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IN SILICO ACTIVITY IDENTIFICATION OF CYCLO PEPTIDE ALKALOIDS FROM *Ziziphus spina-christi* SPECIES AGAINST SARS-COV-2 MAIN PROTEASE

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Abstract. *The COVID-19 has spread worldwide and become an international pandemic. The promising target for drug discovery of COVID-19 was SARS-CoV-2 Main Protease (Mpro), that has been successfully crystallized along with its inhibitor. The discovery of peptide-based inhibitors may present better options than small molecules for inhibitor SARS-CoV-2 Mpro. Natural compounds have such a wide potential and still few explored, Ziziphus spina-christi is one of the medicinal plants that have many pharmacological activities and contains a peptide compound from alkaloids class, i.e. cyclopeptide alkaloids, that is interesting to explore as SARS-CoV-2 Mpro inhibitor. The compound structure was drawn and optimized using density functional theory 3-21G method. The protein chosen was the high resolution of SARS-CoV-2 Mpro receptor (1.45 Å) with PDB ID: 6WNP, in complex with Boceprevir. Molecular docking simulation was performed using AutoDock 4.2 with 100 numbers of GA run, the validation methods assessed by RMSD calculation. Furthermore, the prediction of pharmacological activity spectra was carried out using the PASS Prediction server. The results showed RMSD value was 1.98 Å, this docking method was valid. The binding energy of all compounds showed better results than the native ligand (Boceprevir). The in silico PASS prediction results indicated that all compounds showed antiviral activity. Some compounds showed protease inhibitory activity, i.e Ambiphibine-H, Franganine, and Mauritine-A, and the highest Pa (Predicted activity) value showed by Mauritine-A compounds. It can be concluded that the cyclo peptide compounds of Ziziphus spina-christi were indicated to have a potential as COVID-19 therapy targeting SARS-CoV-2 Mpro.*

Keywords: *COVID-19, in silico, peptide alkaloids, SARS-CoV-2 main protease, Ziziphus spina-christi*

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INTRODUCTION

The COVID-19 pandemic in 2019 has spread from Wuhan China and now spread worldwide and become an international pandemic (Zhu et al., 2020). The disease can lead to severe respiratory illness following an incubation time of 2-14 days (Backer et al., 2020).

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In the present situational report from WHO, released on November 6th, 2020, there are 48,534,508 COVID-19 confirmed cases and 1,231,017 confirmed death globally (World Health Organization, 2020).

One of the promising targets for drug discovery of COVID-19 was SARS-CoV-2 Main Protease (Mpro), which has been obtained its crystal structure along with its inhibitors (Jin et al., 2020; Khaerunnisa et al., 2020; Mirza & Froeyen, 2020; Reiner et al., 2020). The main protease in COVID-19 is not the same as in humans and is the key enzyme of the viral replication cycle (Goyal & Goyal, 2020; Gurung et al., 2020; Ullrich & Nitsche, 2020). The peptide-like anti-HIV-1 drugs are effective against SARS-CoV Main protease (Mpro). Due to the close phylogenetic relationship between SARS-CoV and SARS-CoV-2, many of their main proteases resemblant in structural and functional features (Rut et al., 2020; Yoshino et al., 2020). So, the discovery of peptide-based inhibitors may present better options than small molecules for COVID-19 therapy (Du et al., 2007; Han & Král, 2020).

Natural compounds have broad potential which can still be explored (Dias et al., 2012). So our oriented towards treatments of natural product compounds based on peptide-like from medicinal plants in order to obtain compounds having the ability to inhibit COVID-19 (Benarba & Pandiella, 2020; Khare et al., 2020). One of the medicinal plants and has peptide compounds we chose for this research, i.e. *Ziziphus spina-christi* species (Darusman & Fakih, 2020).

