Potential of Plant Bioactive Compounds as SARS-CoV-2 Main Protease (M^{pro}) and Spike (S) Glycoprotein Inhibitors: A Molecular Docking Study

Trina E. Tallei[1*], Sefren G. Tumilaar[2], Nurdjannah J. Niode[3], Fatimawali[2], Billy J. Kepel[4], Rinaldi Idroes[5], Yunus Effendi[6]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado, Indonesia
[2]Pharmacy Study Program, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado, Indonesia
[3]Department of Dermatology and Venereology, Faculty of Medicine, University of Sam Ratulangi.
[4]Department of Chemistry, Faculty of Medicine, Sam Ratulangi University, Manado, Indonesia
[5]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Syiah Kuala University, Banda Aceh, Indonesia
[6]Department of Biology, Faculty of Mathematics and Natural Sciences, Al Azhar University, Jakarta, Indonesia

Correspondence should be addressed to Trina E. Tallei (trina_tallei@unsrat.ac.id)

Abstract

Background: Since the outbreak of the coronavirus disease 2019 (COVID-19) pandemic, researchers have been trying to investigate several active compounds found in plants that have the potential to inhibit the proliferation of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), the cause of COVID-19. The search for plant-based antivirals against the SARS-CoV-2 is promising, as several plants have been shown to possess antiviral activities against betacoronaviruses (beta-CoVs) **Objective:** The present study aimed to evaluate bioactive compounds found in plants by using a molecular docking approach to inhibit Main Protease (M^{pro}) (PDB code: 6LU7) and Spike (S) Glycoprotein (PDB code: 6VXX) of SARS-CoV-2. **Methods:** Evaluation was performed on the docking scores calculated using AutoDock Vina as a docking engine. For each compound that was docked, a rule of five was calculated to determine whether a compound with certain pharmacological or biological activities might have chemical and physical properties that would make it an active

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drug orally in humans. Determination of the docking score was done by selecting the conformation of the ligand that has the lowest binding free energy (best pose). As a comparison, nelfinavir (an antiretroviral drug), chloroquine and hydroxychloroquine sulfate (anti-malarial drugs recommended by the FDA as emergency drugs) were used. **Results:** The results showed that hesperidine, cannabinoids, pectolinarin, epigallocatechin gallate, and rhoifolin had better poses than nelfinavir, chloroquine and hydroxychloroquine sulfate as spike glycoprotein inhibitors. Hesperidin, rhoifolin, pectolinarin, and cannabinoids had about the same pose as nelfinavir, but were better than chloroquine and hydroxychloroquine sulfate as M^{pro} inhibitors. These plant compounds have the potential to be developed as specific therapeutic agents against COVID-19. **Conclusion**: Several natural compounds of plants evaluated in this study showed better binding free energy compared to nelfinavir, chloroquine and hydroxychloroquine sulfate which so far are recommended in the treatment of COVID-19.

Keywords

Medicinal plants, M^{pro}, $3CL^{pro}$, spike (S) glycoprotein, COVID-19, SARS-CoV-2

Introduction

Coronavirus disease 2019 (COVID-19) is a disease caused by a new type of transmissible pathogenic human severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of Betacoronaviruses (Beta-CoVs) (Lai et al., 2020; Shereen et al., 2020). As of March 11, 2020, WHO has stated that COVID-19 has been characterized as a pandemic. The World Health Organization (2020), as of 3 April 2020, reported 932,166 confirmed cases and 46,764 deaths in 206 countries (WHO, 2020). While in Indonesia, the death toll of COVID-19 reached 170 with the number of positive cases of 1,790 people as of 3 April 2020 (COVID-19, 2020).

COVID-19 infection is characterized by symptoms of acute respiratory distress such as fever 38.1^{o}C - 39^{o}C, dry cough, and shortness of breath with an incubation period of about 5 days (average 2-14 days) (Kamps & Hoffmann, 2020). Until now there is no specific therapy or vaccine available to treat and prevent COVID-19 (NCIRD & Diseases, 2020; WHO, 2020). Therefore, there has been an increase in demand for the availability of medicines, vaccines, diagnostics and reagents, all related to COVID-19. This can lead to opportunities for irresponsible people to distribute falsified medical products.

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Several agents are being used in clinical trials and protocols based on *in vitro* activity against SARS-CoV-2 or related viruses with limited clinical experience, however, the effectiveness of therapy for any type of drug has not been established (Smith et al., 2020). Xu et al. (2020) examined the effectiveness of tocilizumab (atlizumab, an immunosuppressive drug) in a retrospective analysis with the results such as reduced fever, oxygen demand, radiological features and decreased C-reactive protein (CRP) (Xu et al., 2020). Bian et al. (2020) in an open-labeled clinical trial (concurrent controlled add-on clinical trial) of meplazumab found a median virus clearance time, discharge time, and a better repair time (Bian et al., 2020). A study based on molecular dynamics simulation (MDS) of a docked protein-ligand compound, nelfinavir was predicted to be COVID-19 drug candidate as the best potential inhibitor against Main Protease (M^{pro}) (Xu et al., 2020). On the other hand, despite little evidence on the effectivity of chloroquine and hydroxychloroquine, these two antimalarial agents has approved by Food and Drug Administration (FDA) for emergency coronavirus treatment (NCIRD, 2020).

