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Exploring the Capability of Indonesia Natural Medicine Secondary Metabolite as Potential Inhibitors of SARS-CoV-2 Proteins to Prevent Virulence of COVID-19: *In silico* and Bioinformatic Approach

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Abstract

BACKGROUND: SARS-CoV-2 was causing COVID-19 disease resulting in many deaths and being a significant concern in the world today. There is an emergent need to search for possible medications for COVID-19 treatment. The key point to halt SARS-CoV-2 infection through inhibition of the virus-receptor interaction and stimulates the immune system. Utilization of the bioinformatic and *in silico* molecular docking a number of available medications might be proven to be effective in inhibiting SARS-CoV-2 main drug targets including the SARS-CoV2 spike glycoprotein, the 3CL protease SARS-CoV-2 active target, PD-ACE2, 2019-nCoV PLpro, and NF-kβ.

AIM: This present study was conducted to identify the potential target and molecular mechanism of the major compound on *Alpinia galanga* extract and *Citrus sinensis* (L.) extract in circumventing COVID-19 using a bioinformatics approach and *in silico* molecular docking.

RESULTS: Direct protein target of all secondary metabolite and the gene list from PubMed "Severe acute respiratory syndrome coronavirus 2" generated 2 genes (CCL2 and VEGFA) as potential therapeutics target genes (PTTG). The molecular docking was conducted by the Protein-Ligand Ant System (PLANTS) software. The results show that hesperidin, naringenin, and galangin have lower docking score for all five-protein target receptor compared with chloroquine and remdesivir. The lower docking score suggests a high affinity to bind the protein. Moreover, these compounds have a strong affinity in their inhibitory capacity for viral infection.

CONCLUSION: In general, this study's findings show that the compound of *Alpinia galanga* extract dan *Citrus sinensis* (L.) extract exhibit the best potential as an inhibitor to the development of the SARS-CoV-2 and inhibited cytokine storm through inactivation NF-kβ pathway.

Introduction

The spread of coronavirus SARS-CoV-2 (COVID-19) has attracted massive concern around worldwide, due to its progressive implementation in more than 100 countries since December 2019 [1]. The endemic of this virus invites the challenge rapidly to design dan discover any therapeutic drug candidates in concordance with the finding of virus-molecular characteristic [2]. Molecular docking and bioinformatic approach have become successful methods for drug discovery and production [3], [4], [5]. Therefore, to search for potential and specific inhibitors of COVID-19, we carried out the virtual screening to identify novel phytochemicals against COVID-19 from Indonesian medicine plants. In this study, we used spike glycoprotein, the 3CL protease SARS-CoV-2 and 2019-nCoV PLpro of virus and PD-ACE2 of a host cell target as molecular targets against COVID-19. Using molecular docking tools, we evaluated the interaction of drug ligand molecules inside the binding pocket of the target protein [6]. A previous study reported that several proteins that play an important role of the SARS-CoV-2 infection, including the spike glycoprotein, the 3CL protease SARS-CoV-2 active target, PD-ACE2, and 2019-nCoV PLpro [2], [7], [8]. However, exploring the drugs that have binding potential to protein that associated with COVID-19 replication remain unclear. Therefore, in this study, we evaluate the potential target and molecular mechanism of the major compound on *Alpinia galanga* extract and *Citrus sinensis* (L.) extract in circumventing COVID-19 using a bioinformatics approach and *in silico* molecular docking.

