of compounds to achieve the target decreases.11 Furthermore, the

Table 2. Toxicity prediction results using Toxtree

No.	Compound	Mutagen (Amest Fest)	Carcinogenik		Kroes TTC
			Genotoxic	Non Genotoxic	
1.	Rhamnetin	(+)	(-)	(-)	Not At Risk
2.	Isorhamnetin	(+)	(-)	(-)	Not At Risk
3.	Myricetin	(+)	(-)	(-)	Not At Risk
4.	Querstagenin	(+)	(-)	(-)	Not At Risk
5.	Gossypetin	(+)	(-)	(-)	Not At Risk
6.	Isoquercetin	(+)	(-)	(-)	Not At Risk
7.	Rhamnazin	(+)	(-)	(-)	Not At Risk
8.	Pachypodol	(+)	(-)	(-)	Not At Risk
9.	Parietin	(+)	(+)	(-)	low risk
10.	Isoliquiritigenin	(+)	(+)	(-)	low risk
11.	Chalconarigenin	(+)	(+)	(-)	low risk
12.	Butein	(+)	(+)	(-)	low risk
13.	Okanin	(+)	(+)	(-)	low risk
14.	Agarthisflavone	(-)	(-)	(+)	Not At Risk
15	Cupressufkavone	(-)	(-)	(+)	Not At Risk
16.	Ginkgetin	(-)	(-)	(+)	Not At Risk
17.	Sciadopitysin	(-)	(-)	(+)	Not At Risk
18.	Robustaflavone	(-)	(-)	(+)	Not At Risk
19.	Amentoflavone	(-)	(-)	(+)	Not At Risk
20.	Naringin	(-)	(-)	(-)	Not At Risk
21.	Rutin	(-)	(-)	(-)	Not At Risk

Table 3. Result of molecular docking simulation

No.	Ligand	ΔG 5RL4 (kcal/mol)	ΔG 5R7Y (kcal/mol)	ΔG 7BUY (kcal/mol)
1	*N-(4-tert-butylphenyl)-N-[(1R)-2-(methylamino)-2-oxo-1-(pyridin-3-yl) ethyl]propanamide*	-8,47	-	-
2	*N-(2-phenylethyl) methanesulfonamide*	-	-6,91	-
3	Hexyl carbamic acid	-	-	-6,49
4	Lopinavir	-10,86	-9,79	-9,89
5	Remdesivir	-10,39	-9,95	-10,07
6	Tangeretin	-8,57	-8,25	-8,29
7	Rhoifolin	-9,34	-8,58	-9,13
8	Isoquercetin	-8,92	-8,78	-8,67
9	Rutin	-9,57	-8,85	-8,97
10	Naringin	-10,13	-9,84	-9,69
11	Ginkgetin	-9,13	-8,08	-8,96
12	Siadopitisin	-9,51	-8,07	-8,4
13	Hinokiflavone	-8,51	-7,36	-8,02
14	Ochnaflavone	-9,66	-8,17	-8,68
15	Amentoflavone	-8,98	-7,74	-8,4
16.	Phlorizin	-9,12	-8,67	-8,65
17.	Ginkgetin	-9,13	-8,08	-8,96
18.	Pachypodol	-8,38	-7,59	-8,09

donor and acceptor of hydrogen bonds, where both parameters affect the permeability of the bilayer lipid membrane and the capacity of hydrogen bonds. The value of hydrogen bond donors and acceptors in Lipinski's rule of five is hydrogen bond donor <5 and hydrogen bond acceptor <10.[11]

Pharmacokinetic predictions are used to predict absorption with the value parameters of Human Intestinal Absorption (HIA) cells is a parameter for predicting absorption in the human intestine and bioavailability based on the cumulative excretion ratio of urine, bile and feces. HIA value of 70-100% indicates well absorbed compounds. So the higher the value of HIA, the compound is predicted to have a good absorbance. Human colon adenocarcinoma (CaCO2) is a parameter for predicting the permeability of compounds. The expected permeability is moderate permeability, compounds that have moderate permeability can occur because the compound is not too hydrophilic or too lipophilic, caco2 value 4-70 nm / sec medium permeability. *Plasma Binding Protein* (PPB) is a parameter used to predict the ability of compounds to bond with plasma proteins. *Plasma Binding Protein* (PPB) determines the fraction of drugs available in free form distributed to various tissues. The expected bond between compounds and plasma proteins is a weak bond, since in general drugs in free form can more easily penetrate cell membranes and are able to achieve work targets. If the bond between the compound and this protein is strong then the compound is less well distributed in the body. The recommended PPB value of <90% predicts

a bond with weak proteins.[12,13] Based on the results of 80 flavonoid compounds tested, there are 15 compounds that are predicted to have good absorption and distribution capabilities namely tangeretin, rhoifolin, fisetin, kaemferol, kaemferid, ramnetin, isoramnetin, ramnazin, pachypodol, daidzein, formononetin, genistein, orobol, homeriodictyol, fustin and butein.

