

## Active Compounds Activity from the Medicinal Plants Against SARS-CoV-2 using in Silico Assay

Ni Putu Linda Laksmini\*, Luh Putu Febryana Larasanty, Anak Agung Gde Jaya Santika, Putu Agus Andika Prayoga, Anak Agung Intan Kharisma Dewi and Ni Putu Ayu Kristiara Dewi

Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Udayana University, Indonesia 80362.

\*Corresponding Author E-mail : laksmini@unud.ac.id; lindalaksmini@gmail.com

<http://dx.doi.org/10.13005/bpj/1953>

(Received: 02 April 2020; accepted: 28 April 2020)

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), better known as the coronavirus, is a new type of coronavirus that is transmitted to humans. This virus infection is called COVID-19 and was first discovered in the city of Wuhan, China, at the end of December 2019. This virus spread quickly and has spread to other regions in China and several countries, including Indonesia. This disease results in coronavirus pandemic 2019-2020. The objective of this research is to determine the inhibitory ability of several active compounds from natural sources against COVID-19 target protein in silico using molecular docking. In silico research was conducted using autodock 4.2 program by evaluating the binding energy between the active compound with ACE2, TMPRSS2, RdRp, 3CLpro and PLpro as the target proteins. All chemical compounds that evaluated such as asiatic acid, andrographolide, apigenin, brazilein, brazilin, catechin, curcumin, gingerol, hesperidin, hesperetin, kaemferol, luteolin, myricetin, naringenin and quercetin had an affinity to target protein. It reflects that active compounds in medicinal plants can be used as antiviral against COVID-19. Brazilein and brazilin from secang wood (*Caesalpinia sappan* L.) have a superior bond to ACE2 and lower binding energy value than chloroquine, arbidol, remdesivir, ribavirin and lopinavir. Citrus sp containing hesperidin had an excellent affinity to TMPRSS2. Secang wood and citrus sp. could be developed as an anti-SARS-CoV-2 through inhibiting ACE2, TMPRSS2, RdRp and protease (3CLpro and PLpro) that interfered the process of virus infection at the entry, replication and advanced stages, causing worst effect such as pneumonia.

**Keywords:** ACE2; Citrus sp; Protease; RdRp; Secang wood (*Caesalpinia sappan* L.); TMPRSS2.

---

In the last few months, COVID-19 has become a very feared disease caused by a coronavirus. Coronavirus becomes part of a large family of viruses that cause diseases that occur in animals or humans.<sup>1</sup> Humans infected with the virus will show signs of respiratory tract infections ranging from the flu to more serious

ones, such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS).<sup>2</sup> SARS-COV and MERS COV is belong to a beta-coronavirus. In December 2019 a novel beta-coronavirus that infect human was found in Wuhan, China. It was named as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-

CoV-2), while the disease is called Coronavirus Disease-2019 (Covid-19). The SARS-COV-2 not only infected the lower respiratory system induce a severe pneumonia, but also develop other disorders in the digestive system, heart, liver, kidneys, and nervous system, causing multi-organ failure. The elderly and patients with comorbidities such as diabetes, heart, and kidney are very susceptible to COVID-19 disease.<sup>3-5</sup>

Cases of COVID-19 have spread throughout the world, and WHO states COVID-19 is categorized as a pandemic. The spread of this disease also reached Indonesia, not only in imported cases but also in local transmission. It is necessary to develop the discovery of an antiviral drug that can treat COVID-19 effectively. There are two therapeutic strategies for COVID-19, namely the first, therapy to increase the body's resistance of the host or human cells and secondly, is the restriction for the virus itself. Coronavirus is a virus that has glycoprotein spikes arranged like a crown. Coronavirus infects its host with three stages. The first stage is the virus infects its host by attaching the transmembrane spike glycoprotein to host through angiotensin-converting enzyme 2 (ACE2) in the host so that a complex is formed between S-glycoprotein with ACE2 with the help of transmembrane protease, serine 2 (TMPRSS2) produced by host cells.<sup>6,7</sup> The next stage is the replication stage using RNA-dependent RNA polymerase (RdRp). Coronaviruses are RNA viruses that use host cells to replicate. Coronavirus uses RdRp to make new RNA copies. The last stage is the maturation stage of virus replication in the host cell using proteases such as 3CLpro (3C-like protease) and PLpro (Papain-like protease).<sup>8</sup> Some drugs are known to be able to inhibit these three processes, including arbidol as ACE2 inhibitors; camostat mesylate as TMPRSS2 inhibitors; remdesivir and ribavirin as RdRp inhibitors; Lopinavir and ritonavir as protease inhibitors. Chloroquine, Lopinavir, Ritonavir and Remdesivir are an antimicrobials that declared has a potential activity against SARS-CoV<sup>22,9,10</sup>

