Indonesian Journal of Medical Chemistry and Bioinformatics

Volume 1
Number 2 *Biomarker Metabolite Discovery for Pancreatic Cancer using Ma-chine Learning*

Article 2

2-3-2023

Virtual Screening on Indonesian Herbal Compounds as SARS-CoV-2 Spike (S2) Glycoprotein Inhibitors: Pharmacophore Modelling & Molecular Docking Approaches

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Recommended Citation

Sugito, Syailendra Karuna; Cristina, Artha Uli; Harimurti, Putri Saskia; Cendani, Gabriella Regita; Insani, Fauzi Azhar; Erlina, Linda; Paramita, Rafika Indah; and Fadilah, Fadilah (2023) "Virtual Screening on Indonesian Herbal Compounds as SARS-CoV-2 Spike (S2) Glycoprotein Inhibitors: Pharmacophore Modelling & Molecular Docking Approaches," *Indonesian Journal of Medical Chemistry and Bioinformatics*: Vol. 1: No. 2, Article 2.
DOI: 10.7454/ijmcb.v1i2.1006
Available at: https://scholarhub.ui.ac.id/ijmcb/vol1/iss2/2

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Acknowledgements

The authors would like to thank the bioinformatics elective module team, Department of Medical Chemistry, Faculty of Medicine, Universitas Indonesia for the guidance and support for this study.

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INDONESIAN JOURNAL OF MEDICAL CHEMISTRY AND BIOINFORMATICS
Department of Medical Chemistry, Faculty of Medicine, Universitas Indonesia

Article

Virtual Screening on Indonesian Herbal Compounds as SARS-CoV-2 Spike (S2) Glycoprotein Inhibitors: Pharmacophore Modelling & Molecular Docking Approaches

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Abstract: There are still no specific treatments for coronavirus disease (COVID-19) until present days. Several studies have been conducted to determine whether herbal medicine can be an option to be used as a definitive medicine for COVID-19. S2 subunit of spike protein which is responsible for SARS-CoV-2 entry to the host cell, is a potential drug target to inhibit the viral infection. In this study, we aimed to find some compounds from the HerbalDB database that have potential as SARS-CoV-2 spike (S2) glycoprotein inhibitor. The 6LXT protein was used as the target protein. The procedure in this study consisted of these following steps: protein and ligand preparation, pharmacophore modelling and compound screening, molecular docking, ADME, and toxicity analysis. The docking of hit compounds to the target protein were compared to arbidol and quercetin as positive controls. Four hit compounds were screened from HerbalDB compounds. Two of them, octopamine and L-noradrenaline, showed lower binding energies (respectively, -5.19 and -4.98 kcal/mol) than positive controls whereas the other two compounds, mimosine and L-theanine, showed higher binding energies (respectively, -3.99 and -3.62 kcal/mol) compared to positive controls. Mimosine, L-noradrenaline, octopamine, and L-theanine had toxicity classes of IV, II, IV, and IV, respectively. Octopamine shows the best potential as SARS-CoV-2 spike (S2) glycoprotein inhibitor. However, this compound also poses several toxicity risks and therefore, needs a more elaborate consideration upon using.

Keywords: SARS-CoV-2 spike (S2) glycoprotein; pharmacophore modelling; molecular docking; octopamine; L-noradrenaline; mimosine; L-theanine.

Citation: Sugito, S.K.; Cristina, A.U.; Harimurti, P.S.; Cendani, G.R.; Insani, F.A.; Erlina, L.; Paramita, R.I.; Fadilah, F. Virtual screening on indonesian herbal compounds as SARS-CoV-2 spike (S2) inhibitors: pharmacophore modelling & molecular docking approaches. *Ind. J. Med. Chem. Bio. IJMCB.* **2023**, *1*, 2.