Because COVID-19 is a new disease with serious global health problems, research is still needed, including finding specific therapeutic regimens to overcome the morbidity and mortality it causes. Plant is one of the medicinal active compound sources that have been widely used to treat disease caused by microbes (Estevam et al., 2015; Ningsih et al., 2019; Nuraskin et al., 2020). There are many plant bioactive compounds reported to have activities as antifungal (Sardi et al., 2013), antibacterial (Nuraskin et al., 2019; Pratiwi et al., 2015; Rahmad et al., 2019), and antiviral (Calland et al., 2012; Hu et al., 2013). The natural products that have been reported to have antiviral activity can be used as a starting point in finding potential bioactive compound candidates against SARS-CoV-2. Molecular docking can be used to predict how receptor protein interact with bioactive compounds (ligands) (Earlia et al., 2019a; Earlia et al., 2019b). Several previous studies have been performed to investigate bioactive compounds in plants that have the potential to inhibit the proliferation of viruses (Khaerunnisa et al., 2020; Shaghaghi, 2020; Qamar et al., 2020).

Referring to the importance of early screening for the potential of bioactive compounds to find drug candidates or prevention of viral infections, this study aimed to evaluate various bioactive compounds found in several plants known by the community with a molecular docking approach. The results of the study are expected to be one of the references for further research in finding specific regimens to overcome COVID-19.

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Methods

Determination of ligands

The compounds to be docked (ligands) are active compounds from plants that have been known to have antiviral activities. These compounds are quinine, cannabinoids, hesperidin, rhoifolin, pectolinarin, morin, epigallocatechin gallate, herbacetin, ethyl cholate, kaempferol, tangeretin, chalcone, nobiletin, bis (3,5,5-trimethylhexyl) phthalate, 6-gingerol, 6-shogaol, hydroxychloroquine sulfate, myristicin, and eugenol.

Determination of receptors

Two SARS-CoV-2 proteins were chosen as drug discovery targets : Main Protease (M^{pro}) (also called 3C-like protease - $3CL^{pro}$) (PDB code: 6LU7) and Spike Glycoprotein (S) (PDB code: 6VXX).

Ligand and receptor preparation

Three-dimensional (3D) structures of M^{pro} of SARS-CoV-2 were retrieved from Protein Data Bank (http://www.rcsb.org//pdb) in .pdb formats. These proteins were served as receptors in docking process. The files were opened using BIOVIA Discovery Studio Visualizer 2020. Water molecules and ligands that were still attached to the receptors were removed, and the receptors were stored in the .pdb format. Using Autodock Tools, polar hydrogen atoms were added to the receptors. Subsequently, the files were saved in .pdbqt format.

Ligand structures were obtained from the PubChem site (http://pubchem.ncbi.nlm.nih.gov). The search was done by entering the name of the ligand in the search option. Each ligand's file was downloaded and saved. Files in the .sdf format were converted to .pdb using Open Babel. The .pdb format ligand was opened using the Autodock Tools tool. Torque adjustment was done by detecting root and adjusted as desired. The file was saved in .pdbqt format. Properties of active compounds were calculated using Lipinski's rule of five calculated on SWISSADME prediction (http://www.swissadme.ch/) (Lipinski, 2004).

Active site determination

The location of the amino acids as active sites in the receptor region where the ligand was docked was determined using Autodock Tools. For this reason, a three-dimensional map of the gridbox was made in the receptor region. Determination of this map was be based on

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the type of docking used. A three-dimensional map was made as wide as the the size of the receptor (Spike glycoprotein) itself so that the ligand was likely to be docked to all parts of the receptor (blind docking). In M^{pro}/$3CL^{pro}$ docking, the three-dimensional map was only the size of the area to be docked (targeted docking).

Receptor-ligand docking

The docking was performed using Autodock Vina. Ligands and receptors that had been saved in the .pdbqt format were copied into the Vina folder. Then the vina configuration file was typed into notepad, saved with the name 'conf.txt'. Vina program was run through command prompt.

Analysis and visualization

The results of the docking calculation were shown in the output in notepad format. Determination of the docking conformation of the ligand was done by selecting the pose with the highest affinity (most negative Gibbs free energy).

Results

The estimation of free binding energies between potential inhibitors and receptors was calculated using a docking experiment. Table 1 shows the Lipinski's Rule of Five (RO5) of the docking compounds.

Table1. Lipinski's Rule of Five (RO5) of SARS-CoV-2 M^{pro}/$3CL^{pro}$ and S protein potential inhibitors

Compounds	Molecular Formula	Lipinski's Rule of Five	
		Properties	Value
Nelfinavir	$C_{32}H_{45}N_3O_4S$	Molecular weight (<500 g/mol)	567.78
		LogP (<5)	4.41
		H-bond donor (<5)	4
		H-bond acceptor (<10)	5
		Violation	1
		Meet RO5 criteria	Yes
Chloroquine	$C_{18}H_{26}ClN_3$	Molecular weight (<500 g/mol)	319.87
		LogP (<5)	4.15
		H-bond donor (<5)	1
		H-bond acceptor (<10)	2
		Violation	0
		Meet RO5 criteria	Yes