Ziziphus spina-christi is a deciduous tree that generally comes from warm and subtropical climates region, such as North Africa, South Europe, Mediterranean, tropical America, South and East of Asia, and others, including Indonesia (Yossef et al., 2011). There are many names for *Z. spina-christi*, which is known as the thorn of Christ; Syria Christ-thorn in English, on behalf of epine (Saied et al., 2008). *Z. spina-christi* is also a plant mentioned in the Holy Qur'an, the holy book of Islam, as a plant that has many medicinal benefits (Ishrak et al., 2006).

Ziziphus spina-christi has been proven and reported to have various pharmacological activities, including antibacterial, antifungal, antioxidant, antihyperglycemic, and antinociceptive, etc. Flavonoids, alkaloids, and saponins are the main phytochemicals reported from this plant (Asgarpanah, 2012). The *Z. spina-christi* has several peptide compounds in the alkaloids class, i.e. cyclopeptide alkaloids, that have been reported, can be found in stem-bark (Inayat-ur-Rahman et al., 2001).

By looking at the potential of the peptide type compounds that were reported to be the most potent inhibitors of the main protease enzyme of SARS-CoV-2, and also the alkaloid peptide content in the *Z. spina-christi* plant which has not been explored for its activity through SARS-CoV-2 Mpro, So we interested to investigate the potency of cyclopeptide alkaloids from *Z. spina-christi* as an inhibitor of SARS-CoV-2 Mpro.

MATERIALS AND METHODS

The experiment was carried out on October-December 2020, at the Laboratory of Pharmaceutical Chemistry, Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Islam Bandung, Bandung, Indonesia.

Hardware and Software

This research was conducted using hardware in the form of computers and HPC (High-Performance Computing) facili-

ties owned by the Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Islam Bandung. The computer is equipped with software with Windows 7 and Linux Ubuntu 16.04 operating systems equipped with Quantum ESPRESSO v.6.6, MGL Tools 1.5.6 with AutoDock 4.2, Prediction of Activity Spectra for Substances (PASS) web server, and BIOVIA Discovery Studio Visualizer 2020.

Ligand Preparation

The 3D ligand structures were drawn and optimized using Density Functional Theory (DFT) methods with a 3-21G basis set using Quantum ESPRESSO v.6.6. The ligands chose were bioactive compounds that contained in *Ziziphus* species i.e. Amphibine -H, Franganine, Jubanine-A, Mauritine-A, Sativanine-B, and Zizyphine-F. These peptide compounds are reported found in the *Ziziphus spina-christi* plant. The structure of the ligands are presented in Figure 1.