Indonesia has abundant biodiversity. Many active compounds from Indonesia's biological resources have the potential to be developed as medicines. For this reason, research and evaluation of active compounds will be designed to overcome COVID-19. The Active

compounds in natural substances that are explored as candidates for inhibiting and reducing infections from coronavirus cause those compounds boost the immune system and act as antioxidants such as Asiatic acid, andrographolide, apigenin, brazilin, brazilein, catechin, curcumin, gingerol, hesperidin, hesperetin, kaemferol, luteolin, myricetin, naringenin and quercetin.<sup>11-16</sup> The active compound is traced its ability to inhibit the infection process of COVID-19 in silico with molecular docking. The target protein, which is the target of inhibition, are ACE2, TMPRSS2, RdRp, 3CLpro and PLpro. The purpose of this study is to evaluate the inhibitory ability of the active compound in the target protein that plays a role in the process of virus entry in host cells, virus replication and the process of virus maturation in host cells by binding affinity analyze using in silico assay. Hopefully, this research can contribute to the discovery of a drug for the COVID-19 antiviral and provide an overview for the public to use natural materials for daily consumption as a preventative measure for COVID-19 disease.

## MATERIALS AND METHODS

### Materials

The material used in conducting this research is the sample structure of the target protein namely ACE2 (PDB ID: 1O86), TMPRSS2 (PDB ID: 5CE1), RdRp (PDB ID: 6NUR), 3CLpro (PDB ID: 2GTB) and PLpro (PDB ID: 4OW0) downloaded from <http://www.rcsb.org>. Besides, prepared three-dimensional structures of all active test compounds that were downloaded two-dimensional structure of Asiatic acid, andrographolide, apigenin, brazilein, brazilin, catechin, curcumin, gingerol, hesperidin, hesperetin, kaemferol, luteolin, myricetin, naringenin and quercetin from <https://pubchem.ncbi.nlm.nih.gov/compound/>(the active test compound) and prepared in the HyperChem 8 program. Also drugs that used as inhibitor agent to COVID-19 such as Arbidol, chloroquine, camostat mesylate, remdesivir and lopinavir.

### Optimization of Ligand Structure

Preparation and optimization of the three-dimensional structure of the test compounds were carried out on a two-dimensional structure downloaded from <https://pubchem.ncbi.nlm.nih.gov/compound/>(chosen the name of active

compound). The two-dimensional all active compounds files that downloaded were in the sdf format, which will later be converted to pdb format with the Open Babel Gui application and subsequently demonstrated using the HyperChem 8 application.

#### **Preparation of the Target Protein**

Protein preparation begins by selecting the structure of the target protein ACE2, TMPRSS2, RdRp, 3CLpro and PLpro in the active form that binds to the native ligand. The target protein preparation was carried out using the Chimera 1.11.1 program, beginning with the removal of water molecules (H<sub>2</sub>O) contained in the target protein. The next step was to eliminate native ligands.<sup>17,18</sup> The results of the protein preparation were stored in the form of a pdb file, which will later be used in the method validation and docking steps of the test compound.

#### **Molecular Docking Protocol Validation**

Validation of the molecular docking method was done by docking (redocking) the native ligand on the target protein that was previously removed by the native ligand by using the AutoDockTools 1.5.6 application. Validation of molecular docking method aims to ensure the method used has been validated and obtain a suitable method so that it can be used for the next stage of research. The validation parameter of the molecular docking method was the value of RMSD (Root mean square distances) resulting from redocking native ligands with their proteins.

