PubMed

ISSN 2231-4040

Journal of Advanced Pharmaceutical Technology & Research

An Official Publication of Society of Pharmaceutical Education & Research

Volume 12 / Issue 3 / July-September 2021

www.japtr.org

JAPTR

Wolters Kluwer

Medknow

ORIGINAL ARTICLE

Working goal of Brazilein sappan wood as a candidate for SARS-coV-2 antivirus drug against spike (S) glycoprotein, papain-like proteinase, and main protease: *In silico* study

Entuy Kurniawan, Dwi Krihariyani[1]

Departement of Medical Laboratory, Technology Health Polytechnics of Bandung, Ministry of Health, Indonesia, [1]Departement of Medical Laboratory, Technology Health Polytechnics of Surabaya, Ministry of Health, Indonesia

J. Adv. Pharm. Technol. Res.

ABSTRACT

Brazilein sappan wood, played by Spike (S) glycoprotein, Papain-Like proteinase (PLpro), and Main protease (Mpro), is expected to be a candidate for the antiviral drug SARS-CoV-2, which can inhibit viral attachment to the human body, replication, and transcription processes. The aim of this study was to predict *in silico*, using the comparative drug hydroxychloroquine, the working goal of brazilein sappan wood as a candidate for the antiviral drug SARS-CoV-2 against protein S, PLpro, and Mpro. The approach used is the *in silico* docking test using the computer program Molegro Virtual Docker. Receptor used by protein S, Protein Data Bank (PDB) code: 6M0J, NAG_601[E] ligand; PLpro, PDB code: 7JIT, Y95_501[A] ligand; and Mpro, PDB code: 1WOF, I12_1145[A] ligand. Data analysis was carried out by comparing the docking bond energies between the ligands at the target receptor. Silico test results for protein S: ligand bond energy NAG_601 [E] = −59.4555, brazilein = −71.5537, hydroxychloroquine = −79.3704; PLpro protein: Ligand bond energy Y95_501 [A] = −129.561, brazilein = −94.9761, hydroxychloroquine = −100.984; Mpro protein: Ligand bond energy I12 1145 [A] = −141.135, brazilein = −96.6169, hydroxychloroquine = −104.88. The above test results indicate that brazilein sappan wood has potential as a SARS-CoV-2 drug candidate, has a stable bond, and that the biological activity of the compound is stronger against S protein than the proteins of PLpro and Mpro.

Key words: Brazilein, hydroxychloroquine, *in silico* test, main protease, papain-like proteinase, protein S

INTRODUCTION

Brazilein is a secondary metabolite of sappan wood that can be used empirically for the treatment of disease as a medicine.[1] As a candidate for the antiviral drug SARS-CoV-2, Secang wood brazilein may be produced. The *in silico* test revealed that brazilein has immunomodulatory

Address for correspondence:
Mrs. Dwi Krihariyani, MD,
Wiguna Tengah XIX No. 19, Surabaya, East Java, Indonesia.
E-mail: dwikrihariyani@gmail.com

Submitted: 08-Mar-2021 **Revised:** 05-Apr-2021
Accepted: 18-Jun-2021 **Published:** 16-Jul-2021

Access this article online	
Quick Response Code:	Website: www.japtr.org
	DOI: 10.4103/japtr.JAPTR_222_21

How to cite this article: Kurniawan E, Krihariyani D. Working goal of Brazilein sappan wood as a candidate for SARS-coV-2 antivirus drug against spike (S) glycoprotein, papain-like proteinase, and main protease: *In silico* study. J Adv Pharm Technol Res 2021;12:298-304.

properties as well as good pharmacokinetic properties, resulting in low toxicity.[2] As work targets for this antiviral drug candidate, various structural and nonstructural proteins can be targeted.[3,4]

Viral proteins, which play an important role in the mechanism of viral attachment to the human body as well as the replication and transcription processes, can be used as the task objective of this antiviral drug.[5,6] The proteins S, papain-like proteinase (Plpro), and main protease (Mpro) are prospective working targets for candidates for antiviral drugs.[7,8]

There are several designs and working mechanisms for the work goal of the COVID-19 antiviral drug that was used, namely, (a) it may inhibit the operation of virus functional proteins or enzymes such that the process of viral RNA synthesis and replication is inhibited; (b) may interact with the viral S protein, thereby blocking the binding between viruses and receptors of human cells; (c) to restore innate immunity to the host, virulence factors can be produced; and (d) It can interact with host specific enzymes or receptors to prevent viruses from entering the host cells.[3]

