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Relationship Between Phylogenetic of *Apium* and *Foeniculum* Plants from Central Java, Indonesia, and Their Secondary Metabolites Potency against COVID-19 Protease

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Abstract

BACKGROUND: The emergence of COVID-19 in the late of 2019 resulted in the massive screening of drugs, including natural products, to support the current vaccines. Apium and Foeniculum vegetables are members of the Apiaceae family that potentially used to be natural immunosuppressant.

AIM: The purpose of this research is to analyze the phylogenetic position between these two plants as well as find out their secondary metabolites potency against COVID-19 main protease (Mpro) and the papain-like protease (PLpro).

METHODS: The phylogenetic analysis of Apium and Foeniculum from Indonesia was carried out based on internal transcribed spacer (ITS) region and the bioactive virtual screening assay was completed through AutoDock Vina software.

CONCLUSION: Overall, Apium and Foeniculum have close relationships among the members of Apiaceae after maximum likelihood analysis. Furthermore, it also has 70 similar bioactive compounds that some of these potentially inhibit both of COVID-19 proteases.

Introduction

The initial case of COVID-19 pandemic was reported first in the seafood market of Wuhan, China, at the end of 2019. The infected people in the surrounding areas suffered from multiple symptoms such as fever, cough, short of breath, headache, and even diarrhea [1]. It was initially called the novel coronavirus (2019-nCoVs) until the genomic analysis was conducted to obtain the exact phylogenetic position [2]. The analysis showed that the novel coronavirus was taxonomically included in the SARS-CoV group and it was named SARS-CoV-2 as the naturally taxonomic practice in the SARS group [3], [4].

There are two viral cysteine proteases that are encoded by SARS-CoV-2, namely, the main protease (Mpro) and the papain-like protease (PLpro). All of these enzymes have been validated as drug targets to inhibit viral replication [5]. Several drugs such as amprenavir, darunavir, lopinavir, and ritonavir have been developed specifically as viral protease targeting inhibitors [6]. However, the screening of SARS-CoV-2 protease inhibitors, mainly from herbal medicine, is still needed to find the potential alternative compounds to support the massive ongoing vaccination.

Celery plant (*Apium graveolens* L.) is the member of *Apiaceae* family that spreads across Europe and the tropical and subtropical regions of Africa and Asia [7]. In Indonesia, it is usually used as the condiment for salad and soup to strengthen their flavor [8]. Regarding pharmacological activity, several researchers reported that *Apium* has antimicrobial activity, antifungal activity, antioxidant activity, and antidiabetic activity [9]. However, the screening and study about its antiviral activity has not existed yet.

Fennel plant (*Foeniculum vulgare* Mill.) is the consumable plant with medicinal usage to treat various diseases and has an impact on human hormones. In Indonesia, *Foeniculum* is available to grow at an altitude more than 1500 m above sea level [10]. In addition, it also has other pharmacological abilities such as antioxidant, antitumor, chemopreventive, cytoprotective, hepatoprotective, hypoglycemic, and estrogenic activities [11]. There is also no report about the antiviral ability from this plant.

Both *Apium* and *Foeniculum* are the members of *Apiaceae* which are the largest family in the order of Apiales and have been well known as the medicinal and consumable plants [12]. Nevertheless, this family is currently under the major taxonomic rearrangements [13]. Therefore, the aim of this research is to reveal the phylogenetic position as well as the connection with the secondary metabolites content between *Apium* and *Foeniculum*. Furthermore, we also evaluated the ability of their secondary metabolites against COVID-19 main protease and papain-like protease through virtual screening.

Materials and Methods

Plant materials and DNA extraction

The plants used in this study (*Apium* and *Foeniculum*) were obtained from Central Java, Indonesia. The DNA extraction was carried out using Doyle and Doyle methods [14]. The concentration and purity of DNA was measured using Nanodrop2000.

DNA amplification, analysis, and phylogenetic relationship

DNA amplification of the internal transcribed spacer (ITS) gene (ITS5: 5'-GGA AGT AAA AGT CGT AAC AAG G-3' and ITS4: 5'-TCC TCC GCT TAT TGA TAT GC-3') was carried out by a total reaction of 50 μl using MY TAQ HS REDMIX from Bioline. Polymerase chain reaction for 35 cycles was finished with initial denaturation at 94°C for 3 min; denaturation at 94°C for 25 s, annealing at 55°C for 30 s, and extension at 72°C for 50 s; and final extension at 72°C for 2 min, according to our previous research [15]. The phylogenetic relationship was analyzed through Molecular Evolutionary Genetics Analysis (MEGA) X software [16] using the maximum likelihood model with Kimura 2-parameter method.

Secondary metabolites analysis

The secondary metabolites data of *Apium* and *Foeniculum* plant were extracted from two databases comprise of KNApSAcK: A Comprehensive Species-Metabolite Relationship Database (http://www.knapsackfamily.com/KNApSAcK/) and Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/phytochem/search). The similar secondary metabolites between these two plants then used as ligands for further molecular docking analysis. All of SDF format of these ligands were downloaded from PubChem database (http://pubchem.ncbi.nlm.nih.gov) and ZINC (https://zinc20.docking.org/substances/subsets/natural-products/).