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Hydroxy-chloroquine sulfate	$C_{18}H_{28}ClNO_5S$	Molecular weight (<500 g/mol)	439.95
		LogP (<5)	2.13
		H-bond donor (<5)	4
		H-bond acceptor (<10)	7
		Violation	0
		Meet RO5 criteria	Yes
Hesperidin	$C_{28}H_{34}O_{15}$	Molecular weight (<500 g/mol)	610.56
		LogP (<5)	-1.06
		H-bond donor (<5)	8
		H-bond acceptor (<10)	15
		Violation	3
		Meet RO5 criteria	No
Cannabinoids	$C_{42}H_{60}O_4$	Molecular weight (<500 g/mol)	628.92
		LogP (<5)	9.12
		H-bond donor (<5)	3
		H-bond acceptor (<10)	4
		Violation	2
		Meet RO5 criteria	Yes
Pectolinarin	$C_{29}H_{34}O_{15}$	Molecular weight (<500 g/mol)	622.57
		LogP (<5)	-0.09
		H-bond donor (<5)	7
		H-bond acceptor (<10)	15
		Violation	3
		Meet RO5 criteria	No
Epigallocatechin gallate	$C_{22}H_{18}O_{11}$	Molecular weight (<500 g/mol)	458.37
		LogP (<5)	0.95
		H-bond donor (<5)	8
		H-bond acceptor (<10)	11
		Violation	2
		Meet RO5 criteria	Yes
Rhoifolin	$C_{27}H_{30}O_{14}$	Molecular weight (<500 g/mol)	578.52
		LogP (<5)	-0.81
		H-bond donor (<5)	8
		H-bond acceptor (<10)	14
		Violation	3
		Meet RO5 criteria	No
Morin	$C_{15}H_{10}O_7$	Molecular weight (<500 g/mol)	302.24
		LogP (<5)	1.2
		H-bond donor (<5)	5
		H-bond acceptor (<10)	7
		Violation	0
		Meet RO5 criteria	Yes
Kaempferol	$C_{15}H_{10}O_6$	Molecular weight (<500 g/mol)	286.24
		LogP (<5)	1.58
		H-bond donor (<5)	4
		H-bond acceptor (<10)	6
		Violation	0
		Meet RO5 criteria	Yes

Herbacetin	$C_{15}H_{10}O_7$	Molecular weight (<500 g/mol)	302.24
		LogP (<5)	1.33
		H-bond donor (<5)	5
		H-bond acceptor (<10)	7
		Violation	0
		Meet RO5 criteria	Yes
Ethyl Cholate	$C_{26}H_{44}O_5$	Molecular weight (<500 g/mol)	436.62
		LogP (<5)	3.5
		H-bond donor (<5)	3
		H-bond acceptor (<10)	5
		Violation	0
		Meet RO5 criteria	Yes
Quinine	$C_{20}H_{24}N_2O_2$	Molecular weight (<500 g/mol)	324.42
		LogP (<5)	2.81
		H-bond donor (<5)	1
		H-bond acceptor (<10)	4
		Violation	0
		Meet RO5 criteria	Yes
Nobiletin	$C_{21}H_{22}O_8$	Molecular weight (<500 g/mol)	402.39
		LogP (<5)	3.02
		H-bond donor (<5)	0
		H-bond acceptor (<10)	8
		Violation	0
		Meet RO5 criteria	Yes
Tangeretin	$C_{20}H_{20}O_7$	Molecular weight (<500 g/mol)	372.37
		LogP (<5)	3.02
		H-bond donor (<5)	0
		H-bond acceptor (<10)	7
		Violation	0
		Meet RO5 criteria	Yes
Chalcone	$C_{15}H_{12}O$	Molecular weight (<500 g/mol)	402.39
		LogP (<5)	3.30
		H-bond donor (<5)	0
		H-bond acceptor (<10)	1
		Violation	0
		Meet RO5 criteria	Yes
6-Gingerol	$C_{17}H_{26}O_4$	Molecular weight (<500 g/mol)	294.38
		LogP (<5)	3.02
		H-bond donor (<5)	2
		H-bond acceptor (<10)	4
		Violation	0
		Meet RO5 criteria	Yes
Bis(3,5,5-trimethylhexyl) phthalate	$C_{26}H_{42}O_4$	Molecular weight (<500 g/mol)	418.61
		LogP (<5)	6.47
		H-bond donor (<5)	0
		H-bond acceptor (<10)	4
		Violation	1
		Meet RO5 criteria	Yes

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Myristicin	$C_{11}H_{12}O_3$	Molecular weight (<500 g/mol)	192.21
		LogP (<5)	2.49
		H-bond donor (<5)	0
		H-bond acceptor (<10)	3
		Violation	0
		Meet RO5 criteria	Yes
Eugenol	$C_{10}H_{12}O_2$	Molecular weight (<500 g/mol)	164.20
		LogP (<5)	2.25
		H-bond donor (<5)	1
		H-bond acceptor (<10)	2
		Violation	0
		Meet RO5 criteria	Yes
6-Shogaol	$C_{17}H_{24}O_3$	Molecular weight (<500 g/mol)	176.37
		LogP (<5)	3.76
		H-bond donor (<5)	1
		H-bond acceptor (<10)	0
		Violation	0
		Meet RO5 criteria	Yes

Table 2 shows the results of docking analysis between the selected compounds with M^{pro} ($3CL^{pro}$) and S protein. The docking results showed that some compounds from plants which had better binding positions with S protein compared to nelfinavir were hesperidine, cannabinoids, pectolinarin, epigallocatechin gallate, and rhoifolin. Other compounds tend to be better positioned compared to chloroquine and hydroxychloroquine sulfate, except for 6-Shogaol. Binding poses to M^{pro} that were better or equivalent to nelfinavir were hesperidin, rhoifolin, and pectolinarin. Some compounds showed better binding poses than chloroquine and hydroxychloroquine on M^{pro}.

