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Molecular Docking Studies: Activity Of Natural Compounds As SAR-COV-2 Inhibitors In Papain-Like Protease (PL Pro)

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Abstract— SARS-CoV-2 is a respiratory virus often referred to as COVID-19. The challenge of finding SARS-CoV-2 antivirals also attracted many researchers, one of which was by utilizing natural compounds. The purpose of this study was to analyze the potential activity of herbal plants in Indonesia as anti-COVID-19, specifically tasked with inhibiting the target protein, PLpro using the in-silico method. This research is an insilico approach with molecular docking studies. Docking simulation in this study was used to analyze the activity of natural herbal plant compounds against the target protein, namely PLpro. In addition, the docking method is also able to examine the interaction of compounds contained in plants with target receptors seen from a computational-based visual approach. So, the results obtained in this study are that when viewed from the value of the gibbs energy produced, the compound that has the best activity to inhibit the PLpro target protein (SARS CoV-2) is Luteolin. The Gibbs energy generated in the Luteolin docking simulation is -7.3 kcal/mol. The Gibbs energy value produced by the Luteolin ligand is the most negative or the smallest value of the 14 tested ligands. This means that the bond formed between the Papain-like protease (PLpro) protein and the Luteolin ligand is the most stable among the others. In addition to the Luteonin compound, there are also several compounds that have quite good potential when viewed from the amount of gibbs energy owned, namely Mangostin, quercetin compounds and Myricetin compounds with gibbs energy values of -7.1 kcal / mol, -7.0 kcal / mol and -7.0 kcal / mol. Based on these results, it can be concluded that several compounds from typical Indonesian plants such as Luteolin compounds (Compounds found in Celery plants (Apium graveolens)), Mangostin compounds (Compounds found in Mangosteen plants (Garcinia mangostana)), Quercetin compounds (Compounds found in Citrus plants (Citrus aurantium)) and Myricetin compounds (Compounds found in Clove plants (Syzygium aromaticum)) have the potential to be developed as anti-COVID-19.

Keywords— Anti-Virus; Docking; Natural Compound Ingredients

I. INTRODUCTION

The Corona virus was first discovered in the 1930s [1]. This virus then caused the first worldwide outbreak of severe acute respiratory syndrome (SARS) in 2002-2003. Corona virus has a spherical or pleomorphic shape and is the largest RNA genome among other RNA viruses [2-3]. The causative organism of Corona Virus Disease 2019 (COVID-19) is Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2). SARS-CoV-2, like the Corona virus that existed before, is a virus that belongs to the Coronaviridae family. This RNA virus can infect poultry, mammals and humans, then cause acute to chronic diseases [4]. The Coronaviridae family consists of 4 genera, namely: alphacoronavirus, betacoronavirus, gammacoronavirus and deltacoronavirus. SARS-CoV-2 like SARS-CoV belongs to the betacoronavirus genus. SARS-CoV-2 is a betacoronavirus that has a unique phylogenetic position which was later included in the subgenus Sarbecovirus [5].

Most recently SARS-CoV-2 was first discovered in late December 2019 in Wuhan, China. The symptoms shown by the patient are cough, fever, dyspnea (difficulty breathing) accompanied by acute respiratory distress syndrome (ARDS) with an

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unknown cause of infection. ARDS is the leading cause of death for patients infected with SARS-CoV-2 [6]. ARDS is basically an immunopathological response that is common in cases of SARS-CoV-2, SARS-CoV and MERSCoV infection [7].

From the results of research conducted by scientists, it is agreed that SARS-CoV-2 is the same as SARS-CoV, which is an interaction with angiotensin converting enzyme 2 (ACE 2) receptors when entering the body of its host cell. The process of binding the virus to the body of the host cell will determine the pathogenesis of the infection [8]. Viral binding to the ACE2 receptor occurs via the spike protein (S). The entry of viruses into cells causes membrane fusion [9]. There are two protein groups in SARS-CoV-2, namely structural proteins and non-structural proteins (NSP). So to find SAR-CoV-2 antivirals, structural and nonstructural proteins can be used as a target for SAR-CoV-2 antiviral candidates.