Protein and ligand preparation

The structure of SARS coronavirus main protease (COVID-19 $3CL^{pro}/M^{pro}$) and papain-like protease was retrieved from the structure database (PDB) with ID 6LU7 [17] and 6WX4 [18], respectively. The native ligands from both receptors were separated as positive control and the active site of the grid box is adjusted by position of native ligands in the structure through AutoGrid in Autodock 4.0 software [19]. The grid size was set to 25 × 54 × 32 (6LU7) and 30 × 30 × 40 (6WX4) xyz points. The dimension of grid center for 6LU7 was −9.768 (x), 11.436 (y), and 68.904 (z) while for 6WX4 was 9.507 (x), −27.445 (y), and −37.252 (z). The ligand molecules were minimized using MMFF94 and converted into.pdbqt format using Open Babel program in Linux operating system.

Molecular docking analysis

The molecular docking process is carried out using AutoDock Vina (AV) software [20] operated in Windows subsystem Linux throughout the scripting process in Linux terminal. The binding affinity value and RMSD used as the main parameter. In addition, the molecular interaction between ligands and receptors was analyzed using Biovia Discovery Studio software and LIGPLOT.

Lipinski rule of five test, absorption, and toxicology analysis

The physicochemical test of the top 10 metabolites with highest binding against COVID-19 main proteases affinity was carried out by entering the sdf 2D file format (obtained from the PubChem database http://pubchem.ncbi.nlm.nih.gov) on the Lipinski rule of five pages (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). Meanwhile, the absorption and toxicology properties analyzed through pkCSM server (http://biosig.unimelb.edu.au/pkcsm/prediction) and Pro-Tox II (http://tox.charite.de/protox_II/), respectively.

Results and Discussion

In this study, the DNA isolation results of *Apium* and *Foeniculum* plants used the Doyle and Doyle method without the addition of polyvinylpyrrolidone (PVP) are exhibited in Table 1. Sambrook and Russel [21] argue that the use of cetyltrimethylammonium bromide (CTAB) which is a non-ionic detergent that can precipitate nucleic acids and polysaccharides from solutions with low ionic strength has a function to help purify nucleic acids from other organisms that produce polysaccharides in large quantities such as plant. Based on research by Saravanaperumal and La Terza [22], the use of DNA isolation procedures with CTAB without additional antioxidants (PVP) for young *Foeniculum* leaf tissue is able to produce superior DNA quality.

Table 1: The result of DNA isolation of Indonesian *Foeniculum* (A) and *Apium* (B)

Serial number	Plant	DNA concentration (ng/µl)	A_{260}/A_{280}
1	*Apium*	366.7	1.75
2	*Foeniculum*	300	2.04

The two samples have nearly representative DNA purity which ranged from 1.8 to 2.0. The DNA purity <1.8 indicates the presence either of phenolic compounds or protein. Meanwhile, the value more than 2.0 means the contamination of RNA during the isolation process [23]. Previous research by Chaudary *et al*. [24] showed that the DNA extraction of *Foeniculum* from India seed bank using CTAB method had the yield of more than 1000 ng/µl with the purity of 1,8. Regarding the *Apium* plant, Pafundo *et al.* [25] reported that the DNA isolation of *Apium* using various methods yielded <50 ng/µl with purity ranging from 1.6 to 2.0.

The results of DNA amplification showed that the region of internal transcribed spacer (ITS) in the *Apium* and *Foeniculum* is about 700 bp length, as shown in Figure 1. These were confirmed with the DNA alignment in the National Centre of Biotechnological Information (NCBI). The sequence of *Foeniculum* has 634 bp length and is highly similar with *Foeniculum vulgare* voucher 200505069 (99,53%). On the other hand, the *Apium* sequence (711 bp) is closely related with *Apium graveolens* voucher M.V. Chase 2523 (99%). All of these results indicate that all the samples in this research belong to the similar species which has been registered in GenBank.

In a previous study by Zhou *et al.* [26] using several plants belonging to the same family as *Foeniculum*, namely, *Apiaceae* from India, a PCR amplification process was carried out using ITS 4 and ITS 5 primers. The PCR condition comprises of pre-denaturation at 94°C for 3 min, denaturation at 94°C for 3 min 45 s, the annealing temperature is 55°C for 1 min, extension 72°C for 3 min and final extension 72°C for 7 min. The similar annealing temperature in PCR condition with this study gives indication that optimal annealing temperature of *Apiaceae* family for ITS amplification gene is 55°C.

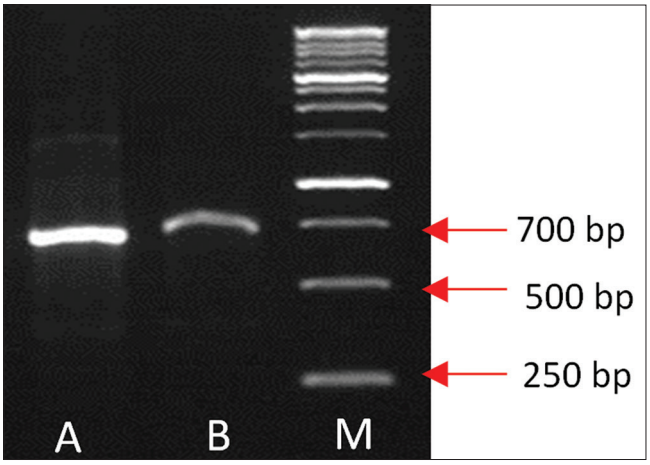

Figure 1: The visualization of the ITS gene PCR product of the Indonesian Foeniculum (a) and Apium (b) using 1 kb DNA ladder (M)

Phylogenetic analysis as illustrated in Figure 2 showed that the original Indonesian *Foeniculum* plant was more closely related to *Foeniculum vulgare voucher* 200505069 (MH645764). The bootstrap value between Indonesian *Foeniculum* and *F. vulgare* voucher was around 92%. It means that the branch between both *Foeniculum* was formed 92 times in phylogenetic tree reconstruction. High bootstrap values can increase the level of trust in phylogenetic tree topologies [27]. Other bootstraps values in different branch also showed the number of ≥90.

