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IN SILICO STUDY: THE BLUE BUTTERFLY PEA FLOWER (CLITORIA TERNATEA L.) COMPOUND HASPOTENTIAL FOR HERBAL MEDICINE FOR COVID-19

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ABSTRACT

The number of outbreaks of COVID-19 cases in the world continues to grow until April 2022. Certain drugs that are officially approved for the treatment of COVID-19 are still under development. Several vaccines have been circulating, but the distribution is not complete. Research on the use of herbs for the treatment of COVID-19 is also ongoing. In Asia, herbs as a treatment have been widely used such as Blue Butterfly Pea Flower (Clitoria ternatea L.) is a plant that is commonly found in Asia with many benefits such as anti-bacterial, antiviral, and so on but has not been widely studied. Blue Butterfly Pea Flower (Clitoria ternatea L.) is a plant that is commonly found in Asia. This study aimed to determine 44 compound contained in the Blue Butterfly Pea Flower, which have the potential as the Main Protease Inhibitor of Sars-Cov-2 based on their Druglikeness, Toxicity Properties and In Silico Study. This research method From 44 compound, has seven compounds have met the druglikeness and toxicity properties requirements then proceed with activity analysis using In Silico methode. The seven compound namely Myrcetin, Kaempferol, Quercetin, Baicalein, Luteolin, Apigenin, and Epicatechin will interact with the SARS-CoV-2-MPro receptor (GDP ID: 7AHA) using the Autodock-Vina program. This study indicates that the original re- validated ligand-receptor RMSD value was 2.3, and the binding energy value was -4.2. The seven compounds have better binding energy values than the original ligands. The best secondary metabolite of Butterfly Flower is Baicalein, with a binding energy of about -8.5. Blue Butterfly Pea flower compound are promising candidates for food applications that are efficacious as Sars-Cov-2 Main Protease inhibitors due to similar drug properties, safety, and effectiveness as a preventive measure against COVID-19.

KEYWORDS: Covid-19; In silico; Main Protease; Bunga Telang; Blue Butterfly Flower; Clitoria ternatea L.

1. INTRODUCTION

The first identification of the impact of SARS-CoV-2 took place in Wuhan-China in December 2019. In February 2020, the World Health Organization named the new severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and Coronavirus disease 2019 (COVID-19) and declared a pandemic.[1] Supported The number of cases of COVID-19 in the world is still increasing. As of April 2022, almost 441 million people were confirmed to have contracted COVID-19, with a relatively high mortality rate of 1,4%.[2] Drugs officially approved for the treatment of COVID-19 are still under development.[3] Several vaccines have been circulated, but it has not been distributed to all humans in the world. Sars-Cov-2 has 2 types of proteins, namely structural and non-structural. Structural proteins consist of Spike (S), Nucleocapsid (N), Matrix (M), and Envelope (E) and non- structural proteins consist of nsp1 - nsp16. The result is translated into mature non-structural protein (NSP) at the proteolytic cleavage stage. The enzymes involved in this translation are two cysteine proteases, namely papain PLpro and chymotrypsin 3CLpro, known as Mpro. Thisenzyme is required to mature viral polyproteins to form new virions. It is also helpful for viralproliferation and infectivity. Drug development for the treatment of COVID-19 is chymotrypsin 3CLpro known as Mpro. It is a target for new drug development and a potentialpoint for tracking phytochemical inhibitors.[4] Clitoria ternatea is a Clitoria plant with the subgenus Clitoria, and represents thearchetypal Clitoria. The etymology of the specific name Ternatea is thought to have come from the island of Ternate in the Indonesian archipelago because this plant was first discovered by Linnaleus at that location. Ternate is the eastern part of Indonesia. The distribution of all othertaxa of the subgenus Clitoria is limited to South and East Africa, India, Madagascar and otherislands of the West Indian Ocean. Therefore, the exact geographic origin of C. ternatea is difficult to determine, but we can conclude from the diversity center of the subgenus Clitoria, that C. ternatea occurs in or around the Indian Ocean and not in the Pacific Ocean or South China Sea where it has benefits. as a food coloring historically. The use of Blue Butterfly Pea Flower (Clitoria ternatea L.) is reported many used.[5] Based on the background, and in silico studies have been done by looking at the druglikeness and toxicity properties associated with the molecular docking method to see the structure- activity relationship (SAR) of physicochemical properties of the Blue Butterfly Pea Flower component with the SARS-Cov-2 Mpro receptor. To obtain predictions of activity, toxicity, and more selective and sensitive

bioavailability to increase self-reliance in herbal medicine.