The SARS-CoV-2 glycoprotein shows little change in the primary structure relative to the beta coronavirus, SARS-CoV, due to mutation, which offers an ideal target candidate for new drugs [9]. The glycoprotein contains receptor binding domain that bind the glycoprotein to the host cell membrane through high affinity for the receptor-mediated angiotensin-converting enzyme 2 (ACE-2) that enables the host cell to join [10], [11]. In addition, 3CL pro main protease is responsible for controlling several major functions of the virus and has a highly conserved catalytic domain from the SARS virus. Some of its roles include virus

replication processes, which make it the perfect target for drug growth [12]. On the other hand, nonstructural protein (NP) is functional protein of high importance to COVID-19. They also take part in the virus replication and human infection through RNA transcription and translation. NP is formed by proteolytic cleavage of replicate polyprotein 1a (pp1a) and replicate polyprotein 1ab (pp1ab) by the action of viral papain-like protease (PLpro) on N-terminus resulting in three products18 and 3 chymotrypsin-like proteases (3CLpro) [13].

All of these proteins (spike, protease, and receptor) are important to the virus transmission and virulence. Through inhibiting anyone of several protein for a higher active therapy, the severity of the infection will be decreased [14], [15], [16]. Therefore, the inhibitory effect of some compounds to these proteins suggests to give protection of the virus recognition. Recently, the research on finding the best protease inhibitor for SARS-CoV-2 treatment has become more comprehensive *in silico* model using the crystal structure of the protease-domain inhibitor complex. This approach of *in silico* study is still challenging to find more accurate candidates efficiently with minimal adverse effects.

Natural plant medicine has been rich source of active secondary metabolite that has had a pivotal role in treating and preventing some diseases [17]. In addition, the use of natural plant medicine also could be more easily utilized by the people [18]. Therefore, we evaluated the docking interaction of several Indonesia natural plant medicine such as *Alpinia galanga* and *Citrus sinensis* (L.) against five target protein, spike glycoprotein, the 3CL protease SARS-CoV-2 active target, PD-ACE2, and 2019-nCoV PLpro is needed. Hopefully, these findings can be used as a guide in the developing of new drug candidates in the COVID-19 prevention with daily consumption without any side effects.

Materials and Methods

Molecular docking

The Protein Data Bank (PDB) was utilized to retrieve the crystal structure of the five SARS-CoV-2 viral proteins; main proteinase or chymotrypsin-like protease (3CLpro, PDB ID:6LU7), papain-like protease (PLpro, PDB ID:4OVZ), spike glycoprotein (s-glycoprotein PDB ID:6VSB), and PD-ACE-2 (PDB ID:6VW1) having resolution < 2 Å, R-Value Free < 0.30, R-Value Work < 0.25. Before testing the ligands against SARS-CoV-2 target proteins, the structures of the small molecules were optimized using the classical MM2 force field. The YASARA software was used to prepare the protein before docking simulation (www.yasara.org/viewdl.htm). The chemical structure of all compounds was obtained from PubChem and prepared using ChemAxon (www.chemaxon.com/marvin/download-user.html). AutoDock Vina program was used for simulation of molecular docking. Furthermore, the visualization of docking simulation in this study was determined under PyMol www.pymol.org (Figure 1).