Table 2. Molecular docking analysis of several plant compounds against S protein (6VXX) and M^{pro} (6LU7)

Ligand Properties	PubChem CID	Binding Energy With Protein 6VXX	Binding Energy With Protein 6LU7
Nelfinavir	64143	-8.8	-8.2
Hydroxychloroquine sulfate	12947	-7.3	-6.6
Chloroquine	2719	-6.1	-5.3
Hesperidin	10621	-10.4	-8.3
Cannabinoids	9852188	-10.2	-8
Pectolinarin	168849	-9.8	-8.2
Epigallocatechin gallate	65064	-9.8	-7.8
Rhoifolin	5282150	-9.5	-8.2

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Morin	5281670	-8.8	-7.8
Kaempferol	5280863	-8.5	-7.8
Herbacetin	5280544	-8.3	-7.2
Ethyl Cholate	6452096	-8.1	-6.7
Nobiletin	72344	-8.1	-6.4
Tangeretin	68077	-7.9	-6.5
Chalcone	637760	-7.5	-6.2
Quinine	3034034	-7.5	-6.9
6-Gingerol	442793	-6.3	-5.8
Bis(3,5,5-trimethylhexyl) phthalate	34277	-6.1	-5.6
Myristicin	4276	-6.1	-5.3
Eugenol	3314	-6.1	-5.4
6-Shogaol	5281794	-5.5	-5.8

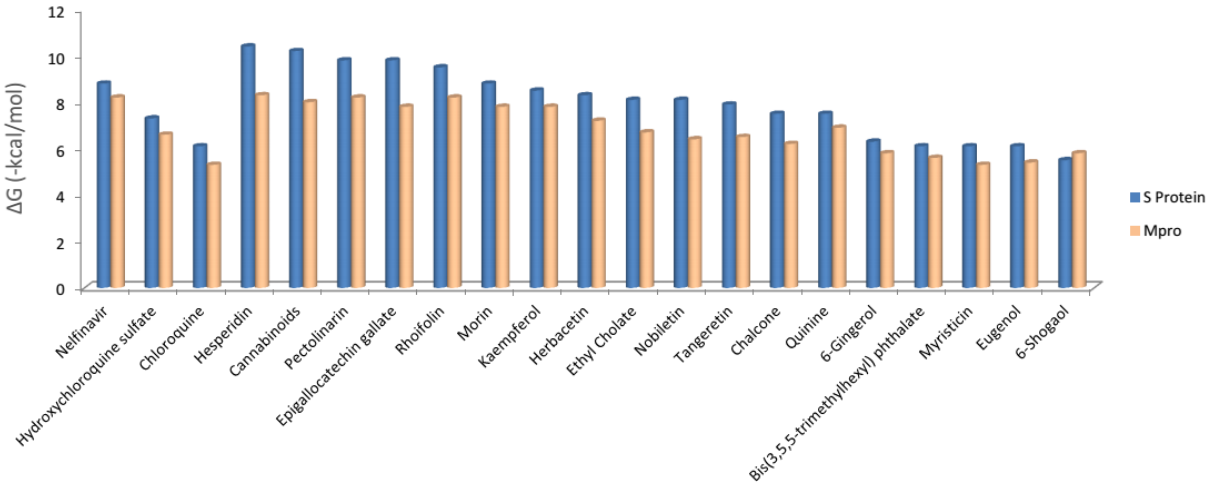

Figure 1. Histogram showing the energy binding value of ΔG (-kcal/mol) of S protein and M^{pro} with several inhibitor compound candidates

The list of plants that have active compounds used as ligands is presented in Table 3. It can be seen that Citrus fruit has many active compounds which are potential as anti-SARS-CoV-2, including hesperidin, rhoifolin, nobiletin, tangeretin, and chalcone. The table shows that only pectolinarin, epigallocatechin gallate, myristicin and eugenol have high bioavailability when administered orally.

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Table 3. List of plants that have active compounds used as ligands and their bioavailability

Compounds	Oral Bioavailability	Sources
Hesperidin	Low	Citrus fruit (*Citrus* spp.), Peppermint (*Mentha* spp.), Yellow Toadflax (*Linaria vulgaris*)
Cannabinoids	Low	Marijuana (*Cannabis* spp.)
Pectolinarin	High	Plume thistles (*Cirsium* spp.), Yellow toadflax (*Linaria vulgaris*)
Epigallocatechin gallate	High	Tea (*Camellia sinensis*) (green tea), skin of Apple (*Malus domestica*), Plum (*Prunus domestica*), Onion (*Allium cepa*), Hazelnut (*Corylus avellana*)
Rhoifolin	Low	Rhus plant (*Rhus succedanea*), Bitter orange (*Citrus aurantium*), Bergamot (*Citrus bergamia*), Grapefruit (*Citrus paradisi*), Lemon (*Citrus limon*), Lablab beans (*Lablab purpureus*), Tomato (*Lycopersicon esculentum*), Artichoke (*Cynara scolymus*), Bananas (Musa spp.), Grape (*Vitis vinifera*)
Morin	Very low	Osage orange (*Maclura pomifera*), Almond (*Prunus dulcis*), Old fustic (*Chlorophora tinctoria*), Guava (*Psidium guajava*)
Kaempferol	Low to good	Kale (*Brassica oleracea* var. *sabellica*), Beans (*Phaseolus vulgaris*), Tea (*Camellia sinensis*), Spinach (*Spinacia oleracea*), Broccoli (*Brassica oleracea* var. *Italica*)
Herbacetin	Good	Golden root (*Rhodiola* spp.), Gossypium (*Gossypium hirsutum*), Common horsetail (*Equisetum arvense*), Common boneset (*Eupatorium perfoliatum*)
Ethyl Cholate	N/A	Leaf of football fruit / keluak (*Pangium edule*)
Nobiletin	Low	Citrus fruit (*Citrus* spp.)
Tangeretin	Low	Citrus fruit (*Citrus* spp.)
Chalcone	Very good	Citrus fruit (*Citrus* spp.)
6-Gingerol	Low	Fresh Ginger (*Zingiber officinale*)
Bis (3,5,5-trimethylhexyl) phthalate	Low	Leaf of football fruit / keluak (*Pangium edule*)
Myristicin	High	Nutmeg (*Myristica fragrans*)
Eugenol	High	Clove (*Syzygium aromaticum*)
6-Shogaol	Low	Ginger (*Zingiber officinale*)