Toxicity prediction is performed using Toxtree by ames test toxicity, carcinogenicity and Kroes TTC decision tree.[18] Ames toxicity is a method of predicting toxicity to predict the mutagen of test compounds based on in vitro testing of *S. thypimurium* bacteria. The second test used The Benigni Bossa Rulebase method to predict carcinogenic and mutagenic properties based on genotoxic and nongenotoxic structures. The positive results shown from both methods mean that the predicted compounds are mutagen and act as carcinogenic. Kroes TTC decision tree is a method used in toxicity prediction by estimating the threshold of exposure to compounds in humans. Results shown in the form of possible levels of risk of evasion.19,20 Based on the results of the study, of the 80 flavonoid compounds tested there were only five compounds that have a low risk of exposure and genotoxic carcinogens namely parietin, isoliquiritigenin, chalconarigenin, butein and okanin. While the other 75 flavonoid compounds are not at risk of exposure but there are six predicted non genotoxic carcinogenic compounds namely agathisflavone, cupressuflavone, ginkgetin, sciadopitysin, robustaflavone and amentoflavone. In addition, there are 13 flavonoid compounds that are predicted to have mutagen properties based on ames toxicity test against S. thypimurium bacteria namely ramnetin, isoramnetin, myricetin, querstagenin, gossypetin, isoquercetin, ramnazin, pachypodol, parietin, isoliquiritigenin, chalconarigenin, butein and okanin.

The first stage in molecular docking is to download macromolecules from the Protein Data Bank (PDB) (http://www.rscb.org/) website. The selected macromolecule must have non-mutated characteristics, resolution ≤2Å and complex with native ligands. The downloaded macromolecules are 3D SARS CoV-2 main protease structures with PDB ID codes 5RL4, 5R7Y and 7BUY, the three macromolecules are downloaded in the form of formats (.pdb). Macromolecules with PDB ID code 5RL4 are complex macromolecules with native ligand *N-(4-tert-butylphenyl)-N-[(1R)-2-(methylamino)-2-oxo-1-(pyridin-3 yl) ethyl]-propanamide* and have a resolution of 1.53Å. Macromolecules with PDB ID code 5R7Y have a resolution value of 1.65Å and are complex with *N-(2-phenylethyl) methane sulfonamide*. As for macromolecules with PDB ID code 7BUY has a resolution of 1.60Å and complex with *Hexyl carbamic acid*.[21–23]

Macromolecules that have been downloaded on PDB are prepared using the Discovery Studio Visualizer. The purpose of macromolecular preparation is for the docking process to run optimally. Preparation is done by separating the target receptors with native ligands as well as eliminating water molecules and other residues. These receptors and native ligands that have been separated will be used in the validation phase of the method. Validation of molecular docking method is done by re-docking native ligands on receptors that have been separated in macromolecular preparation. Validation of molecular docking methods is performed to ensure that the method used has Qualify requirements. Method validation parameter that uses Root Mean Square Deviation RMSD value <2Å.[24]

The re-docking process is done by inputting native ligands and receptors and then the grid-box settings are performed. This setting is intended to provide ligand search suggestions on the active side of the receptor with grid-center positions x, y and z. In macromolecules PDB ID code 5RL4 is set to volume box 26×26×26 with spacing 0.375 and grid-center position x = 9236, y= -1.254 and z= 22.639. In macromolecules PDB ID code 5R7Y is set to volume box 22×22×20 with spacing 0.375 and grid-center position

x= 10.301, y= -2.386, and z= 24.735. While macromolecules with PDB ID code 7BUY are set at box volume 12×12×22 with spacing 0.375 and grid-center position x= -11.912, y= 16.194 and z= 68.[16]

Based on the results of the study conducted validation methods of the three macromolecules declared valid because the RMSD value generated Qualify parameter requirements. In the validation of the method of results that analysis is not only RMSD but must analyze the value of free energy (ΔG) and need to visualize the interaction of chemical bonds and also amino acid residues. Method validation on PDB ID 5RL4 returns a RMSD value of 0.647Å and a ΔG value of -8.93 kcal/mol. A PDB ID of 5R7Y returns an RMSD value of 1.592Å and a ΔG value of -4.85 kcal/mol and a PDB ID of 7BUY resulting in a value of RMSD of 1.839Å and a value of ΔG of -2.22 kcal/mol.