1.1 Plant itroduction

The Blue Butterfly Pea Flower (Clitoria ternatea L.) is a vine usually found in yards or forest edges. This plant originates from tropical Asia but has now spread throughout the tropics. The Blue Butterfly Pea Flower belongs to the Papilionaceae or Fabaceae (legumes) family. Clitoria belongs to the legume group. They are planted in the yard as ornamental plants. This flower has various names in Indonesia, such as in Sumatra it is called Bunga Biru, Bunga Klentit, or Bunga Telang, in Java it is called Bunga Telang, Menteleng, and in Sulawesi it is called bunga talang, bunga temen raleng, and in Maluku it is called bisi, seyamagulele.[6] In

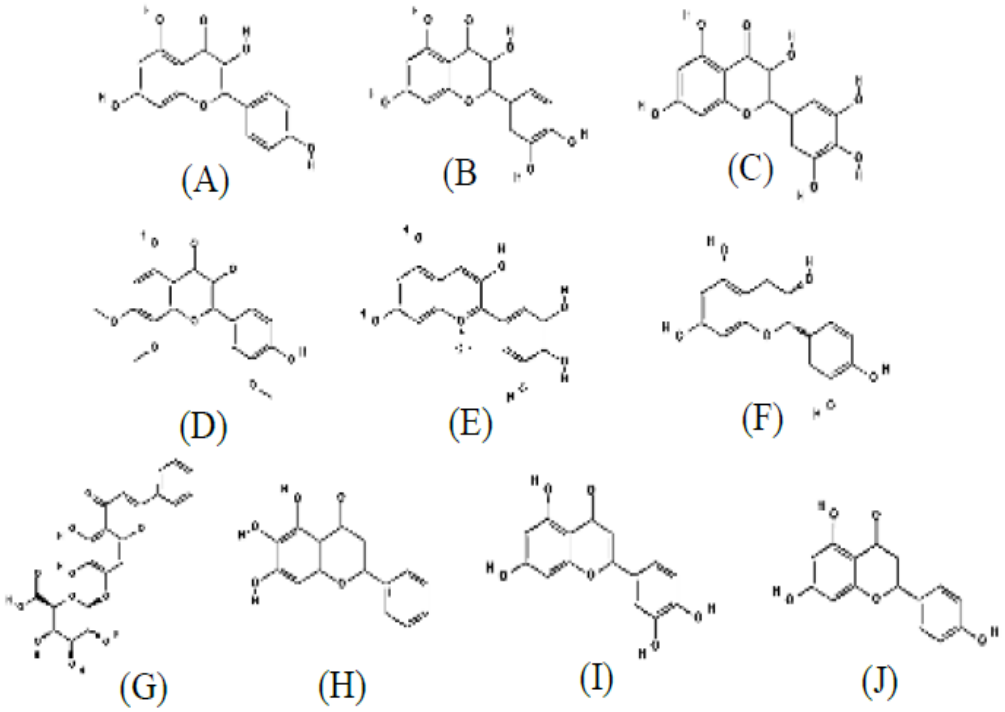


Fig. 1: Kaempferol; (B) Quercetin; (C) Myricetin; (D) Ternatin; (E) Delphinidin; (F) Epicatechin; (G) Scutellaria; (H) Baicalein; (I) Luteolin; (J) Apigenin.

Indonesia, Blue Butterfly Pea Flower is used as an herbal drink. Besides producing a beautiful color, it also has many benefits. The Blue Butterfly Flower image is in figure 1.

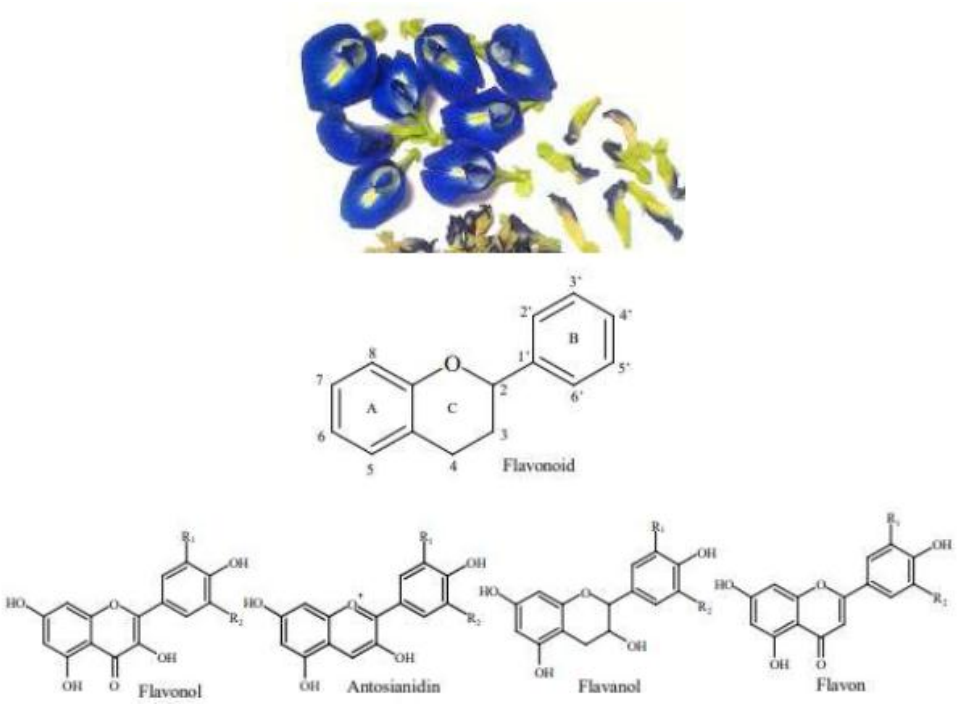


Fig. 3: The basic structure of flavonoids and their derivatives: flavonols, anthocyanidins, flavanols, and flavones.

