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Research Article
In silico Study Phytosterol *Cymbopogon citratus* and *Curcuma longa* as Inhibitor Agent 3C-Like Protease SARS-CoV-2

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Abstract

Background and Objective: Lemongrass (*Cymbopogon citratus*) and turmeric (*Curcuma longa*) are widely used by the community for traditional medicinal spices and cooking spices. In the era of the COVID-19 pandemic, people use lemongrass and turmeric to increase immunity and protect the body from infection with the SARS-CoV-2 virus. However, the antiviral mechanisms have not been studied much. This study aims to predict the bioactivity of the phytosterol compounds of lemongrass and turmeric for COVID-19 therapy through inhibition of 3C-like protease (3CLPro) *in silico*. **Materials and Methods:** The 3CLPro protein 3D structure was downloaded from the PDB database with the access code 2ZU2 and the phytosterol compounds of lemongrass and turmeric were taken from PubChem. A total of 59 total phytosterol compounds from turmeric and lemongrass were screened for their bioactivity as an antiviral by using online PASS. Compounds with a high activating potential (Pa) were interacted with 3CLPro protein with the PyRx program and analyzed by Discovery Studio version 19.0 and LigPlus. **Results:** A total of 22 total phytosterol compounds were identified as potential antiviral agents. Based on the Pa value, 15 phytosterol compounds have the potential to act as inhibitor agents for 3CLPro SARS-CoV-2. The phytosterol compounds of lemongrass and turmeric bind to the 3CLPro protein in the N-finger domain region and the A and B domain inhibitors connect residues of the 3CLPro protein. The phytosterols of lemongrass and turmeric show a low binding affinity with 3CLPro SARS-CoV-2, indicating a strong interaction between ligand and protein. The inhibition of phytosterols against 3CLPro protein can be used as a basis for determining candidates for COVID-19 therapeutic agents. **Conclusion:** The phytosterol compounds contained in lemongrass and turmeric have the potential to act as 3CLPro inhibitors. Further studies both *in vitro* and *in vivo* need to be done to prove the inhibitory potential of phytosterol compounds.

Key words: *Cymbopogon citratus*, COVID-19, *Curcuma longa*, *in silico*, SARS-CoV-2 protein 3C-like protease, apoptotic cells, body immunity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Coronavirus Disease (COVID-19) is a disease caused by the coronavirus, one of the SARS-CoV-2 beta coronaviruses[1]. Currently, COVID-19 is still a pandemic in several countries and causes a death rate of up to 5%. Symptoms experienced by people with COVID-19 vary, including fever, cough, flu, rash on the skin, fatigue, confusion and diarrhoea. Various treatment efforts are being developed to treat COVID-19 patients such as vaccines. In addition, various researchers in the world are exploring drugs that act as antiviral for the treatment of COVID-19 and exploring the potential of compounds from natural sources for prevention and treatment[2-6].

The target of COVID-19 treatment can be done in two ways, namely by preventing viral infections and increasing body immunity. Prevention of viral infection can be done by inhibiting the compound through the SARS-CoV-2 virus protein[5,7,8]. The SARS-CoV-2 virus consists of four main structures, namely spike, membrane, nucleocapsid and envelope[9]. In addition, SARS-CoV-2 also has 16 Non-Structural Proteins (NSP), one of which is a 3C-like protease (3CLPro). The protein 3C-like protease (3CLPro) is a SARS-CoV-2 protease that plays an important role in the replication process of the viral genome[9,10-12]. In addition, 3CLPro also causes dysregulation of the immune system in the body by inhibiting the type 1 interferon pathway, decreasing p53 protein expression, activating TGF-β signalling and inducing apoptotic cells[13,14]. Various herbal spices in Indonesia have been known to be able to increase immunity, including ginger, lemongrass, meniran, cinnamon, garlic, secang wood and other spices[15-19].

Lemongrass (*Cymbopogon citratus*) is reported to contain flavonoids, alkaloids, terpenoids, tannins and phytosterols[20,21]. Until now, lemongrass is used as a spice in cooking and the production of aromatherapy oil. The content of phytosterols in lemongrass consists of several types of compounds, including Selina-6-en-4-ol, α-cadinol, 3,7-dimethyl-1,3,6-octatriene, decanal, naphthalene, elemol, β-eudesmol, cubebol, humulene, citral acetate, citral diethyl acetal, verbenone, sabinene, geranyl acetate, citronella, mentha-1 (7), limonene, 8-dien-2-ol cis, mentha-2,8-diene-1- oltrans-para , mentha-1 (7), 8-dien-2-ol trans and mentha-2,8-diene-1-ol cis-para[22]. The total phytosterol compounds of lemongrass have been studied as anti-inflammatory, antioxidant, anti-carcinogenic, antimicrobial and antimutagenic[20,22]. Turmeric (*Curcuma longa*) is also known to be used as a cooking spice and herbal medicine in the community, containing active compounds such as alkaloids, flavonoids, tannins and phytosterols[23,24]. The flavonoids and alkaloids of turmeric are widely explored for various treatments such as anticancer, antimicrobial, antioxidant and antimutagenic[23-26]. However, the phytosterols contained in turmeric have not been studied much. At present, people use various spices to prevent further COVID-19 infection, especially in terms of increasing body immunity. However, the mechanisms and effectiveness of these spices have not been studied much to date. The *in silico* approach is one study that uses computation to open up the mechanisms that occur in cells[19,27,28]. This *in silico* study has several advantages including that it can be used for initial screening in biological activity and can be used to explore compounds as drug candidates[16].