#### **Docking the Active Test Compound to the Target Protein**

The optimized test compound was then docking to the prepared target protein (eliminated its native ligand) using a validated method. Docking was done using the AutoDockTools 1.5.6 Application program, equipped with the Autodock and Autogrid programs. The docking process of the active compound on the target protein results in the form of bond energy values and intermolecular interactions (in this case the type of bond formed) that stabilizes the interaction between the test compound and the target protein.<sup>17,19</sup>

#### **Data Analysis**

Data analysis was performed using descriptive methods. The results obtained from molecular docking are the bond energy and the type of bond between the compound and the

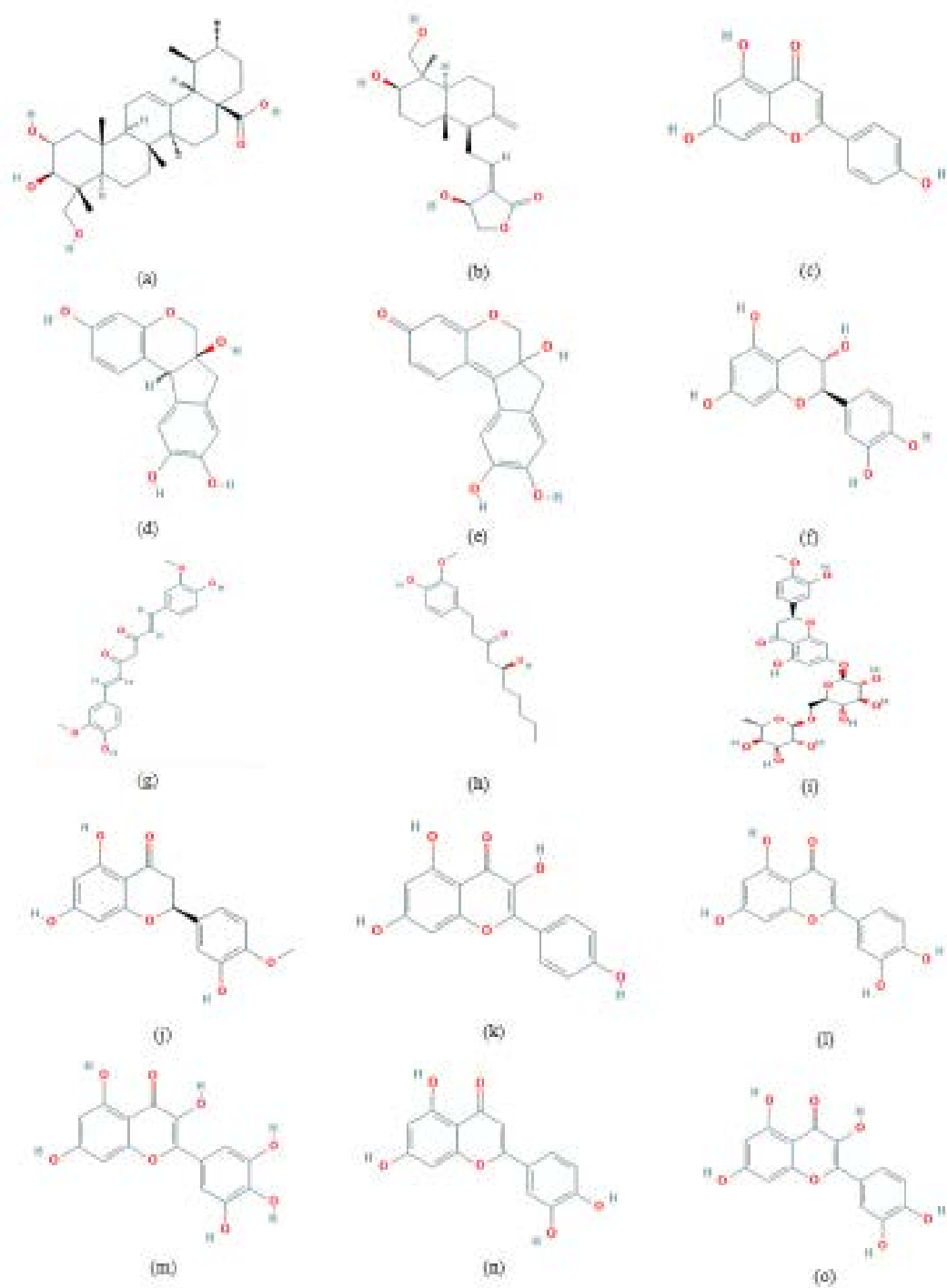
target protein. Bond energy values indicate affinity (bond strength) between compounds and target proteins. The binding energy between the active test compound and the target protein is negative, meaning that the test compound has an affinity for the target protein and potentially to inhibit the target protein, whereas if it is positive, it indicates that the active test compound has no affinity for the target protein. The negative bond energy value indicates a spontaneous reaction and indicates a stable system that allows the formation of bonds, while the positive energy value suggests a system that has very little or even no tendency for the reaction to occur so that the bond was not formed.<sup>20,21</sup>