Received: Wed Feb 2, 2022
Accepted: Fri Feb 3, 2023
Published: Fri Feb 3, 2023

1. Introduction

To date, there are still no specific treatments for coronavirus disease (COVID-19). Several studies have been conducted to determine whether herbal medicine can be an option to be used as a definitive medicine for COVID-19. Some of the drugs that have been researched as potential drugs for COVID-19 are herbal medicines that are used to treat flu and cold symptoms [1]. Usually, these herbal medicines contain flavonoids (quercetin, kaempferol, eriodictyol, hesperetin, naringenin, and luteolin), alkaloids (pyridine, pyrrolidine, quinoline, indole, isoquinoline, and quinazoline), terpenes (curcumin, betulinic acid, chrysanthemumin B, savinin, iguesterin, cryptotanshinone, dihydrotanshinone I, 3β-friedelanol), saponins (glycyrrhizin, escinidin, saikosaponin B2), coumarins (leptodactylon, xanthoangelol E), organosulfur compounds, glycosides, tannins, secoiridoids, mucus, lignans, lipid anthraquinones, phenolics carotenoids, aromatics, steroids, and other ingredients [2].

The spike protein is an important protein for SARS-CoV-2 binding and entry into the host. The spike protein contains the N-terminal S1 and the C-terminal S2 subunits. The S1 subunit consists of a signal peptide, N-terminal domain, and receptor binding domain (RBD). Downstream to the S1, there is S2 subunit that consists of fusion peptide (FP), heptad repeat 1, central helix, connector domain, and heptad repeat 2. The RBD in the S1 subunit has a receptor binding motive (RBM) that recognizes angiotensin-converting enzyme 2 (ACE2) as its receptor. After recognition and binding to ACE2, the S2 subunit of the spike glycoprotein dissociates from S1 subunit and exposes FP to the host membrane. Insertion of FP into the host membrane allows the fusion between viral and host membrane, therefore viral entry can occur and start its replication cycles. Since the S2 subunit plays a role in early stage of viral replication process, blocking this subunit is an effective way to suppress the viral infection [3].

In this study, we aimed to find some compounds from the HerbalDB database that have potential as SARS-CoV-2 replication inhibitors by binding to the S2 subunit of the viral spike protein.

2. Results and Discussion

In this study, we used crystal structure of SARS-CoV-2 spike (S2) glycoprotein obtained Protein Data Bank (https://rcsb.org/) with PDB ID: 6LXT as target protein. The 6LXT protein does not have a small molecule as its native ligand. Therefore, we used two different compounds as positive controls that had been shown by previous studies could bind avidly to the protein. Those two compounds were arbidol and quercetin [4]. Arbidol, also known as umifenovir, is a broad-spectrum antiviral drug that has been used against influenza A and B viruses and other respiratory viral infections in Russia and China for decades. It works by inhibiting the fusion process of viral and host lipid membranes [5]. A recent study showed that arbidol could inhibit SARS-CoV-2 infection by blocking viral entry and post-entry stages [6]. One case-control study also showed that arbidol could reduce the incidence rate of SARS-CoV-2 infection [7]. Quercetin is carbohydrate-free flavonoid, found abundantly in fruits and vegetables [8]. It is known for its antioxidant, anti-inflammatory, and immunomodulatory properties, making it a potential drug to treat SARS-CoV-2 patients [9].

Table 1. The predicted binding affinity of positive controls and hit compounds against the protein target with their inhibition constants.