By forming bonds between protein S and angiotensin-converting enzyme 2 (ACE2), which is played out by 2 functional subunits, viruses invade human cells. The functional subunit S1 plays a role in the formation of ACE2 receptor bonds on the host cell, while the functional subunit S2 plays a role in the combination of the virus with the membrane of the host cell.[9,10] Sequences of receptor-binding domain (RBD) such as receptor-binding motif with certain amino acid residues have the ability to transmit between humans.[11,12] The function of PLpro is required after entering the human body for the cleavage process of nsp1, nsp2, and nsp3, which are essential in viral replication.[13] In determining the viability of CoV that mediates the replication and transcription of proteins in viruses, one of the essential enzymes is Mpro. The essential role of proteins S, Plpro, and Mpro in the design of antiviral drugs for COVID-19 is what makes them important targets.[14,15]

The *in silico* test is carried out by docking the molecules, the behavior of which is predicted with the target cells selected. The results from the *in silico* test are in the form of a bond energy value or Rerank Score (RS). The amount of energy needed to form bonds between ligands and receptors is shown by bond energy. The lower the energy of the bond, the more stable it is. The more stable the bond between the ligand and the receptor, the greater the activity can also be predicted. When docking, ligands with biological activity and the ability to bind to biological targets well in the Protein Data Bank (PDB) are used.[16]

A comparator drug that has been used and has a similar effect is needed to demonstrate the activity of the brazilein compound in sappan wood as a candidate for the antiviral drug SARS-CoV-2 against protein S, PLpro, and Mpro. Hydroxychloroquine, which has proven potential for the treatment of COVID-19 but does not support its use in hospitalized patients with COVID-19 who need oxygen, was used as a comparison drug.[17,18]

In a design for the antiviral drug SARS-CoV-2, sappan wood brazilein may be produced. Current drugs are designed using drug designs. Using molecular modification achieves new forms of drugs with improved activity and less to no side effects.[19]

MATERIALS AND METHODS

Materials

Three-dimensional (3D) structure Crystal structure of SARS-CoV-2 spike RBD bound with ACE2, code PDB: 6M0J; The crystal structure of Papain-Like Protease of SARS CoV-2, C111S mutant, in complex with PLP_Snyder495, code PDB 7JIT; and Crystal structure of SARS-CoV Mpro in complex with an inhibitor N1, code PDB; 1WOF from http://www.rcsb.org/pdb/home.do/.

Tools

Windows 8 64-bit, ChemDraw Professional 16.0, Chem3D 16.0, and Molegro Virtual Docker 5 are the device requirements.

Methods

The structure of the molecule target protein S, code PDB: 6M0J, ligand NAG_601 [E]; PLpro, code PDB 7JIT, ligand Y95_501 [A]; Mpro, PDB; 1WOF, ligand I12_1145 [A]. ChemDraw Professional 16.0.0 is used to draw the 2D structure of the compound to be docked, which is then transformed to 3D using Chem3D 16.0.0. The conformation that was found to be the most stable was identified and determined. After calculating the minimum power, it is stored in the form of mol2 {SYBYL2(*.mol2)}. The docking process is carried out against the target receptor target Spike (S) glycoprotein, code PDB: 6M0J, PLpro, code PDB 7JIT, Mpro, PDB; 1WOF, using the Molegro Virtual Docker 5 computer software. The results are expressed as a RS, which is the amount of energy required during the ligand-receptor interaction phase.[20]

RESULTS

Figure 1 depicts the effects of forming a 2D structure with ChemDraw Professional 16.0. 2Dstructures are then used to construct a 3D structure in Chem3D 16.0.0. Figure 2 displays the next 3D structure used at all levels of docking.

Interaction visualization of the target molecule protein S with NAG_601[E], brazilein, and hydroxychloroquine ligands is shown in Figure 3; PLpro with Y95_501[A], brazilein, and hydroxychloroquine ligands are shown

in Figure 4; Mpro with I12_1145[A], brazilein, and hydroxychloroquine ligands are shown in Figure 5.