Discussion

In determining that a compound has the potential as a drug, one of which is to follow the rule of five (RO5). Therefore, each docking compound was checked whether it met the Lipinski's RO5. Some compounds that show violations towards RO5 are nelfinavir (1), hesperidin (3), cannabinoids (2), pectolinarin (3), epigallocatechin gallate (2), rhoifolin (3), and bis(3,5,5-trimethylhexyl) phthalate (1) (Tabel 1). The rule is used for the evaluation the drug-likeness, as well as determination if any certain chemical compound possesses chemical

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and physical properties to be used as an active drug which can be consumed orally in humans (Lipinski, 2004). It also acts as a basis for the prediction of high probability of success or failure of one compound with certain pharmacological or biological activity to be developed as a drug. This rule also suggests that if a compound shows two or more RO5 violations, then the compound shows low solubility or permeability (Benet et al., 2016)

Dozens of proteins are coded by coronavirus, some of which are involved in viral replication and entry into cells. Main protease (M^{pro}/$3CL^{pro}$) is a key enzyme for coronavirus replication (X. Liu & Wang, 2020), and surface Spike (S) glycoprotein (S protein) is an important binding protein for fusion of the virus and cellular membrane via cellular receptor angiotensin-converting enzyme 2 (ACE2) (Song et al., 2018). SARS-Cov-2 is easily transmitted because the S protein on the surface of the virus binds very efficiently to ACE2 on the surfaces of human cells. Therefor, M^{pro} and S protein are ideal targets for drug design and development.

Efforts have been made globally to obtain vaccines or drugs for the prevention or treatment of COVID-19 infections. So far, remdesivir is the most promising COVID-19 drug, although the FDA has also approved the use of chloroquine and hydroxychloroquine. Coutard et al. (2020) suggested finding an inhibitor for furin, because the S protein sequence has a specific furin-like cleavage. In addition, some researchers have targeted $3CL^{pro}$ for treating coronaviral infection (Qamar et al., 2020; Wu et al., 2020).

The results of this study which aimed at predicting the inhibition ability of compounds found in some plants against M^{pro} and S proteins have revealed several results showing that these compounds have a better docking pose than nelfinavir, chloroquin, and hydroxychloroquine sulfate (Tabel 2 and Figure 1). If the results are juxtaposed, the potential candidates to become drugs targeting S protein and M^{pro} were hesperidin, cannabinoids, rhoifolin, pectolinarin, morine, epigallocatechin gallate, and herbacetin. Some of the plants producing compounds which are docked with the target protein can be seen in Table 3. Table 3 also contains information on oral bioavailability of the compounds used as ligands in this analysis. Only a few compounds have high bioavailability when administered orally, i.e. pectolinarin, epigallocatechin gallate, myristicin and eugenol. The low oral bioavailability has become a common problem in drug design, since it may pose failure to a new drug in a clinical trials, eventhough the compounds have high eficacy in *in vitro* and/or *in vivo* tests (Kim et al., 2014). This may incure problem faced by scientists in the pharmaceutical industry (Lin & Wong, 2017). Therefore, oral bioavailability of a compound is essential to

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be taken into account when predicting the compound as a drug candidate. Oral availability of some compounds can be low if administered together with food.

The major flavanone glycosides in the citrus peel is hesperidin (Brett et al., 2008). Docking scores in this study of S protein and M^{pro} were -10.4 and -8.3, respectively. Utomo et al. (2020) have docked this compound against S protein (-9.6) and M^{pro} (-13.51). Chen et al (2020) revealed that the best hesperidin position against SARS-CoV-2 3C-like protease ($3CL^{pro}$) was -10.1 (Chen et al., 2020). Adem et al. (2020) found that the ability of hesperidin was better than nelfinavir (Adem et al., 2020).

Cannabinoids are active compounds of *Cannabis sativa* and *C. indica*. Docking score of this compound against M^{pro} and S protein was -8 and -10.2, respectively. Besides being known as an anti-herpes simplex virus (Blevins & Dumic, 1980), this compound also has an anti-inflammatory activity (Reiss, 2010). However, some researches show that this compound can increase the pathogenesis of the virus to the host (Liu et al., 2020; Reiss, 2010; Tahamtan et al., 2018).

The docking results using rhoifolin as ligan were -9.5 and -8.2 for S protein and M^{pro}, respectively. Rhoifolin is a flavon that was first discovered in fresh leaves of *Rhus succedanea* in 1952 (Hattori & Matsuda, 1952). In addition, this compound was also found in *Citrus grandis* (Rao et al., 2011). The results of rhoifolin docking on S protein were -9.5 and M^{pro} was -8.2. The rhoifolin binding score for SARS-CoV $3CL^{pro}$ shows a value of -9.565 (Jo et al., 2020).