Compounds	Binding energy (kcal/mol)	Inhibition constant (μM)
Arbidol *	-4.91	250.2
Quercetin *	-4.80	305.45
Mimosine	-3.99	1,190
L-Noradrenaline	-4.98	224.53
Octopamine	-5.19	157.75
L-Theanine	-3.62	2,220

* Positive control

Based on the molecular docking analysis, arbidol and quercetin showed binding energy of -4.91 kcal/mol and -4.8 kcal/mol, respectively (Table 1). Based on the pharmacophore modelling analysis, arbidol showed interactions with three amino acid residues in the receptor, namely GLN949B, ASP950B, ASN953B (Figure 1a), whereas quercetin showed interactions with two different amino acid residues, namely VAL1176E and GLU1182E (Figure 1b). Their pharmacophore models were then used as references for compound screening using HerbalDB as the compound database. Four hit compounds were screened and then docked into 6LXT protein. The order of hit compounds based on the lowest to highest binding energy was octopamine, L-noradrenaline, mimosine, and L-theanine (Table 1). Octopamine and L-noradrenaline showed lower binding energy than both positive controls whereas mimosine and L-theanine did not. Table 1 also shows the

inhibition constant (Ki) of each compounds. The inhibitory constant (Ki) is the inhibitor concentration required to reduce the maximum rate of a reaction by half, hence smaller value of Ki indicates stronger inhibitory potential. Figure 2a-d shows the 3D and 2D visualization of molecular interaction between the four hit compounds against S2 glycoprotein.

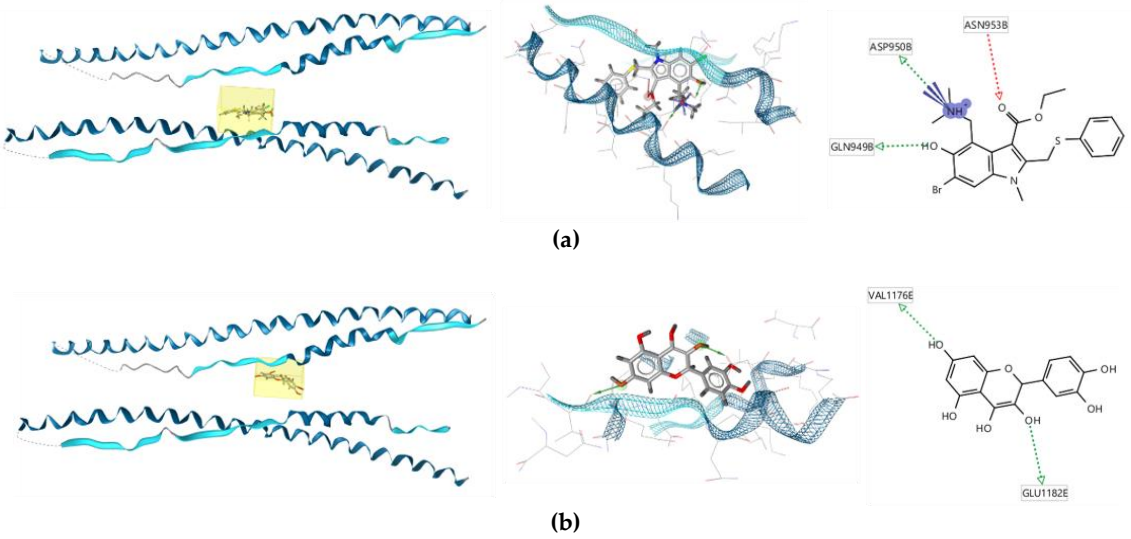

Figure 1. Complex structures and pharmacophore features between positive controls**, (a)** arbidol and **(b)** quercetin, against S2 glycoprotein. Pharmacophore features: red = hydrogen bond acceptor, green = hydrogen bond donor, purple = positive ionizable area.

Mimosine is a non-protein amino acid found in the *Fabaceae* family, such as *Mimosa* spp. and *Leucaena* spp [10]. This compound is known to be toxic due to its ability in reducing divalent metal ions availability and preventing their binding with metal-dependent enzymes [11]. However, this compound also has beneficial biological properties such as anti-inflammation, anti-fibrosis, anti-cancer [10], and antioxidants [11]. Based on the absorption, distribution, metabolism, and excretion (ADME) and toxicity analysis, the molecular weight of mimosine was 198.18 g/mol and LogP of -1.96 with 5 hydrogen bond acceptors and 3 hydrogen bond donors. Mimosine had high gastrointestinal (GI) absorption and had neither CYP inhibitor nor toxicity properties. This compound was classified as class IV (harmful if swallowed) in toxicity analysis with LD_{50} value of 2000 mg/kg (Table 2 and 3). According to the docking result, mimosine had binding energy value of -3.99 kcal/mol which was higher than both positive controls. This finding showed that mimosine was not a good candidate as SARS-CoV-2 spike (S2) glycoprotein inhibitor.