Figure 6 and Table 1 indicate the amino acids involved in the interaction of the protein S receptor with ligand NAG_601 [E], brazilein, and hydroxychloroquine; Figure 7 and Table 2 show PLpro with ligand Y95_501 [A], brazilein, and hydroxychloroquine; Figure 8 and Table 3 show the Mpro with ligand I12_1145 [A], brazilein, and hydroxychloroquine.

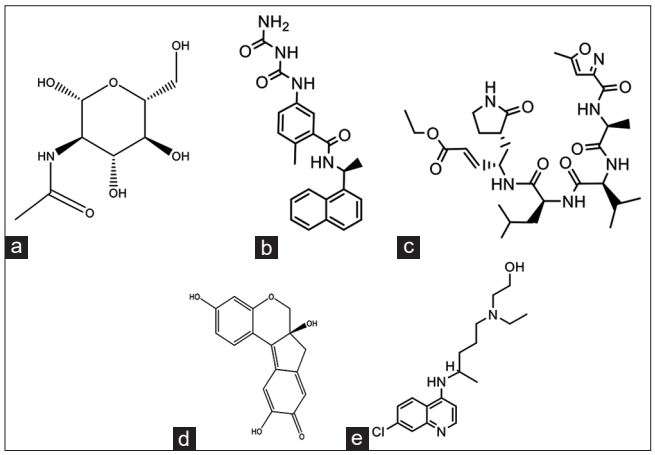

Figure 1: Two-dimensional design (a) Ligand NAG; (b) Ligand Y95; (c) Ligand I12; (d) Brazilein; (e) Hydroxychloroquine

Protein S, PDB code: 6M0J, PLpro, code 7JIT PDB, Mpro, PDB; 1WOF receptor re-docking effects, using the computer program Molegro Virtual Docker 5, can be seen in Table 4.

DISCUSSION

Protein S receptor with ligand NAG_601[E], Y95_501[A], I12_1145[A] is the target molecular receptor structure used. The three target receptors that were downloaded from the PDB were model proteins that were homologous to the target proteins of the SARS-CoV-2 drug candidate.

The ligands NAG_601[E], Y95_501[A], I12_1145[A] are compounds with known molecular structure and biological activity that have been present in the protein bank results. The three ligands were chosen because they have shown strong biological activity and are capable of binding during the docking process to biological targets, namely, the desired receptors, as well as specifying the parameters of the molecule's chemical and physical properties. The activity groups (pharmacophores) and groups that can decrease activity, as well as the lipophilic, electronic, and steric/geometric properties of the groups, are searched with the aid of a computer program so that they can be used to design the minimum structural characteristics necessary for growth. Supplementary medicine.

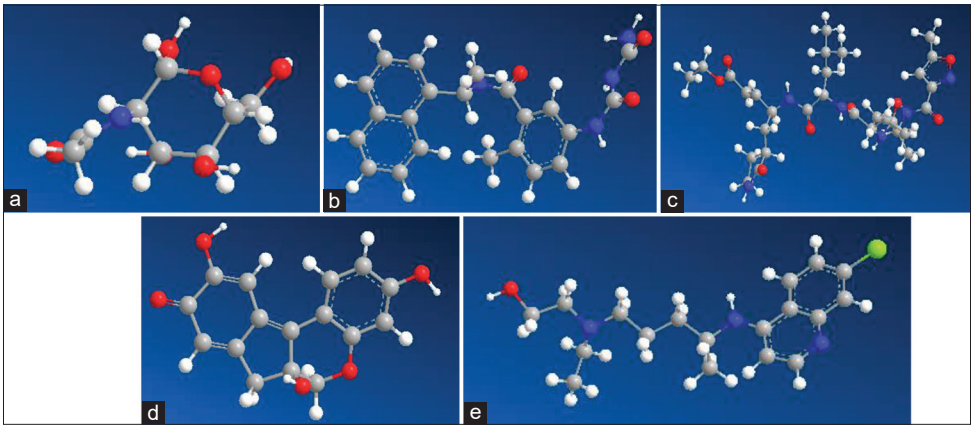

Figure 2: The form of the three-dimensional structure that is stored in SYBYL2. (a) Ligand NAG; (b) Ligand Y95; (c) Ligand I12; (d) Brazilein; (e) Hydroxychloroquine