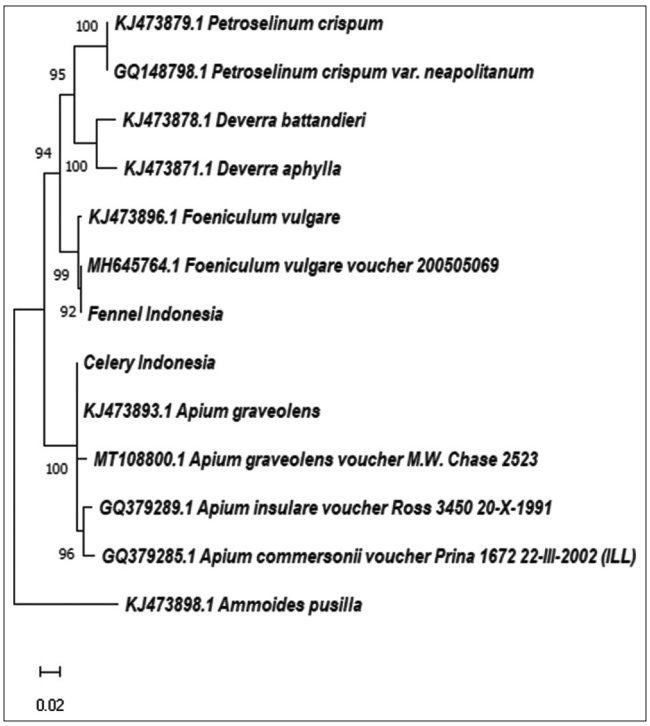

Figure 2: The phylogenetic reconstruction of the Indonesian Apium and Foeniculum with several member of family [13] using maximum likelihood tree with 100 bootstrap replications. Ammoides pusilla is used as out-group

In addition, Indonesian *Apium* was closely related to *Apium graveolens* (KJ473893) and *Apium graveolens voucher* M. W. Chase 2523 (MT108800), with bootstrap value around 100% meaning that they

Table 2: The result of virtual screening of secondary metabolites from *Apium* and *Foeniculum* against COVID-19 main protease (6LU7) using Autodock Vina

Serial number	Bioactive compound	Molecular formula	Binding energy (Kcal/mol)	RMSD upper/lower bound	Lipinski rule of five
-	N3 (native ligand)	-	−11.4	0.0	-
1	Rutin	$C_{27}H_{30}O_{16}$	−9.7	0.0	Not fulfill
2	Stigmasterol	$C_{29}H_{48}O$	−9.5	0.0	Not fulfill
3	Campesterol	$C_{28}H_{48}O$	−9.4	0.0	Not fulfill
4	Beta-carotene	$C_{40}H_{56}$	−9.0	0.0	Not fulfill
5	Beta-sitosterol	$C_{29}H_{50}O$	−8.9	0.0	Not fulfill
6	Phytosterol	$C_{29}H_{50}O$	−8.8	0.0	Not fulfill
7	Chlorogenic Acid	$C_{16}H_{18}O_9$	−8.0	0.0	Not fulfill
8	Riboflavin	$C_{17}H_{20}N_4O_6$	−8.0	0.0	Fulfill
9	Colombianite	$C_{14}H_{14}O_4$	−7.7	0.0	Fulfill
10	Beta-caryophyllene	$C_{15}H_{24}$	−7.4	0.0	Fulfill

RMSD: Root mean square deviation.

are in the same branch (monophyletic) or in the same taxa. Based on the phylogenetic analysis above, it can be seen that the scale substitution rate (scale bar) is 0.02, this value means that for every 100 bases, there are two different nucleotide substitutions. The existence of genetic distances affects the level of gene differences (genomic differences) in a population [28]. The result in Figure 2 also gives indication that *Apium* and *Foeniculum* are the closely related species among the group of *Apiaceae* even though separated in two distinct clades.

Besides the data of phylogenetic relationship, we obtained hundreds secondary metabolites contained in *Apium* and *Foeniculum* plant, respectively (Supplementary 1) collected from both KNApSAcK and Dr. Duke's Database Phytochemical and Ethnobotanical Databases. KNApSAcK database was built in the year of 2011 for metabolomics research purpose [29] and currently containing 141.486 of metabolite-species pair. Meanwhile, the Dr. Duke's database has existed since the year of 1992 and stores thousands of biologically active phytochemicals from higher plants. Until now, there are about 49.000 plant secondary metabolites that can be explored [30]. Interestingly, this research found 87 compounds that exactly match between these two plants and 70 compounds that could be used for further virtual screening against COVID-19 main protease and papain-like protease. Others categorized as elements that could not be used for molecular docking. We selected the 10 out of 70 compounds based on the lower binding energy and root mean square deviation (RMSD) results as can be seen from Table 2 (6LU7) and Table 3 (6WX4). The complete virtual screening results are available in Supplementary 2.

The virtual screening results in Table 2 showed that rutin has the closest binding affinity towards 6LU7 receptor (N3) followed by stigmasterol, campesterol, and beta-carotene (binding energy ≤ 9). The binding between protein and ligand occurs when the required energy is low so that the more negative binding affinity number gives higher possibility regarding the stability interaction between ligands and receptor [31]. However, all of these compounds did not meet the criteria of Lipinski's rule of five. Regarding the rutin, it only meets the criteria of logP (1.8) while others exceed maximum number (5) (Supplementary 3). It can also be observed that only three compounds in the top 10 of the virtual screening, namely, riboflavin, colombianite, and beta-caryophyllene that fulfill Lipinski's rule of five. All of these compounds have differences of about 3.0 kcal/mol of binding energy compared to N3.