Table 2. Absorption, distribution, metabolism, and excretion **(**ADME) properties of all hit compounds.

Ligands	Druglikeness					Pharmacokinetics				
						CYP inhibitor				
	MW (g/mol)	HBA	HBD	LogP	GI Abs	CYP1 A2	CYP2 C19	CYP2 C9	CYP2 D6	CYP3 A4
Mimosine	198.18	5	3	-1.96	high	no	no	no	no	no
L-Noradrenaline	169.18	4	4	-0.17	high	no	no	no	no	no
Octopamine	153.18	3	3	0.20	high	no	no	no	no	no
L-Theanine	174.2	4	3	-1.37	high	no	no	no	no	no

MW = molecular weight; HBA = number of hydrogen bond acceptors; HBD = number of hydrogen bond donors; GI Abs = gastrointestinal absorbtion.

Table 3. Toxicity properties of all hit compounds.

Ligands	Toxicities				
	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Class
Mimosine	-	-	-	-	IV(Harmful if swallowed)
L-Noradrenaline	-	-	-	-	II (Fatal if swallowed)
Octopamine	-	-	-	-	IV (Harmful if swallowed)
L-Theanine	-	-	-	-	IV (Harmful if swallowed)

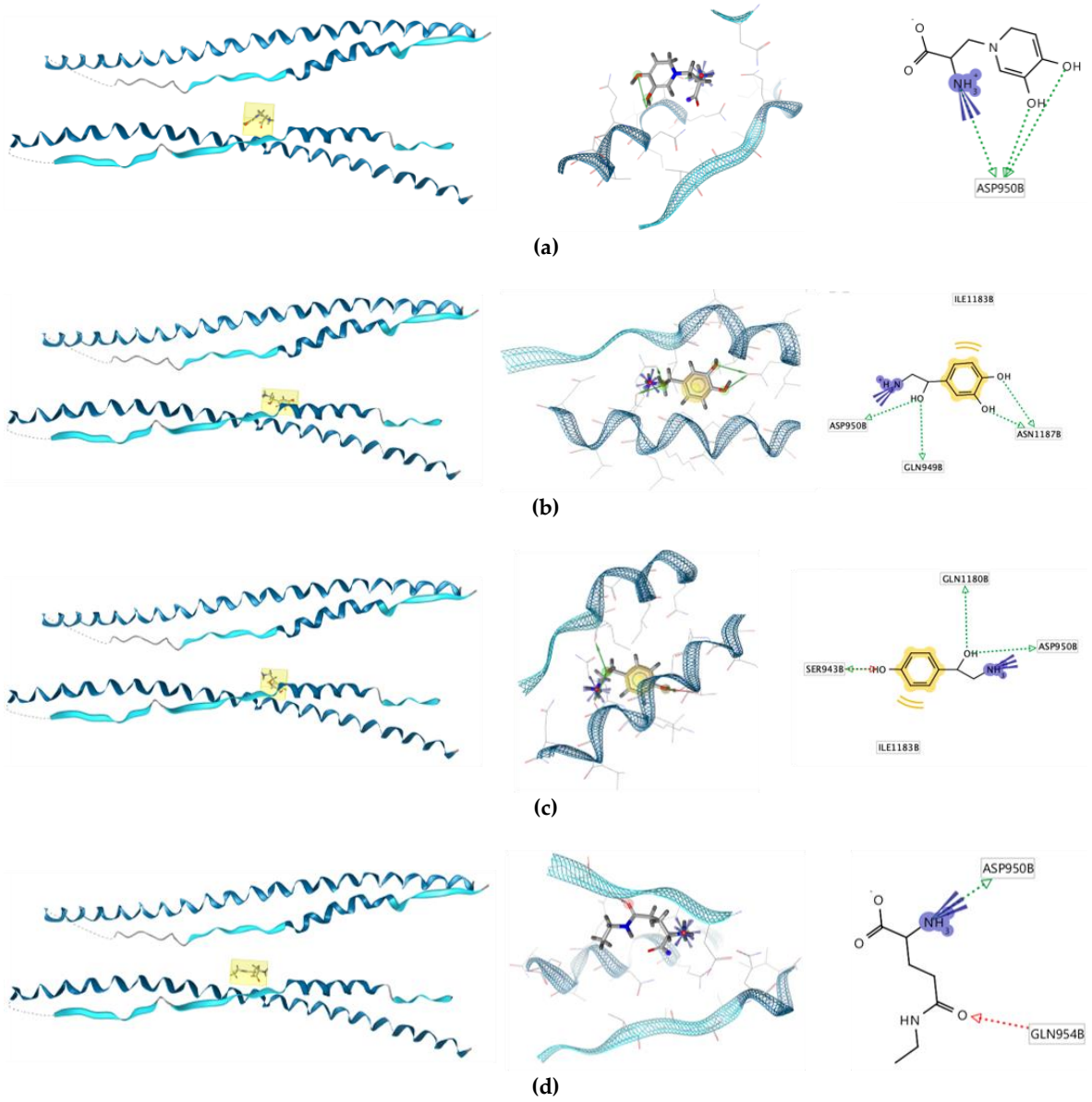

Figure 2. Complex structures and pharmacophore features between hit compounds**: (a)** mimosine, **(b)** L-noradrenaline, **(c)** octopamine, and **(d)** L-theanine, against S2 glycoprotein. Pharmacophore features: red = hydrogen bond acceptor, green = hydrogen bond donor, yellow = hydrophobic interaction, purple = positive ionizable area.