Therefore, this study aimed to explore and analyze the potential of the phytosterol compounds of lemongrass and turmeric oil as antiviral through inhibition of the 3C-like protease (3CLPro) SARS-CoV-2 in handling the COVID-19 pandemic through an in silico study.

MATERIALS AND METHODS

Study area: The research was conducted at the Department of Biology, Bioinformatics Laboratory, Brawijaya University, Malang, East Java, Indonesia from March to August, 2020.

Sampling and preparation: The sample used in this study is secondary data in the form of three-dimensional structures of protein and phytosterol compounds. The three-dimensional structure of the 3C-like protease (3CLPro) protein was downloaded from the Protein Data Bank (PDB) database with ID 2ZU2, then the protein was cleaned of solvents and ligands that bind to the Discovery Studio Program Version 19.0. The phytosterol compounds of lemongrass and turmeric (Table 1) were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format. Phytosterol compounds were prepared by converting the SDF format to pdb and minimizing ligand energy with PyRX 0.8 software[29].

Table 1: Phytosterol compounds of lemongrass and turmeric

Compound	ID PubChem	Compound	ID PubChem
Selina- 6-en-4-ol	527220	Zingiberene	92776
A-cadinol	6431302	Camphor	2537
3,7-dimethyl-1,3,6-octatriene	5281553	1,8-cineole	2758
Decanal	8175	Germacrone	6436348
Naphthalene	931	Isoborneol	64685
Elemol	92138	Camphene	6616
β-eudesmol	91457	Limonene	22311
Ar-curcumene	92139	ar-turmerone	160512
β-curcumene	6428461	β-selinene	10123
Curzerene	572766	P-cymene	7463
Curzerenone	3081930	α-terpineol	8748

Prediction of the biological function of the phytosterol compounds of lemongrass and turmeric: The phytosterol compounds of lemongrass and turmeric were screened by predicting their bioactivity as antiviral agents. The atomic code for compounds (canonical SMILES) is used for the prediction of compound bioactivity with the online Way2Drug PASS Tool (http://www.way2drug.com/passonline/). Bioactivity predictions are presented in the form of a dendrogram which is integrated with the Heatmap[28].

Data from the results of the analysis using the Discovery Studio Client 3.5 software are described in tabular form according to the research adaptation of the previous research[27]. The analysis of the Discovery Studio Client 3.5 software that was carried out was adapted to the method that was already used with several modifications[30]. The table provides information on the types of bonds formed, amino acid residues, types of bonds resulting from interactions and interactions with target compounds and proteins[15]. Table 1 has information about the names of the compounds, amino acid residues and types of bonds formed. There are three types of bonds formed from the interaction of ligands and proteins, namely hydrophobic, hydrogen bond and electrostatic. This inhibition process is also supported by several external factors, one of which is the bond that is formed during the interaction. The bonds formed between ligands and proteins are predominantly hydrogen and hydrophobic (Table 1).

Analysis of ligand-protein interactions: Protein 3C-like protease (3CLPro) interacted with phytosterol compounds of lemongrass and turmeric using the PyRx 0.8 program[29] and visualized in 3D using Discovery Studio version 19.0. Ramachandran plot analysis using Discovery Studio version 19.0, 2D structures were analyzed by LigPlus.

RESULTS AND DISCUSSION

A total of 20 types of citronella phytosterol compounds and 39 types of turmeric phytosterol compounds that have been identified in previous studies were screened for biological function activity. Based on the prediction of biological activity with a pa (potential activate) value of the online PASS program of more than 0.7, 22 phytosterol compounds of lemongrass and turmeric have potential as antiviral agents. These compounds, among others selina-6-en-4-ol, α-cadinol, 3,7-dimethyl-1,3,6-octatriene, decanal, naphthalene, elemol, β-eudesmol, ar-curcumene, β-curcumene, curzerene, curzerenone, zingiberene, camphor, 1,8-cineole, germacrone, isoborneol, camphene, limonene, ar-turmerone, β-selinene, p-cymene and α-terpineol (Fig. 1).