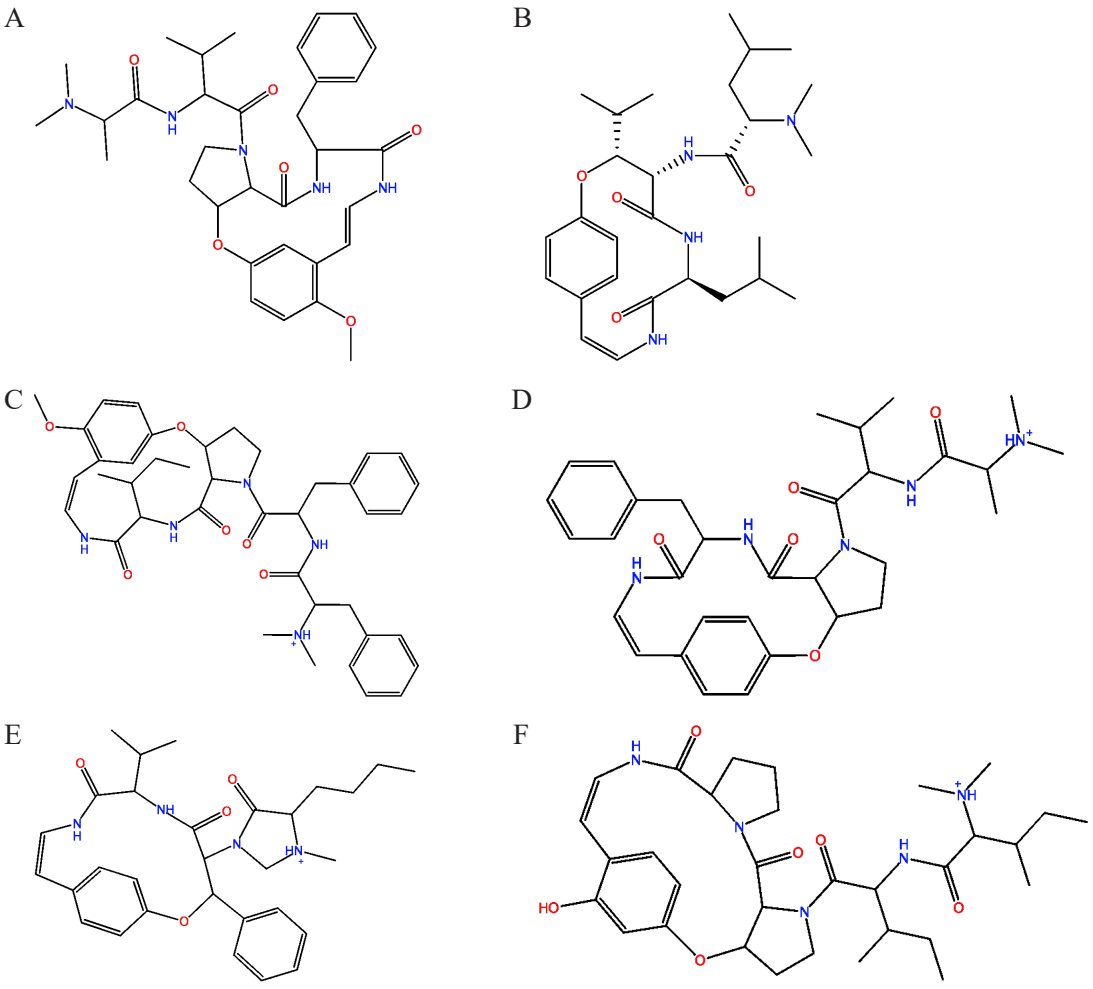

Figure 1. Chemical structure of Amphibine-H (A); Franganine (B); Jubanine-A (C); Mauritine-A (D); Sativanine-B (E); and Zizyphine-F (F).

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Reseptors Preparation

The crystallography structures of SARS-CoV-2 Mpro were obtained from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb/). The high resolution of the SARS-CoV-2 Mpro receptor (1.45 Å) with PDB ID: 6WNP was chosen. The 6WNP was chosen because of its high resolution compared to other types of SARS-CoV-2 Mpro crystal protein in PDB. The receptor was complexed with Boceprevir an HCV protease inhibitor as a co-crystal ligand. Furthermore, the unique ligands and water molecules were removed from the receptor. Then, polar hydrogen and a charge (Kollman charge) was added to the receptor structure. All preparation procedures were performed using MGLTools 1.5.6. with AutoDock 4.2 (Morris et al., 2010).

In Silico PASS Prediction

The in silico Prediction of Activity Spectra for Substances (PASS) was carried out to get biological activity spectra of compounds accessed through PASS web server (http://www.way2drug.com/PASSOnline/predict.php) (Lagunin et al., 2000). The predicted spectrum as probable activity (Pa) and probable inactivity (Pi) that was estimated based on structure-activity relationship analysis of the training set containing more than 205,000 compounds exhibiting more than 3750 kinds of biological activities. Being probabilities, the Pa and Pi values vary from 0.000 to 1.000. The PASS prediction was interpreted and used in a flexible manner: (i) only activities with Pa > Pi are considered as possible for a particular compound; (ii) if Pa >0.7, the chance to find activity is experimentally high; (iii) if Pa is >0.5 but less than <0.7, the chance to find activity is experimentally low, but the compound is probably different to known pharmaceutical agents; (iv) if Pa <0.5, the chance to find activity is experimentally is low, but the chance to find new chemical entities is high (Anzali et al., 2001).