L-noradrenaline, also known as norepinephrine, is a tyrosine-derived sympathomimetic amine that naturally exists as a catecholamine hormone in the human body. It works as a neurotransmitter in the sympathetic nervous system with the fight-or-flight response. It is secreted by the medulla part of adrenal glands and functions as the main alpha-1 and beta-1 receptors agonist (it has little to no effect on alpha-2 or beta-2 receptors). It is predominantly used as a peripheral vasoconstrictor and vasoactive agent, and it is the first-

line agent on treating hypotension caused by distributive shock that does not respond to fluid resuscitation [12,13]. Through the ADME-TOX analysis, the molecular weight of L-noradrenaline was 169.18 g/mol and LogP of -0,17 with 4 hydrogen bond acceptors and 4 hydrogen bond donors (Table 2 and 3). L-noradrenaline had high GI absorption and had neither CYP inhibitor nor toxicity properties. This compound was classified as class II (fatal if swallowed) in toxicity analysis with LD_{50} value of 20 mg/kg. From the molecular docking analysis, the binding energy and inhibition constant, respectively, were -4.98 kcal/mol and 224.53 μM. It was the second best out of the four compounds, provided that it had the second lowest binding energy, thus L-noradrenaline had the potential to become one of the candidates as the inhibitory agent against SARS-Cov-2 spike (S2) glycoprotein. However, elaborate safety measures must be taken since it has the narrowest LD_{50} out of four compounds in causing toxicity.