The RMSD value for all screened ligands showed the value <2 which indicates that the docking method used has been well validated [32]. Interestingly, the virtual screening against 6WX4 papain-like proteases has almost the similar result regarding the top 10 bioactive compounds with lowest binding energy. Nevertheless, almost all of the bioactive compounds had lower or similar energy numbers compared to VIR251 as native ligand. It means that almost all of these ligands can bind spontaneously to protein [31]. Although there are only three out of 10 compounds that fulfill Lipinski's rule of five, the presence of seselin might become the promising compound to inhibit the 6WX4 papain-like protease. Besides the lower binding affinity compared to the native ligand, previous *in vitro* research revealed the antiviral ability of seselin compound against nuclear polyhedrosis virus infection in the larvae silkworm [33].

The result of drugs oral availability in Table 4 based on water solubility, Caco-2 cell permeability (Papp), and human intestinal absorption (HIA) indicating

Table 3: The result virtual screening of secondary metabolites from *Apium* and *Foeniculum* against COVID-19 papain-like protease (6WX4) using Autodock Vina

Serial number	Bioactive compound	Molecular formula	Binding energy (Kcal/mol)	RMSD upper/lower bound	Lipinski rule of five
-	VIR251 (Native Ligand)	-	−8.3	0.0	-
1	Rutin	$C_{27}H_{30}O_{16}$	−8.9	0.0	Not fulfill
2	Seselin	$C_{14}H_{12}O_3$	−8.7	0.0	Fulfill
3	Stigmasterol	$C_{29}H_{48}O$	−8.6	0.0	Not fulfill
4	Campesterol	$C_{28}H_{48}O$	−8.6	0.0	Not fulfill
5	Beta-carotene	$C_{40}H_{56}$	−8.4	0.0	Not fulfill
6	Beta-sitosterol	$C_{29}H_{50}O$	−8.3	0.0	Not fulfill
7	Phytosterol	$C_{29}H_{50}O$	−8.3	0.0	Not fulfill
8	Chlorogenic Acid	$C_{16}H_{18}O_9$	−8.3	0.0	Not fulfill
9	Riboflavin	$C_{17}H_{20}N_4O_6$	−8.3	0.0	Fulfill
10	Beta-caryophyllene	$C_{15}H_{24}$	−8.0	0.0	Fulfill

RMSD: Root mean square deviation.

Table 4: Absorption and toxicology data of selected ligands

Bioactive compound	pkCSM				Pro Tox II						Oral rat acute toxicity	Acute toxicity
	Water solubility (log mol/L)	Caco-2 permeability (cm/s)	HIA (% absorbed)	P-glycoprotein inhibitor/substrate	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity	(LD50) (mg/kg)	class
Rutin	−2.892	−0.949	23.446	Non-inhibitor/substrate	Inactive	Inactive	Active	Inactive	Inactive	5000	5
Stigmasterol	−6.682	1.213	94.97	Inhibitor/non-substrate	Inactive	Inactive	Active	Inactive	Inactive	890	4
Campesterol	−7.068	1.223	94.543	Non-inhibitor/substrate	Inactive	Inactive	Active	Inactive	Inactive	890	4
Beta-carotene	−7.39	1.262	91.732	Inhibitor/non-substrate	Inactive	Inactive	Inactive	Active	Inactive	1510	4
Beta-sitosterol	−6.773	1.201	94.464	Inhibitor/non-substrate	Inactive	Inactive	Active	Inactive	Inactive	890	4
Phytosterols	−6.773	1.201	94.464	Inhibitor/non-substrate	Inactive	Inactive	Active	Inactive	Inactive	890	4
Chlorogenic acid	−2.449	−0.84	36.377	Non-inhibitor/substrate	Inactive	Inactive	Active	Inactive	Inactive	5000	5
Riboflavin	−2.029	−0.679	36.107	Non-inhibitor/non-substrate	Inactive	Inactive	Inactive	Inactive	Inactive	10000	6
Beta-caryophyllene	−5.555	1.423	94.845	Non-inhibitor/non-substrate	Inactive	Inactive	Active	Inactive	Inactive	5300	6
Colombianite	−3.172	1.18	96.577	Non-inhibitor/non-substrate	Inactive	Inactive	Inactive	Active	Inactive	832	4
Seselin	−3.401	1.128	97.946	Non-inhibitor/non-substrate	Inactive	Active	Active	Inactive	Inactive	3850	5

HIA: Human intestinal absorption.

its movement across intestinal epithelial barrier, and the inhibitory effect of P-glycoprotein in regulating the efflux process of the cell. Riboflavin is shown to have the highest water solubility (–2.029 log mol/L), compared to beta-carotene as the least soluble compound (–7.39 log mol/L). Despite being the most soluble compound in water, riboflavin has the poorest permeability in Caco-2 human cell (–0.679 cm/s). Most selected compounds have comparatively positive value of Papp, except for rutin, chlorogenic acid, and riboflavin. This value correlated with HIA that showed the three compounds to have the least percent absorbed molecule in human intestinal. Lower human intestinal absorption may indicate that the compounds are better consumed from other than gastrointestinal tract [34], [35]. The inhibitory effect of P-glycoprotein (P-gp) could enhance the bioavailability because non-inhibitor and substrates would pump the compound back into the lumen and may promote the elimination of that drug into the bile and urine. Among the 10 compounds, only stigmasterol, beta-carotene, beta-sitosterol, and phytosterols act as inhibitor.