The prediction of the potential bioactivity of lemongrass and turmeric oil in Fig. 1 shows a function as an anti-virus with a function that appears predominantly in blue, with an indication of very high antiviral activity. This determination process is carried out by taking the online Way2Drug PASS Tool data, these data report the potential of compounds against various diseases[31]. The same study conducted by Sari and Bare[28] reported the biological activity of compounds contained in black pepper using the online software Way2Drug PASS Tool, showing research on this compound as an inhibitor of several genes encoding various diseases. Research by Lagunin *et al.*[32] shows that the data obtained is classified in advance to obtain a value of Pa>0.7 or 0.5<Pa>0.7 so that very significant data is obtained as one of the very high pharmacological compounds. At this stage, the selection of compounds that have the highest level of inhibition of an agent will be given a blue label, therefore from Fig. 1, we can predict that the compounds contained in lemongrass and turmeric oil have an antiviral function, especially as a viral entry. Inhibitors and 3C-like proteases. The two functions of lemongrass and turmeric oil compounds have the potential to inhibit virus entry and inhibit the working system of the 3C-like protease virus.

Antiviral parameters used in screening are antivirals against CMV, adenovirus, picornavirus, herpes, influenza, rhinovirus and human coronavirus. In addition, antiviral parameters were also used by inhibiting virus entry and inhibiting 3CLPro protease activity. The blue colour on the heatmap indicates higher activity, while the pink colour indicates lower activity. The cladogram of the compound's potential as an antiviral shows that 22 phytosterol compounds of lemongrass and turmeric are divided into two major groups, the first group is potential as a viral entry inhibitor and a 3CLPro protein protease inhibitor. The second group has potential as antiviral against CMV, adenovirus, picornavirus, herpes, influenza and rhinovirus and protease inhibitor simian immunodeficiency virus.

Fifteen of the 22 phytosterol compounds of lemongrass and turmeric interacted with 3CLPro protein which produced several active residues of 3CLPro protein (Fig. 2a-r and Table 2). The 3,7-dimethyl-1,3,6-octatriene, decanal, naphthalene, elemol, curzerene, curzerenone, 1,8-cineole, germacrone, isoborneol, camphene, limonene, ar-turmerone, β-selinene, p-cymene and α-terpineol binds to the 3CLPro protein on the same side, namely between the A and B domain inhibitors of the 3CLPro protein. The residue that binds the fifteen phytosterol compounds of lemongrass and turmeric, namely MET6, GLN8, ASN112, TYR117, ILE140, ALA246, ILE249, LEU250, LYS253, GLU291, VAL293, LYS294, PHE297, VAL299 and LEU301 (Fig. 2a-r, Table 2).

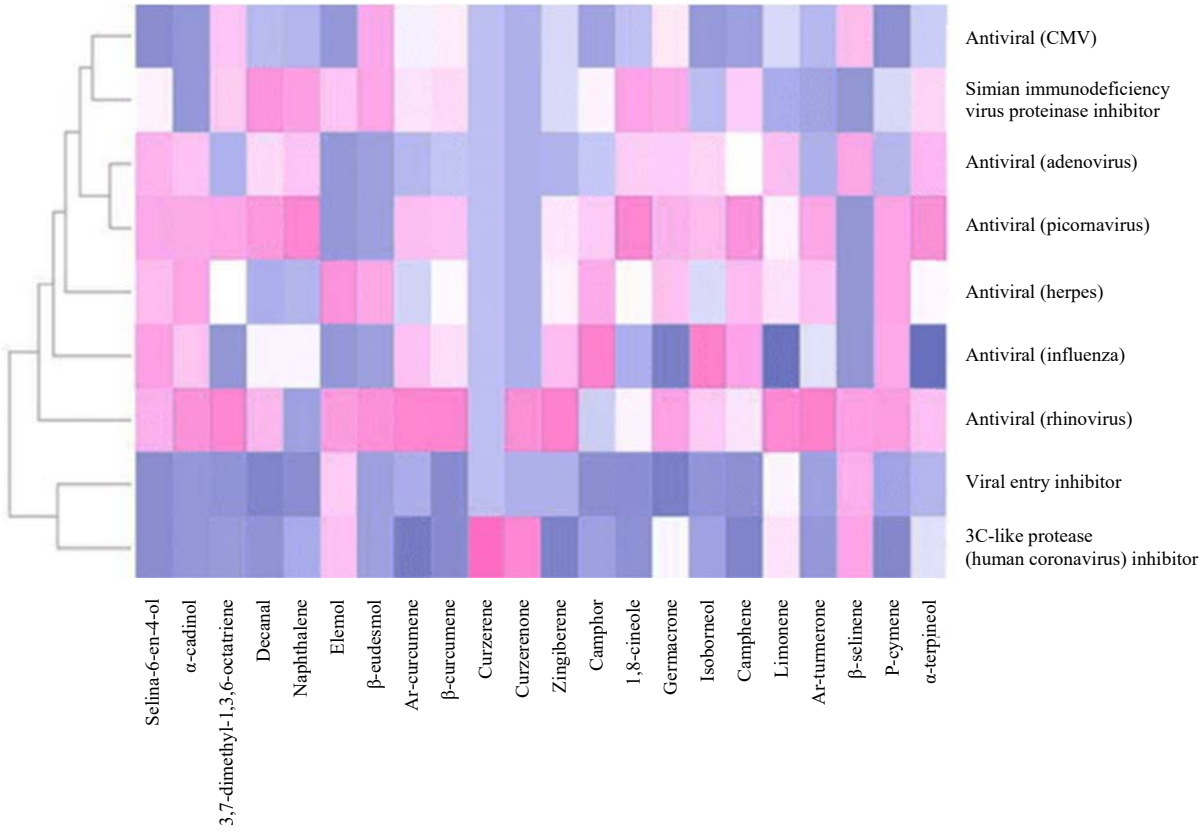

Fig. 1: Predicted bioactivity of lemongrass and turmeric oil as an anti-virus

Blue colour indicates higher antiviral activity and while pink has low antiviral potential