## **RESULTS AND DISCUSSION**

### **The Active Test Compounds Have Been Optimized and Prepared using Hyperchem 8**

The preparation of the structure of the test compound is done by first changing the two-dimensional structure of the compound (Figure 1) into a three-dimensional structure. Three-dimensional structure optimization of compounds was done by single-point calculations and continued with geometry optimization. Single point calculation is a calculation used to determine the total molecular energy of a structure before the optimization process of the test compound was carried out. Geometry optimization was a process to minimize total energy so that the structure of the most stable test compound was obtained, characterized by a decrease in the overall energy value of the structure of the test compound.<sup>22-23</sup> In geometry optimization results a shift in the structure of compounds into the most stable structure, so that there was a decreasing energy value of the structure of the test compound. Three-dimensional structure of the compound test results of single-point calculations and geometry optimization.

This study used several active compounds of medicinal plants that are known to have high antioxidant content and enhance the immune system. These active compounds may have the potential to inhibit infection from coronavirus. Asiatic acid contained in *Centella asiatica*; andrographolide from *Andrographis paniculata* NEES; apigenin, luteolin, quercetin contained in *Moringa oleifera*; brazilin and brazilin from



**Fig. 1.** Chemical structure of the active test compounds. Asiatic acid (a), andrographolide (b), apigenin (c), brazilin (d), brazilein (e), catechin (f), curcumin (g), gingerol (h), hesperidin (i), hesperetin (j), kaempferol (k), luteolin (l), myricetin (m), naringenin (n) and quercetin (o)

Caesalpinia sappan L.; catechin from Uncaria and Camellia sinensis; curcumin in Curcuma longa Linn.; gingerol from Zingiber officinale; Citrus sp. containing hesperetin, hesperidin and naringenin; Psidium guajava containing kaemferol, quercetin and myricetin.

#### **The ACE2, TMPRSS2, RdRp, 3CLpro and PLpro as Target Protein Were Prepared Using Chimera 1.11.1. Program**

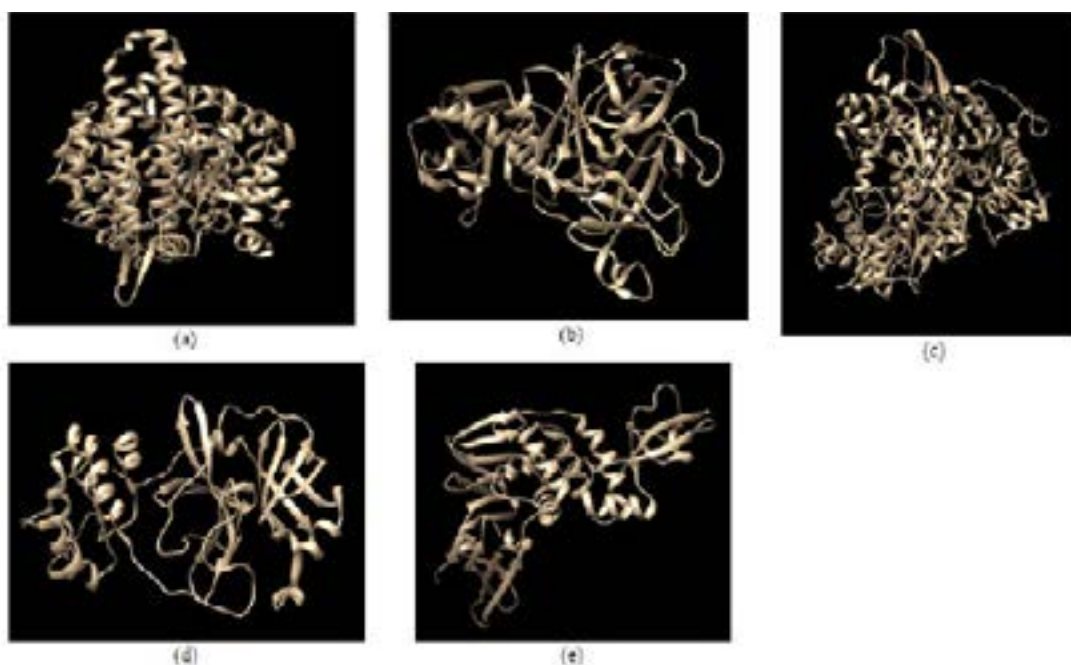
Protein preparation aim to separate the native ligand from the target protein, thereby providing pocket or binding sites that will be used during the docking process. Protein preparation was to choose a chain that binds with the appropriate native ligand. The use of one chain aims to make it easier to determine the coordinates of the space (pocket cavity) of the ligand that will be used for docking. Figure 2 demonstrated the results of protein preparation.