Grid-boxes are generated from validation methods using AutoDock Tools® used on docking using SwissDock. So it is necessary to re-dock the receptors with native ligands, because to analyze the results of docking must be done by comparing data from the same software. Re-docking uses SwissDock with the same grid-box as AutoDock Tools resulting in a smaller RMSD value.24 Re-docking results using SwissDock generate RSMD values that are calculated using Discovery Studio Visualizer. The RMSD value of re-docking SARS-CoV-2 receptor main protease ID PDB 5RL4 is 0.069 Å with a value of ΔG= -8.47 kcal/mol. The RMSD value of re-docking SARS-CoV-2 receptor main protease ID PDB 5R7Y is 0.080 Å with a value of ΔG= -6.91 kcal/mol. While the RMSD value of re-docking SARS-CoV-2 receptor main protease ID PDB 7BUY is 0.083 Å with a value of ΔG= -6.49 kcal/mol.

Chemical bond interactions and amino acid residues that occur in re-docking native *N-(4-tert-butylphenyl)-N-[(1R)-2-(methylamino)-2-oxo-1-(pyridin-3-yl)ethyl] propane-mide* with SARS-CoV-2 main protease receptors PDB ID 5RL4 are hydrogen bonds, hydrophobic bonds and other bonds. Hydrogen bonds include carbon hydrogen bonds (PHE 140) and conventional hydrogen bonds (GLU 166, GLY 143), Pi-Cation (ASN 142, GLU 166). Meanwhile, hydrophobic bonds are alkyl (CYS 145) and other bonds in the form of unfavorable bump bonds (ASN 142). The interaction of chemical bonds and amino acid residues that occur in the process of re-docking native ligand *N-(2-phenylethyl) methane sulfonamide* on SARS-CoV-2 receptors main protease ID PDB 5R7Y is a conventional hydrogen bond (GLU 166, THR 190), Pi-Cation (MET 165), Pi-bond hydrogen donor (GLU 166) and metal-acceptor (GLN 189, LEU 167). Chemical bond interactions and amino acid residues that occur in the re-docking native process of Hexyl carbamic acid ligands on SARS-CoV-2 receptors main protease ID PDB 7BUY is alkyl (PRO168).

Preparation of comparison ligands and test ligands is carried out by performing optimizations and protonation. The purpose of optimization using *ChemDraw software* is to minimize energy so that ligands are able to find positions with a stable state. After optimization, ligand test was conducted protonation using Marvin Sketch software® with the aim of ligand condition adjusted to the pH of the human body is 7.4.12 Based on the results of predictions of antiviral activity using PASS online, out of 80 flavonoid compounds only 71 flavonoid compounds show predictions of antiviral activity. So only 71 flavonoid compounds were conducted docking simulations. Seventy-one flavonoid compounds performed docking simulations on SARS CoV-2 main protease receptors.

Analysis of comparison ligand and ligand docking results on SARS-2-CoV-2 main protease receptors only includes ΔG values, chemical bond interactions, and amino acid residues that can be visualized using the Discovery Studio Visualizer. Gibbs free energy value (ΔG) is a molecular docking simulation parameter that shows the stability of interactions between ligands and receptors. Negative ΔG values are expressed simulated to run spontaneously and steadily. The smaller the ΔG value, the stronger the bond formed between ligands and receptors. While the

interaction of chemical bonds is a parameter that affects affinity and inhibition activity against receptors. The expected bond is the hydrogen bond, the more hydrogen bonds are formed the better the test compound is used as an inhibitor. In addition to hydrogen bonds, non-bond interactions such as electrostatic interactions and van der Waals interactions can affect affinity.[25]

The docking of comparing ligands and flavonoid test compounds with SARS CoV-2 receptors main protease with different PDB ID codes (5RL4, 5R7Y, 7BUY) shows negative values so that it means that docking simulations run spontaneously and stable. Data shown in Table 3.

Simulation molecular docking of flavonoid compounds with SARS CoV-2 receptors main protease ID PDB 5RL4 conducted analysis on compounds that have the lowest ΔG values namely naringin, ochna flavone and rutin. Naringin flavonoid compounds tethered to SARS-CoV-2 main protease 5RL4 GDP ID receptors have a value of ΔG=-10.13 kcal/mol. Chemical bond interactions that occur are the interaction of hydrogen bonds, hydrophobic bonds and other interactions. Hydrogen bonds formed are conventional hydrogen bonds (GLU 166, THR 24, PHE 140) and hydrogen carbon bonds (GLU 166), hydrophobic interactions that occur namely Pi-cation (THR 25) and alkyl (MET 49) and other interactions namely metal-acceptor (ASN 142). Visualization interaction shown in Figure 1.