Table 2: Interaction of the bioactive compounds of lemongrass and turmeric

Ligand	3CLPro protein amino acids	Type of bond	Bond affinity (kcal mol^{-1})
3,7-dimethyl-1,3,6-octatriene	LYS294, MET6	Hydrophobic	-4.9
Decanal	ILE140	Hydrophobic	-5.1
Naphthalene	**ILE249**, PHE297, LEU250, LYS253, VAL299	Hydrophobic	-6.2
Elemol	LYS294	Hydrophobic	-6.4
Curzerene	**GLU291**	Electrostatic	-6.6
	LYS294, LYS294	Hydrophobic	
Curzerenone	GLU291	Electrostatic	-6.5
	LYS294	Hydrophobic	
Camphor	GLN8, LYS294	Hydrogen bond	-6.1
	MET6, LYS294	Hydrophobic	
1,8-cineole	**MET6**, LYS294, MET6	Hydrophobic	-5.8
Germacrone	**ASN112**	Hydrogen bond	-7.3
	LYS294, LYS294, MET6	Hydrophobic	
Isoborneol	**GLN295**, MET6, GLU291	Hydrogen bond	-5.5
	MET6, LYS294	Hydrophobic	
Camphene	MET6, **LYS294**	Hydrophobic	-5.5
Limonene	**ALA246**, **ILE249**, **VAL293**, ILE249, LEU250, VAL293, LEU301, VAL299, PHE297	Hydrophobic	-5.9
β-selinene	MET6, LYS294	Hydrophobic	-6.8
P-cymene	**TYR117**, LYS294, ILE140	Hydrophobic	-6.1
α-terpineol	ASN112	Hydrogen bond	-5.9
	LYS294	Hydrophobic	