The bioactive components in Blue Butterfly Pea Flower, which are thought to have functional benefits come from various phytochemical compounds, such as phenol groups (flavonoids, phenolic acids, tannins, and anthraquinones), terpenoids (triterpenoids, tocopherol saponins, phytosterols), and alkaloids. The flavonoid components in Blue Butterfly Flower areflavonols, anthocyanidins, flavonols, and flavones.[5] Basic structure flavonoids on figure 2.

Flavonols of Blue Butterfly Pea Flower are found in glycosides, namely flavonol glycosides, which consist of kaempferol 3-glucoside (kaempferol 3- (2-rhamnosilrutinoside) is the most found about 87% in flower, kaempferol 3-neohesperidoside, kaempferol 3-(2- rhamnosil-6-malonyl) glucoside, kaempferol 3-rutinoside), quercetin 3-glucoside (quercetin 3(2-rhamnosylrutinoside), quercetin 3- neohesperidoside, quercetin 3-rutinoside, quercetin 3-glucoside) and myrisetin 3-glycoside (myrisetin 3-(2-rhamnosilrutinoside).[7] Structure of flavonol glycosides, flavonol and flavons on figure 3.

Anthocyanidin in glycone form, called anthocyanins, is about 27% contained in flowers. The blue color of the flower is due to anthocyanins. Anthocyanins can be used as antiviral, anti-inflammatory, antioxidant, anti-allergic, antimicrobial, anticancer, anti-arterial atherosclerosis, anti-hypertensive, prevent diabetes, protect the cardiovascular system from

damage, and many other health benefits. Many benefits are obtained from anthocyanins because of their ability to donate hydrogen to radicals and stop radical chain reactions. The anthocyanin in Blue Butterfly Pea flower is given a unique name, ternatin. The most complex anthocyanin is ternatin A1, while the most abundant in flowers are ternatin B2 and B1.[5]

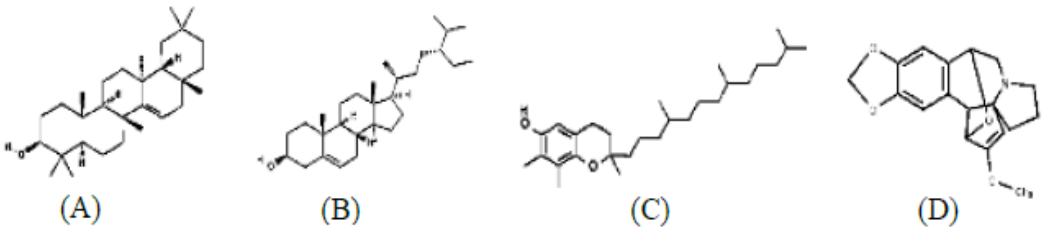


Fig. 4: Taraxerol; (B) Phytosterols; (C) Tocopherols; (D) 3-deoxy-3,11-epoxy cephalotaxine.

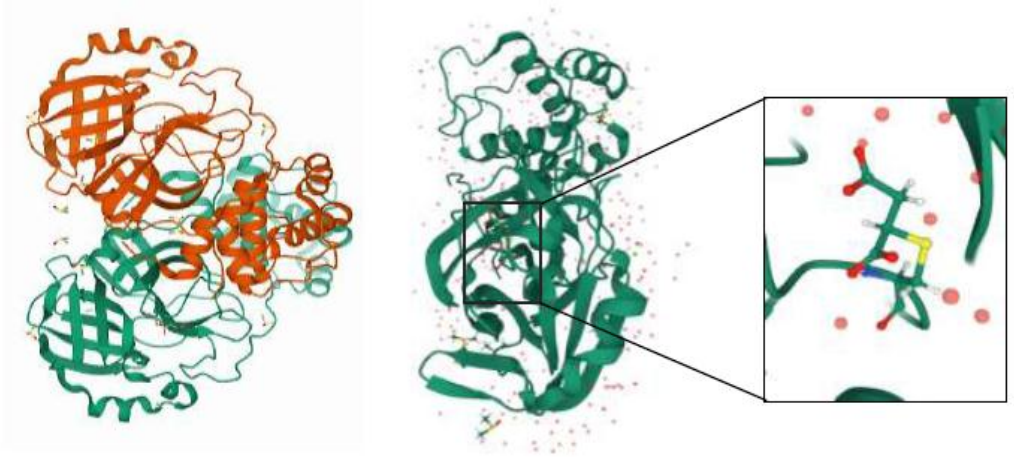
Other anthocyanins are Delphinidin 3-O-(2″-O-a-rhamnosyl-6″-O-malonyl)-β- glucoside, Delphinidin 3-(6″-malonyl) glucoside, Delphinidin 3-neohesperidoside, Delphinidin 3-glucoside (8). Four flavons compounds were found in the methanol extract of Blue Butterfly Pea Flower. The flavons are Scutellaria was the most abundant (36.9%), Baicalein (12.6%), Luteolin (9.3%), and Apigenin (6.3%). The only flavanol compound confirmed to be present in Blue Butterfly Pea Flower is epicatechin (9). Catechins are the most potent flavonoids to protect the body from reactive oxygen reactions (10). The terpenoid compounds found in the Blue Butterfly Pea Flower are triterpenoids (taraxerol), phytosterols, and tocopherols (α-tocopherols dan γ- tocopherols). The alkaloid compound isolated from the chloroform extract of Blue Butterfly Pea Flower is 3-deoxy-3, 11-epoxy cephalotaxine which can be used as an antibacterial (11). Sructure of terpenoid in figure 4.