There are several types of non-structural protein 3 (NSP 3)) that have a role in the replication of the SAR-CoV-2 virus, namely: NSP3b, NSP3c, PLpro (papain likecysteine protease), NSP3e [10]. Among the types of non-structural protein 3 proteases that are most important in the process of viral division is PLpro (papain like cysteine protease). So this protein is widely used as a potential drug target to treat SAR-COV-2 [11-13]. PLpro has an important role in the cleavage of the N-terminal polyprotein (pp) to release NSP1, NSP2 and NSP3 which are needed in the viral replication process [14]. Papain-like protease is an enzyme with a structure resembling papaya carica that has cysteine, histidine and asparagine with an effective ability to inhibit the covid-19 virus in viral polyproteins and suppress the host's immunity [15].

Several treatment therapies based on research conducted found that Favipiravir and Remdesivir markedly inhibited SARS-CoV-2 infection in normal Vero E6 cells [16]. In addition, reports were also found stating that several antiviral drugs such as Hydroxychloroquine [17], Lopinavir-Ritonavir and Ribavirin [18], Remdesivir [19], and Tocilizumab have been tested on SARS-CoV-2 patients.

The next challenge is to find antivirals derived from natural ingredients for the treatment of SAR-COV-2. This has the potential to be done considering that Indonesia is one of the countries that has abundant biological wealth. There are 30,000 plant species in Indonesia's tropical forests. Of the 30,000 plant species, only about 9,600 species have known uses. And the usefulness of these medicinal plants has not been utilized optimally [20]. China is a country that has utilized herbal medicine prescriptions to treat COVID-19 and has submitted applications for the use of herbal medicines to treat COVID-19 patients [21]. It's just that there haven't been many studies reporting the effectiveness of using herbal medicines as anti-SARS-CoV-2. So this study will try to explore insilico related to the activity of herbal plants in Indonesia as an anti-COVID-19 alternative.

II. EXPERIMENT

This research uses an in-silico predictive approach, commonly known as Molecular Docking studies. This research is included in experimental research. The use of the molecular docking method of ligand bonding is also used for the interaction of proposed compounds against target proteins. The equipment used in this study consists of software and hardware. The software consists of: Operating System Windows 10, Autodock vina version 1.1.1, LigPlot version 4.5.3, Avogadro version 1.2, AutoDock Tools version 1.5.7, Gimp version 2.10.24, Open Babel version 3.1.1 and PyMOL version 2.5.1. The hardware used includes an AMD Ryzen 5 4500U processor, 8.00 GB RAM, 500 GB hard drive, Lenovo motherboard.

The materials used in this study are materials in the form of data that can be downloaded from the website page. The PLpro target protein receptor data used was downloaded from the Protein Data Bank website [22]. For materials in the form of data on compounds from Indonesian plants that have antiviral properties, taken from the results of previous studies, which have the potential to inhibit target proteins. The data on Indonesian plant compounds used were downloaded from the PubChem website which contains the three-dimensional structure of active compounds of natural ingredients.

The stages carried out in this study began with protein preparation by downloading protein data from the protein data bank (www.pdb.org), while the target protein used was PLpro. The target protein data obtained from the website was first prepared and adjusted to the most optimal conditions. Target protein preparation was carried out using the Pymol software. Target protein preparation is carried out by first removing the Oxygen compound (O2) present in the target protein data. This is also done on the plant compounds used (Ligan) so that ligand data will be obtained that is as desired. Ligands that have been downloaded from the pubchem web are converted from 2D to 3D structures using Pymol software, then stored in *.pdb form. After that, the optimization process is carried out by adding hydrogen ions using AutoDock Tools 1.5.7. software then the file is saved in *.pdbqt format. Furthermore, the target protein data and ligands are placed in a box containing water so that the simulation

conditions given are the same as the real conditions. The size of the box is adjusted to the size of the target protein or commonly known as blind docking.