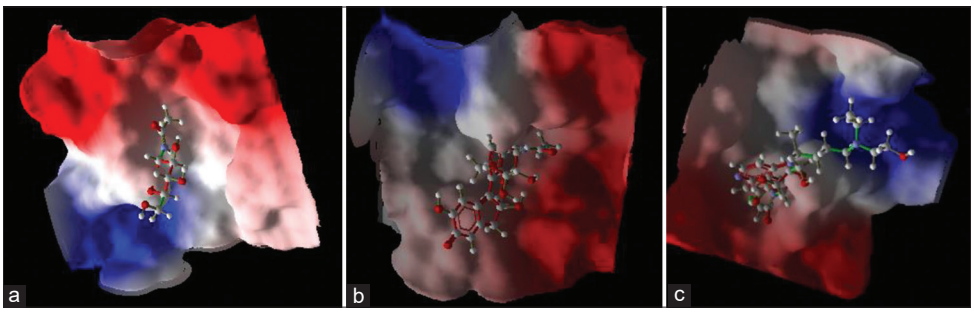

Figure 3: Interactions with receptors protein S dengan (a) Ligand NAG_601[E]; (b) Brazilein; (c) Hydroxychloroquine

Table 1: The amino acids involved in the hydrogen-bond, electronic, and steric bond interaction of the protein spike receptor with ligands

Ligand	Hydrogen bonds		Electrostatic bonds		Steric bond	
NAG_601[E]	0	-	0	-	1	Asn 343(E)
Brazilein	3	Val 341(E) Gly 339(E) Glu 340(E)	0	-	6	Phe 338(E) Val 341(E) Phe 342(E) Glu 340(E) Asn 343(E)
Hydroxychloroquine	0	-	0	-	3	Phe 374(E) Ser 373(E) Ser 371(E)

Table 2: The amino acids involved in the hydrogen-bond, electronic, and steric bond interaction of the papain-like proteinase receptor with ligands

Ligand	Hydrogen bonds		Electrostatic bonds		Steric bond	
Y95_501[A]	4	Asp 164 Gln 269 Tyr 264 Tyr 268	0	-	5	Asp 164 Gln 269 Tyr 264 Glu 167 Tyr 268
Brazilein	2	Gln 269 Glu 167	0	-	6	Tyr 264 Tyr 273 Glu 167 Gln 269 Leu 162 Gly 271
Hydroxychloroquine	4	Asp 164 Gln 269 Tyr 264 Tyr 268	0	-	5	Asp 164 Gln 269 Tyr 264 Glu 167 Tyr 268

Table 3: The amino acids involved in the hydrogen-bond, electronic, and steric bond interaction of the main protease receptor with ligands

Ligand	Hydrogen bonds		Electrostatic bonds		Steric bond	
I12_1145[A]	5	Thr 190(A) Glu 166(A) Gln 189(A) His 164(A) Cys 145(A)	0	-	11	Thr 190(A) Ala 191(A) Glu 166(A) Met 165(A) Gln 189(A) His 164(A) Gly 143(A) Thr 26(A) Cys 145(A) His 163(A)
Brazilein	1	Ala 191(A)	0	-	3	Gln 192(A) Ala 191(A) Thr 190(A)
Hydroxychloroquine	3	Asn 142(A) Ala 191(A) Gln 192(A)	0	-	5	Glu 166(A) Met 165(A) Thr 190(A) Gln 192(A) Ala 191(A)

In order to meet the receptors, physical chemistry plays an essential role in transporting drugs. Drug physical chemistry (lipophilic and electronic) plays a part in the drug's absorption and delivery such that the drug content is very high when it hits the receptor. Only drugs that have a high specificity structure can interact and cause activity with the biological receptors. The physical chemistry of the drug (electronic and steric) plays a role in promoting

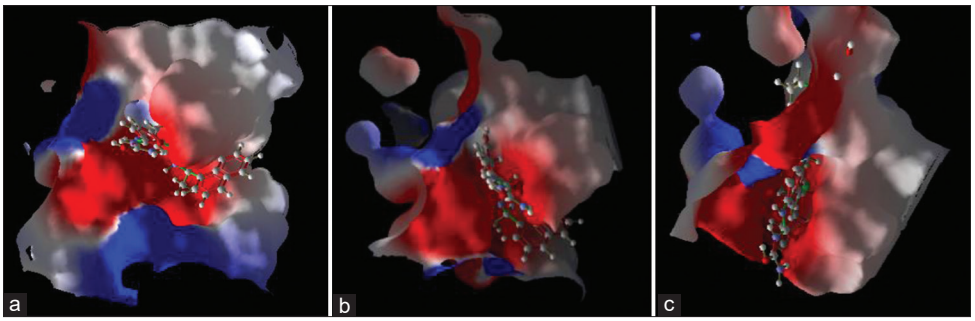

Figure 4: Interactions with receptors papain-like proteinase dengan (a) Ligan Y95_501[A]; (b) Brazilein; (c) Hydroxychloroquine