1.2 SARS-CoV-2-MPro

One of the important components of the coronavirus plays a role in the proteolytic cleavage stage. The major protease (Mpro), also known as the 3C-like protease (3CLpro or nsp5), is a polyprotein encoded by the genome of the coronavirus. The primary function of Mpro is to release functional polypeptides from polyproteins through a proteolytic process. Two polyproteins are required for coronavirus replication and transcription, namely pp1a and pp1b. Mpro cleaves the viral polyprotein pp1ab at 11 different sites. The cleavage motif is Leu Gln↓(Ser/Ala/Gly). Mpro has a chymotrypsin-like fold added to a C-terminal helical domain and harbors a catalytic dyad (Cys145 and His41) at its active site forming four main, labeled

pockets that follow the scissile bond sequence of the substrate. The active site is located in the gap between the two N-terminal domains of the monomeric three-domain structure. Mpro is a prime target for antiviral drug discovery because of its significant involvement in the viral replication process. This aims to identify and optimize drugs that can overcome coronavirus infections.[12]

On April 2, 2021, Sebastian Guenther and other authors published a journal that found the Structure of SARS-CoV-2 Main Protease bound to Maleate with PDB ID 7AHA. This journal show that most compounds with high resolution structures can bind at the active site of Sars-Cov-2-Mpro. The ligand used in this study is easily obtained, namely succinic acid. The resolution of structure is 1.68 Å.[13] Figure 5 (A) Biological assembly structure of Sars-Cov-2-Mpro reveals that there are two allosteric drug binding sites; figure 5 (B) explains the structure of Mpro and interaction with ligand (succinic acid). Figure 5(C) show ligand structure (Succinic acid) interaction.



A **B** **C**

Fig. 5: Biological Assembly of Mpro; (B) 3D structure Asymmetric Unit Mpro with ligand; (C) The Ligand (Succinic Acid).

2. MATERIALS AND METHODS

2.1 Drug-likeness and Toxicity Properties

From SMILES Format, the druglikeness and toxicity properties of the secondary metabolites compound Blue Butterfly Pea Flower (Clitoria ternatea L.) were predicted with the PKCSM program by entering the SMILES format via web http://biosig.unimelb.edu.au/pkcsm/. The druglikeness property follows Lipinski's theory (the Lipinski rule of five), i.e., the molecular weight <500 da, Rotable Bond range is 1-15, not more than five hydrogen bond donors, not

more than ten hydrogen bond acceptors, partitioncoefficient (log P) value <5 and Total Polar Surface Area (TPSA) <140.[14] From the toxicity, a parameter is AMES toxicity to know the phytochemical has a carcinogenic effect(Yes/No), hERG I, hERG II inhibitor to determine the toxic effect on the heart (Yes or No), Hepatotoxicity (Yes or No), Max. Tolerated dose (human) (log mg/kg/day) to determine the level of toxicity from an "acceptable" quantity or if the amount exceeding the limit can cause a risk to animals test, Lethal Dose 50 (LD50) is a value that indicates administration of drug dose to experimental animals but can because 50% death in animals tests acutely and Lowest Observed Adverse. Effect Level (LOAEL) in a rat is the lowest concentration of a substance found through testing that causes adverse changes in normal test animals under the specified exposure conditions.[15]

2.2 Preparation of Ligands and Receptor

The secondary metabolites of Blue Butterfly Pea Flower (Clitoria ternatea L.) as a ligand,based on reference information.[7,5] The ligand structure was taken from PubChem in. The SDF format for the 3D structure was converted to SMILES format by Marvin Sketch.[16] Thelowest conformation of the structure ligand is selected using the Marvin sketch, and it is saved with.Mol2 format. SMILES format is used for the prediction of druglikeness and toxicity while mol.2 format Used for docking method.[16] The macromolecules SARS-Cov-2-MPro was prepared from Protein Data Bank (https://www.rcsb.org) with.PDB format. The SARS-CoV-2 Main Protease receptor PDB ID has used is ID:7AHA, and the original ligand is Succinic Acid. The specifications of the SARS macromolecule are as follows: Total Structure Weight is 34.48 kDa, has 2820 Atomic Numbers, 1 Unique Protein Chain and Resolution is 1.68.[13]