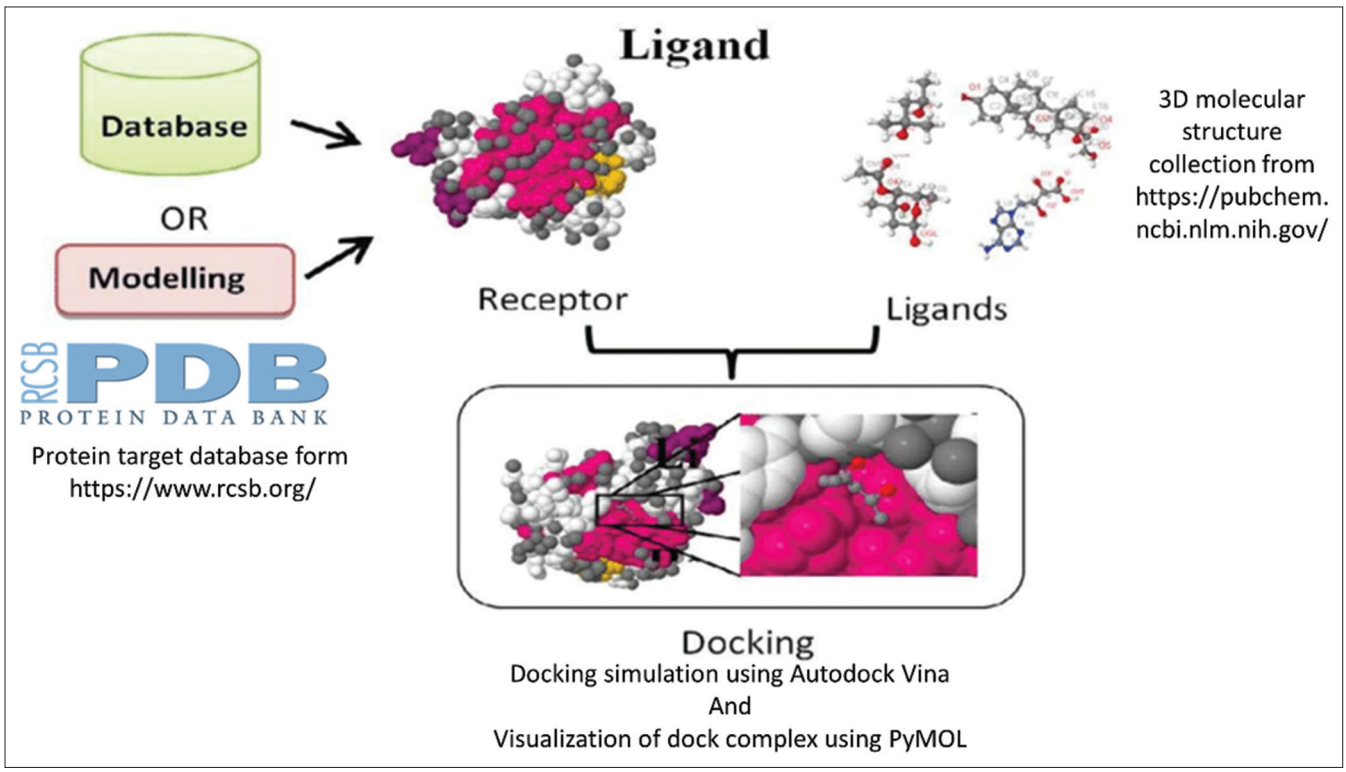

Figure 1: Schematic molecular docking method

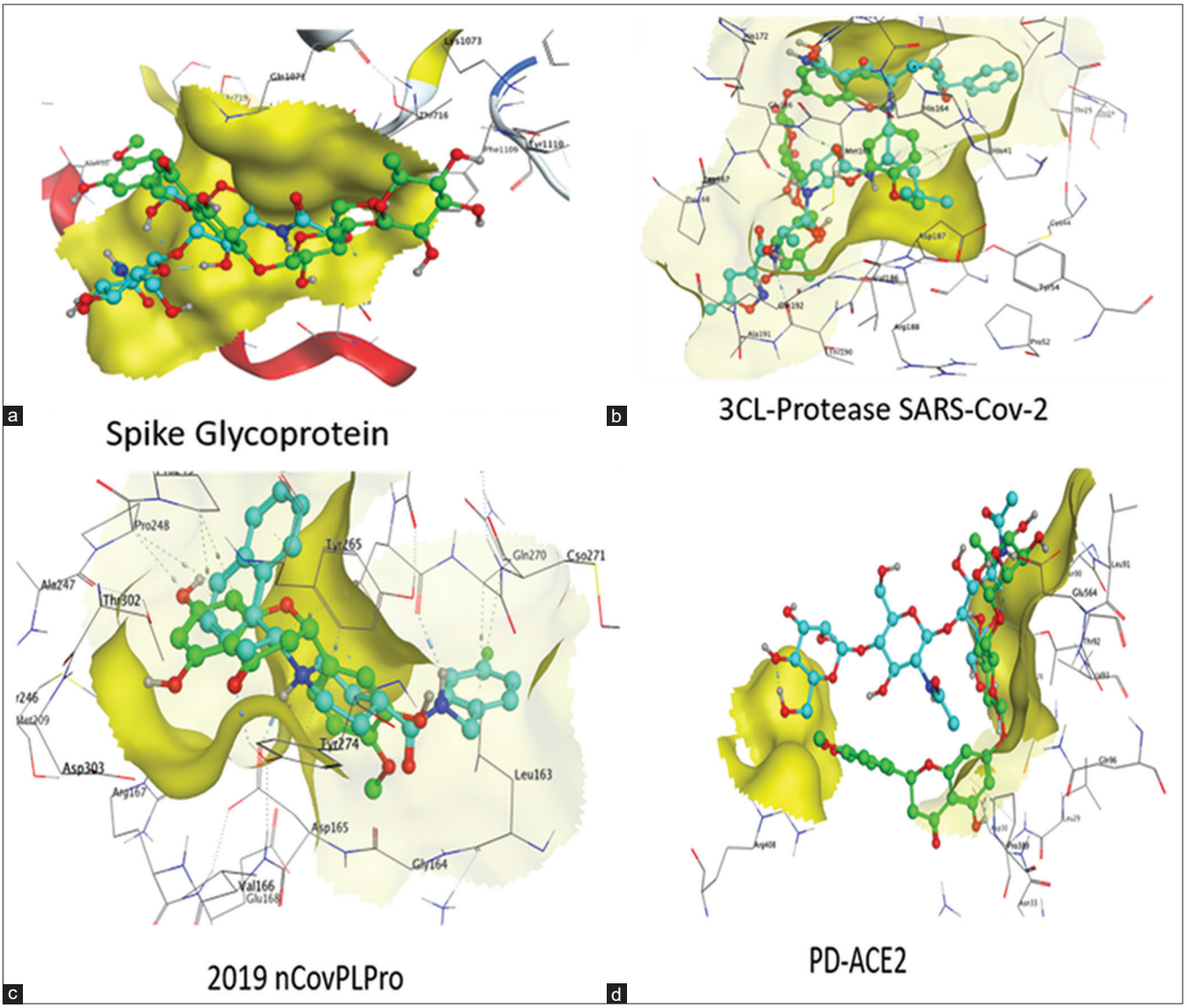

Figure 2: The visualization of compound interaction between Hesperidin and five proteins target SARS-Cov-2. Molecular interaction was evaluated using PyMol. Compound is represented as gold/green balls and sticks, while the native ligand is represented as Tosca balls and sticks

Bioinformatic data collection of direct protein target and COVID-19 regulatory genes

Direct genes protein (DTP) of *Alpinia galanga* and *Citrus sinensis* (L.) secondary metabolite including was search from STITCH having coefficient correlating >0.7. Cellular senescence regulatory genes were retrieved from PubMed with the keywords "COVID-19." A venn diagram between DTP and COVID-19 regulatory genes was constructed using Venny 2.1. The overlapping genes were considered as *Alpinia galanga* and *Citrus sinensis* (L.) secondary metabolite targets in COVID-19. Analysis of protein-protein interaction network was constructed with STRING-DB v11.0 with confidence scores > 0.9 and visualized by Cytoscape software (version 3.7.1). Genes with a degree greater than 10, analyzed by CytoHubba plugin, were selected as hub genes.

