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In Silico Inhibitory Potential Of Xestoquinone From Sea Sponge Xestospongia Testudinaria Against Plasmodium Falciparum Plasmepsin Ii (Pfpm-Ii)

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Abstract

Plasmodium falciparum resistance to artemisinin derivatives is increasing in Southeast Asian countries, prompting researchers to search for alternative antimalarial drugs in natural resources.The discovery of the active compound xestoquinone in sea sponge (Xestospongia testudinaria) as a novel regiment for malaria parasites. The objective of this study is to explore and observe the inhibitory outcomet of xestoquinone in sea sponges (Xestospongia testudinaria) against PfPM-II based on in silico method. As one-shot experimental study research, this research used three predictive analysis tests includes of: predictive analysis of active compound content, predictive analysis of the active compound mechanism of action, and predictive analysis of absorption, distribution, metabolism, and excretion of active substances. The analysis test used the PASS prediction method, STITCH DB version 5.0, Autodock Vina, Ligplot 2.1, and the SWISS ADME webserver. Based on the PASS prediction method shows that xestoquinone has a lower antimalarial potential than artemisinin. Xestoquinone's "binding affinity" was almost the same as artemisinin. The path analysis prediction proved that the inhibitory effect of the active compound xestoquinone on sea sponge (Xestospongia testudinaria) against PfPM-II was not proven. ADME analysis showed that xestoquinone met Lipinski's criteria, but the toxicity test showed toxicity class 4 which was more toxic than artemisinin. In conclusion, this study has proven that xestoquinone has anti-malarial effects, but not inhibiting PfPM-II and has a higher toxicity than artemisinin.

Keywords: Malaria, sea sponge, xestoquinone, PfPM-II , in silico.

Introduction

Malaria is a mosquito borne illness due to blood apicomplexa, blood protozoa, Plasmodium sp (Prastiawan, 2019). Malaria was also one of the deadliest diseases in the world. Malaria infection was responsible for an estimated 405,000 deaths in 2018 and 93% mortality in the African continent, the rest are spread over two areas such as Southeast Asian, and East Mediterranean (WHO, 2019). In Indonesia, malaria is well known in various regions, especially in eastern Indonesia. The east territory of Indonesia (Papua Barat, Papua, and Nusa Tenggara Timur/NTT) had the highest incidence rates.(Kemenkes RI, 2014; Susilowati, 2018). In NTT Province, out of 90% of villages, almost 100% are malaria -endemic villages, especially in remote areas (Susilowati, 2018). Commonly, there are 5 species from Plasmodium's genus that can infect and cause malaria in humans. The infection from Plasmodium falciparum is the most dangerous, because it can cause complications of cerebral malaria, which often causes death (Alami et al., 2016).

Since 2004, ACT or Artemisinin-based Combination Therapy had been used to treat all types of malaria in Indonesia (Kinansi, Mayasari and Pratamawati, 2018). Artemesinin (qinghaosu) is made from the cinnamon plant Artemisia annua However, artemisinin resistance to P. falciparum began to emerge in Cambodia in 2008 and several neighboring countries in Southeast Asia can be said to be failing clinical treatment of malaria again (Duru, Witkowski and Ménard, 2016).

Indonesia is a maritime country that contains abundant marine biodiversity. The waters and coastal areas of Indonesia and its surroundings (Indo-Pacific Coral Triangle) are recognized as the center of world biodiversity (Jompa et al., 2019). Marine biotas in Indonesian waters is a natural wealth containing various types of novel compounds that are not found in terrestrial biotas. The health benefits of marine biotas are the basic ingredients for making cosmetics and medicines. One of the marine biotas that has the potential to be developed into health products is a sea sponge (Luissandy et al., 2017).

The marine sponge, Xestospongia testudinaria belongs to the genus Xestospongia, and to the Demospongiae class., which contains various secondary metabolites and also has several antimalarial alkaloids, anti-fungal alkaloids, cytotoxics, antimalarial quinones, and also antiplasmodial benzaldehyde (Murtihapsari et al., 2019). According to a previous study, Xestospongia testudinaria has the inhibitory effect on the development of P. falciparum with an inhibitor concentration 50 (IC50) <50 µg / mL, while the fractionation results <25 µg / mL (Murtihapsari et al., 2013). From those previous in vivo studies, active substance xestoquinone has been found as antimalarial compound inside of Xestospongia testudinaria.