2.3 Molecular docking method

The ligands were prepared in. mol2 format from marvinsketch converted to pdbqt formatwith autodock 4.5.6 program. The main macromolecular structures were taken from the RSCB Protein Data Bank (PDB ID: 7AHA). Then the SARS-Cov-2-Mpro macromolecule was optimized and checked by the software package Swiss-PDB viewer (version 4.1.0) based on their lowest energy. Several important factors, such as improper bond order, side-chain geometry, and missing hydrogens, were observed in the crystal structure of proteases. The software package used to remove all the heteroatoms, water molecules, and inhibitors present in the structure is Autodock 4.5.6. Finally, the non-bond interaction of phytochemicals-Macromolecule was calculated using the Autodock Vina software package for the docking analysis. The molecular docking result is binding energy values (kcal/mol).[17]

2.4 Validation Docking Method, Virtual Prediction Analysis and Visualization

The validation of the docking method using Mpro macromolecules (PDB ID: 7AHA) byre-validating the original ligand (succinic acid) from the macromolecules. Then the ligand was released from the docking bond with the Sars-Cov-2-MPro macromolecule, then redocking again with SARS-Cov-2-MPro macromolecules. A good result will show an RMSD value <2.5. Protein-ligand complexes from the docking step were analyzed and visualized using Discovery Studio 2020. The site of interaction was analyzed based on ligand-macromolecule interaction and structural conformation. The binding energy from the ligand-receptor will be used to compare secondary metabolites Blue Butterfly Pea Flower to be docked.[18]

3. RESULT AND DISCUSSION

3.1 Validation and Visualization docking method original ligand-receptor

Validation of the docking method MPro receptor (PDB ID: 7AHA) with original ligand, namely succinic acid, is using a grid box size of 14x14x14 and grid center x (7.027), y (-2.204), z (20.039). The result shown in Figure 6 is visualization revalidation Succinic Acid-Mpro with an RMSD value is 2.32. In Figure 6, the blue-red chain represents the Redocked Ligand, while the gray-pink chain is the Original Ligand. The binding energy value of interaction Succinic Acid-Mpro is -4.2 kcal/mol. Figure 7 shows an amino acid interaction Mpro receptor with Succinic Acid.

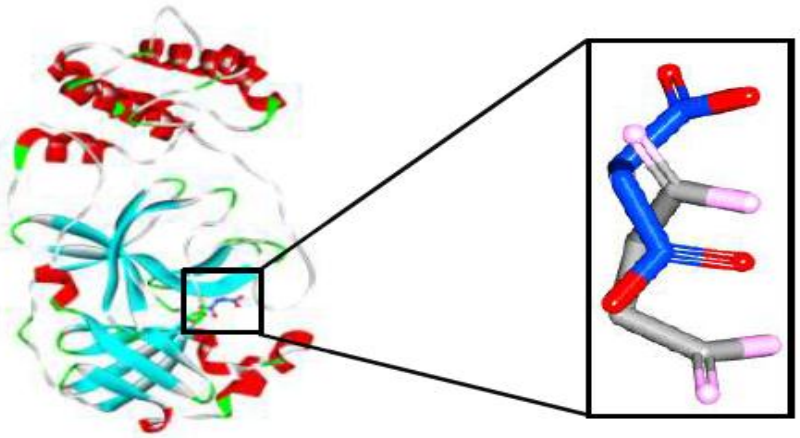


Fig. 6: Receptor-Structure Visualization, Original Ligand with Redocked Ligand.

Based on figure 7, the interaction of Succinic Acid and Mpro is Conventional HydrogenBond and Attractive Charge (Electrostatic). Hydrogen bond interactions occur in amino acids such as ASN142, SER 144, CYS 145, GLU 166. And Electrostatic interaction in amino acid HIS 163. Hydrogen bonding is an intermolecular or dipole-dipole attraction between two partial

electric charges of opposite polarity. The intermolecular forces resulting from the hydrogen bonds are strong. It indicates that the ligand-receptor bonds are vital.[19]

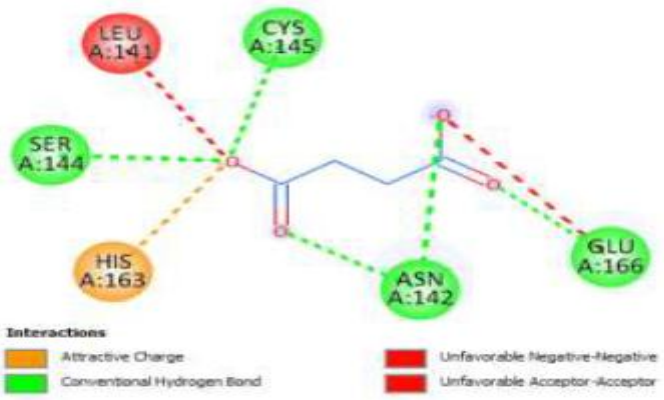


Fig. 7: 2D Visualization Molecular Docking Result of Succinic Acid with Mpro.