Results and Discussion

Molecular docking study of secondary metabolite of Alpinia galanga and Citrus sinensis peels extract

Alpinia galanga and *Citrus sinensis* have various metabolite compounds that are supposed to have bioactivity to inhibit the spreading of SARS-CoV-2. They are Galangin, Kaempferitrin, and ACA for *Alpinia galanga* and for *Citrus sinensis* they are Hesperidin, Hesperitin, Naringenin, Nobiletin, and Tangeretin. All ligands from *Alpinia galanga* and *Citrus sinensis* metabolite compounds have been targeted to the SARS-CoV-2 protein using docking studies to combat SARS-CoV-2 protein interaction with other cells in human body. The Docking score between the four SARS-CoV-2 proteins

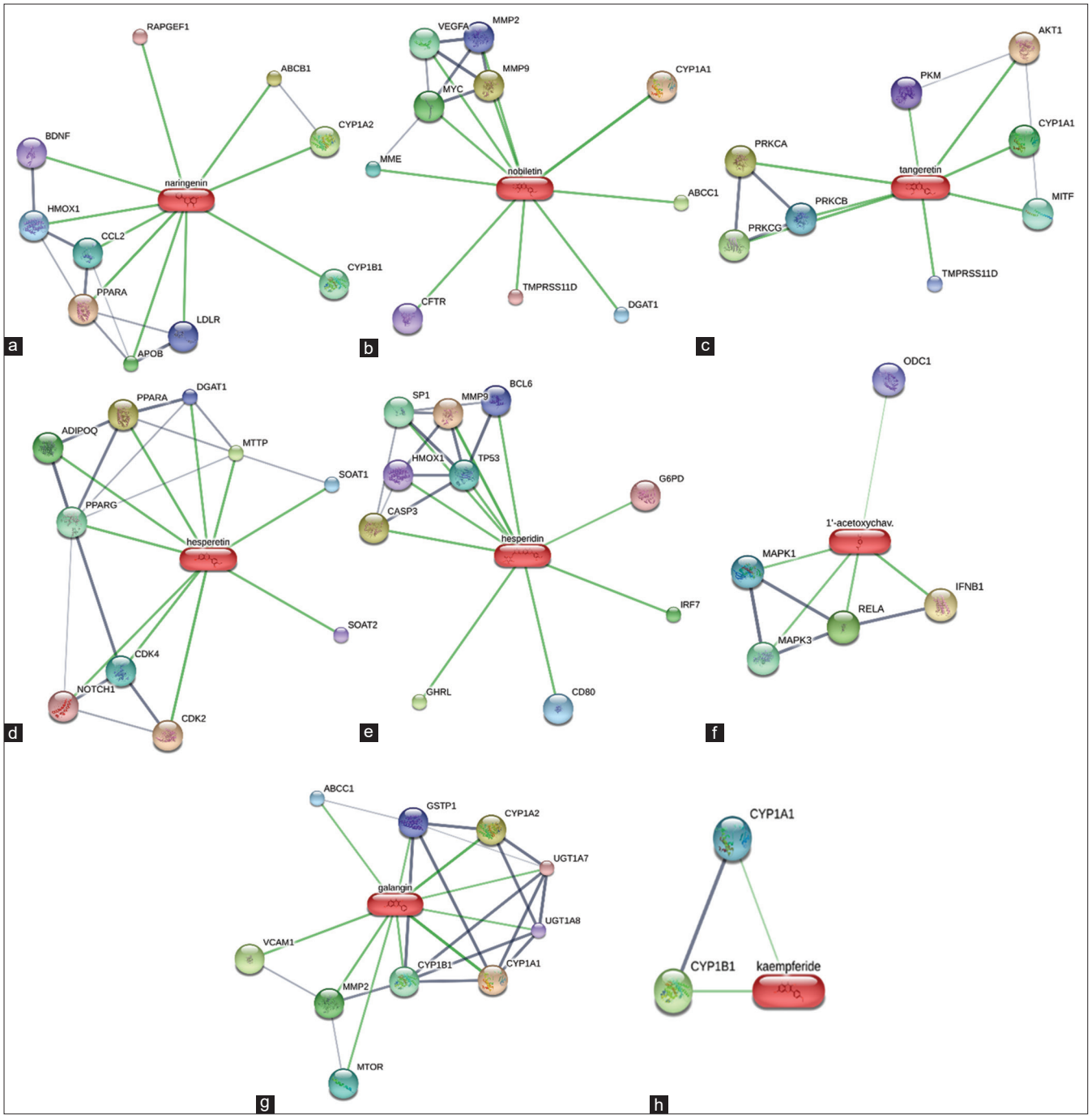

Figure 3: Direct protein target of (a) naringenin (b) nobiletin (c) tangeretin (d) hesperidin (e) Hesperetin (f) 1'-acetochavicol acetate (g) galangin and (h) kaempferitrin