#### **Caesalpinia Sappan Containing Brazilin; Brazilein and Citrus sp. Containing Hesperetin, Hesperidin and Naringenin Exhibit Strong Affinity to the Target Protein that Plays a Role in Coronavirus Infection**

Docking the active test compounds on the five target proteins, namely ACE2, TMPRSS2,

RdRp, 3CLpro and PLpro are carried out after the docking method has been validated with a fulfilling RMSD value of = 3 Å.<sup>24</sup> The docking process was done using the AutoDockTools 1.5.6 application, which equipped with Autodock4 and Autogrid4. Docking the test compound was carried out at the binding site of each target protein using the same grid box coordinate settings during the validation process. Using the same grid box coordinates at the time of validation with the docking process can ensure that the interactions that occur between the test compounds are indeed at the binding site of each target protein. Table 1 presented the docking score of natural compounds breaking up the target protein. The overall data gives negative values for binding energy, except Asiatic acid. Asiatic acid has no affinity to ACE2 and RdRp.

In this *in silico* study, we seen that brazilin in secang (*Caesalpinia sappan* L.) wood exhibited the most negative value of docking score toward ACE2, means the best affinity was formed. In TMPRSS2 protein, the compound that has the most stable bond is hesperidin in Citrus sp, while quercetin shows the best bond with RdRp. The chemical compound in natural materials that have the potential to bind the most strongly with 3CLpro



**Fig. 2.** The target protein structure without native ligand that were made using Chimera 1.11.1 program. ACE2 (a); TMPRSS2 (b); RdRp (c); 3CLpro (d); PLpro (e)

is andrographolide. Besides that curcumin has the strongest bond compared to other chemical compounds in PLpro. Curcumin and quercetin also were reported as protease inhibitor with PDB ID (6LU7),<sup>25</sup> and these two compounds had an antiaging effect.<sup>26-28</sup> Thus this invention will increase the usefulness of active compounds for developing antiviral agents, which are also rich in antioxidants and act as antiaging.

The binding energy obtained between the active compounds and the five target proteins was also compared to the bond energy of the drug that acts as ACE2; TMPRSS2; RdRp; 3CLpro and PLpro inhibitors. If the binding energy value of the chemical compound with the five target proteins was more negative than the inhibitors of the five proteins, the ability or affinity of the active compound to bind to the target protein was more exceptional. This shows that more robust and

more stable interactions that occur between active compounds and target protein.

Among all the data in table 1, it appears that brazilin and brazilein in secang wood bond to all target proteins and have lower binding energy compared to arbidol, chloroquine, camostat mesylate, remdesivir, ribavirin and lopinavir and have the best bond with ACE2. This shows that it can act as an entry inhibitor of SARS-Cov-2 by inhibiting ACE2 and TMPRSS2. Secang wood also inhibit RdRp which plays a role in RNA replication in the host cell. Proteases was slowed down too, so that the coronavirus maturation process was stopped. Another compound that also has the inhibiting ability in three critical processes of coronavirus infecting its host was Citrus sp. Citrus sp contains hesperidin, which has the highest ability on TMPRSS2 so that the process of forming the S-glycoprotein complex with ACE2