The induced-fit docking result of pectolinarin against SARS-CoV $3CL^{pro}$ was -8.054 (Jo et al., 2020). In this study, the best pose between pectolinarin and S protein was -9.8 and -8.2 with M^{pro}. Pectolinarin can be found in Plume thistles (*Cirsium* spp). Morin docking results by Jo et al (2020) against SARS-CoV $3CL^{pro}$ was -8,930. In this study, the best docking scores of morin against S protein and M^{pro} were -8.8 and -7.8, respectively. Almond, Old fustic, and Guava contain high quantity of this compound. Kaempferol can be found in spinach and kale. The best position of kaempferol against S protein was -8.5 and -7.8 against M^{pro}, while Jo et al. (2020) found that -8,526 was the best binding position of this compound against SARS-CoV $3CL^{pro}$.

Epigallocatechin gallate is found in high quiantity in tea (*Camellia sinensis*), especally in the form of green tea. The best binding position of this compound against S protein was -9.8 and against M^{pro} was -7.8. It has ben reported previously that this compound was able to inhibit the proteolytic activity of SARS-CoV $3CL^{pro}$ (Nguyen et al., 2012). Herbacetin which can be found *Rhodiola* sp. (golden root) has antiviral activity against

vesicular stomatitis virus (VSV), a prototype of negative-strand RNA viruses, such as rabies and influenza viruses (Ahmed et al., 2015). The best binding pose of this compound against SARS-3CL^{pro} was -9.263 as reported by Jo et al. (2020), while in this study, binding score of -8.3 against S protein and -7.2 against M^{pro} were obtained. They also stated that herbacetin might act as an MERS-CoV 3CL^{pro} inhibitor.

In a very limited time, we have been able to assess some potential M^{pro} and S protein of SARS-CoV-2 plant-derived inhibitors using molecular docking method. These results are only preliminary screening to facilitate subsequent tests starting from *in vitro* and *in vivo* (in animal models or human clinical trials).

Conclusion

Our study revealed that natural compounds hesperidine, cannabinoids, pectolinarin, epigallocatechin gallate, and rhoifolin had better binding free energies with M^{pro} and S protein of SARS-CoV-2. These compounds have a potential as anti-viral phytochemicals that may inhibit the replication of the virus. However, further *in vitro* and later *in vivo* tests are needed to evaluate these potential inhibitors as clinical drugs. The oral bioavailability of several compounds were indicated low, therefore this also needs attention.

References

Adem, S., Eyupoglu, V., Sarfraz, I., Rasul, A., & Ali, M. (2020). Identification of Potent COVID-19 Main Protease (Mpro) Inhibitors from natural polyphenols: An *in silico* strategy unveils a hope against CORONA. *Preprints*, *2020030333*. https://doi.org/10.20944/preprints202003.0333.v1

Ahmed, M., Henson, D. A., Sanderson, M. C., Nieman, D. C., Zubeldia, J. M., & Shanely, R. A. (2015). *Rhodiola rosea* exerts antiviral activity in athletes following a competitive marathon race. *Frontiers in Nutrition*, *2*. https://doi.org/10.3389/fnut.2015.00024

Benet, L. Z., Hosey, C. M., Ursu, O., & Oprea, T. I. (2016). BDDCS, the Rule of 5 and drugability. *Advanced Drug Delivery Reviews*, *101*, 89–98. https://doi.org/10.1016/j.addr.2016.05.007

Bian, H., Zheng, Z.-H., Wei, D., Zhang, Z., Kang, W.-Z., Hao, C.-Q., … Zhu, P. (2020). Meplazumab treats COVID-19 pneumonia: an open-labelled, concurrent controlled add-on clinical trial. *MedRxiv*, 2020.03.21.20040691. https://doi.org/10.1101/2020.03.21.20040691

Blevins, R. D., & Dumic, M. P. (1980). The effect of delta-9-tetrahydrocannabinol on herpes

simplex virus replication. *The Journal of General Virology*, *49*(2), 427–431. https://doi.org/10.1099/0022-1317-49-2-427

Brett, G. M., Hollands, W., Needs, P. W., Teucher, B., R. Dainty, J., Davis, B. D., … Kroon, P. A. (2008). Absorption, metabolism and excretion of flavanones from single portions of orange fruit and juice and effects of anthropometric variables and contraceptive pill use on flavanone excretion. *British Journal of Nutrition*, *101*(5), 664–675. https://doi.org/10.1017/S000711450803081X

Calland, N., Dubuisson, J., Rouillé, Y., & Séron, K. (2012). Hepatitis C virus and natural compounds: a new antiviral approach? *Viruses*, *4*(10), 2197–2217. https://doi.org/10.3390/v4102197

Chen, Y. W., Yiu, C.-P. B., & Wong, K.-Y. (2020). Prediction of the SARS-CoV-2 (2019-nCoV) 3C-like protease (3CLpro) structure: virtual screening reveals velpatasvir, ledipasvir, and other drug repurposing candidates. *F1000Research*, *9*, 129. https://doi.org/10.12688/f1000research.22457.1

Coutard, B., Valle, C., de Lamballerie, X., Canard, B., Seidah, N. G., & Decroly, E. (2020). The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. *Antiviral Research*, *176*, 104742. https://doi.org/10.1016/j.antiviral.2020.104742