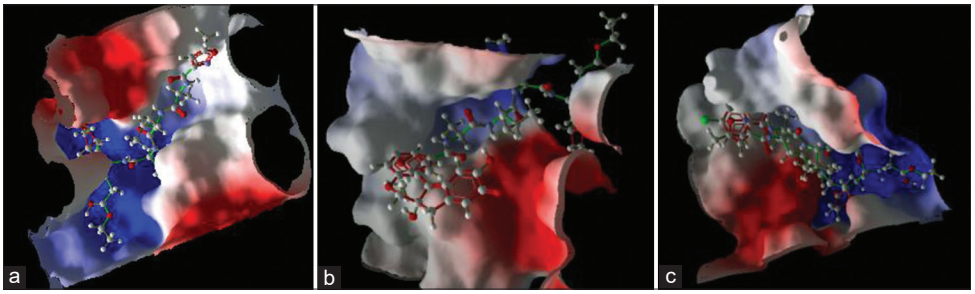

Figure 5: Interactions with receptors main protease dengan (a) Ligand I12_1145[A]; (b) Brazilein; (c) Hydroxychloroquine

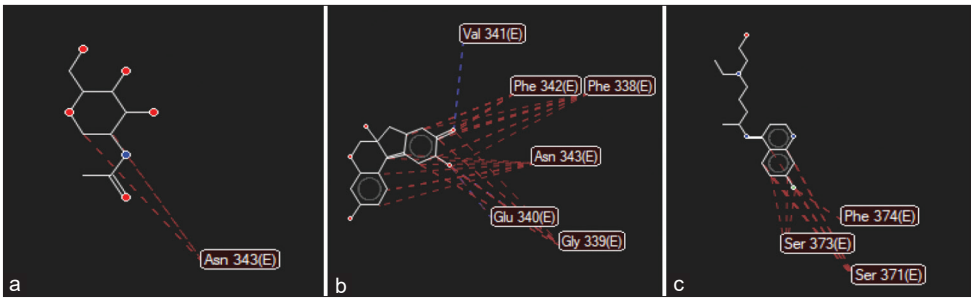

Figure 6: Amino acids involved in the interaction of the protein S receptor with (a) ligand NAG_601[E]; (b) Brazilein; (c) Hydroxychloroquine

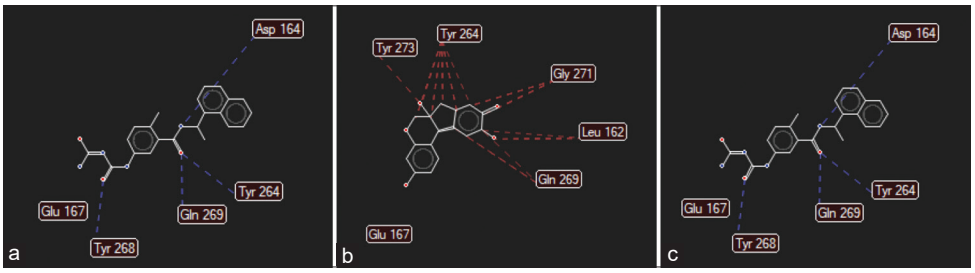

Figure 7: Amino acids involved in the interaction of the papain-like proteinase with (a) Ligand Y95_501[A]; (b) Brazilein; (c) Hydroxychloroquine

the precise orientation of the molecule at the receptor surface.[20]

Through lipophilic/hydrophobic, electronic, and steric bonds, amino acids involved in receptor interactions with ligands occur. There are numerous interactions between each compound of the ligand and the target receptor, since there are variations in the compound structure's spatial configuration. The large number of amino acid residues produced suggests that there is a good potential for the expected ligands to bind to the target protein.[21,22]