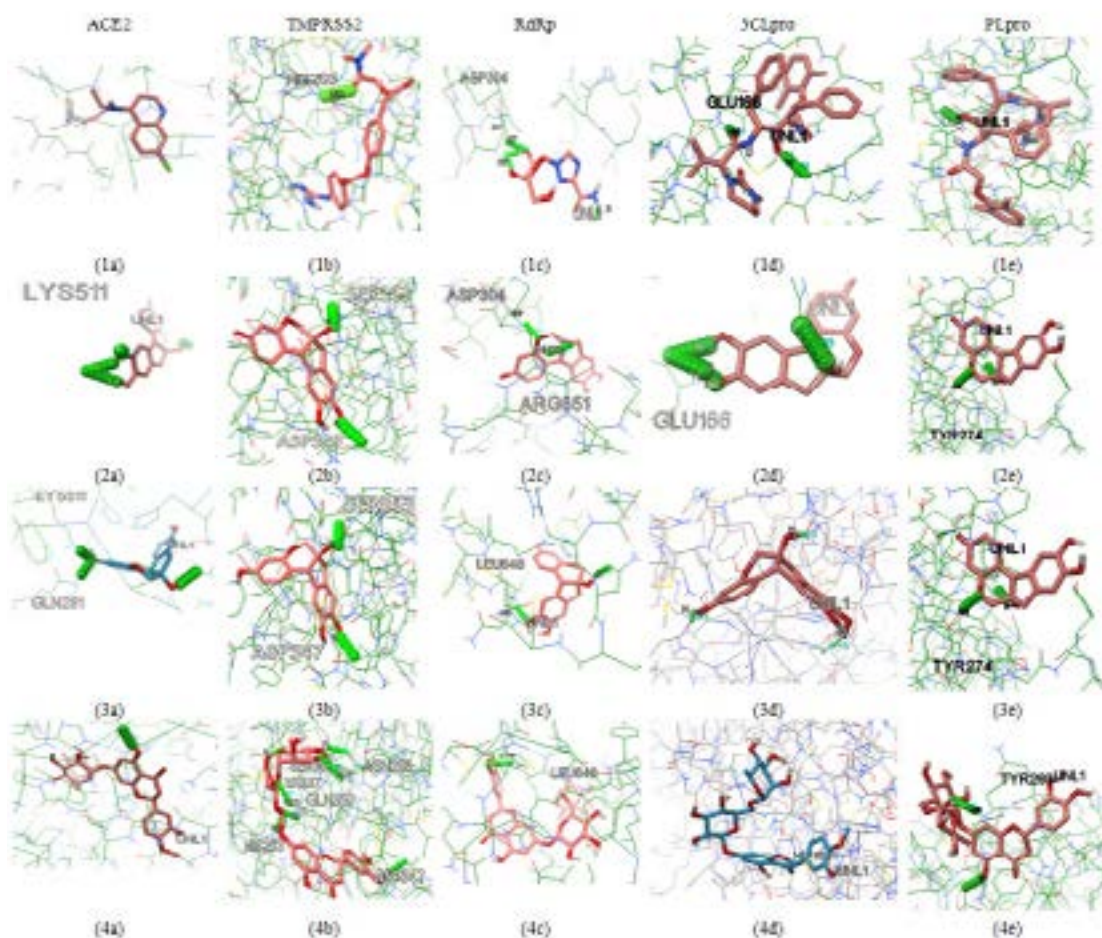
**Table 1.** The binding energy of the chemical compound from natural sources with proteins related to the infectious process of coronavirus in COVID-19.

Medicinal plant source	Ligand	Binding energy(kcal/mol)				
		ACE2 (1O86)	TMPRSS2 (5CE1)	RdRp (6NUR)	3CLpro (2GTB)	PLpro (4OW0)
-	Arbidol	-4.22	-	-	-	-
-	Chloroquine	-7.69	-	-	-	-
-	Camostat mesylate	-	-8.35	-	-	-
-	Remdesivir	-	-	-4.21	-	-
-	Ribavirin	-	-	-3.69	-	-
-	Lopinavir	-	-	-	-9.01	-4.19
<i>Centella asiatica</i>	Asiatic acid	-	-8.76	-	-7.82	-7.97
<i>Andrographis paniculata</i>	Andrographolide	-7.67	-7.92	-3.18	-7.85	-7.79
<i>Moringa oleifera</i>	Apigenin	-8.14	-7.08	-6.14	-7.4	-6.60
	Luteolin	-7.90	-7.01	-5.37	-7.12	-6.90
	Quercetin	-8.05	-6.95	-6.83	-6.83	-6.60
<i>Caesalpinia sappan</i> L	Brazilin	-8.32	-7.25	-4.31	-7.05	-7.18
	Brazilein	-7.73	-7.86	-3.81	-7.73	-7.87
<i>Uncaria; Camellia sinensis</i>	Catechin	-7.91	-7.50	-3.5	-6.80	-7.00
<i>Curcuma longa</i> Linn	Curcumin	-7.99	-7.19	-5.3	-7.24	-8.45
<i>Zingiber officinale</i>	Gingerol	-7.45	-5.57	-5.09	-6.32	-7.09
<i>Citrus sp</i>	Hesperetin	-7.94	-7.00	-5.39	-7.49	-6.99
	Hesperidin	-7.13	-9.74	-5.39	-6.79	-6.47
	Naringenin	-8.31	-6.98	-5.66	-7.59	-6.83
<i>Psidium guajava</i>	Kaemferol	-6.66	-6.94	-4.63	-6.86	-6.33
	Myricetin	-7.08	-6.25	-3.38	-6.65	-6.57
	Quercetin	-8.05	-6.95	-6.83	-6.83	-6.60

in the host cell was be bothered. Other research also states that Citrus sp can be developed for the discovery of anti-SARS-CoV-2 drugs because it is able to inhibit S-glycoprotein and proteases.<sup>29</sup> The interaction of hydrogen bonds between the target protein with the drug and brazilein, brazilein and hesperidin was shown in figure 3. Further research must be conducted to evaluate more deeply about the potential activities of secang wood and Citrus sp to eradicate SARS-CoV-2. People can use these medicinal plants for prevention and combination therapy with drugs against coronavirus.