Electrostatic bonding is a chemical bond in which one atom loses electrons to form positive ions and the other atom gains electrons to form negative ions. Electrostatic interactionsare the key to determining the orientation of the intermolecular structures adsorbed on the wall surface.[20]

3.2 Drug-likeness Properties of Secondary metabolites Blue Butterfly Pea Flower (Clitoria ternatea L.)

The initial stage of this research is the analysis of druglikeness properties of the secondary metabolites of the Blue Butterfly Pea Flower as many as 44 compounds. The results of the druglikenes study are shown in table 1.

Table 1: The result of druglikeness properties of secondary metabolites blue butterfly pea flower.

No.	Primary Metabolit	Secondary Metabolite	MW[1]	LogP[2]	RB[3]	HBA[4]	HBD[5]	TPSA[6]	Lipinski
1	Flavonols glycoside	Kaempferol	286	2	1	6	4	117	Yes, 0 violation
2		Kaempferol 3-robinoside-7-rhamnoside (robinin)	741	-3	8	19	11	293	No, 4 violation: MW>500, HBA>10, HBD>5, TPSA>140
3		Kaempferol 3-glucoside	448	-0,2	4	11	7	179	No, 3 violation:HBA>10, HBD>5, TPSA>140
4		Kaempferol 3-rutinoside	595	-1	6	15	9	236	No, 4 violation: MW>500, HBA>10, HBD>5, TPSA>140
5		Kaempferol 3-neohesperidoside	595	-1	6	15	9	236	No, 4 violation: MW>500, HBA>10, HBD>5, TPSA>140

No.	Primary Metabolit	Secondary Metabolite	MW[1]	LogP[2]	RB[3]	HBA[4]	HBD[5]	TP SA[6]	Lipinski
6		Kaempferol-3-*O*-rhamnosyl-(1 → 2)-*O*-[rhamnosyl-1(1 → 6)]glucoside	757	-3	8	20	12	298	No, 4 violation: MW>500, HBA>10, HBD>5, TPSA>140
7		Kaempferol 3-*O*-(2″-*O*-*a*-rhamnosyl-6″-*O*-malonyl)-*b*-glucoside	695	-1	11	17	9	275	No, 4 violation: MW>500, HBA>10, HBD>5, TPSA>140
8		Kaempferol 3-(2^{G}-rhamnosylrutinoside)	773	-3	8	21	13	303	No, 4 violation: MW>500, HBA>10, HBD>5, TPSA>140
9		Quercetin	302	2	1	7	5	122	Yes, 0 violation
10		Quercetin 3-glucoside	463	-1	4	12	7	184	No, 3 violation: HBA>10, HBD>5, TPSA>140
11		Quercetin 3-*O*-(2″-*O*-*a*-rhamnosyl-6″-*O*-malonyl)-*b*-glucoside	711	-2	11	18	10	280	No, 4 violation: MW>500, HBA>10, HBD>5, TPSA>140
12		Quercetin 3-rutinoside;	611	-2	6	16	10	241	No, 4 violation: MW>500, HBA>10, HBD>5, TPSA>140
13		Quercetin 3-(2G-rhamnosylrutinoside)	775	-3,7	8	20	12	303	No, 4 violation: MW>500, HBA>10, HBD>5, TPSA>140
14		Quercetin 3-neohesperidoside	611	-2	6	16	10	241	No, 4 violation: MW>500, HBA>10, HBD>5, TPSA>140
15		Quercetin 3-glucoside	463	-1	4	12	7	184	No, 3 violation: HBA>10, HBD>5, TPSA>140
16		Myrcetin	318	2	1	8	6	127	yes, 1 violation: HBD>5
17		Myricetin 3-neohesperidoside	627	-2	6	17	11	246	No, 4 violation: MW>500, HBA>10, HBD>5, TPSA>140
18		Myricetin 3-(2G-rhamnosylrutinoside)	757	-3	8	20	12	298	No, 4 violation: MW>500, HBA>10, HBD>5, TPSA>140
19		Myricetin 3-*O*-(2″,6″-di-*O*-*a*-rhamnosyl)-β-glucoside	773	-31	8	21	13	303	No, 4 violation: MW>500, HBA>10, HBD>5, TPSA>140
20		Myricetin 3-glucoside	480	-0,8	4	13	9	189	No, 3 violation: HBA>10, HBD>5, TPSA>140
21		Myricetin 3-rutinoside	627	-2	6	17	11	246	No, 4 violation: MW>500, HBA>10, HBD>5, TPSA>140
22	Anthocyanins	ternatin A1 (mayor)	2.109	-7	37	51	27	839	No, 5 violation: MW>500, RB>15, HBA>10, HBD>5, TPSA>140
23		ternatin A2 (mayor)	1.801	-7	31	44	24	714	No, 5 violation: MW>500, RB>15, HBA>10, HBD>5, TPSA>140
24		ternatin A3	1.492	-6	25	37	21	590	No, 5 violation: MW>500, RB>15, HBA>10, HBD>5, TPSA>140
25		ternatin B1 (mayor)	1.855	-6	33	45	23	737	No, 5 violation: MW>500, RB>15, HBA>10, HBD>5, TPSA>140
26		ternatin B2 (mayor)	1.638	-4	28	39	21	652	No, 5 violation: MW>500, RB>15, HBA>10, HBD>5, TPSA>140