The toxicity data derived from Pro-Tox II (http://tox.charite.de/protox_II/) and it comprises hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity, and their acute toxicity class based on oral rat acute toxicity or LD_{50} value [36], [37]. The compounds reported have various LD_{50} ranged from 800 to 10,000 mg/kg. Riboflavin and beta-caryophyllene are classified in Class 6 ($LD_{50} > 5000$) that considered as non-toxic. Rutin, chlorogenic acid, and seselin classified as Class 5 ($2000 < LD_{50} < 5000$), which may be harmful if swallowed. Stigmasterol, campesterol, beta-carotene, beta-sitosterol, phytosterols, and colombianite were fall into Class 4 ($300 < LD_{50} < 2000$) which harmful if swallowed. Among all of the compounds, riboflavin is the only one that would not trigger any hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity as adverse effects.

Since only riboflavin which meets the criteria of drug-like properties, absorption, and toxicology data, we evaluate the molecular interaction to this ligand. All of the ligands form hydrophobic interaction with Met165, His41, and Gln189 in 6LU7 receptor (Supplementary 4). However, only riboflavin has hydrogen-binding interactions and more hydrophobic interactions compared to N3 (Figure 3). These hydrogen interactions consist of Gly143 and Glu166. Based on the previous report, the amino acid of Gly143 in 6LU7 receptor is the strongest residue to create hydrogen interaction with ligands. This was followed by Cys145 and His163 [38] as we also can observe in the riboflavin interaction residues. In addition, Gly143 and Glu166 reported playing an important role in stabilizing the ligand-receptor complex [39].

Although seselin has the lowest binding energy of the best three compounds against 6WX4 protein, it did not contain any hydrogen interactions. All of the ligands form hydrophobic interaction with Tyr264

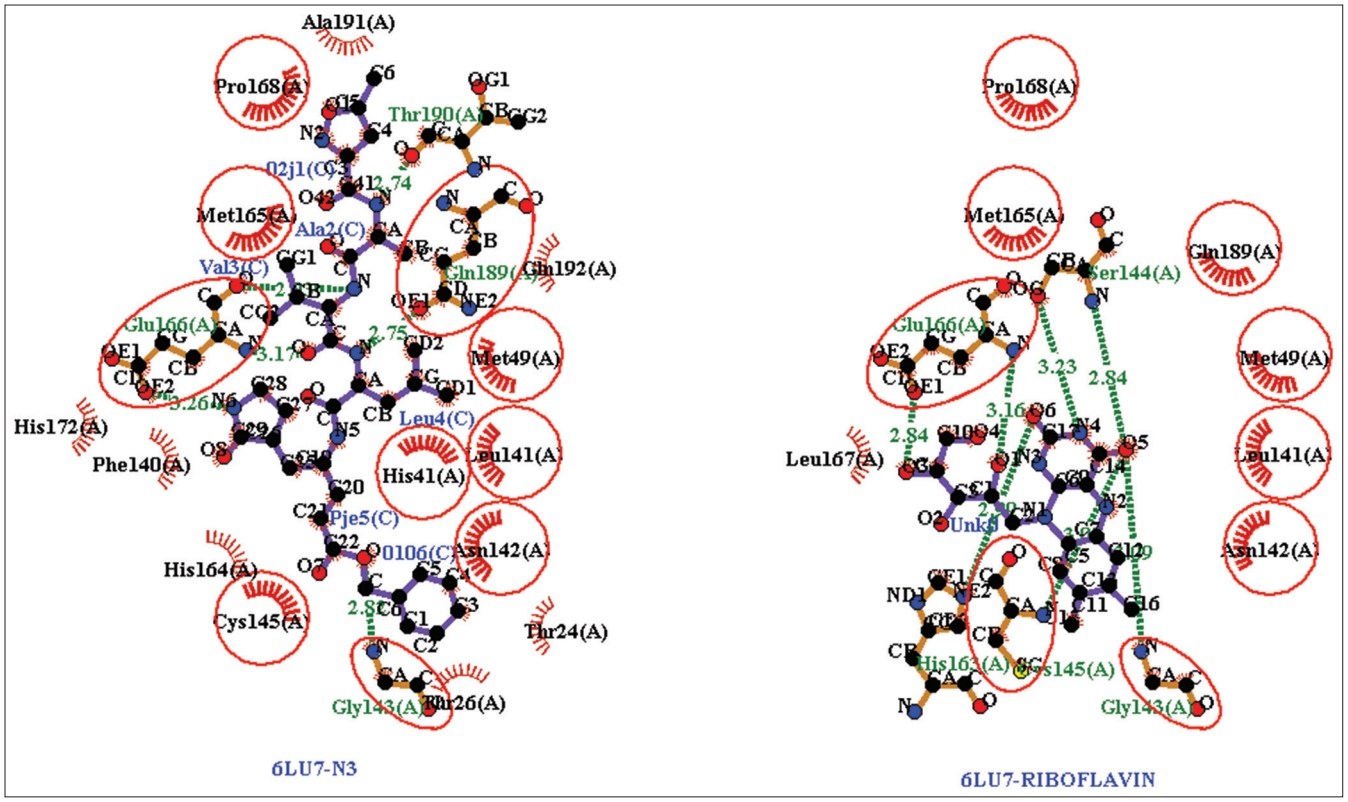

Figure 3: The comparison of molecular interaction between N3 as native ligands of 6LU7 and riboflavin

and Pro248 (Supplementary 5) in 6WX4 protein. Only riboflavin has hydrogen interactions through Asp164 that we can also find in VIR251 (Figure 4). Based on Rut *et al.* [18], a deep hydrophobic pocket in papain-like protease, formed in the residue of Tyr264, Tyr 268, Pro247, Pro248, Tyr273, Thr301, and Met208. The previous study [40] found that the hydrogen interaction with Asp164 increased the stability of the complexes. Therefore, riboflavin based on this study forms the most stable interaction with 6WX4.