Octopamine is named as such due to its original discovery in the salivary glands of octopuses. This compound is generally synthesized from the amino acid tyrosine and can be detected in nervous tissue using radioenzymatic assay. It was pointed out by one study that octopamine posed a potential as an important amine that could affect the therapeutic effects of inhibitors, namely monoamine oxidase (MAO) inhibitors, due to the increasing number of octopamine levels in the urine samples of mammals treated with this inhibitor [14,15]. MAO inhibitors were the first antidepressant developed. This inhibitor works by altering brain chemistry activities that are active in depression [16]. A study by Cuperlovic-Culf (2020) found that there were significant differences in a number of mono- and polyamines in operative delirium and delirium caused by SARS-CoV-2. This supported the hypothesis of SARS-CoV-2 having influences on MAOB activity, which could be linked to the many neurological and platelet-based complications of the infection. Thus, using this theory, inhibition of monoamine oxidase can help prevent the neurological implications from SARS-CoV-2 [15]. Based on the ADME-TOX analysis, the molecular weight of octopamine was 153.18 g/mol and LogP of 0.2 with 3 hydrogen bond acceptors and donors (Table 2 and 3). Octopamine had high GI absorption and had neither CYP inhibitor nor toxicity properties. Octopamine was classified as class IV (harmful if swallowed) in toxicity analysis with LD_{50} value of 1090 mg/kg. Through the molecular docking process, it was found that, out of the four compounds, octopamine had the lowest binding energy (-5.19 kcal/mol) and inhibition constant (157.75 μM). This finding showed that octopamine presented the best inhibitory capacity against SARS-CoV-2 spike (S2) glycoprotein.

L-theanine (-glutamylethylamide) is a non-protein amino acid present in green tea (Camellia sinensis), a popular beverage known for its health benefits. Due to its L-structure that is similar to L-glutamic acid, its mode of action might be mediated through glutamate receptors, a theory supported by its partial co-agonistic impact on the N-methyl-D-aspartate receptor. Because L-theanine is a phytochemical consumed on a daily basis, it has the potential to be a nutraceutical element that alleviates and avoids stress-related, psychological disorientation in modern culture. L-theanine has been advocated in rats as a neuroprotective and cognitive-enhancing agent. Peripherally- and centrally-given L-theanine have been shown to have an effect on the brain, modulating monoamine levels in the central nervous system as well as glutamate and glycine neurotransmissions [17]. To some extent, L-theanine can also oxidize LDL cholesterol. The combination of L-theanine and L-cystine can increase IgG serum and levels of antigen-specific IgM. In the human body system, combining L-theanine and L-cystine improves immunity to influenza vaccination in older people with low hemoglobin levels, as well as alleviates post-gastrectomy inflammation, and promotes healing post surgery in the perioperative period [18]. Molecular docking analysis of this compound found that it had the largest number for binding energy (-3.62 kcal/mol) and inhibition constant (2.22 mM) out of the four compounds studied. This generally means that L-theanine is not recommended to be used as an inhibitor for SARS-Cov-2 spike (S2) glycoprotein. Through the ADME-TOX analysis, the molecular weight of L-theanine was 174.2 g/mol and LogP of -1.37 with 4 hydrogen

bond acceptors and 3 hydrogen bond donors (Table 2 and 3). L-theanine had high GI absorption and had neither CYP inhibitor nor toxicity properties. L-theanine was classified as class IV (harmful if swallowed) in toxicity analysis with LD_{50} value of 1750 mg/kg.

Therefore, based on our findings, it was shown that octopamine has the lowest binding energy and inhibition constant out of all four hit compounds. The binding energy and inhibition constant of octopamine were also found to be lower than those of the positives controls. These findings indicate octopamine has a high affinity and great inhibitory potential for S2 glycoprotein. As previously mentioned, the S2 subunit of the SARS-CoV spike protein has a function in the viral fusion process and contributes to the initial process of viral replication [3]. Therefore, octopamine is expected to suppress the viral replication process by preventing viral fusion through inhibition of S2 subunit. Further *in silico* or *in vitro* studies can be conducted to predict and determine the inhibitory mechanism and activity. Despite its potential as an inhibitor of S2 glycoprotein, octopamine also poses some degree of toxicity based on the ADME-TOX analysis where it classified as class IV (harmful if swallowed) with LD_{50} value of 1090 mg/kg. Considering its low inhibition constant, a small dose of octopamine is expected to be able to provide inhibitory activity, thus not exceed the toxicity threshold. However, further *in vitro* and *in vivo* studies are needed to determine the optimum dose that provides the best efficacy and minimum toxic effects of octopamine.