COVID-19. (2020). *COVID-19 Hotline 119 ext 9*. Retrieved from www.covid19.go.id

Earlia, N., Muslem, Suhendra, R., Amin, M., Prakoeswa, C. R. S., Khairan, & Idroes, R. (2019a). GC/MS analysis of fatty acids on Pliek U oil and its pharmacological study by molecular docking to filaggrin as drug candidate in atopic dermatitis treatment. *The Scientific World Journal*, Article ID 8605743, 7 pp. https://doi.org/10.1155/2019/8605743

Earlia, N., Rahmad, R., Amin, M., Prakoeswa, C., Khairan, K., & Idroes, R. (2019b). The potential effect of fatty acids from Pliek U on epidermal fatty acid binding protein: Chromatography and bioinformatic studies. *Sains Malaysiana*, *48*(5), 1019–1024. https://doi.org/10.17576/jsm-2019-4805-10

Estevam, E.C., Griffin, S., Nasim, J., Zieliński, D., Aszyk, J., Osowicka, M., … Jacob, C. (2015). Inspired by nature: The use of plant-derived substrate/enzyme combinations to generate antimicrobial activity *in situ*. *Natural Product Communications*, *10*, 1733–1738. https://doi.org/10.1177/1934578X1501001025.

Hattori, S., & Matsuda, H. (1952). Rhoifolin, a new flavone glycoside, isolated from the leaves of *Rhus succedanea*. *Archives of Biochemistry and Biophysics*, *37*(1), 85–89.

https://doi.org/10.1016/0003-9861(52)90164-1

Hu, Q. F., Zhou, B., Huang, J. M., Gao, X. M., Shu, L. D., Yang, G. Y., & Che, C. T. (2013). Antiviral phenolic compounds from *Arundina gramnifolia*. *Journal of Natural Products*, *76*(2), 292–296.

Jo, S., Kim, H., Kim, S., Shin, D. H., & Kim, M. (2019). Characteristics of flavonoids as potent MERS-CoV 3C-like protease inhibitors. *Chemical Biology & Drug Design*, *94*(6), 2023–2030. https://doi.org/10.1111/cbdd.13604

Jo, S., Kim, S., Shin, D. H., & Kim, M.-S. (2020). Inhibition of SARS-CoV 3CL protease by flavonoids. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *35*(1), 145–151. https://doi.org/10.1080/14756366.2019.1690480

Kamps, B. S., & Hoffmann, C. (2020). *Covid Reference*. Steinhauser Verlag. Retrieved from http://zums.ac.ir/files/socialfactors/files/CovidReference._ENGLAND_(1).pdf

Khaerunnisa, S., Kurniawan, H., Awaluddin, R., & Suhartati, S. (2020). Potential inhibitor of COVID-19 main Protease (M pro) from several medicinal plant compounds by molecular docking study. *Preprints*, (March), 1–14. https://doi.org/10.20944/preprints202003.0226.v1

Kim, M. T., Sedykh, A., Chakravarti, S. K., Saiakhov, R. D., & Zhu, H. (2014). Critical evaluation of human oral bioavailability for pharmaceutical drugs by using various cheminformatics approaches. *Pharmaceutical Research*, *31*(4), 1002–1014. https://doi.org/10.1007/s11095-013-1222-1

Lai, C.-C., Shih, T.-P., Ko, W.-C., Tang, H.-J., & Hsueh, P.-R. (2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. *International Journal of Antimicrobial Agents*, *55*(3), 105924. https://doi.org/10.1016/j.ijantimicag.2020.105924

Lin, L., & Wong, H. (2017). Predicting Oral Drug Absorption: Mini review on physiologically-based pharmacokinetic models. *Pharmaceutics*, *9*(4), 41. https://doi.org/10.3390/pharmaceutics9040041

Lipinski, C. A. (2004). Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discovery Today: Technologies*, *1*(4), 337–341. https://doi.org/10.1016/j.ddtec.2004.11.007

Liu, C., Sadat, S. H., Ebisumoto, K., Sakai, A., Panuganti, B. A., Ren, S., … Califano, J. A. (2020). Cannabinoids promote progression of HPV positive head and neck squamous cell carcinoma via p38 MAPK activation. *Clinical Cancer Research*, clincanres.3301.2018. https://doi.org/10.1158/1078-0432.CCR-18-3301

Liu, X., & Wang, X.-J. (2020). Potential inhibitors for 2019-nCoV coronavirus M protease from clinically approved medicines. *BioRxiv*, 2020.01.29.924100. https://doi.org/10.1101/2020.01.29.924100

NCIRD. (2020). Clinical Guidance Management Patients. *National Center for Immunization and Respiratory Diseases, Diseases, Division of Viral*. Retrieved from https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-guidance-management-patients.html

Nguyen, T. T. H., Woo, H.-J., Kang, H.-K., Nguyen, V. D., Kim, Y.-M., Kim, D.-W., … Kim, D. (2012). Flavonoid-mediated inhibition of SARS coronavirus 3C-like protease expressed in Pichia pastoris. *Biotechnology Letters*, *34*(5), 831–838. https://doi.org/10.1007/s10529-011-0845-8

Ningsih, D. S., Idroes, R., Bachtiar, B. M., & Khairan. (2019). The potential of five therapeutic medicinal herbs for dental treatment : A review. *IOP Conference Series: Materials Science and Engineering*, *523*(1), 12009. https://doi.org/10.1088/1757-899X/523/1/012009