## CONCLUSION

Brazilein and brazilin from secang (*Caesalpinia sappan* L.) wood can be developed as an angiotensin-converting enzyme 2 (ACE2) and protease inhibitor. This also occurs in the content of compounds in Citrus sp, especially hesperidin. Secang wood and Citrus sp could be used as a prevention agent and supportive therapy against SARS-CoV-2.



**Fig. 3.** Chemical interaction between active compounds in secang (*Caesalpinia sappan* L.) wood and *citrus* sp. with the target protein. Chloroquine with ACE2 (1a), camostat mesylate with TMPRSS2 (1b), Ribavirin with RdRp (1c), Lopinavir with 3CLpro (1d), Lopinavir with PLpro (1e); Brazilein with ACE2 (2a), TMPRSS2 (2b), RdRp (2c), 3CLpro (2d), PLpro (2e); Brazilin with ACE2 (3a), TMPRSS2 (3b), RdRp (3c), 3CLpro (3d), PLpro (3e); Hesperidin with ACE2 (4a), TMPRSS2 (4b), RdRp (4c), 3CLpro (4d), PLpro (4e)

### ACKNOWLEDGEMENT

This research was supported by Udayana University PNB Fund in 2020

### REFERENCES

- Fehr A and Perlman S. 2015. Coronaviruses: An overview of their replication and pathogenesis. *Methods Mol Biol.* **1282**: 1-23.
- Chhikara B.S, Rathi B, Singh J and Poonam JS. Coronavirus SARS-CoV-2 disease COVID-19: Infection, prevention and clinical advances of the prospective chemical drug therapeutics. *Chem. Biol. Lett.* **7**(1): 63-72 (2020).
- Liu C, Zhou Q, Li Y, Garner L.V, Watkins S.P, Carter L.J, Smoot J, Gregg A.C, Daniels A.D, Jervey S and Albaiu D. Research and development on therapeutic agents and vaccines for COVID-19 and related human coronavirus diseases. *ACS Cent. Sci.* <https://dx.doi.org/10.1021/acscentsci.0c00272>.
- Elfiky A and Ibrahim N.S. 2020. Anti-SARS and anti-HVC drugs repurposing against the Papain-like protease of the newly emerged coronavirus (2019-nCoV). *ResearchSquare*. DOI:10.21203/rs.2.23280/v1.
- Santos Y.M.B, Barraza S.J, Wilson M.W, Agius M.P, Mielech A.M, Davis N.M, Baker S.C, Larsen S.D and Mesecar A.D. X-ray structural and biological evaluation of series of potent and highly selective inhibitors of human coronavirus papain-like proteases. *J. Med. Chem.* **57**: 2393-2412 (2014).
- Shahid S.M.A, Kausar M.A, Khalid M.A, Tewari S, Alghassab T.A, Acar T, Ahmed M.Q and Alenazi F.S.H. Analysis of binding properties of angiotensin-converting enzyme 2 through in silico molecular docking. *J.Exp. Zool. India.* **21** (1): 559-563 (2018).
- Hoffman M, Weber H.K, Schroeder S, Muller M.A, Drosten C and Pohlmann S. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell.* **181**: 1-10 (2020).
- Kong R, Yang G, Xue R, Liu M, Wang F, Hu J, Guo X and Chang S. COVID-19 docking server: An interactive server for docking small molecules, peptides and antibodies against potential targets of COVID-19. Q-bio. *BM. arXiv:2003.00163* (2020).
- Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, Shi Z, Hu Z, Zhong W and Xiao G. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res.* **30**: 269-271 (2020).
- Wu C, Liu Y, Yang Y, Zhang P, Zhong W, Wang Y, Wang Q, Xu Y, Li M, Li X, Zheng M, Chen L and Li H. Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. *Acta Pharm. Sin. B.* **20**: 1-44 (2020).
- Brinkhaus B, Lindner M, Schuppan D and Hahn E.G. Chemical, pharmacological and clinical profile of the east Asian medical plant *Centella asiatica*. *Phytomedicine.* **7**(5): 427-448 (2000).
- Pestariati, Edy H and Dwi K. Bioactive wood potential (*Caesalpinia sappan* L.) in the proliferation of wistar in infection *Salmonella Typhimurium*. *Indian J Med Forensic Med Toxicol.* **13**(4): 1579-1581 (2019).
- Puig E.R and Castell M. Cocoa: antioxidant and immunomodulator. *Br. J. Nutr.* **101**: 931-940 (2008).
- Kathal R and Rawat P. Immunity booster herbs and their conservation-a review. *International Conferences on Public Health: Issues, challenges, opportunities, prevention, awareness.* *Public Health.* 135-140 (2016).
- Senchina D.S, Hallam J.E, Kohut M.L, Nguyen N.A, Ann M and Perera N. Alkaloids and athlete immune function: Caffeine, theophylline, gingerol, ephedrine and their congeners. *Exerc Immunol Rev.* **20**: 68-93 (2014)
- Rosyid A, Timur W.W and Vania P.A.M. Comparative long hospitalization treatment to the children using supportive medicine containing extract of guajava leaves (*Psidium guajava* Linn.) and immunomodulatory medicine. *Borneo J Pharm.* **1**(2): 1-8 (2017).
- Ferreira L.G, Santos R.N.D, Oliva G and Andricapulo A.D. Molecular docking and structure-based drug design strategies. *Molecules.* **20**: 13384-13421 (2015).
- Prieto M.F.D, Arciniega M and Medina F.J.L. Molecular docking: current advances and challenges. *TIP Revista Especializada en Ciencias Quimico Biologicas.* **21**:65-87 (2018).
- Kitchen D.B, Decornez H, Furr J.R, Bajorath J. Docking and scoring in virtual screening for drug discovery: methods and applications. *Nat. Rev. Drug Discov.* **3**:935-949 (2004).
- Mukesh and Rakesh. Molecular docking: a review. *Int J Res Ayurveda Pharm.* **2**:1746-1751 (2011).
- Murray, R.K., Bender, D.A., Botham, K.M., Kennelly, P.J., Rodwell, V.W., and Weil, P.A. 2012, Herper biochemistry. Edisi 29. EGC, Jakarta.
- Laksmiani N.P.L, Reynaldi K.R, Widiastari M.I, Nugraha I.P.W, Suyadnya I.M.K and Maharani