Molecular Docking Simulation

The co-crystal ligand was re-docked on the binding pocket of the protein to validated the docking method. The best conformation of the co-crystal ligand was taken and superimposed with the co-crystal ligand before docked, and the Root-Mean-Square Deviation (RMSD) was assessed. The acceptable RMSD value must be less than 2.0 Å (Bell & Zhang, 2019). Furthermore, we docked all of the ligands to the binding pocket of the SARS-CoV-2 Mpro. The grid box was set with coordinates 7.579, 26.283, and 23.017 (x, y, and z), and the dimensions of the grid box were 64, 60, and 60 (x, y, and z), and 100 numbers of GA run. Furthermore, the conformation of each ligand was analyzed for binding energy and amino acid interaction.

RESULTS AND DISCUSSION

In Silico PASS Prediction

PASS prediction was carried out on Boceprevir, and cyclopeptide alkaloids compound to see and compare their probability level as a COVID-19 main protease inhibitor (Table 1). Boceprevir was predicted to have activity as an antiviral and protease inhibitor, with Pa values of 0.730 and 0.347, respectively. The cyclopeptide alkaloids compounds, Ambiphine-H, Franganine, Jubanine-A, Mauritine-A, Sativanine-B, and Zizyphine-F, showed antiviral activity with Pa values of 0.263, 0.201, 0.203, 0.277, 0.162, and 0.300, respectively. Protease inhibitor activity predicted by Ambiphine-H (Pa 0.118), Franganine (Pa 0.097), and Mauritine-A (Pa 0.131) compounds. Overall, all compounds showed potency as antiviral activity and some as a protease inhibitor, and Mauritine-A

compounds showed the best results of PASS prediction as antiviral and protease inhibitor.

Molecular Docking Simulation

Validation was carried out to see the strength of binding mode prediction through re-docking the co-crystal ligand into its binding site. It showed that the RMSD value of the co-crystal ligand was 1.069 Å. The RMSD value below 2.0 Å shows that the molecular docking method used is valid. The overlay of the docked co-crystal ligand and docked boceprevir can be seen in Figure 2. The cyclopeptide alkaloids after docked showed varied binding energies (Table 2). In general, the cyclopeptide alkaloids of *Ziziphus spina-christi* from the docking results, showed high binding energy and Ki compared to the co-crystal ligand. The negative sign or the lowest binding energy is considered to be stable to interact with receptors (Ramadhan et al., 2020). The binding energy of the co-crystal ligand was −7.93 Kcal/mol. The cyclopeptide alkaloids binding energy sort by highest to lowest were –9.88 Kcal/mol (Mauritine-A), −9.12 Kcal/mol (Amphibine-H), −8.98 Kcal/mol (Sativanine-B), −8.61 Kcal/mol (Jubanine-A), −8.08 Kcal/mol (Zizyphine-F), and –7.97 Kcal/mol (Franganine).

Table 1. In silico PASS prediction results

Ligands	Activities prediction	Pa	Pi
Boceprevir	Antiviral	0.730	0.004
	Protease inhibitor	0.347	0.006
Ambiphibine-H	Antiviral	0.263	0.052
	Protease inhibitor	0.118	0.070
Franganine	Antiviral	0.201	0.095
	Protease inhibitor	0.097	0.092
Jubanine-A	Antiviral	0.203	0.093
	Protease inhibitor	-	-
Mauritine-A	Antiviral	0.277	0.045
	Protease inhibitor	0.131	0.060
Sativanine-B	Antiviral	0.162	0.146
	Protease inhibitor	-	-
Zizyphine-F	Antiviral	0.300	0.037
	Protease inhibitor	-	-