Thick residue indicates donor and while, thick residue is an acceptor

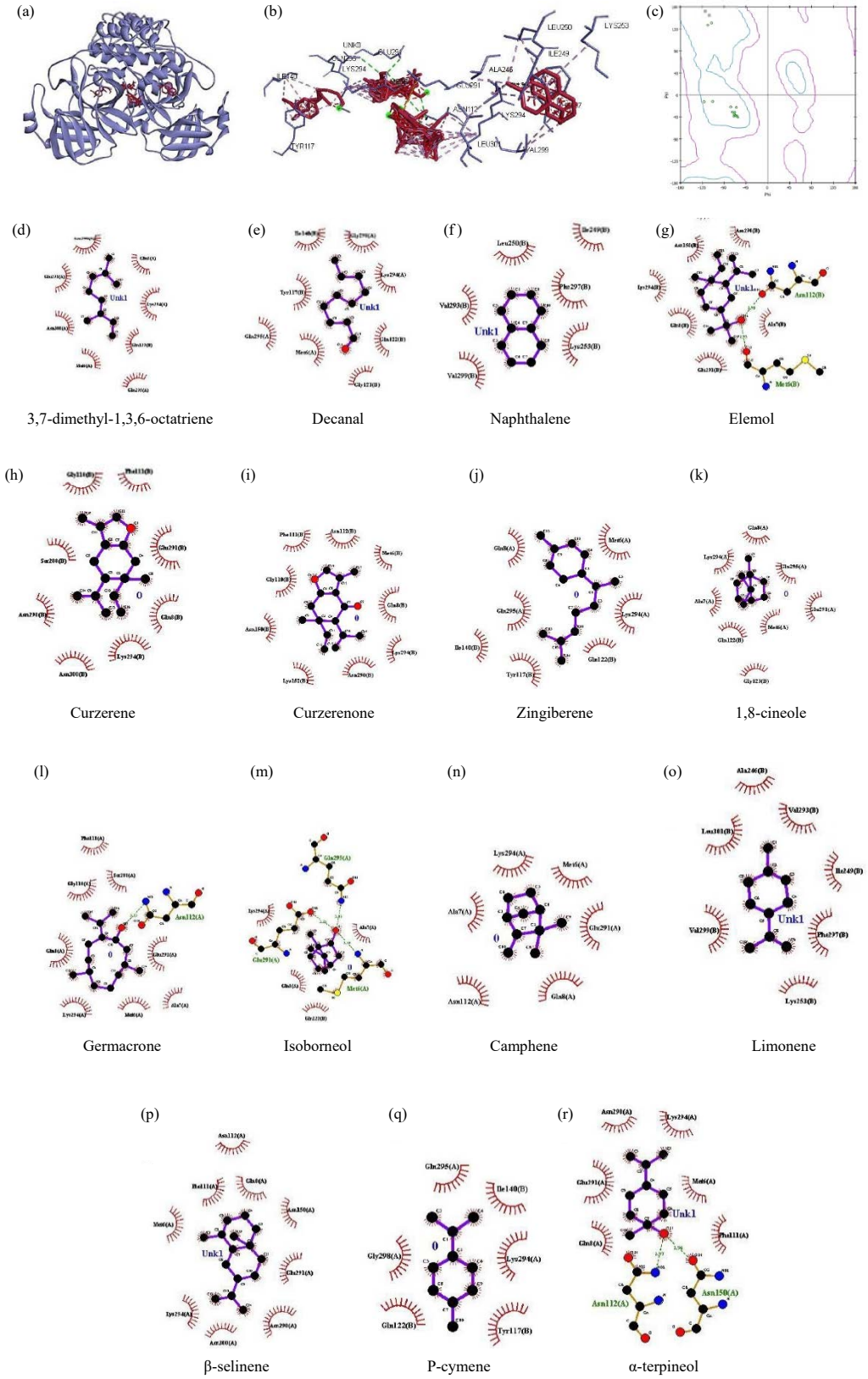

Fig. 2(a-r): Potential of lemongrass and turmeric phytosterol compounds as antiviral through inhibition of 3C-like protease SARS-CoV-2

In addition, in this study, phytosterol compounds that have low polarity and high hydrophobicity can easily enter through the cell membrane. In this study, an interesting thing was also found, namely the presence of electrostatic bonds in the amino acid residue of GLU291. This electrostatic bond will provide additional energy to the ligand and protein interactions, especially in the Curzerene and Curzerenone compounds. The combination of lemongrass and turmeric compounds can be used for COVID-19 therapy through inhibition of 3C-like protease.

Inhibition of 3CLPro will interfere with the replication process of the SARS-CoV-2 virus genome so that it can be used as a candidate for COVID-19 herbal therapy. In addition, based on the prediction of biological function, phytosterols also act as antivirals such as influenza, picornavirus, adenovirus, herpes and others.

The target used in the prevention and therapy of COVID-19 in this study is the 3C-like protease, which is a protease enzyme that plays an important role in coronavirus replication. The compounds that are predicted to have the potential to inhibit the activity of the enzyme, among others: 3,7-dimethyl-1,3,6-octatriene, decanal, naphthalene, elemol, β-eudesmol, curzerene, curzerenone, 1,8-cineole, germacrone, isoborneol, camphene, limonene, ar-turmerone, β-selinene, p-cymene, dan α-terpineol. Curcumin, which is an alkaloid of turmeric, has antiviral potential by binding to SARS-CoV-2 spike glicoprotein and Angiotensin-Converting Enzyme (ACE) receptors[33].

Ramachandran plot shows the active site of the binding of the fifteen compounds in quadrant 1 and quadrant 3 areas which are favourable (Fig. 2c). Interestingly, decanal and p-cymene bind to ILE140 which is the N-finger side of the 3CLPro protein (Fig. 2d). The N-Finger side of 3CLPro protein is an important side of protein for dimerization and protein activation. Inhibition in the N-Finger region can be used as a candidate for SARS-CoV-2 protease inhibitor[13,34,35].

Previous *in silico* studies reported that flavonoids tended to bind to the S1 and S2 sites of the 3CLPro protein. Kaempferol binds to 3CLPro on the residues of Ile188, Glu166 and ASP142. Herbacetin binds to Ile188, Asp187, Gln189, His41 and Glu166, whereas morine interacts with the residues of Asp142, Glu166 and Ile188. The residue is the S1 and S2 pocket areas of the 3CLPro protein. Likewise, pectolinarin and rhoifolin bind to the S1 and S2 pocket proteins of 3CLPro[9,11,14]. In addition, several natural compounds have also been reported as potential therapeutic agents for COVID-19, such as theaflavins, catechins, epigallocatechin, calzone, pyrazolone and others[10,36-40]. The findings in this study indicate that the phytosterol compounds of lemongrass and turmeric bind the active side of 3CLPro. Previous research identified residues in pocket S1 namely PHE140, ASN142, GLU166, HIS163 and HIS172. The S2 pocket sides are HIS41, MET49, TYR54 and MET165. The catalytic site of 3CLPro is HIS41 and Cys145[39-42].

The interaction between the fifteen phytosterol compounds of lemongrass and turmeric showed almost the same bonds as the hydrogen, hydrophobic and electrostatic bonds (Table 2). The more bonds that occur in compounds and proteins, the lower the binding affinity value. The lower the binding affinity, the stronger the ligand and protein interactions[43,44].

The strong ligand and protein bonds will stabilize the interaction[19]. The hydrophobic side contains long hydrocarbons and has a very high level of permeability to the cell membrane[45].