Molecular docking simulation of flavonoid compounds with SARS CoV-2 main protease receptor PDB ID code 5R7Y shows compounds that have the lowest ΔG values of naringin, rutin and isoquercetin. Naringin compounds tethered to SARS-CoV-2 receptors main protease ID PDB 5R7Y has a value of ΔG=-9.84 kcal/mol with the interaction of chemical bonds and amino acid residues produced in the form of hydrogen bonds in the form of conventional hydrogen bonds (PHE 140) and hydrophobic bonds in the form of Pi-cation bonds (GLN 189) and Pi-alkyl (MET 165). The ΔG value of naringin is not only lower than the native ligand but also lower than the comparison ligand of lopinavir. Visualization interaction shown in Figure 2.

The docking of flavonoid compounds with SARS CoV-2 receptors main protease CODE ID PDB 7BUY shows compounds that have the lowest ΔG values namely naringin, rutin and ginkgetin. Naringin compounds tethered to SARS-CoV-2 receptors main protease ID PDB 7BUY has a value of ΔG=-

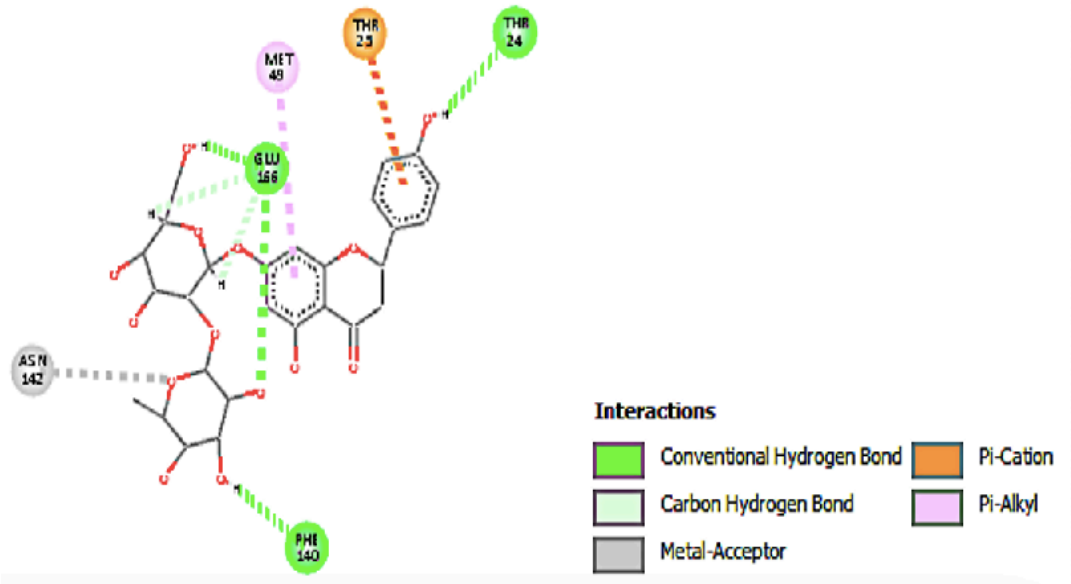

Figure 1. Visualization of amino acid residues and interaction of naringin compound chemical bonds against SARS-CoV-2 main protease 5RL4 receptors