After completing the preparation stage, the next step is the docking simulation stage. The software used to run docking simulations is Autodock Vina. Target protein file data and ligand data after preparation will be used as input at this stage. In addition to these two files, a config file is needed which contains the size of the box that includes the target protein and ligand. Ukuran kotak disesuaikan dengan besar protein target. Furthermore, docking simulations can be run. The length of the docking simulation process depends on the size of the box in the config file. The next stage is to do running docking and then wait until all processes are complete. After the running process is complete, data will be obtained in the form of docking mode generated between the target protein and ligand as well as the affinity value (Energy Value). Based on these data, it can be interpreted whether the proposed compounds have bioactivity that can be used as anti-COVID-19. This can be done by analyzing running data using the Pymol application. The interaction between the target protein and the proposed compound can be seen and confirmed. The more negative or low-energy bonds formed, the stronger the bond between the ligand and the target protein. Natural compounds with more negative bond energy are selected as compounds with anti-COVID-19 potential and then interaction visualization will be carried out [23].

III. Results And Discussion

This study used an insilico test approach, namely molecular docking studies. The software used in this study is AutoDock Vina. AutoDock Vina software is an open-source application for simulating molecular docking. The application was originally designed and implemented by Dr. Oleg Trott at the Molecular Graphics Lab (now CCSB) at The Scripps Research Institute [24].

The target protein used in this study is the PLpro protein. The PLpro protein was previously downloaded from the Protein Data Bank (PDB) on the www.rcsb.org page. The determination of the target protein used in this simulation is due to several reasons, namely the PLpro target protein has a role in cutting N-terminal on polyprotein replication to secrete Nsp1, Nsp2, and Nsp 3, which play an important role in correcting SARS-CoV-2 replication [25].

The PBD code of the downloaded PLpro protein is 6WX4. This data is in the form of experimental data from X-Ray Diffraction which contains coordinate data on the three-dimensional structure of the PLpro protein with Chain: A and Resolution: 1.66 Å. The selection of the PDB code must also comply with the rules in receptor determination, namely resolution ≤ 3 and on the Ramachandran plot > 90% of the amino acid residues are in the most favored regions. Figure 1 shows the 3D structure of the PLpro protein.

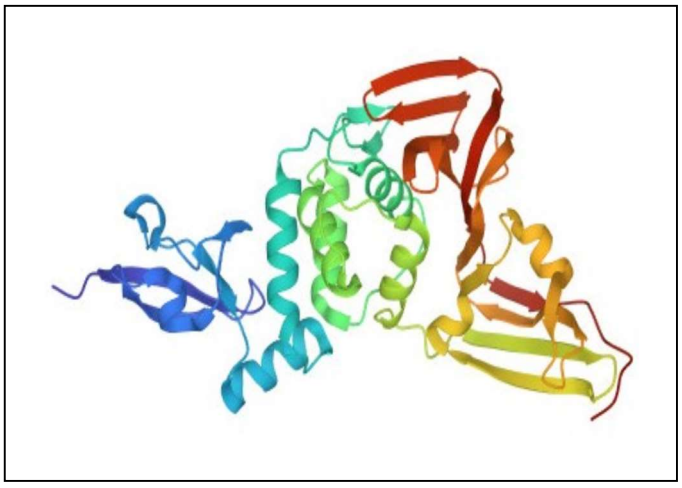

Fig. 1.PLpro 3D Structure

The downloaded target protein is then prepared so that the target protein obtained is the target protein according to the desired conditions. Usually the target protein downloaded from the website is still bound to water and also its natural ligands. Both of these are not needed when docking simulations will be carried out, so before the simulation is carried out, it must be

removed first. If water and natural ligands are not removed first, it will disrupt the interaction of the target protein with the proposed compound ligand. Furthermore, the addition of hydrogen atoms is given to the target protein with the aim of bringing up H atoms in the protein structure.