Nuraskin, C. A., Marlina, Idroes, R., Soraya, C., & Djufri. (2019). Activities inhibition methanol extract laban leaf (*Vitex pinnata*) on growth of bacteria S. mutans Atcc 31987. *IOP Conference Series: Materials Science and Engineering*, *523*(1). https://doi.org/10.1088/1757-899X/523/1/012008

Nuraskin, C., Marlina, Idroes, R., Soraya, C., & Djufri. (2020). Identification of secondary metabolite of laban leaf extract (*Vitex pinnata* L.) from geothermal areas and non-geothermal of agam mountains in Aceh Besar, Aceh province, Indonesia. *Rasayan Journal of Chemistry*, *13*(1), 18–23. https://doi.org/10.31788/RJC.2020.1315434

Pratiwi, S. U. T., Lagendijk, E. L., de Weert, S., Idroes, R., Hertiani, T., & den Hondel, C. Van. (2015). Effect of *Cinnamomum burmannii* Nees ex Bl. and *Massoia aromatica* Becc. essential oils on planktonic growth and biofilm formation of *Pseudomonas aeruginosa* and *Staphylococcus* aureus in *vitro*. *International Journal of Applied Research in Natural Products*, *8*(2), 1–13.

Rahmad, R., Earlia, N., Nabila, C., Inayati, I., Amin, M., Prakoeswa, C. R. S., … Idroes, R. (2019). Antibacterial cream formulation of ethanolic Pliek U extracts and ethanolic residue hexane Pliek U extracts against *Staphylococcus aureus*. *IOP Conference Series: Materials Science and Engineering*, *523*, 12011. https://doi.org/10.1088/1757-899X/523/1/012011

Rao, Y. K., Lee, M.-J., Chen, K., Lee, Y.-C., Wu, W.-S., & Tzeng, Y.-M. (2011). Insulin-

Mimetic Action of Rhoifolin and Cosmosiin Isolated from Citrus grandis (L.) Osbeck Leaves: Enhanced Adiponectin Secretion and Insulin Receptor Phosphorylation in 3T3-L1 Cells. *Evidence-Based Complementary and Alternative Medicine*, *2011*, 1–9. https://doi.org/10.1093/ecam/nep204

Reiss, C. S. (2010). Cannabinoids and Viral Infections. *Pharmaceuticals (Basel, Switzerland)*, *3*(6), 1873–1886. https://doi.org/10.3390/ph3061873

Sardi, J. C. O., Scorzoni, L. B., & T. Fusco-Almeida, A M Giannini, M. . (2013). Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. *Journal of Medical Microbiology*, *62*(1), 10–24.

Shaghaghi, N. (2020, March 5). Molecular Docking Study of Novel COVID-19 Protease with Low Risk Terpenoides Compounds of Plants. https://doi.org/10.26434/chemrxiv.11935722.v1

Shereen, M. A., Khan, S., Kazmi, A., Bashir, N., & Siddique, R. (2020). COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. *Journal of Advanced Research*, *24*, 91–98. https://doi.org/10.1016/j.jare.2020.03.005

Smith, T., Bushek, J., & Prosser, T. (2020). COVID-19 Drug Therapy Highlights : Antimicrobials with potential activity against SARS-CoV-2 : *Clinical Drug Information*, (Cdc).

Song, W., Gui, M., Wang, X., & Xiang, Y. (2018). Cryo-EM structure of the SARS coronavirus spike glycoprotein in complex with its host cell receptor ACE2. *PLOS Pathogens*, *14*(8), e1007236. https://doi.org/10.1371/journal.ppat.1007236

Tahamtan, A., Samieipoor, Y., Nayeri, F. S., Rahbarimanesh, A. A., Izadi, A., Rashidi-Nezhad, A., … Salimi, V. (2018). Effects of cannabinoid receptor type 2 in respiratory syncytial virus infection in human subjects and mice. *Virulence*, *9*(1), 217–230. https://doi.org/10.1080/21505594.2017.1389369

Qamar, M. T., Alqahtani, S. M., Alamri, M. A., & Chen, L.-L. (2020). Structural basis of SARS-CoV-2 3CLpro and anti-COVID-19 drug discovery from medicinal plants. *Journal of Pharmaceutical Analysis*. https://doi.org/10.1016/j.jpha.2020.03.009

Utomo, R. Y., Ikawati, M., & Meiyanto, E. (2020). Revealing the Potency of Citrus and Galangal Constituents to Halt SARS-CoV-2 Infection. *Preprints.Org*, *2*(March), 1–8. https://doi.org/10.20944/preprints202003.0214.v1

WHO. (2020a). Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected. Retrieved April 1, 2020, from https://www.who.int/publications-detail/clinical-management-of-severe-acute-

 doi:10.20944/preprints202004.0102.v2

respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected

WHO. (2020b). World Health Organization. Coronavirus disease 2019 (COVID-19) Situastion Report – 72. Retrieved April 1, 2020, from https://experience.arcgis.com/experience/685d0ace521648f8a5beeeee1b9125cd

Wu, C., Liu, Y., Yang, Y., Zhang, P., Zhong, W., Wang, Y., … Li, H. (2020). Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. *Acta Pharmaceutica Sinica B*. https://doi.org/10.1016/j.apsb.2020.02.008

Xu, X., Han, M., Li, T., Sun, W., Wang, D., Fu, B., … Wei, H. (2020). Effective Treatment of Severe COVID-19 Patients with Tocilizumab. *ChinaXiv*, 202003.00026v1. Retrieved from http://www.chinaxiv.org/abs/202003.00026v1