3. Materials and Methods

3.1. Protein receptors preparation

Crystal structure of SARS-CoV-2 spike (S2) glycoprotein with PDB ID: 6LXT (2.90 Å) was retrieved from Protein Data Bank (https://rcsb.org/) in .pdb format. Protein preparation was conducted using AutoDockTools 4.2 by removing water, ligand molecules, and unnecessary chains, and by adding polar hydrogen and chargers.

3.2. Pharmacophore modelling and compound screening

Arbidol and quercetin were used as positive controls in this study. The pharmacophore binding sites identified from the two positive controls were then used for compound screening by using LigandScout version 4.3. A total of four hit compounds were screened from the HerbalDB compound database without omitting any of the pharmacophore features.

3.3. Ligand preparation and drug-likeness activity

The 2D structures of the ligands were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in .sdf format and converted into 3D structures in .pdb format using MarvinSketch. Ligand preparation was conducted using AutoDockTools 4.2 by setting torsion. Drug-likeness activity was inquired from absorption, distribution, metabolism, excretion (ADME), and toxicity analysis. ADME analysis was conducted using SwissADME (https://swissadme.ch/) whereas toxicity was analyzed using ProtoxII (https://tox-new.charite.de/protox_II/).

3.4. Molecular docking

Molecular docking was done using a laptop with an Intel Core i3-7100U CPU @ 2.40GHz, 4.00GB RAM as the working device, and with Windows 10 Home Single Language ver 20H2 installed as the Operating System. Molecular docking was firstly performed using the positive controls to validate and ensure the reliability of the process. This produced grid coordinates of (X, Y, Z), respectively, -11.707, 3.425, -10.425, with Grid Box dimensions 40×40×60. The grid coordinates were obtained from a previous study that had performed the binding pocket analysis of the protein receptors. The docking process was performed using AutoDockTools 4.2 software. SARS-CoV-2 spike (S2) glycoprotein (PDB ID: 6LXT) was docked with two positive controls and four hit compounds, and analyzed using AutoDockTools 4.2.

4. Conclusions

The search for COVID-19 and its complication treatments is still on-going, and it is crucial to thoroughly search for potential inhibitors to this virus. After scouring the HerbalDB database, analyzing the molecular docking, ADME, and toxicity results, we recommend octopamine as the compound that shows the best potential as SARS-Cov-2 spike (S2) glycoprotein inhibitor. However, octopamine also poses several toxicity risks and therefore, needs a more elaborate consideration upon using. Further *in vitro* and *in vivo* research needs to be done to define the efficacy and safety of said inhibitor.

Author Contributions: Conceptualization, S.K.S, A.U.C., P.S.H., G.R.C., and F.A.I.; methodology, S.K.S, A.U.C., P.S.H., G.R.C., and F.A.I.; software, S.K.S, A.U.C., and G.R.C.; validation, S.K.S, A.U.C., P.S.H., G.R.C., and F.A.I.; writing—original draft preparation, S.K.S, A.U.C., P.S.H., G.R.C., and F.A.I.; writing—review and editing, S.K.S, A.U.C., P.S.H., and G.R.C.; supervision, L.E., R.I.P., and F.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The authors would like to thank the bioinformatics elective module team, Department of Medical Chemistry, Faculty of Medicine, Universitas Indonesia for the guidance and support for this study.

Conflicts of Interest: The authors declare no conflict of interest.

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