In this study, compounds from Indonesian plants were used that have antiviral properties, taken from previous research reports, which have the potential to inhibit target proteins. Senyawa ligan yang digunakan dalam penelitian di unduh dari Database PubChem [26]. The ligands used are ligands that have been reported to have activity against SARS-CoV-2. The ligands to be used are Emodin (Compounds found in Ketepeng rhinoceros plants (*Cassia alata*)), Luteolin (Compounds found in Celery plants (*Apium graveolens*)), Curcumin (Compounds found in Turmeric plants (*Curcuma sp.)),* Kaemferol (Compounds found in Guava plants (*Psidium guajava*)), Quercetin (Compounds found in Citrus plants (*Citrus aurantium*)), Myricetin (Compounds found in Clove plants (*Syzygium aromaticum*)), Scutellarein (Compounds found in Sweet Broom plants (Scoparia dulcis)), 10-Gingerol (Compounds found in Ginger plants (*Zingiber officinalis*)), Shogaol (Compounds found in Ginger plants (*Zingiber officinalis*)), Mangostin (Compounds found in Mangosteen plants (*Garcinia mangostana*)), Piceatannol (Compounds found in Grape plants (*Vitis vinifera*)), Diallyl disulfide (Compounds found in Garlic plants (*Allium cepa*)), Cyperotundone (Compounds found in Puzzle grass plants (*Cyperus rotundus*)) and Eugenol (Compounds found in laurel plants (*Syzygium polyanthum*)) [27]. These fourteen ligands have passed testing with Lipinski's Rule of Five. The requirements for Lipinski's Rule of Five are that the molecular weight of the ligand is less than 500 g / mol, the ligand has a hydrogen atom donor less than 5, the ligand has a hydrogen atom acceptor less than 10, and the MLOGP value is less than 4.15 [28].

The next stage is the docking simulation using the Autodock Vina application. The results of this docking simulation are in the form of the resulting docking mode between the target protein and the ligand and the affinity value (free energy value) obtained when the bond forms between the target protein and the ligand. The docking results for the PLpro protein target using test compounds selected based on Gibbs energy generated in the docking simulation can be seen in table 1.

TABLE I. GIBBS FREE ENERGY (ΔG BINDING) TARGET PROTEIN PAPAIN-LIKE PROTEASE (PL PRO) AND LIGANDS

No.	**Ligands**	**PubChem ID**	**Gibbs free energy (ΔG*binding)***
1	Emodin	3220	-6.8 kcal/mol
2	Luteolin	5280445	-7.3 kcal/mol
3	Curcumin	969516	-5.9 kcal/mol
4	Kaemferol	5280863	-6.8 kcal/mol
5	Quercetin	5280343	-7.0 kcal/mol
6	Myricetin	5281672	-7.0 kcal/mol
7	Scutellarein	5281697	-6.7 kcal/mol
8	10-Gingerol	168115	-6.0 kcal/mol
9	Shogaol	5281794	-5.3 kcal/mol
10	Mangostin	5281650	-7.1 kcal/mol
11	Piceatannol	667639	-6.6 kcal/mol
12	Diallyl disulfide	16590	-3.1 kcal/mol
13	Cyperotundone	12308615	-6.2 kcal/mol
14	Eugenol	3314	-5.1 kcal/mol

From table 1 it can be observed that the gibbs energy values obtained to fourteen test compounds (ligands) allow it to be used as inhibitors of PLpro target proteins. Hal ini dikarenakan nilai energi gibbs yang dihasilkan semuanya bernilai negatif. This means that the bond formed between the target protein and the test compound is quite stable. If observed, the most negative or smallest Gibbs energy value is obtained by the Luteolin ligand with a Gibbs energy value of -7.3 kcal/mol. This means that the bond formed between the Papain-like protease (PLpro) receptor and the Luteolin ligand is the most stable among the others. Besides the Luteolin ligand, there are also Mangostin, Quercetin and Myricetin ligands with Gibbs energy values of -7.1 kcal/mol and -7.0 kcal/mol, respectively. So that when viewed from the Gibbs energy value produced, compounds that have activity to inhibit the PLpro target protein (SARS CoV-2) are Luteolin compounds (Compounds found in Celery plants (*Apium graveolens*)), Mangostin compounds (Compounds found in Mangosteen plants (*Garcinia mangostana*)), Quercetin compounds (Compounds found in Citrus plants (*Citrus aurantium*)) and Myricetin compounds (Compounds found in Clove plants (*Syzygium aromaticum*)). In addition to gibbs free energy, docking analysis also shows the pose of ligands and proteins formed. The pose of the Luteolin ligand and the PLpro target protein formed is shown in figure 2.