The biological activity of the compound can be predicted using a comparison of docking bond energies between ligands at the target receptor. The lower the ligands' bond energy to the target receptor, the more the bonds are secure. The effects of re-docking the protein S receptor with the ligands NAG_601[E], brazilein, and hydroxychloroquine, PLpro with ligands Y95_501 [A],

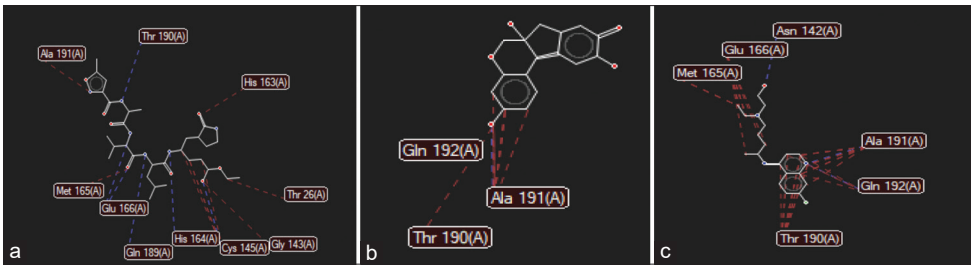

Figure 8: Amino acids involved in the interaction of the main protease with (a) Ligand I12_1145[A]; (b) Brazilein; (c) Hydroxychloroquine

Table 4: Effects of re-docking using the application molegro virtual docker 5

Action target for SARS-CoV-2 antiviral drugs	Ligand	Rerank Score
S glycoprotein	NAG_601[E]	−59.4555
	Brazilein	−71.5537
	Hydroxychloroquine	−72.1194
PLpro	Y95_501[A]	−129.561
	Brazilein	−94.9761
	Hydroxychloroquine	−100.984
Mpro	I12_1145[A]	−141.135
	Brazilein	−96.6169
	Hydroxychloroquine	−104.88

S: Spike, PLpro: Papain-like proteinase, Mpro: Main protease

brazilein, and hydroxychloroquine; Ligand I12_1145 [A], brazilein, and hydroxychloroquine, Mpro using the computer software Molegro Virtual Docker 5, can be seen in Table 4.

The target protein S ranking indicates that brazilein provides lower energy than the NAG_601[E] ligand, making it more stable to bind to the receptor, but higher than hydroxychloroquine, making it less stable to bind to the receptor. Rank scores on the PLpro target suggest that brazilein supplies higher energy than Y95_501[A] and hydroxychloroquine ligands so that receptor binding is less stable. The Mpro goal rank score indicates that brazilein provides higher energy than I12_1145[A] ligand and hydroxychloroquine, rendering it less stable to bind to the receptor.

CONCLUSION

The comparison of ligand, brazilein, and hydroxychloroquine bond energy values with the target receptor protein S, PLpro, and Mpro indicates that the ability of wood brazilein as a SARS-CoV-2 drug candidate is stable bonding and that the biological activity of the compound is stronger against glycoprotein S than that of PLpro and Mpro by the molecular docking method.