Plasmepsin is a P. falciparum protease enzyme found in the erythrocyte stage that aids in the degradation of Hb-infected erythrocytes. (Kashyap et al., 2016). There are similarities between Plasmepsin I (PfPM-I) and Plasmepsin II (PfPM-II) Plasmepsin I and Plasmepsin II which share 73% of their sequence. The two enzymes hydrolyze Hb at different rates, with PfPM-II hydrolyzing Hb to globin three times faster than PfPM-II (Luker et al., 1996). PfPM-II has 2 catalytic aspartases, namely D34 and D214, it degrades hemoglobin (Hb) by proteolytic cutting. PfPM-II has been shown in numerous studies to be an effective therapeutic target for malaria treatment (Aberathne et al., 2008; Nasamu et al., 2020). Previously, in-vivo research had been carried out on the active substance xestoquinone as an antimalarial but not against PfPM-II but Pfnek-1 (protein kinase)(Laurent et al., 2006).

In silico method is needed to determine the inhibitory activity of xestoquinone substances inside of sea sponge (Xestospongia testudinaria) against the PfPM-II from P. falciparum, based on the Structure-Based Virtual Screening. Each tested-substance will bind virtually to the target molecule via binding software, which computationally models the ligand with the target for optimal physical and chemical properties (Wadood et al., 2013). The primary objective of the in silico method is to determine the inhibitory potential of xestoquinone against the development of P.falciparum as a first step in the exploration for a novel antimalarial drug.

Materials and Methods

The study methods used “a One-Shot Experimental Study’s design” by using in silico approach to analyse the potential of active compounds in sea sponges (Xestospongia testudinaria) against the PfPM-II. The research began on March 2020 until October 2020 which was carried out at the INBIO Biomolecular and Bioinformatics Laboratory, Lowokwaru, Malang, Indonesia.

The independent variable was the active compound from sea sponges (Xestospongia testudinaria), xestoquinone, meanwhile, the PfPM-II of P. falciparum was the dependent variable. The three-dimensional structure of 7- (4-chloro-2-hydroxyphenoxy)-4-methyl-2H-chromen-2-one from the INBIO Indonesia Laboratory Biomolecular and Bioinformatics Laboratory’s database was used as the control variable in this study.

Protein structure and ligand

PubChem's program database was used to be the source of the active compound, xestoquinone. PubChem itself is known as the largest database of chemical elements and compounds that can be accessed freely to identify specific molecular structures (Lagunin et al., 2020). This research also obtained a three-dimensional (3D) structure PfPM-II and three-dimensional structure of control variable from INBIO Indonesia Laboratory Biomolecular and Bioinformatics Laboratory's database.

Potential prediction of the compounds

The WAY2DRUG PASS prediction program will be used to assess the antimalarial potential of the compounds obtained. The results of the analysis will be measured using the Probability to be active value to assess the activity related to the potency of compound from sea sponge as Plasmodium's inhibitors. The determination of this value is done by comparing the structure of the sea sponge compounds inputted with compounds that have been proven to be anti-malarial. The thresholds for the predicted Pa are Pa> 0.7 and 0.5 <Pa <0.7. If Pa is more than 0.7, the compound is predicted to have high potential as antiprotozoa (Plasmodium), because it has a high similarity with compounds that have been proven to be anti-inflammatory (Parasuraman, 2011). However, if the Pa is higher than 0.5 but still lower than 0.7, then the compound has the potential as an antiprotozoa (Plasmodium), although its similarity to compounds that have been proven to be antiprotozoa (Plasmodium) is low (Rowe, 2014).

Prediction of the pathway

After searching the bioactivity of the compound from sea sponge, the pathway prediction is done using STITCH DB with a maximum prediction of 50 interactions. The STITCH DB Version 5.0 webserver with a P. falciparum model and confident medium was used to analyze all herbal compounds for their interaction with the target protein (Szklarczyk et al., 2016).