Pa: Probable activity, Pi: Probable inactivity

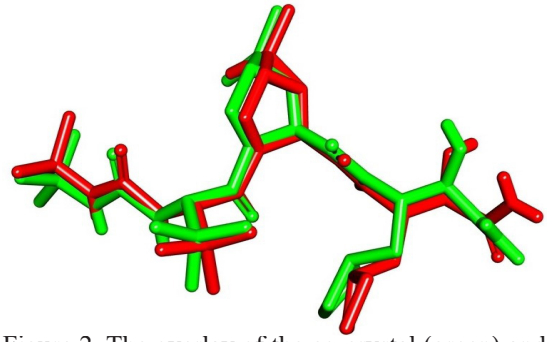

Figure 2. The overlay of the co-crystal (green) and docked (red) conformation of Boceprevir

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Table 2. Binding energy of the cyclopeptide alkaloids of *Ziziphus spina-christi* to the SARS-CoV-2 Mpro receptor

Compounds	Binding energy (Kcal/mol)	Ki (nM)
Co-crystal ligand (Boceprevir)	−7.93	1540
Amphibine-H	−9.12	207.44
Franganine	−7.97	1440
Jubanine-A	−8.61	492.2
Mauritine-A	−9.88	57.52
Sativanine-B	−8.98	263.62
Zizyphine-F	−8.08	1190

Molecular Interactions

The amino acid interactions of cyclopeptide alkaloids with the SARS-CoV-2 Mpro were observed and compared to reference co-crystal ligand (Boceprevir) binding mode. The tabulation data of the interactions is presented in Table 3 and the 2D interaction can be seen in Figure 3. Co-crystal ligand in its interactions, form hydrogen bonds with amino acids Glu:166, His:163, Leu:141, and Cys:145. Co-crystal ligand also forms several types of pi interaction with His:41 (pi-sigma and pi-alkyl) and alkyl interaction with Met:165 and Met:49. The cyclopeptide alkaloids in SARS-CoV-2 Mpro showed richer interaction than co-crystal ligand, but the types of bonds formed are slightly different. Amphibine-H showed 5 similar residues, i.e. Glu:166, His:163, Cys:145, His:41, Met:49, and 3 different residues, i.e. Gly:143, Leu:27, and Phe:140. Franganine showed 5 similar residues, i.e. Glu:166, His:163, Cys:145, His:41, Met:49, and 3 different residues, i.e. Pro:168, Gln:189, and Arg:188. Jubanine-A showed 4 similar residues, i.e. Glu:166, His:41, Met:165, and Met:49, and 1 different residue, i.e. Arg:188. Mauritine-A showed 3 similar residues, i.e. Glu:166, His:41, Met:49, and Met:49, and 2 different residues, i.e. Arg:188, and Phe:140. Sativanine-B showed 4 similar residues, i.e. His:163, Cys:145, His:41, Met:165, and 1 different residue, i.e. Arg:188, and His:164. Zizyphine-F showed 3 similar residues, i.e. Glu:166, His:41, Met:165, and 6 different residues, i.e. His:164, Gln:189, Pro:168, Leu:167, and Arg:188.

The hydrogen bond is a bond between an H partial positive charge atom with other nearby electronegative atoms and has lone pairs of free electrons that have a complete octet, such as O, N, and F, and is the strongest non-bond interaction of ligand-receptor (Megantara et al., 2017). Co-crystal ligand still shows the highest intensity of hydrogen bonding (4 hydrogen bonds), followed by Sativanine-B (3 hydrogen bonds), and Ampibhine-H, Jubanine-A, Mauritine-A, and Zizyphine-F (2 hydrogen bonds, respectively). The other interactions, i.e. Pi-sigma, Pi-alkyl, and Pi-Sulfur, etc., mostly involve charge transfer assisting in intercalating the drug at the receptor-binding site. The highest number of amino acid interactions that form Pi interaction are dominated by Zizyphine-F, then Amphibine-H, Franganine, Sativanine-B, Mauritine-A, and Jubanine-A, respectively.