No.	Primary Metabolit	Secondary Metabolite	MW[1]	LogP[2]	RB[3]	HBA[4]	HBD[5]	TPSA[6]	Lipinski
27		ternatin B4	1.326	-232	22	30	16	531	No, 5 violation: MW>500, RB>15, HBA>10, HBD>5, TPSA>140
28		ternatin D1	1.785	-3	31	40	21	715	No, 5 violation: MW>500, RB>15, HBA>10, HBD>5, TPSA>140
29		ternatin D3	1.168	-1	19	27	15	466	No, 5 violation: MW>500, RB>15, HBA>10, HBD>5, TPSA>140
30		Preternatin A3	1.406	-6	22	35	21	557	No, 5 violation: MW>500, RB>15, HBA>10, HBD>5, TPSA>140
31		Preternatin C4	1.098	-6	16	28	18	433	No, 5 violation: MW>500, RB>15, HBA>10, HBD>5, TPSA>140
32		Delphinidin 3-*O*-(2″-*O*-*a*-rhamnosyl-6″-*O*-malonyl)-β-glucoside	698	-16	10	18	11	274	No, 4 violation: MW>500, HBA>10, HBD>5, TPSA>140
33		Delphinidin 3-(6″-malonyl) glucoside	551	0,11	7	13	9	217	No, 3 violation: MW>500, HBA>10, HBD>5, TPSA>140
34		Delphinidin 3-neohesperidoside	596	-0,8	6	14	10	237	No, 4 violation: MW>500, HBA>10, HBD>5, TPSA>140
35		Delphinidin 3-glucoside	465	0,09	4	11	9	185	No, 3 violation: HBA>10, HBD>5, TPSA>140
36	Flavons	Scutellaria	446	0,14	4	10	6	178	No, 2 violation: HBD>5, TPSA>140
37		baicalein	270	3	1	5	3	113	Yes, 0 violation
38		luteolin	286	2	1	6	4	117	Yes, 0 violation
39		apigenin	270	3	1	5	3	113	Yes, 0 violation
40	Flavanols	epicatechin	290	2	1	6	5	120	Yes, 0 violation
41	terpenoid	taraxerol	427	8	0	1	1	192	No, 2 violation: LogP>5, TPSA>140
42		phytosterols	415	8	6	1	1	187	No, 2 violation: LogP>5, TPSA>140
43		α-tocopherols	431	9	12	2	1	193	No, 2 violation: LogP>5, TPSA>140
44		γ-tocopherols	417	9	12	2	1	186	No, 2 violation: LogP>5, TPSA>140

Table description

1. *MW: Molecular Weight (<500 Da)*
2. *LogP: Prediction Octanol-Water Partition Coefficient (<5)*
3. *RB: Rotable Bound (1-10)*
4. *HBA: Hydrogen Bond Acceptor (<10)*
5. *HBD: Hydrogen Bond Donor (<5)*
6. *TPSA: Topological Polar Surface Area (<140 Å)*

Compounds meet Lipinski's requirements

According to Lipinski Rules of Five, 7 druglikeness parameters include Molecular Weight, Prediction Octanol-Water Partition Coefficient, Rotable Bound, Hydrogen Bond Acceptor, Hydrogen Bond Donor, Topological Polar Surface Area at least 5 parameters to qualify the druglikeness properties. The analysis results showed that 7 compounds were prepared to druglikenes properties. The seven compounds are kaempferol; quercetin are included in flavonol glycosides; baicalein, luteolin, apigenin are included in flavones, and epicatechin are included in flavanols.

3.3 Toxicity Prediction of Seven Compound of Blue Butterfly Pea Flower

These Seven compound are analyzed for their toxicity properties in the next step—the toxicity prediction in table 2. Seven compounds showed that they qualify the toxicity requirements on ames toxicity, hERG I Inhibitor, hERG II Inhibitor, and hepatotoxicity parameters. So, the next step is docking the 7 compounds with the Mpro receptor.

Table 2: Toxicity prediction of 7 compound of blue butterfly pea flower.

No.	Secondary Metabolite	AMES toxicity	hERG I inhibitor	hERG II inhibitor	Hepato toxicity	MTD (log mg/kg/day)	LD50 (mol/kg)	LOAEL (log mg/kg_bw/day)
1	Myrcetin	No	No	No	No	0.51	2	3
2	Kaempferol	No	No	No	No	0.531	2	25,05
3	Quercetin	No	No	No	No	0.499	2	3
4	baicalein	No	No	No	No	0.498	2	3
5	luteolin	No	No	No	No	0.499	2	2
6	apigenin	No	No	No	No	0.328	2,45	2
7	epicatechin	No	No	No	No	0.438	2	2,5

3.4 Molecular Docking Ligand-Receptor

The activity parameter is the binding energy value in kcal/mol units obtained through the AutoDock Vina program. The lower the binding energy value, the stronger the bond between the drug and the receptor and the more stable the bond. Figure 8 shows the binding energy value molecular docking ligand-receptor 7 compound Blue Butterfly Pea Flower is a ligand docked with Mpro receptor.