Several studies have also stated that the content of compounds in rhizomes such as ginger and turmeric can increase immunity and as a therapy for metabolic diseases[23,24,46,47].

The phytosterol compounds contained in lemongrass and turmeric are very potential 3CLPro inhibitors. Further research needs to be carried out both *in vitro* and *in vivo* to prove the potential for inhibition of phytosterol compounds through antiviral tests for total phytosterol compounds of lemongrass and turmeric. The phytosterol compounds of lemongrass and turmeric have potential as COVID-19 therapeutic agents through inhibition of N-finger 3C-like SARS-CoV-2 protease.

CONCLUSION

The phytosterol compounds of lemongrass and turmeric have the potential as COVID-19 therapeutic agents through inhibition of N-finger 3C-like SARS-CoV-2 protease. Further *in vitro* and *in vivo* research needs to be carried out to test the antiviral compounds of total phytosterols in lemongrass and turmeric.

SIGNIFICANCE STATEMENT

This study found that the phytosterol compounds contained in lemongrass and turmeric have the potential to act as 3CLPro inhibitors which can be beneficial for increasing immunity and protecting the body from infection with the SARS-CoV-2 virus. This study will help researchers to uncover critical areas of bioactivity of the phytosterol compounds of lemongrass and turmeric for COVID-19 therapy through the inhibition of 3C-like protease (3CLPro) *in silico* which cannot be explored by many researchers. Thus, a new theory about the potential of the phytosterol compounds of lemongrass and turmeric oil as antiviral through inhibition of the SARS-CoV-2. The 3C-like protease (3CLPro) protein in the handling of the COVID-19 pandemic through an *in silico* study, can be obtained.