Molecular docking analysis

Autodock Vina was used in the PyRx 9.5 program to perform molecular docking analysis.. The target protein was the crystal structure of P. falciparum FabI forms a complex with NAD and the inhibitor 7- (4-Chloro-2-hydroxyphenoxy) -4-methyl-2H-chromen-2-one (PDB 4IGE Chain B). Meanwhile, the ligands used were artemisinin, xestoquinone and control ligands. Specific molecular docking was applied by mimicking the binding between the PfPM-II protein and inhibitor control. The presence of bound conformations, as well as the prediction of binding affinity, describes the strength of the interaction between the ligand and the receptor. The stronger the bond between the ligand and the receptor, the lower the binding affinity value. If the results of the tested bioactive compounds are close to the regulate scoring system, it is possible that the bioactive compounds have inhibitory action against the target molecule (Forli et al., 2016).

Prediction of Pharmakokinetic

The pharmacokinetic phase started with absorption (absorption), then spreads to all body tissues via the blood (distribution), then is metabolized in specific organs, particularly the liver (biotransformation), and finally the remainder or products of this metabolism are excreted (elimination) from the body (abbreviated to ADME). The SWISS ADME webserver was used in this study to forecast the active compound's ADME. The results of this prediction can be reviewed using the Lipinski parameter (Lipinski's five-point rule) has the following criteria:

1. Fewer than or equivalent to five hydrogen bond donors (total amount of nitrogen-hydrogen and oxygen-hydrogen bonds).

2. Fewer than or equivalent to ten hydrogen bond acceptors (all oxygen or nitrogen atoms); and finally,
3. Molecular weight ≤ 500 Da (Chagas et al., 2018).

The next step was to use blood-brain barrier (BBB) analysis to predict the ability of these compounds to cross the BBB. In addition, analysis of absorption in the human intestine (Human Intestinal Absorption / HIA) is also carried out to predict the pharmacokinetic activity of a compound, to determine its bioavailability in the absorption process in the small intestine (Radchenko et al., 2016).

Prediction of toxicity

The Pro-Tox program was used to predict toxicity. The quantitative parameter is the toxic dose or LD50 with a value in mg / kg body weight. LD50 is the deadly dose of a substance / compound, which is given at one time and can make 50% of the tested animal objects die after being exposed to the substance / compound (Rang & Hill, 2013). A substance / compound with an LD50 less than five mg/Kg is considered very poisonous, whereas a substance with an LD50 greater than 15,000 mg/Kg is considered relatively safe (Loomis & Hayes, 1996; Erhirhie et al., 2018). Toxic can be classified as the following:

1. If the LD50 is fewer than or equivalent to 5 mg/kg, it is defined as enormously poisonous.
2. If the LD50 ranging from 5 to 50 mg/kg, it is defined as as highly poisonous
3. If the LD50 ranging from 50 to 500 mg/kg, it is defined as as moderately poisonous
4. If the LD50 ranging from 500 to 5000 mg/kg, it is defined as slightly poisonous
5. If the LD 50 ranging from 5000 to 15,000 mg/kg, it is defined as nearly non-poisonous
6. If the LD 50 greater than or equivalent to 15,000 mg/kg, it is defined as relatively harmless

Result and Discussion

Active compounds in sea sponge (Xestospongia testudinaria) and its potential prediction

Sea sponge (Xestospongia testudinaria) contains of various active compounds, including: sapinofuranone A, sapinofuranone B and xestoquinone (Table 1).

Table 1. Antiparasitic-Antiprotozoal/Antiplasmodial Prediction Results

NO	COMPOUND	Antiprotozoal (Antiplasmodial) (Pa)	Antiparasitic (Pa)
1	Sapinofuranone A	none	0.642
2	Sapinofuranone B	none	0.642
3	Xestoquinone	0.284	none
4	Artemisinin	0.954	0.857

Sapinofuranone A and sapinofuranone B have no potential as antiprotozoal. However, they have potential as antiparasitic (Pa value <0.642), although not as high as artemisinin (Pa value <0.857). Meanwhile, the active compound xestoquinone from the sea sponge (Xestospongia testudinaria) has potential as an antiprotozoa, according to the outcomes of data study using the Pa value, although its value (Pa value <0.3) is not as high as the predicted artemisinin (Pa value <0.954).