and the ligands from *Alpinia galanga* and *Citrus sinensis* peels secondary metabolite are shown in Table 1. The docking score from metabolite compounds of *Alpinia galanga* and *Citrus sinensis* compared to Chloroquin and Remdesivir. Chloroquine and Remdesivir used as standard compounds because they are well known that have bioactivity to inhibit SARS-CoV-2 spreading. From the data docking score, Hesperidin has the lowest docking score compared all compounds except in 2019nCov PLPro protein, which mean the more negative the docking score, the easier interaction between the ligand and the SARS-CoV-2 protein target happen. Hesperidin has docking score of -18.077, -25.3755, and -20.3862 to the

Table 1: Binding energy of representative compound with four SARS-CoV-2 pivotal protein

Compound	Formula	Docking score (Kcal/mol)			
		Spike glycoprotein	3CL protease Sars Cov-2	PD-ACE2	2019nCov PLPro
Alpinia galanga					
Galangin	$C_{15}H_{10}O_5$	−13.6435	−17.8989	−13.1661	−9.6039
Kaempferitrin	$C_{16}H_{12}O_6$	−13.2716	−18.0857	−15.2229	−12.8756
ACA	$C_{13}H_{14}O_4$	−8.7356	−18.5500	−12.1135	−18.3345
Citrus sinensis					
Hesperidin	$C_{28}H_{34}O_{15}$	−18.0779	−25.3755	−20.3862	−17.2118
Hesperetin	$C_{16}H_{14}O_6$	−10.7967	−19.0874	−13.8868	−10.9538
Naringenin	$C_{15}H_{12}O_5$	−10.6489	−20.1127	−14.1507	−15.3689
Nobiletin	$C_{21}H_{22}O_8$	−14.1871	−23.1904	−18.1176	−9.7388
Tangeretin	$C_{20}H_{20}O_7$	−13.1613	−21.2513	−17.3559	−14.7756
First line therapy COVID−19 in Indonesia					
Chloroquine	$C_{18}H_{26}ClN_3$	−17.6572	−19.2622	−14.8980	−14.8952
Remdesivir	$C_{27}H_{35}N_6O_8P$	−12.5798	−23.1358	−20.3354	−16.7460

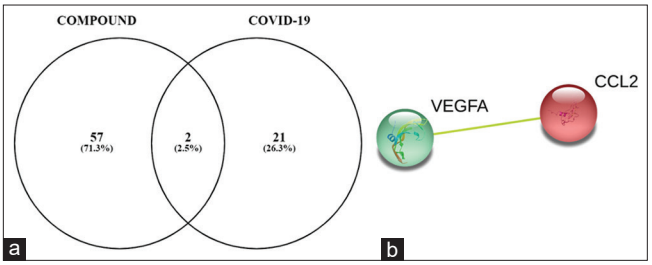

Figure 4: (a) Venn diagram of Alpinia galanga and Citrus sinensis (L.) secondary metabolite protein targets towards COVID-19. (b) interaction of 2 genes that were regulated by Alpinia galanga and Citrus sinensis (L.) secondary metabolite and related to COVID-19