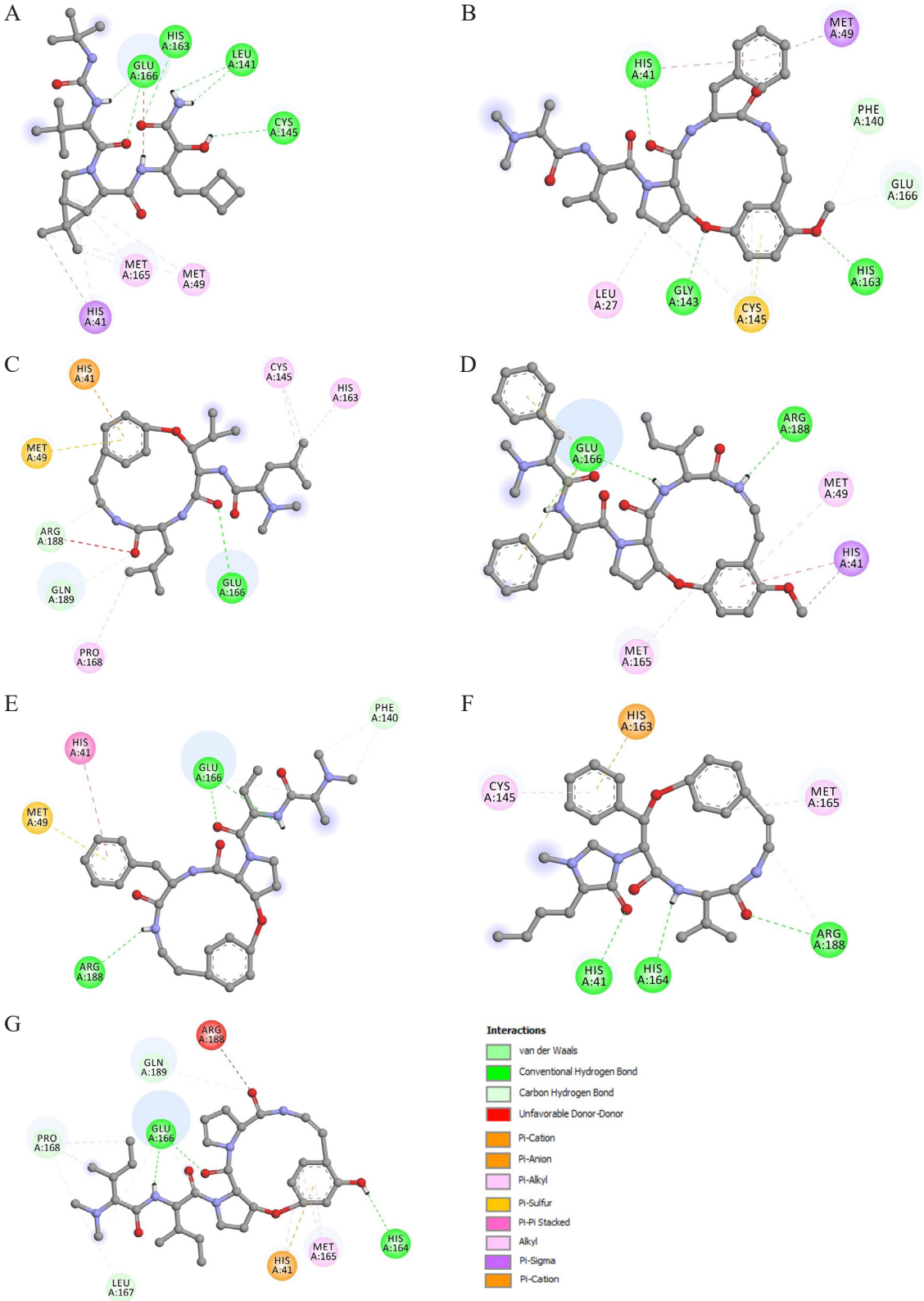

Figure 3. Two-dimensional interactions of co-crystal ligand (A), Amphibine-H (B), Franganine (C), Jubanine-A (D), Mauritine-A (E), Sativanine-B (F), and Zizyphine-F (G).