Research on the antimalarial activity of the active substance xestoquinone against PfPM-II has not been available before, but based on previous in vitro studies with the bioassay fraction it appears that xestoquinone inhibits the PfNEK-1 protein kinase (P.falciparum NIMA-related kinase)(Laurent et al., 2006; Fattorusso and Scafati, 2009). The protein kinase PfNEK-1 has a role in cell's division (mitosis and meiosis), in the previous research on 2006 which was conducted by Laurent, xestoquinone has IC50 ±1 µM, which means that its inhibitory activity can be categorized as quite good. Xestoquinone also has inhibitory activity against PfPK5 (Plasmodium falciparum protein kinase 5) although it is lower, while in PfPK7 (P.falciparum atypical MEK-related protein kinase) and PfGSK-3 (P.falciparum glycogen synthase-3) has no inhibitory activity of xestoquinone (Laurent et al., 2006). This conforms to Table 1's Pa value of 0.284., which shows the potential of xestoquinone as an antiprotozoa (antiplasmodium), although it still does not outperform the artemisinin Pa value of 0.954.

Sapinofuranone A and sapinofuranone B have the same potential as antiparasitic, namely 0.642. Although the results are not as high as the Pa value of artemisinin which reaches 0.857. These results indicate the hidden potential of sapinofuranone A and sapinofuranone B and need further research, because there is no research that has examined the potential of these two compounds as antiparasitic. Antiparasitic drugs are drugs which used to treat infestations caused by helminths, protozoa and other ectoparasites (Kuhlmann & Fleckenstein, 2017). There are several classes of antiparasitic drugs depending of the drugs target, in this case, these active compounds potency might have been more effective to helminths or ectoparasites.

Therefore, from the results of the potential test for the active compound xestoquinone in Table 1, xestoquinone has the potential to be an antimalarial, even though its values aren't as significant as artemisinin's.

Pathway prediction for active compounds against PfPM-II

This analysis used the STITCH DB program with a maximum prediction of 50 interactions, for visualizing the interaction pathway xestoquinone from sea sponge (Xestospongia testudinaria) against PfPM-II which can be seen in Figure 1. The results from Figure 1 has shown no interaction between the active substance xestoquinone from the sea sponge (Xestospongia testudinaria) and the PfPM-II. The analysis could not be carried out because the research with this target has not been done much on these compounds and affecting the STITCH DB program, which its mechanism is mainly depended with the previous datas and researchs (Szklarczyk et al., 2016).

Overall, this test's result has shown us that neither xestoquinone nor artemisinin have any interaction with PfPM-II, which means the mechanism of action (pathway) is still unknown. This result might be occur because the target receptor for xestoquinone is more precisely PfNEK-1(Laurent et al., 2006; Fattorusso & Taglialatela-Scafati, 2009). Previous research has identified endoplasmic reticulum Ca2 + -ATPase (SERCA) and P.falciparum ATPase6/PfATP6 as the artemisinin receptor target (Yuan et al., 2017). Therefore, although xestoquinone can damage the erythrocyte cycle of plasmodium, as can be seen in aspartate protein inhibitor (API), xestoquinone cannot inhibit PfPM-II.

Although there is no interaction has been found, molecular docking can still be done. Regarding the previous research, it has been found that the active compounds from sea sponge (Xestospongia testudinaria) has the potential to be anti-malarial(Murtihapsari & Chasanah, 2010; Murtihapsari et al., 2013, 2018). In addition to analyze the predictive mechanism of action (pathway) the active compounds from sea sponge (Xestospongia testudinaria) against PfPM-II, a molecular docking approach was also used in this research.

Molecular docking is performed after carrying out the pathway prediction test. It shows the strong interaction between the receptor and the ligand, where the negative value, the stronger the interaction (Meng et al., 2012). Furthermore, the closer the value to the control value, it means that the inhibitory's potential of the compound against the target protein is higher. The results of the molecular docking test for xestoquinone (-8.6), artemisinin (-7.2) with control (-8.5) (Table 2).

Based on the Table 3, the active compound xestoquinone has 5 hydrophobic bonds in the same amino acid residue as the control-comparison. The combination of the results of the LigPlot analysis (Table 2) and the prediction of binding affinity (Table 3) shows that xestoquinone has the strongest bond with PfPM-II. The binding affinity value of xestoquinone is the highest, indicating xestoquinone's binding to PfPM-II is stronger than artemisinin and control compounds' bindings to PfPM-II.

The most negative value was also held by xestoquinone and even higher than the control's and artemisinin's values. Therefore, from all those samples, xestoquinone has the largest chance to inhibit PfPM-II because of its interaction with PfPM-II is the strongest. This result was due to xestoquinone having many residues in common with the controls.