respected receptor of spike glycoprotein, 3CL protease Sars Cov-2, and PD-ACE2, respectively. In addition, ACA have the lowest docking score of -18.3345 toward 2019nCov PLPro. The lower of the docking score indicated that the lower energy required for a compound to bind/interact with another compounds [19]. Hesperidin also has lower docking score compared to Chloroquine and Remdesivir. RMSD calculation wass used to determine how well specific docking/scoring combination pose and score ligand in the pterin site. In this study, all off docking scores have RMSD value under 2 Å depending on ligand size. The RMSD value 1.5–2 Å are considered the good performed of docking [20]. From the docking studies, Hesperidin can have great ability to interact with SARS-Cov-2 protein, so it is a potential candidate to inhibit the spreading of SARS-Cov-2 (Figure 2 and Table 2).

Direct protein target and network analysis of Alpinia galanga and Citrus sinensis (L.) secondary metabolite and COVID-19 regulatory genes

The molecular target that regulated between secondary metabolite of *Alpinia galanga* and *Citrus*

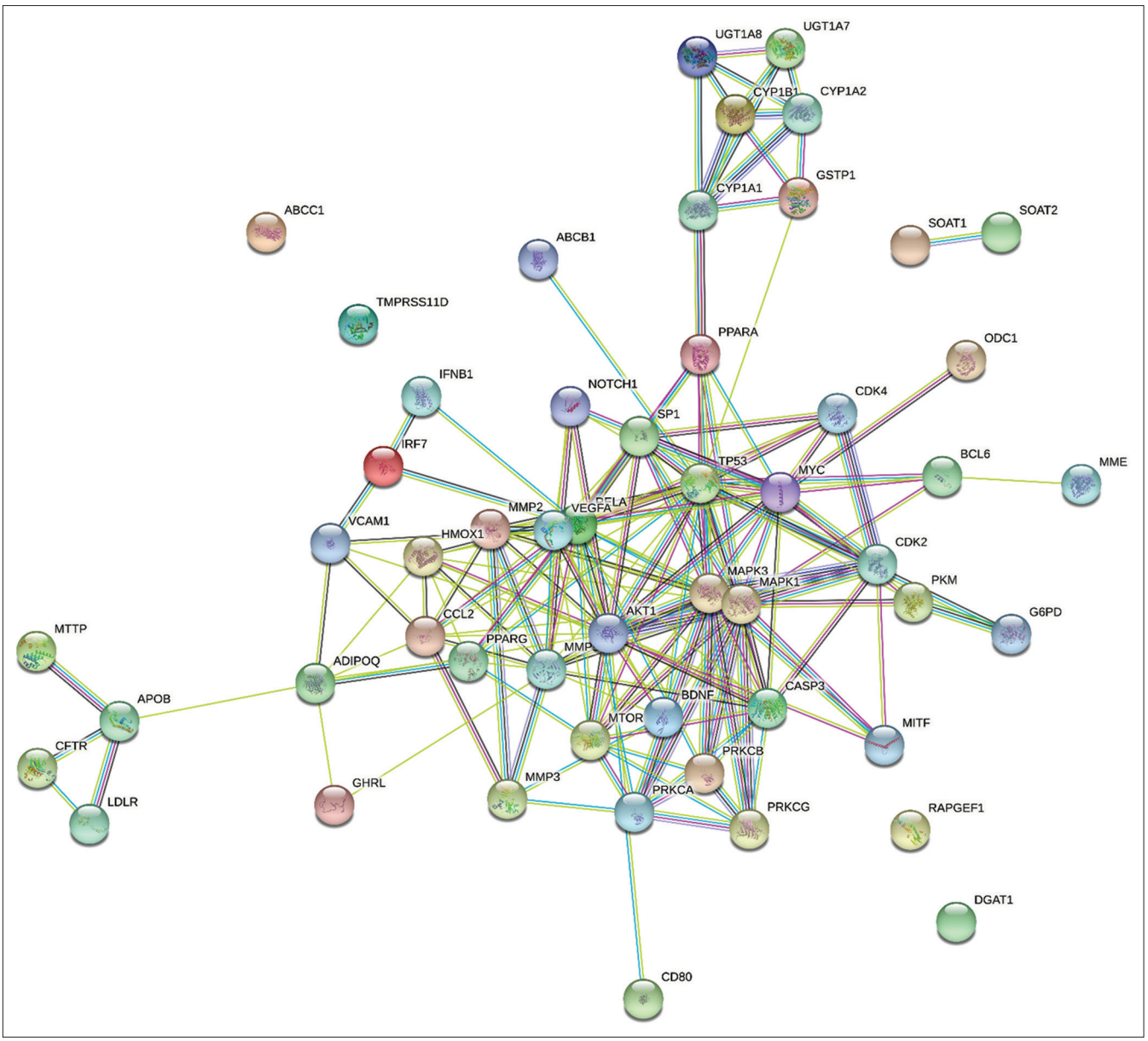

Figure 5: Protein network of genes that regulate of isolate compound Alpinia galanga dan Citrus sinensis (L.)

Table 2: RMSD score of representative compound with four SARS-CoV-2 pivotal protein

Compound	Formula	RMSD (°A)			
		Spike glycoprotein	3CL protease Sars Cov-2	PD-ACE2	2019nCov PLPro
Alpinia galanga					
Galangin	$C_{15}H_{10}O_5$	1.8745	1.6453	1.7607	1.6308
Kaempferitrin	$C_{16}H_{12}O_6$	0.9699	1.3783	1.6256	1.0760
ACA	$C_{13}H_{14}O_4$	1.7858	1.9183	1.7923	1.7199
Citrus sinensis					
Hesperidin	$C_{28}H_{34}O_{15}$	1.6869	1.7676	1.4388	1.5290
Hesperetin	$C_{16}H_{14}O_6$	1.8633	0.7263	1.6810	1.4458
Naringenin	$C_{15}H_{12}O_5$	1.3104	1.6419	1.4508	1.8059