Acknowledgments

Prof. Dr. Siswandono, Apt., M.S. The Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia, which has a Molegro Virtual Docker software license, is greatly appreciated.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Nirmal NP, Rajput MS, Prasad RG, Ahmad M. Brazilin from Caesalpinia sappan heartwood and its pharmacological activities: A review. Asian Pac J Trop Med 2015;8:421-30.
2. Krihariyani D, Sasongkowati R, Haryanto E. Studi *in silico* sifat farmakokinetik, toksisitas, dan aktivitas imunomodulator brazilein kayu secang terhadap enzim 3-chymotrypsin-like cysteine protease coronavirus. J Indones Med Lab Sci 2020;116:32-47.
3. Purwaniati, Asnawi A. Target kerja obat antivirus Covid-19: Review. Jurnal Farmagazine 2020;VII: 30-42.
4. Pandey P, Rane JS, Chatterjee A, Kumar A, Khan R, Prakash A, *et al.* Targeting SARS-CoV-2 spike protein of COVID-19 with naturally occurring phytochemicals: An *in silico* study for drug development. J Biomol Struct Dyn [Internet]. 2020;0(0):1-11. Available from: https://doi.org/10.1080/07391102.2020.1796811.
5. Prastyowati A. Mengenal karakteristik virus SARS-CoV-2 penyebab penyakit COVID-19 sebagai dasar upaya untuk pengembangan obat antivirus dan vaksin. BioTrends 2020;11:1-10.
6. Tahir Ul Qamar M, Alqahtani SM, Alamri MA, Chen LL. Structural basis of SARS-CoV-2 $3CL^{pro}$ and anti-COVID-19 drug discovery from medicinal plants. J Pharm Anal 2020;10:313-9.
7. Huang J, Song W, Huang H, Sun Q. Pharmacological Therapeutics Targeting RNA-Dependent RNA Polymerase, Proteinase and Spike Protein: From Mechanistic Studies to Clinical Trials for COVID-19. J Clin Med. 2020;9:1-23.
8. Romeo A, Iacovelli F, Falconi M. Targeting the SARS-CoV-2 spike glycoprotein prefusion conformation: virtual screening and molecular dynamics simulations applied to the identification of potential fusion inhibitors. Virus Res [Internet]. 2020;286(198068):1–10. Available from: https://doi.org/10.1016/j.virusres.2020.198068.
9. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 2020;181:281-92.e6.
10. Shi Y, Wang Y, Shao C, Huang J, Gan J, Huang X, *et al.* COVID-19 infection: The perspectives on immune responses. Cell Death Differ 2020;27:1451-4.
11. Burhan E, Isbaniah F, Susanto AD, Aditama TY, Soedarsono, Sartono TR, *et al.* Pneumonia Covid-19, Diagnosis & Penatalaksanaan Di Indonesia. Perhimpunan Dokter Paru Indonesia. 2020:1-67.
12. Wang Q, Zhang Y, Wu L, Niu S, Song C, Zhang Z, *et al.* Structural

and functional basis of SARS-CoV-2 entry by using human ACE2. Cell 2020;181:894-904.e9.

13. Klemm T, Ebert G, Calleja DJ, Allison CC, Richardson LW, Bernardini JP. "Mechanism and inhibition of the papain-like protease, PLpro, of SARS-CoV-2. EMBO J 2020;39:1-17.
14. Dai W, Zhang B, Jiang XM, Su H, Li J, Zhao Y, *et al.* Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. Science 2020;368:1331-5.
15. Fu L, Ye F, Feng Y, Yu F, Wang Q, Wu Y, *et al.* Both Boceprevir and GC376 efficaciously inhibit SARS-CoV-2 by targeting its main protease. Nat Commun 2020;11:4417.
16. Krihariyani D, Wasito EB, Isnaeni I, Siswodihardjo S, Yuniarti WM. In Silico Study on Antibacterial Activity and Brazilein ADME of Sappan Wood (Caesalpinia Sappan L .) Against Escherichia coli (Strain K12). Syst Rev Pharm. 2020;11:290-6.
17. Mahévas M, Tran VT, Roumier M, Chabrol A, Paule R, Guillaud C, *et al*. Clinical efficacy of hydroxychloroquine in patients with covid-19 pneumonia who require oxygen: Observational comparative study using routine care data. BMJ 2020;369:m1844.
18. Kesuma D, Siswandono S, Purwanto BT, Hardjono S. Uji in silico Aktivitas Sitotoksik dan Toksisitas Senyawa Turunan N-(Benzoil)-N'- feniltiourea Sebagai Calon Obat Antikanker. JPSCR J Pharm Sci Clin Res. 2018;3:1.
19. Hardjono S, Siswodihardjo S, Pramono P, Darmanto W. Quantitative structure-cytotoxic activity relationship 1-(Benzoyloxy) urea and its derivative. Curr Drug Discov Technol 2016;13:101-8.
20. Siswandono S. Kimia Medisinal 1. Edisi 2. Kampus C Unair, Mulyorejo Surabaya 60115: Airlangga University Press; 2016:554 .
21. Mardianingrum R, Endah SRN, Suhardiana E, Ruswanto R, Siswandono S. Docking and molecular dynamic study of isoniazid derivatives as anti-tuberculosis drug candidate. Chem Data Collect [Internet]. 2021;32:100647. Available from: https://doi.org/10.1016/j.cdc.2021.100647.
22. Ruan Z, Liu C, Guo Y, He Z, Huang X, Jia X, *et al.* SARS-CoV-2 and SARS-CoV: Virtual screening of potential inhibitors targeting RNA-dependent RNA polymerase activity (NSP12). J Med Virol. 2021;93-:389-400.