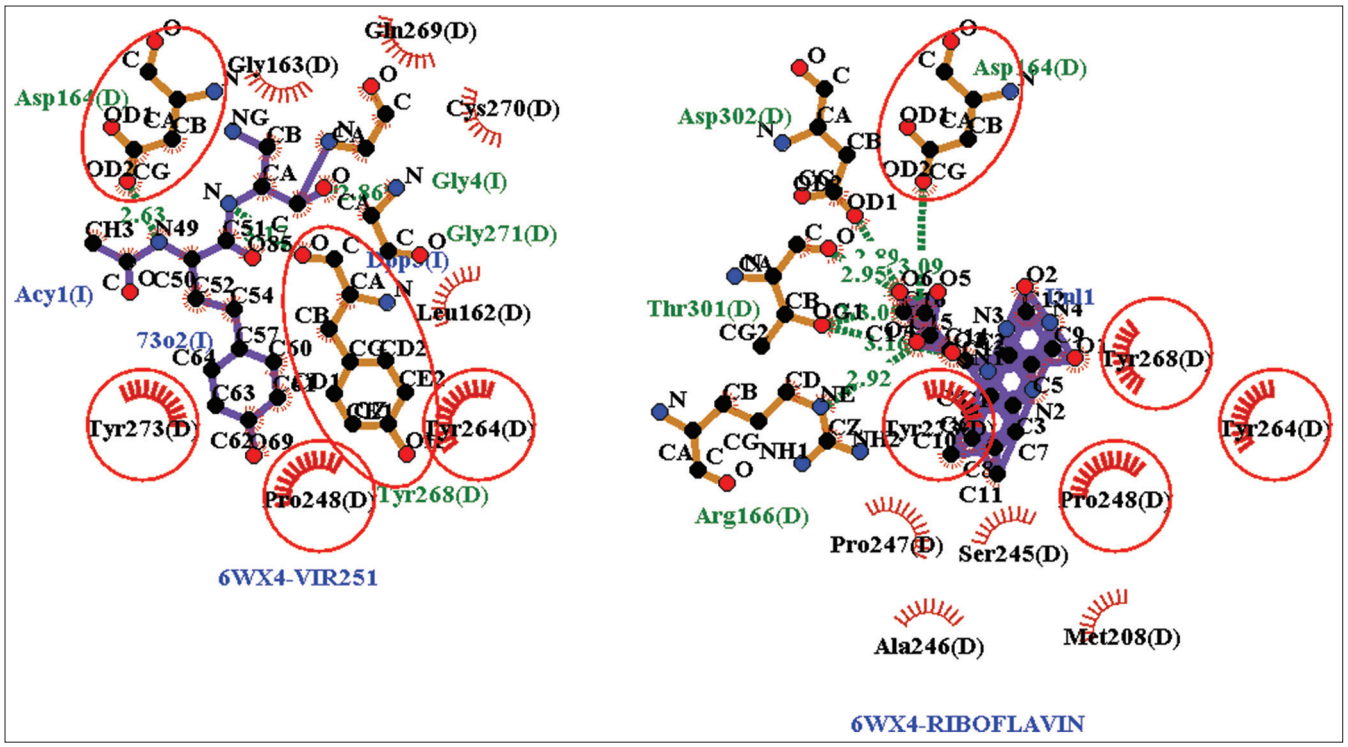

Figure 4: The comparison of molecular interaction between VIR251 as native ligands of 6WX4 and riboflavin

Based on the research result, the close phylogenetic analysis between *Apium* and *Foeniculum* plants was followed by the similarity of several secondary metabolites which have promising ability to inhibit both of COVID-19 protease and resulted riboflavin as the best compound which meets drug-like properties and toxicology criteria. These results supported the previous study which shows that natural immunosuppressant compounds derived from plant sources are known to inhibit the production and release of pro-inflammatory cytokines and chemokines in COVID-19 [41], [42], [43], [44]. The use of natural immunosuppressants from these two plants was used as an adjuvant to ameliorate cytokine storms, as was other plants from the previous studies. The results show the potential of the two plants which when combined with other currently used antiviral agents and drugs will have the potential as a new, synergistic approach for more effective COVID-19 treatment and cure.

Conclusion

Phylogenetic analysis indicates that Indonesian *Apium* and *Foeniculum* have close relationships inside of the family *Apiaceae*. Besides, it also shares similar bioactive compounds that potentially bind strongly to the COVID-19 protease receptors. Further *in vitro* assay is needed to validate this study.