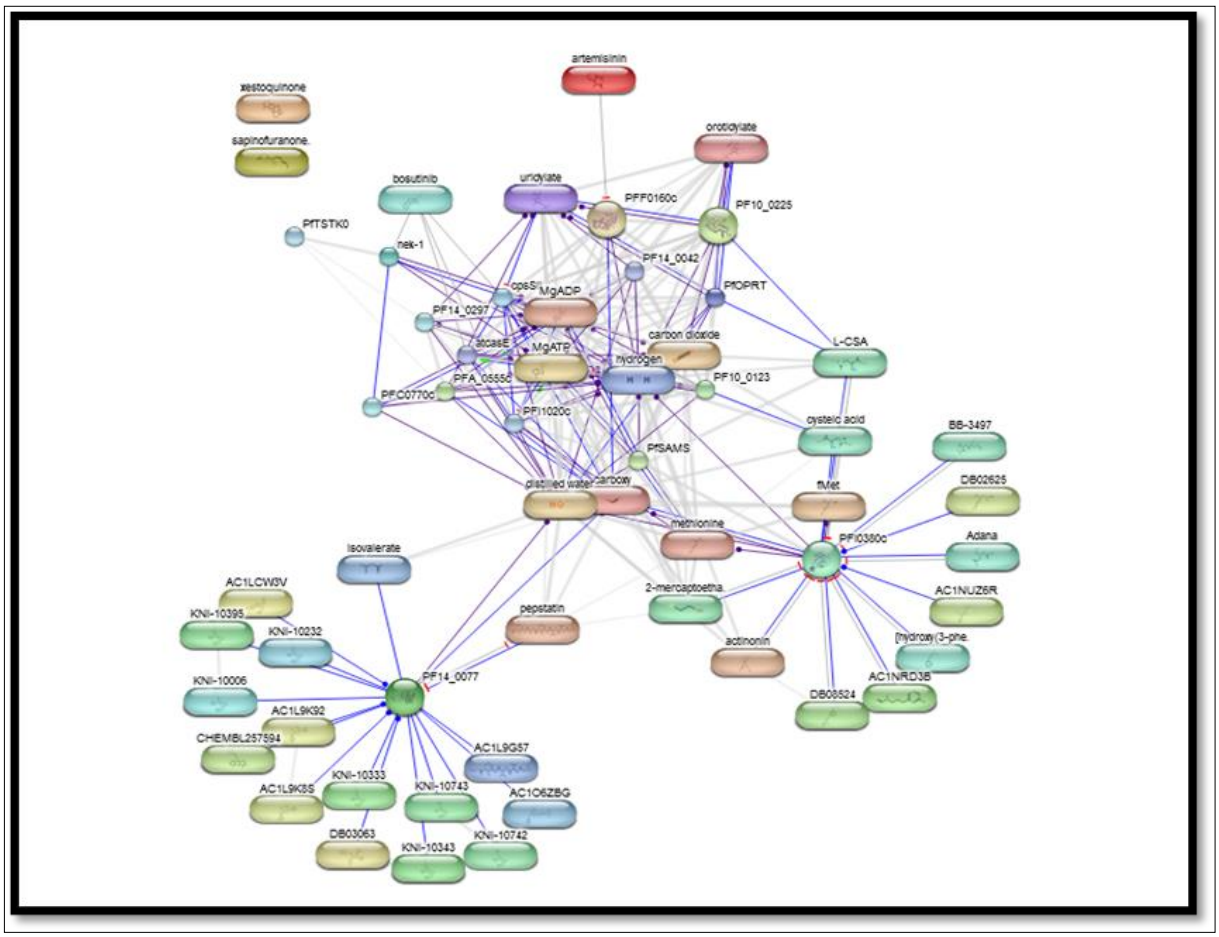

Figure 1. Prediction of Pathway Active Substance of Xestospongia testudinaria Marine Sponge Against Plasmepsin II (PfPM-II)

Table 2. The Active Substance of Xestospongia testudinaria Against Plasmepsin II (PfPM-II by Molecular Docking Prediction Assay

Compound	Scor Binding affinity (kcal.mol-1)	Visualizing colors
Xestoquinone	-8.6	Pink
Artemisinin	-7.2	Yellow
Kontrol	-8.5	Red