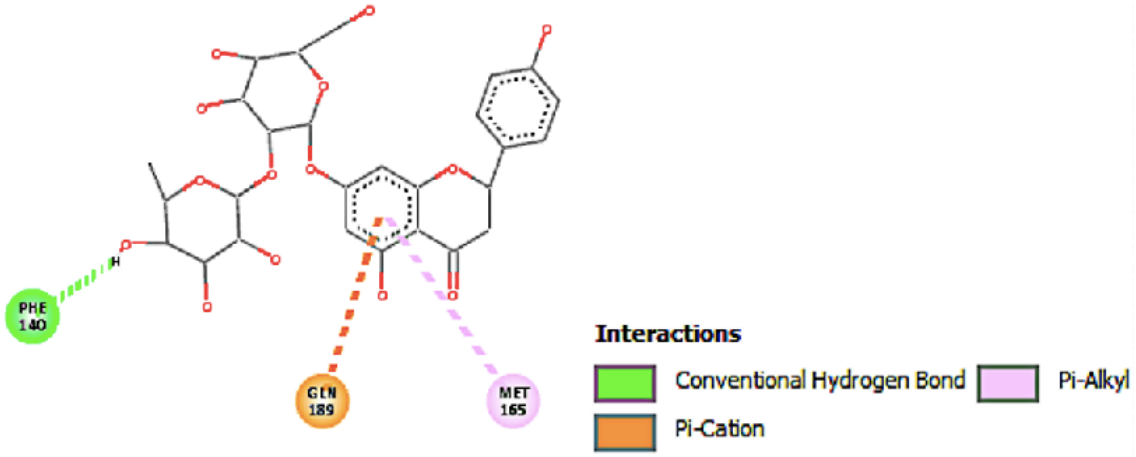

Figure 2. Visualization of amino acid residues and interaction of naringin compound chemical bonds against SARS-CoV-2 main protease ID 5R7Y receptors

9.69 kcal/mol with the interaction of chemical bonds and amino acid residues produced i.e. hydrogen bonds in the form of conventional hydrogen bond (CYS 44, ARG 188, GLU 166, GLY 143, SER 144, LEU 141) and carbon hydrogen bond (GLU 166) in addition to hydrophobic bonds in the form of alkyl and pi-alkyl bonds (MET 49, CYS 145). Visualization interaction shown in Figure 3.

The final analysis of naringin and rutin compound research results had lower ΔG values than native ligands but not lower than comparison ligands. But in docking with SARS-CoV-2 receptors main protease ID PDB 5RL4, naringin compounds have a lower ΔG value of the lopinavir comparison ligand. Based on the results of overall virtual screening, naringin and rutin compounds have good results on antiviral activity predictions and toxicity predictions, for drug likeness predictions both compounds do not qualify Lipinski's rule of five while for pharmacokinetic predictions produced less absorption and good distribution. Molecular mass (less than 500 Dalton), lipophilicity (expressed as LogP less than 5), hydrogen bond donors (less than 5) hydrogen bond acceptors (less than 10), molar refractivity (should be between 40-130).

5. Conclusion

Virtual screening of 80 flavonoid compounds is conducted including screening based on predictions of antiviral activity produced 71 compounds that show antiviral activity, 26 of them have a low probability and

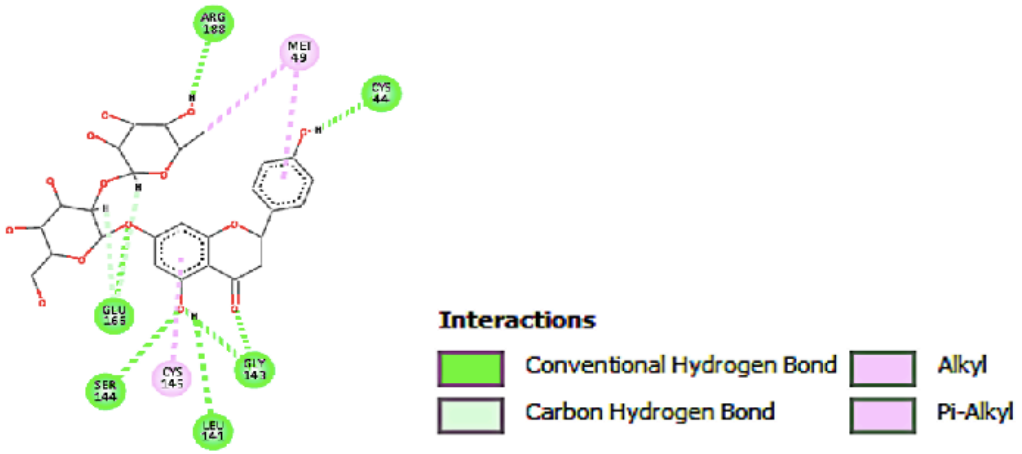

Figure 3. Visualization of amino acid residues and interaction of naringin compound chemical bonds against SARS-CoV-2 main protease 7BUY receptors

five compounds among them are rhoifolin, isoquercetin, rutin, epigallocatechin and naringin have a high probability. On drug likeness predictions produced 64 flavonoid compounds qualify rules Lipinski's rule of five. On pharmacokinetic predictions produced 15 flavonoid compounds that are predicted to have absorbance and good distribution. On the prediction of toxicity produced five compounds that are predicted to have a low risk of exposure and carcinogenic genotoxic, five other compounds predicted non-genotoxic carcinogenic as well as 13 compounds predicted to have mutagen properties. While in molecular docking simulations compounds that become anti-SARS-CoV-2 candidates that work as inhibitors of main protease are naringin and rutin compounds that have a better affinity value than native ligands. Naringin and rutin compounds also have predictions of antiviral activity and predictions of good toxicity as well as a good pharmacokinetic profile of distribution.

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