References

1. Wu YC, Chen CS, Chan YJ. The outbreak of COVID-19: An overview. J Chin Med Assoc. 2020;38(3):217-20. https://doi.org/10.1097/JCMA.0000000000000270
   PMid:32134861
2. Tan W, Zhao X, Ma X, Wang W, Niu P, Xu W, *et al*. A novel coronavirus genome identified in a cluster of pneumonia cases-whuan, China 2019-2020. China CDC Wkly. 2020;2(4):61-2. https://doi.org/10.46234/ccdcw2020.017
   PMid:34594763
3. Wu Y, Ho W, Huang Y, Jin DY, Li S, Liu SL, *et al*. SARS-CoV-2 is an appropriate name for the new coronavirus. Lancet. 2020;395(10228):949-50. https//doi.org/10.1016/S0140-6736(20)30557-2
   PMid:32151324
4. Sah R, Rodrigues-Morales AJ, Jha R, Chu DK, Gu H, Peiris M, *et al.* Complete genome sequence of novel coronavirus (SARS-CoV-2) strain isolated in Nepal. Microbiol Res Announc. 2020;9(11):e00169-20. https://doi.org/10.1128/MRA.00169-20
   PMid:32165386
5. Ma C, Wang J. Validation and invalidation of SARS-CoV-2 papain-like protease inhibitors. ACS Pharmacol Transl Sci. 2022;5(2):102-209. https://doi.org/10.1021/acsptsci.1c00240
   PMid:35178512
6. Mengist HM, Dilnessa T, Jin T. Structural basis of potential inhibitors targeting SARS-CoV-2 main protease. Front Chem. 2021;9:622898. https://doi.org/10.3389/fchem.2021.622898
   PMid:33889562
7. Kooti W, Daraei N. A review of antioxidant activity of *Apium* (*Apium graveolens* L). J Evid Based Complement Altern Med. 2017;22(4):1029-34. https://doi.org/10.1177/2156587217717415
   PMid:28701046
8. Wijaya CH, Epriliati I. Daily Vegetables in Indonesia. Indonesian: Gramedia Pustaka Utama; 2019. p. 61.
9. Khairullah AR, Solikhah TI, Ansori AN, Hidayatullah AR, Hartadi EB, Ramandinianto SC, *et al*. Review on the pharmacological and health aspects of *Apium graveolens* or *Apium*: An update. Syst Rev Pharm. 2021;12(1):606-12. http://dx.doi.org/10.31838/srp.2021.1.87
10. Rifqiyanti N, Wahyuni A. *Foeniculum* (*Foeniculum vulgare*) leaf infusion effect on mamary gland activity and kidney function of lactating rats. Nusantara Biosci. 2019;11(1):101-5. https://doi.org/10.13057/nusbiosci/n110117
11. Badgujar SB, Patel VV, Bandivdekar AH. *Foeniculum vulgare* mill: A review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. Biomed Res Int. 2014;2014:842674. https://doi.org/10.1155/2014/842674
    PMid:25162032
12. Kahered J. The family pennaticeae and its relationships to apiales. Bot J Linn Soc. 2003;141(1):1-24. https://doi.org/10.1046/j.1095-8339.2003.00110.x
13. Jimenez-Mejias P, Vargas P. Taxonomy of the tribe apieae (*Apiaceae*) revisited as revealed by molecular phylogenies and morphological characters. Phytotaxa. 2015;212(1):57-79. http://doi.org/10.11646/phytotaxa.212.1.2
14. Mawarni SN, Khairunnisa D, Larasati I, Rizqo N, Erfianti T, Kusumaningrum HP, *et al*. Application of doyle and doyle method for DNA isolation from pamelo yellow orange (*Citrus maxima* Merr), lime orange (*C. limon*) and sunkist orange (*C. sinensis*). J Phys Conf Ser. 2021;1943(1):012079. https://doi.org/10.1088/1742-6596/1943/1/012079
15. Kusumaningrum HP, Budiharjo A, Suprihadi A, Eshananda Y, Fadillah A, Pangestuti DR. The characterization of *Citrus* sp. from Parang Island Karimunjawa based on morphological, DNA barcoding and nutritional analysis. Int J Genet Mole Biol. 2018;10(3):26-38. https://doi.org/10.5897/IJGMB2018.0167
16. Kumar S, Stecher G, Knyaz C, Tamura K. MEGA X: Molecular evolutionary genetics across computing platforms. Mole Biol Evol. 2018;35(6):1547-9. https://doi.org/10.1093/molbev/msy096
    PMid:29722887
17. Jin Z, Du X, Xu Y, Deng Y, Liu M, Zhao Y, *et al*. Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors. Nature. 2020;582(7811):289-93. https://doi.org/10.1038/s41586-020-2223-y
    PMid:322724481
18. Rut W, Lv Z, Zmudzinski M, Patchett S, Nayak D, Snipas SJ, *et al*. Activity profiling and crystal structures of inhibitor-bound SARS-CoV-2 papain-like protease: A framework for anti-COVID-19 drug design. Sci Adv. 2020;6(42):1-12. https://doi.org/10.1126/sciadv.abd4596
    PMid:33067239
19. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, *et al.* Autodock4 and autodocktools4: Automated docking with selective receptor flexibility. J Comput Chem. 2009;30(16): 2785-91. https://doi.org/10.1002/jcc.21256
    PMid:19399780
20. Trott O, Olson AJ. AutoDock vina: Improving the speed

and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem. 2010;31(2):455-61. https://doi.org/10.1002/jcc.21334
PMid:19499576