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Table 3. Data of amino acid interactions of reference ligand (co-crystal ligand) compared to cyclo peptide alkaloids in SARS-CoV-2 Mpro

	Co-crystal ligand (Boceprevir)	Amphibine-H	Franganine	Jubanine-A	Mauritine-A	Sativanine-B	Zizyphine-F
Residues	Glu:166 (a)	Glu:166 (g)	Glu:166(a)	Glu:166 (a, k)	Glu:166 (a,g)	-	Glu:166 (a,g)
	His:163 (a)	His:163 (a)	His:163(c)	-	-	His:163 (h)	-
	Leu:141 (a)	-	-	-	-	-	-
	Cys:145 (a)	Cys:145 (d,e)	Cys:145(d)	-	-	Cys:145 (c)	-
	His:41 (b,c)	His:41 (a,f)	His:41 (h, i)	His:41 (b,f)	His:41 (f)	His:41(a)	His:41 (c,h,i)
	Met:165 (d)	-	-	Met:165 (d)	-	Met:165 (d)	Met:165 (c,d)
	Met:49 (d)	Met:49 (b)	Met:49 (e)	Met:49 (c)	Met:49 (e)	-	-
		Gly:143 (a)	Pro:168(e)	Arg:188 (a)	Arg:188 (a)	Arg:188 (a,g)	His:164 (a)
		Leu:27 (d)	Gln:189(g)		Phe:140 (g)	His:164 (a)	Gln:189 (g)
		Phe:140 (g)	Arg:188(g, j)				Pro:168 (g,d)
							Leu:167 (g)
							Arg:188 (j)

a: conventional hydrogen-bond, b: pi-sigma, c: pi-alkyl, d: alkyl, e: pi-sulfur, f: pi-pi T-shaped, g: carbon-hydrogen bond, h: pi-cation, i: pi-pi Stacked, j: unfavorable bond, k: pi-anion

Synthetic peptides have been extensively researched and proven to be able to inhibit the activity of the SARS-CoV-2 Mpro enzyme, however, peptides from natural compounds are still lacking in exploration while their potential is still very wide. One of the plants known to contain metabolites in the form of peptides which are classified as alkaloids group, e.g. cyclopeptide alkaloids, is *Ziziphus spina-christi*. In this study we tried to predict whether the cyclopeptide alkaloids from *Ziziphus spina-christi* have potential as an antiviral for SARS-CoV-2, specific to the main protease receptor, using the in silico method. This research revealed that cyclopeptide alkaloids from *Ziziphus spina-christi* species have potential as inhibitor candidates for the SARS-CoV-2 Mpro receptor. This is supported by the activity prediction results, In silico PASS prediction, that showed all cyclopeptide alkaloid compounds have antiviral activity and some compounds showed protease inhibitory activity, i.e Ambiphibine-H, Franganine, and Mauritine-A. The highest Pa value (Predicted

activity) showed by Mauritine-A compounds. Further, the molecular docking of Amphibine-H, Franganine, Jubanine-A, Mauritine-A, Sativanine-B, and Zizyphine-F, showed better binding affinity against SARS-CoV-2 Mpro compared to co-crystal ligand (Boceprevir). These compounds form interactions that are similar in some residues with the co-crystal ligand. It is suspected that the large binding energy of these ligands is due to the amino acid interactions, which are more numerous than Boceprevir. Where it is known, the number of amino acid interactions will affect the bond strength of the ligand to the active site of the receptor.

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