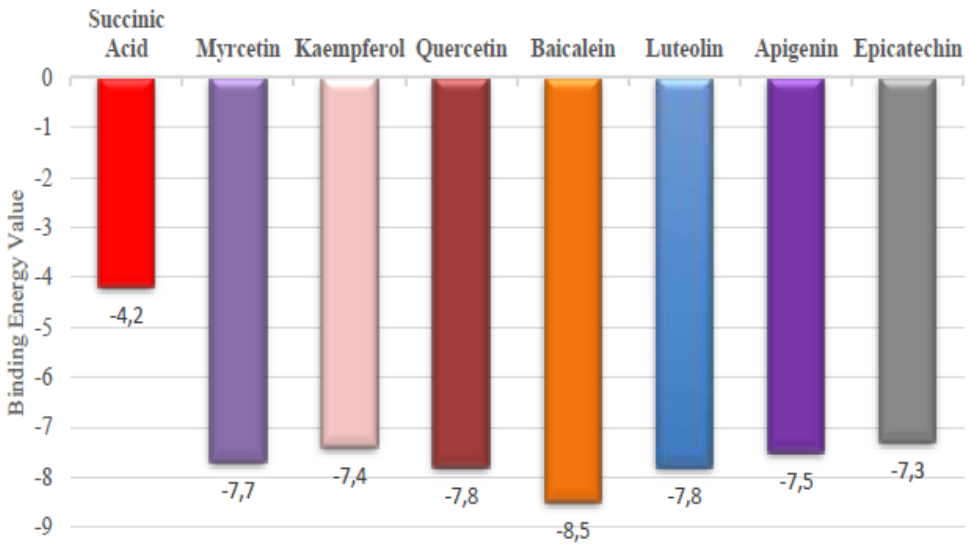


Fig. 8: Binding energy value of the native ligand, 7 secondary metabolites with the mpro receptor.

According to Figure 8, the binding energy value of 7 compound (Myricetin, Kaempferol, Quercetin, Baicalein, Luteolin, Apigenin) shows a better deal than the original ligand, succinic acid. The best binding energy value is baicalein, so the next step is to visualize the interaction between baicalein and the mpro receptor using Discovery Studio 2020.

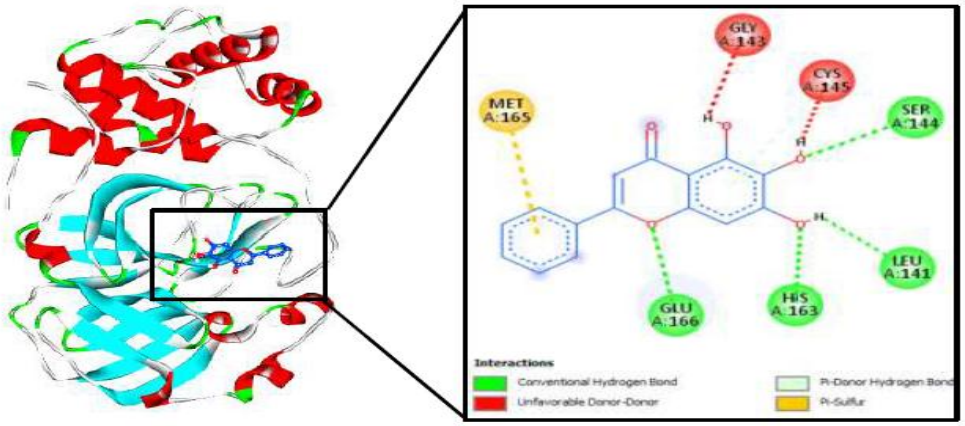


Fig. 9: Receptor-Structure Visualization, Baicalein-Mpro Interaction.

The visualization of the baicalein-Mpro interaction shows that the baicalein compound works in the active site of Sars-Cov-2-Mpro which is found in the amino acid LEU141, SER 144, HIS163, GLU 166, and CYS 145 like in figure 9. The type of interaction for the 5 aminoacids is a hydrogen bond. Compared with the succinic acid-Mpro interaction, hydrogen bondsoccur at the active amino acid sites such as ASN142, SER 144, CYS 145, GLU 166. At the same time, baicalein acts on the same 3 amino acids, namely SER 144, CYS 145, GLU 166. This

causes the baicalein value to be stronger and more stable than succinic acid, indicating that the baicalein bond with the receptor is stronger and more stable than succinic acid, so it can be used as a target for herbal treatment for COVID-19.

4. CONCLUSIONS

The results showed that the secondary metabolites of the Blue Butterfly Flower, namely Myrcetin, Kaempferol, Quercetin, Baicalein, Luteolin, and Apigenin, had better binding energy values than the original ligand (succinic acid). The best secondary metabolite is baicalein, potentially for Sars-Cov-2 Main Protease Inhibitor. This result is an initial screening for potential compound, so continuing with in vitro and in vivo tests is necessary.

5. ACKNOWLEDGMENTS

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6. Conflicts of interest

Covid-19 is no longer a pandemic disease to be afraid of, some countries have made peace with COVID-19.

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