REFERENCES

1. Bohn, M.K., A. Hall, L. Sepiashvili, B. Jung, S. Steele and K. Adeli, 2020. Pathophysiology of COVID-19: Mechanisms underlying disease severity and progression. Physiology, 35: 288-301.
2. Erlanson, D.A., 2020. Many small steps towards a COVID-19 drug. Nat. Commun., Vol. 11. 10.1038/s41467-020-18710-3.
3. Kasozi, K.I., G. Niedbała, M. Alqarni, G. Zirintunda and F. Ssempijja *et al*., 2020. Bee venom-A potential complementary medicine candidate for SARS-CoV-2 infections. Front. Public Health, Vol. 8. 10.3389/fpubh.2020.594458.
4. Paraiso, I.L., J.S. Revel and J.F. Stevens, 2020. Potential use of polyphenols in the battle against COVID-19. Curr. Opin. Food Sci., 32: 149-155.
5. Shehroz, M., T. Zaheer and T. Hussain, 2020. Computer-aided drug design against spike glycoprotein of SARS-CoV-2 to aid COVID-19 treatment. Heliyon, Vol. 6. 10.1016/j.heliyon.2020.e05278.
6. Zhang, S., M. Krumberger, M.A. Morris, C.M.T. Parrocha, A.G. Kreutzer and J.S. Nowick, 2021. Structure-based drug design of an inhibitor of the SARS-CoV-2 (COVID-19) main protease using free software: A tutorial for students and scientists. Eur. J. Med. Chem., Vol. 218. 10.1016/j.ejmech.2021.113390.
7. Poduri, R., G. Joshi and G. Jagadeesh, 2020. Drugs targeting various stages of the SARS-CoV-2 life cycle: Exploring promising drugs for the treatment of COVID-19. Cell. Signalling, Vol. 74. 10.1016/j.cellsig.2020.109721.
8. Xiu, S., A. Dick, H. Ju, S. Mirzaie and F. Abdi *et al*., 2020. Inhibitors of SARS-CoV-2 entry: Current and future opportunities. J. Med. Chem., 63: 12256-12274.
9. Lee, C.C., C.J. Kuo, M.F. Hsu, P.H. Liang, J.M. Fang, J.J. Shie and A.H.J. Wang, 2007. Structural basis of mercury- and zinc-conjugated complexes as SARS-CoV 3C-like protease inhibitors. FEBS Lett., 581: 5454-5458.
10. Chen, Y.W., C.P.B. Yiu and K.Y. Wong, 2020. Prediction of the SARS-CoV-2 (2019-nCoV) 3C-like protease ($3CL^{pro}$) structure: Virtual screening reveals velpatasvir, ledipasvir, and other drug repurposing candidates. F1000Research, Vol. 9. 10.12688/f1000research.22457.2.
11. Fan, K., L. Ma, X. Han, H. Liang, P. Wei, Y. Liu and L. Lai, 2005. The substrate specificity of SARS coronavirus 3C-like proteinase. Biochem. Biophys. Res. Commun., 329: 934-940.
12. Huang, C., P. Wei, K. Fan, Y. Liu and L. Lai, 2004. 3C-like proteinase from SARS coronavirus catalyzes substrate hydrolysis by a general base mechanism. Biochemistry, 43: 4568-4574.
13. Pillaiyar, T., M. Manickam, V. Namasivayam, Y. Hayashi and S.H. Jung, 2016. An overview of Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV) 3CL protease inhibitors: Peptidomimetics and small molecule chemotherapy. J. Med. Chem., 59: 6595-6628.
14. Jo, S., S. Kim, D.H. Shin and M.S. Kim, 2019. Inhibition of SARS-CoV 3CL protease by flavonoids. J. Enzyme Inhib. Med. Chem., 35: 145-151.
15. Bare, Y., D.R.T. Sari, Y.T. Rachmad, G.C. Krisnamurti, A. Elizabeth and A. Maulidi, 2020. *In silico* insight the prediction of chlorogenic acid in coffee through cyclooxygenase-2 (COX2) interaction. Biogenesis: J. Ilmiah Biol., 7: 100-105.
16. Bare, Y., L.I.N. Indahsari, D.R.T. Sari and T. Watuguly, 2021. *In silico* study: Potential prediction of *Curcuma longa* and *Cymbopogon citratus* essential oil as lipoxygenase inhibitor. JSMARTech: J. Smart Bioprospecting Technol., 2: 75-80.
17. Tiring, S.S.N.D., Y. Bare, A. Maulidi, S. Mansur and F.A.D. Nugraha, 2019. Studi *In silico*: Prediksi potensi 6-shogaol dalam *Zingiber officinale* sebagai inhibitor JNK. Al-Kimia, 7: 147-153.
18. Mansur, S., Y. Bare, M. Helvina, A.P. Pili and G.C. Krisnamurti, 2020. *In silico* study: Potential activity of 10-shogaol in ginger (*Zingiber officinale*) against the ACE gene. Spizaetus: J. Biol. Pendidikan Biol., 1: 12-18.
19. Bare, Y., S. Mansur, S.S.N.D. Tiring, D.R.T. Sari and A. Maulidi, 2020. Virtual screening: Prediksi potensi 8-shogaol terhadap c-Jun n-terminal kinase (JNK). J. Res. Edu. Stud.: E-Saintika, 4: 1-6.
20. Olorunnisola, S.K., H.T. Asiyanbi, A.M. Hammed and S. Simsek, 2014. Biological properties of lemongrass: An overview. Int. Food Res. J., 21: 455-462.
21. Tongnuanchan, P. and S. Benjakul, 2014. Essential oils: Extraction, bioactivities, and their uses for food preservation. J. Food Sci., 79: R1231-R1249.
22. Oladeji, O.S., F.E. Adelowo, D.T. Ayodele and K.A. Odelade, 2019. Phytochemistry and pharmacological activities of *Cymbopogon citratus*: A review. Sci. Afr., Vol. 6. 10.1016/j.sciaf.2019.e00137.
23. Amalraj, A., A. Pius, S. Gopi and S. Gopi, 2017. Biological activities of curcuminoids, other biomolecules from turmeric and their derivatives-A review. J. Traditional Complementary Altern. Med., 7: 205-233.
24. Kim, S., M. Kim, M.C. Kang, H.H.L. Lee and C.H. Cho *et al*., 2021. Antioxidant effects of turmeric leaf extract against hydrogen peroxide-induced oxidative stress *in vitro* in vero cells and *in vivo* in zebrafish. Antioxidants, Vol. 10. 10.3390/antiox10010112.
25. Tanvir, E.M., M.S. Hossen, M.F. Hossain, R. Afroz, S.H. Gan, M.I. Khalil and N. Karim, 2017. Antioxidant properties of popular turmeric (*Curcuma longa*) varieties from Bangladesh. J. Food Qual., Vol. 2017. 10.1155/2017/8471785.