Nobiletin	$C_{21}H_{22}O_8$	1.6990	1.7958	1.8444	1.5433
Tangeretin	$C_{20}H_{20}O_7$	1.2411	0.9760	1.0197	1.4599
First line therapy COVID-19 in Indonesia					
Chloroquine	$C_{18}H_{26}ClN_3$	1.7881	1.3678	1.7934	1.7622
Remdesivir	$C_{27}H_{35}N_6O_8P$	1.9081	1.7181	1.9557	1.7955

sinensis (L.) and gene related COVID-19 was analyzed using bioinformatic approach. The direct target protein of all secondary metabolite was collected using STITCH. From STITCH analyses, we obtained 57 direct target protein of secondary metabolite (Figure 3). Thus, the use of A PubMed (keyword “Severe acute respiratory syndrome coronavirus 2”) resulted in 23 genes associated with COVID-19. Interestingly, under venny 2.1. analyses, we obtained 2 genes including C-C motif chemokine 2 (CCL2) and Vascular endothelial growth factor A (VEGFA) that was regulated by *Alpinia galanga* and *Citrus sinensis* (L.) and related to COVID-19 (Figure 4a and b). VEGFA is genes that play important role for viral infection and its associated with promotion of SARS-CoV viral entry [21], [22], [23]. In addition, CCL2 significantly enhances the pathogenesis and replication of viruses [24], [25]. Based on bioinformatic study indicated that secondary metabolite of *Alpinia galanga* and *Citrus sinensis* (L.) can prevent from SARS-CoV-2 infection through VEGFA and CCL2 regulation (Figure 5).

Conclusion

In summary, all the ten secondary metabolite compounds showed better binding affinity than the positive standard. Form the bioinformatic study conducted, it could be understood that in most of compounds the interaction of the VEGFA and CCL-2 gene that regulates the viral infection and viral replication. Furthermore, the most effective hesperidin, naringenin, and galangin as an antiviral agent could be tested against COVID-19.

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