21. Sambrook J, Russel D. Molecular Cloning: A Laboratory Manual. 3rd ed., Vol. 3. New York: Cold Spring Harbor Laboratory Press; 2001. p. 130.
22. Saravanaperumal SA, La Terza A. Polyphenolics free DNA isolation and optimization of PCR-RAPD for *Foeniculum* (*Foeniculum vulgare* Mill.) from mature and young leaves. Afr J Biotechnol. 2021;11(35):8622-31. https://doi.org/10.5897/AJB11.1676
23. Barbas CF 3rd, Burton DR, Scott KJ, Silverman GJ. Quantitation DNA and RNA. CSH Protoc. 2007;2007:pdb.ip47. https://doi.org/10.1101/pdb.ip47
PMid:21356961
24. Choudary S, Meena RS, Jethra G, Sharma R, Panwar A. Optimised methodology for high quality DNA isolation from leaves and seeds of *Foeniculum* (*Foeniculum vulgare*). J Plant Dev Sci. 2015;7(2):173-5.
25. Pafundo S, Gulli M, Marmiroli N. Comparison of DNA extraction methods and development of duplex and real-time to detect tomato, carrot, and *Apium* in food. J Agri Food Chem. 2011;59(19):10414-24. https://doi.org/10.1021/jf202382s
PMid:21894887
26. Zhou J, Peng H, Downie SR, Liu ZW, Gong XA. Molecular phylogeny of Chinese *Apiaceae* subfamily *Apioideae* inferred from nuclear ribosomal DNA internal transcribed spacer sequences. Taxon. 2008;57(2):402-16. https://doi.org/10.2307/25066012
27. Hall BG. Phylogenetic Trees Made Easy: A How to Manual. 5th ed. Oxford: Oxford University Press; 2018. p. 148.
28. Nei M. Molecular Evolutionary Genetics. New York: Colombia University Press; 1987. https://doi.org/10.7312/nei-92038
29. Afendi FM, Okada T, Yamazaki M, Hirai-Morita A, Nakamura Y, Nakamura K, *et al*. KNApSAcK family databases: Integrated metabolite-plant species databases for multifaceted plant research. Plant Cell Physiol. 2012;53(2):e1. http://doi.org/10.1093/pcp/pcr165
PMid:22123792
30. Duke JA. Database of Biologically Active Phytochemicals and their Activity. Boca Raton: CRC Press; 2020. p. 30. https://doi.org/10.1201/9780429332869
31. Afriza D, Suriyah WH, Ichwan SJ. *In silico* analysis of molecular interactions between the anti-apoptotic protein survivin and dentatin, nordentatin, and quercetin. J Phys: Conf Ser. 2018;1073(3):032001. https://doi.org/10.1088/1742-6596/1073/3/032001
32. Pantsar T, Poso A. Binding affinity via docking: Fact and fiction. Molecules. 2018;23(8):1899. https://doi.org/10.3390/molecules23081899
PMid:30061498
33. Shomu C, Karuppiah H, Sundaram J. Antiviral activity of seselin from *Aegle marmelos* against nuclear polyhedrosis virus infection in the larvae of silkworm. J Ethnopharmacol. 2019;245:112155. https://doi.org/10.1016/j.jep.2019.112155
PMid:31449858
34. Finch A, Pillans P. P-glycoprotein and its role in drug-drug interactions. Aust Prescr. 2014;37(4):137-9. https://doi.org/10.18773/austprescr.2014.050
35. Srivastava V, Yadav A, Sarkar P. Molecular docking and ADMET study of bioactive compounds of *Glycyrrhiza glabra* against main protease of SARS-CoV2. Mater Today Proc. 2020;49:2999-3007. https://doi.org/10.1016/j.matpr.2020.10.055
PMid:33078096
36. Banerjee P, Eckert AO, Schrey AK, Preissner R. ProTox-II: A webserver for the prediction of toxicity of chemicals. Nucleic Acids Res. 2018;46(W1):257-63. https://doi.org/10.1093/nar/gky318
PMid:29718510
37. Pratama MR, Hadi P, Siswandono S. ADMET properties of novel 5-O-benzoylpinostrobin derivatives. J Basic Clin Physiol Pharmacol. 2019;30(6):251. https://doi.org/10.1515/jbcpp-2019-0251
PMid:31851612
38. Nguyen DD, Gao K, Chen J, Wang R, Wei GW. Unveiling the molecular mechanism of SARS-CoV-2 main protease inhibition from 137 crystal structures using algebraic topology and deep learning. Chem Sci J. 2020;11(44):12036-46. https://doi.org/10.1039/D0SC04641H
39. Farhat N, Khan AU. Repurposing drug molecule against SARS-CoV-2 (COVID-19) through molecular docking and dynamics: A quick approach to pick FDA approved drugs. J Mole Model. 2021;27(11):312. https://doi.org/10.1007/s00894-021-04923-w
PMid:34601658
40. Alfaro M, Alfaro I, Angel C. Identification of potential inhibitors of SARS-CoV-2 papain-like protease from tropane alkaloids from *Schizantus porrigens*: A molecular docking study. Chem Phys Lett. 2020;761:138068. https://doi.org/10.1016/j.cplett.2020.138068
PMid:33052144
41. Peter AE, Sandeep BV, Rao BG, Kalpana VL. Calming the storm: Natural immunosuppressants as adjuvants to target the cytokine storm in COVID-19. Front Pharmacol. 2021;11:583777. https://doi.org/10.3389/fphar.2020.583777
PMid:33708109
42. Babaei F, Nassiri-Asl M, Hosseinzadeh H. Curcumin (a constituent of turmeric): New treatment option against COVID-19. Food Sci Nutr. 2020;8(10):5215-27. https://doi.org/10.1002/fsn3.1858
PMid:33133525
43. Bachiega TF, De Sousa JP, Bastos JK, Sforcin JM. Clove and eugenol in noncytotoxic concentrations exert immunomodulatory/anti-inflammatory action on cytokine production by murine macrophages. J Pharm Pharmacol. 2020;64(4) 610-6. https://doi.org/10.1111/j.2042-7158.2011.01440.x
PMid:22420667
44. Bachiega TF, Sforcin JM. Lemongrass and citral effect on cytokines production by murine macrophages. J Ethnopharmacol. 2011;137(1):909-13. https://doi.org/10.1016/j.jep.2011.07.021
PMid:21782918