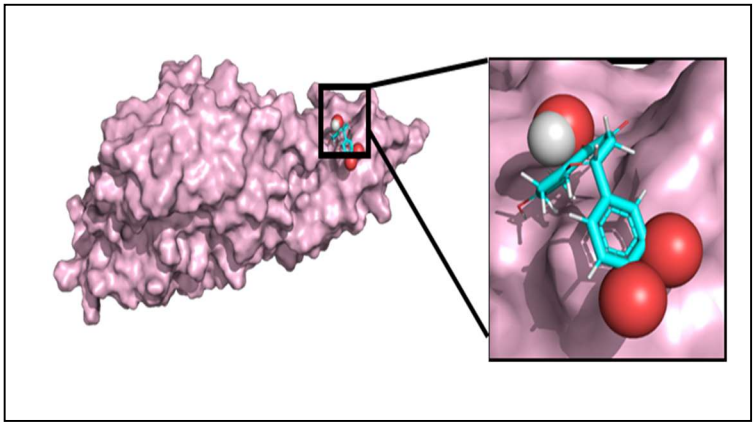

Fig. 2. Papain-like protease Receptor (PLpro) and Luteolin Ligand Pose

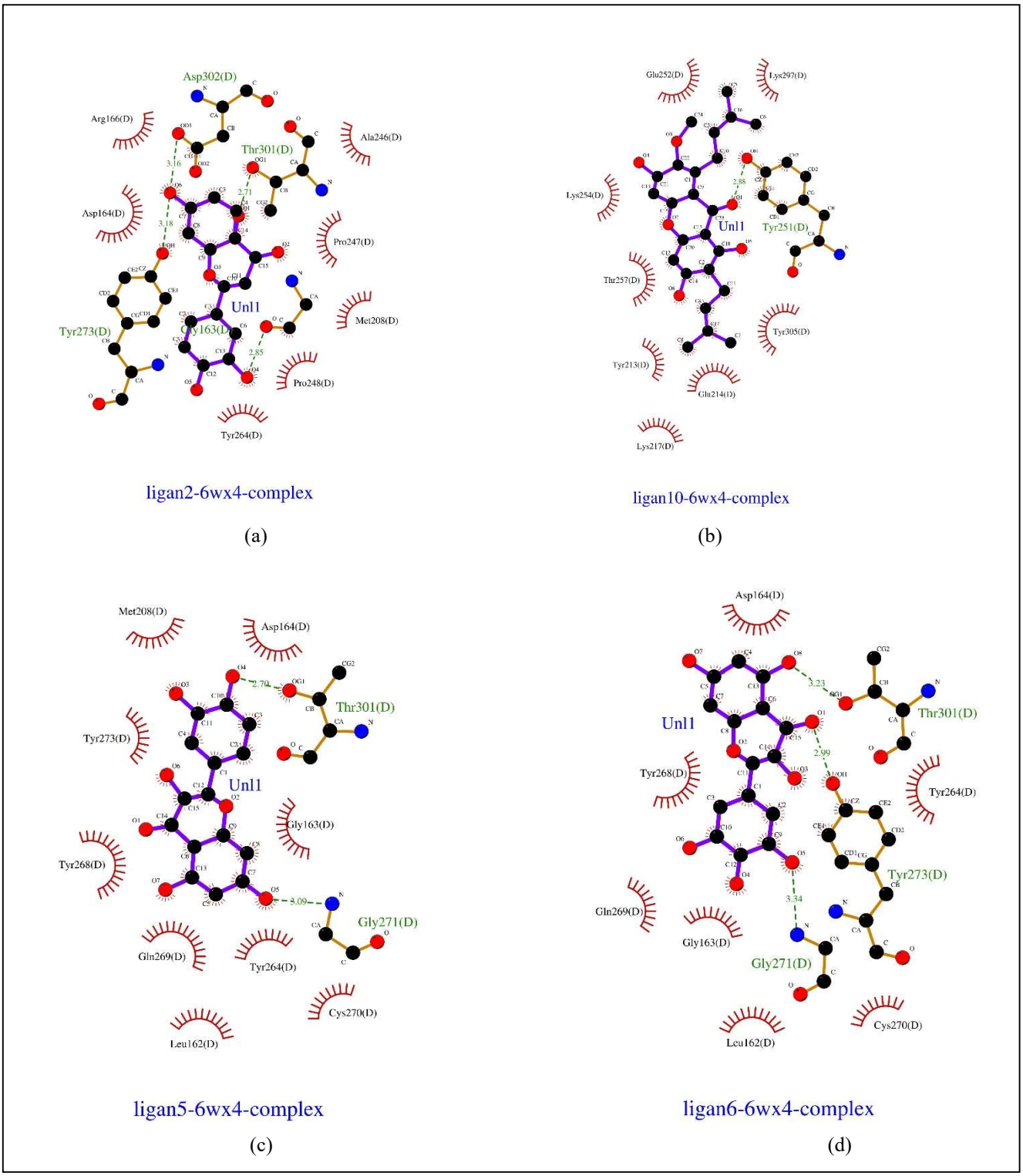

Fig. 3.2D visualization test compounds and PLpro target proteins (a) Luteolin (b) Mangostin (c) Quercetin and (d) Myricetin

Based on figure 3, in the visualization of luteolin compounds and PLpro target proteins there are 4 hydrogen bonds, namely with Asp302 (3.16), Tyr273 (3.18), Thr301 (2.71) and Gly163 (2.85), there are hydrophobic contacts observed in Ala246, Pro247, Met208, Pro248, Tyr264, Asp164, and Arg116 (7 amino acids). For the interaction between Mangostin compounds and

PLpro target proteins, there is only 1 hydrogen bond namely Tyr251 (2.88) in addition to hydrophobic contact observed in Lys297, Tyr305, Glu214, Lys217, Tyr213, Thr257, Lys254 and Glu252 (8 amino acids). For the interaction between Quercetin compounds and PLpro target proteins, there are 2 hydrogen bonds, namely Thr301 (2.70) and Gly271 (3.09) in addition to hydrophobic contacts observed in Asp164, Gly163, Cys270, Tyr264, Leu162, Gln269, Tyr268, Tyr273, and Met208 (9 amino acids). As for the interaction between Myricetin compounds and PLpro target proteins, there are 3 hydrogen bonds, namely Tyr273 (2.99), Gly271 (3.34) and Thr301 (3.23) besides that there are also hydrophobic contacts observed in Tyr264, Cys270, Leu162, Gly163, Gln269, Tyr268, and Asp164 (7 amino acids).

IV. CONCLUSION

Based on the research that has been done, it can be concluded that some compounds from typical Indonesian plants when viewed from the gibbs energy value produced, the compounds that have activity to inhibit the PLpro target protein (SARS CoV-2) are Luteolin compounds (Compounds found in Celery plants (*Apium graveolens*)), Mangostin compounds (Compounds found in Mangosteen plants (*Garcinia mangostana*)), quercetin compounds (compounds found in citrus plants (*Citrus aurantium*)) and Myricetin compounds (compounds found in clove plants (*Syzygium aromaticum*)). The amount of Gibbs energy produced in the Luteolin docking simulation is -7.3 kcal / mol. While Mangostin ligand, quercetin ligand and Myricetin ligand have gibbs energy values of -7.1 kcal/mol, -7.0 kcal/mol and -7.0 kcal/mol, respectively. The gibbs energy value produced by the Luteolin ligand is the most negative or the smallest of the 14 ligands tested. This means that the bond formed between the Papain-like protease (PLpro) receptor and the Luteolin ligand is the most stable among the others.

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