26. Umar, N.M., T. Parumasivam, N. Aminu and S.M. Toh, 2020. Phytochemical and pharmacological properties of *Curcuma aromatica* salisb (wild turmeric). J. Appl. Pharm. Sci., 10: 180-194.
27. Sari, D.R.T., J.R.K. Cairns, A. Safitri and F. Fatchiyah, 2019. Virtual prediction of the delphinidin-3-O-glucoside and peonidin-3-O-glucoside as anti-inflammatory of TNF-α signaling. Acta Inf. Med., 27: 152-157.
28. Sari, D.R.T. and Y. Bare, 2020. Physicochemical properties and biological activity of bioactive compound in *Pepper nigrum*: *In silico* study. Spizaetus: J. Biol. Pendidikan Biol., 1: 1-6.
29. Dallakyan, S. and A.J. Olson, 2015. Small-Molecule Library Screening by Docking with PyRx. In: Chemical Biology, Hempel, J.E., C.H. Williams and C.C. Hong (Eds.), Humana Press, New York, ISBN: 978-1-4939-2269-7, pp: 243-250.
30. Tapiory, A.A., K.O. Pertiwi, K. Fadilla, D. Reyhanditya and F. Fatchiyah, 2020. *In-silico* analysis of methoxyl pectin compounds from banana peels as HMG-CoA reductase inhibitor complexes. JSMARTech: J. Smart Bioprospecting Technol., 1: 46-50.
31. Filimonov, D.A., A.A. Lagunin, T.A. Gloriozova, A.V. Rudik, D.S. Druzhilovskii, P.V. Pogodin and V.V. Poroikov, 2014. Prediction of the biological activity spectra of organic compounds using the pass online web resource. Chem. Heterocycl. Compd., 50: 444-457.
32. Lagunin, A., A. Stepanchikova, D. Filimonov and V. Poroikov, 2000. PASS: Prediction of activity spectra for biologically active substances. Bioinformatics, 16: 747-748.
33. Choudhury, A. and S. Mukherjee, 2020. *In silico* studies on the comparative characterization of the interactions of SARS-CoV-2 spike glycoprotein with ACE-2 receptor homologs and human TLRs. J. Med. Virol., 92: 2105-2113.
34. Muramatsu, T., C. Takemoto, Y.T. Kim, H. Wang and W. Nishii *et al*., 2016. SARS-CoV 3CL protease cleaves its C-terminal autoprocessing site by novel subsite cooperativity. Proc. Nat. Acad. Sci., 113: 12997-13002.
35. Moustaqil, M., E. Ollivier, H.P. Chiu, S.V. Tol and P. Rudolffi-Soto *et al*., 2021. SARS-CoV-2 proteases PLpro and 3CLpro cleave IRF3 and critical modulators of inflammatory pathways (NLRP12 and TAB1): Implications for disease presentation across species. Emerging Microbes Infec., 10: 178-195.
36. Chen, C.N., C.P.C. Lin, K.K. Huang, W.C. Chen, H.P. Hsieh, P.H. Liang and J.T.A. Hsu, 2005. Inhibition of SARS-CoV 3C-like protease activity by theaflavin-3,3'-digallate (TF3). Evidence-Based Complementary Altern. Med., 2: 209-215.
37. Nguyen, T.T.H., H.J. Woo, H.K. Kang, V.D. Nguyen and Y.M. Kim *et al*., 2012. Flavonoid-mediated inhibition of SARS coronavirus 3C-like protease expressed in *Pichia pastoris*. Biotechnol. Lett., 34: 831-838.
38. Jo, S., H. Kim, S. Kim, D.H. Shin and M.S. Kim, 2019. Characteristics of flavonoids as potent MERS CoV 3C like protease inhibitors. Chem. Biol. Drug Des., 94: 2023-2030.
39. Liu, P., H. Liu, Q. Sun, H. Liang and C. Li *et al*., 2020. Potent inhibitors of SARS-CoV-2 3C-like protease derived from *N*-substituted isatin compounds. Eur. J. Med. Chem., Vol. 206. 10.1016/j.ejmech.2020.112702.
40. Rajpoot, S., M. Alagumuthu and M.S. Baig, 2021. Dual targeting of $3CL^{pro}$ and PL^{pro} of SARS-CoV-2: A novel structure-based design approach to treat COVID-19. Curr. Res. Struct. Biol., 3: 9-18.
41. Lin, C.W., F.J. Tsai, C.H. Tsai, C.C. Lai and L. Wan *et al*., 2005. Anti-SARS coronavirus 3C-like protease effects of *Isatis indigotica* root and plant-derived phenolic compounds. Antiviral Res., 68: 36-42.
42. Liu, Y., C. Liang, L. Xin, X. Ren and L. Tian *et al*., 2020. The development of coronavirus 3C-like protease ($3CL^{pro}$) inhibitors from 2010 to 2020. Eur. J. Med. Chem., Vol. 206. 10.1016/j.ejmech.2020.112711.
43. Raharjo, S.J., C. Mahdi, N. Nurdiana, T. Kikuchi and F. Fatchiyah, 2014. Binding energy calculation of patchouli alcohol isomer cyclooxygenase complexes suggested as COX-1/COX-2 selective inhibitor. Adv. Bioinform. 10.1155/2014/850628.
44. Sari, D.R.T., A. Safitri, J.R.K. Cairns and F. Fatchiyah, 2020. Anti-apoptotic activity of anthocyanins has potential to inhibit caspase-3 signaling. J. Trop. Life Sci., 10: 15-25.
45. Chen, D., N. Oezguen, P. Urvil, C. Ferguson, S.M. Dann and T.C. Savidge, 2016. Regulation of protein-ligand binding affinity by hydrogen bond pairing. Sci. Adv., Vol. 2. 10.1126/sciadv.1501240.
46. Maurya, A.K. and N. Mishra, 2021. *In silico* validation of coumarin derivatives as potential inhibitors against main protease, NSP10/NSP16-methyltransferase, phosphatase and endoribonuclease of SARS CoV-2. J. Biomol. Struct. Dyn., 39: 7306-7321.
47. Bare, Y., A. Marhendra, T. Sasase and F. Fatchiyah, 2018. Differential expression of IL-10 gene and protein in target tissues of rattus norvegicus strain wistar model type 2 diabetes mellitus (T2DM). Acta Inform. Med., 26: 87-91.