- R.A.I.K. Cytotoxic Activity of Andrographolide in Colon Cancer Through Inhibition Cox-2 by In Silico Study. *J. Phys. Conf. Ser.* **1040**:1-6 (2018).
23. Korb O, Stützle T and Exner T.E., Plants: Application of ant colony optimization to structure-based drug design. *Lect. Notes Comput. Sci.* 247-258 (2006).
24. Jain A.J and Nicholls A. Recommendations for evaluational methods. *J. Comput. Aided Mol. Des.* **22**: 133-139 (2008).
25. Khaerunnisa S, Kurniawan H, Awaluddin R, Suhartati S, Soetjipto S. 2020. Potential inhibitor of COVID-19 main protease (M<sup>pro</sup>) from several medicinal plant compounds by molecular docking study. Preprints. DOI:10.20944/preprints202003.0226.v1.
26. Lima C.F, Wilson C.P and Rattan S.I.S. Curcumin induces heme oxygenase-1 in normal human skin fibroblasts through redox signaling: Relevance for anti-aging intervention. *Mol Nutr Food Res.* **55** (3): 430-442 (2010).
27. Jadoon S, Karim S, Asad M.H.H.B, Akram M.R, Khan A.K, Malik A, Chen C and Murtaza G. Anti-aging potential of phytoextract loaded-pharmaceutical creams for human skin cell longetivity. *Oxidative med. cell. longev* (2015). <http://dx.doi.org/10.1155/2015/709628>.
28. Jantan I, Ahmad W and Bukhari S.N.A. Plant-derived immunomodulators: an insight on their preclinical evaluation and clinical trials. *Front. Plant Sci* (2015). DOI: 10.3389/fpls.2015.00655.
29. Utomo R.Y, Ikawati M, Meiyanto E. Revealing the potency of citrus and galangal constituents to halt SARS-CoV-2 infection. Preprints (2020). DOI: 10.20944/preprints202003.0214.v1.