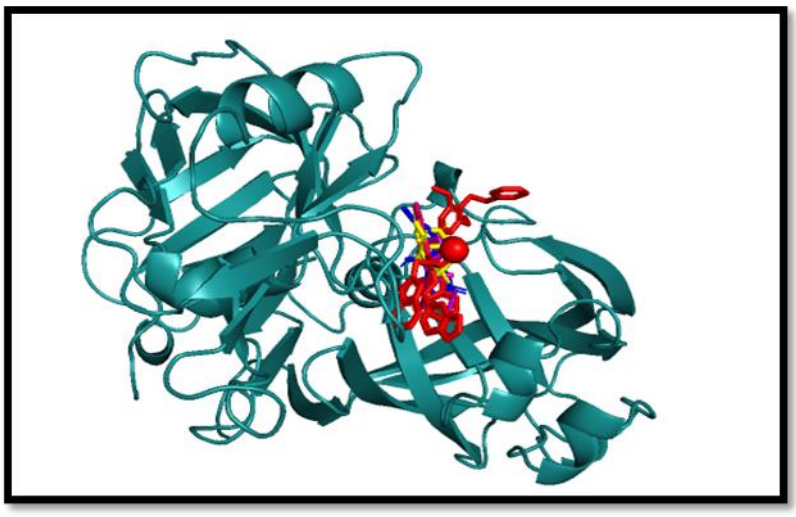

Figure 2. Visualization of molecular docking results between PfPM-II with the best ligand (color indicators are in table 2)

Prediction of Pharmakokinetic

Predictive analysis of farmakokinetics using The SWISS ADME program and the Lipinski parameter (Table 3).

Table 3. Visualization of LigPlot Results between PfPM-II and Ligands

Compound	Hydrophobic bond	Hydrogen bond
Control	THR114 ILE14 PHE111 ILE32 MET15 GLY216 ILE123 ASP34 PHE120 GLY36 ASN76 SER37 TYR77 TYR192	SER218 VAL78 SER79
Xestoquinone	TYR77 **ILE32 ASP34** ILE300 ASP214 GLY36 **VAL78 SER79 GLY216**	Not found

Note: The effect of the bold lettes is the identical amino acid residue that the control and comparison ligands share.

Table 4. ADME Prediction Results between PfPM-II and ligands

	Xestoquinone	**Artemisinin**
Molecular weight (g.mol-1)	318.32 g. mol-1	**282.33 g.mol-1**
Acceptors of Hydrogen Bond	4	**5**
Hydrogen Bond Donors	0	**0**
Atoms	24	**20**
Solubility in Water	Soluble Moderately	**Soluble**
Absorption in the Gastrointestinal Tract	High absorbtion	High absorbtion
Blood Brain Barier Permeant	+	**+**
Bioavailability	0.55	**0.55**

Predicting the toxicity of active compounds using the Pro-tox program to evaluate the toxicology category of a substance/compound/drug based on the chemical labeling classification, namely the globally harmonized system (GHS), with toxic's dose parameters / LD50, which its value is measured in mg / kgBW. From all those active compounds which have been tested, xestoquinone was a compound that met all the Lipinski criteria, had a fairly good solubility, had a high gastrointestinal (GI) absorption score and biovaibility, as well as "Hydrogen Bond Acceptors" that were almost close to artemisinin. Xestoquinone can penetrate the blood brain barrier and dissolves in water, which means it can be used as a medicine for cerebral malaria

Toxicity Prediction Results

Table 5. Toxicity Prediction Results

	Xestoquinone	Artemisinin
LD50 estimation (mg.kg-1 wt)	500	4.228
Toxicity estimation	Class 4	Class 5

The results of the toxicity prediction showed that xestoquinone has a lower Lethal Dose (LD50), which is far below artemisinin, which is 500 mg / kgBW. Xestoquinone belongs to toxic effects category 4, which means it is toxic if consumed (300<LD50≤2000). The result shows that xestoquinone is more toxic than artemisinin.

Conclusion

There is inhibitory potential of xestoquinone from the marine sponge (Xestospongia testudinaria) against the PfPM-II on Plasmodium falciparum through in silico method. Three active compounds which were obtained from the test, namely: xestoquinone, sapinofuranone A and sapinofuranone B. Using WAY2DRUG PASS prediction program, out of those three compounds, xestoquinone has the highest inhibitory potential against malaria, although the potency is still lower than artemisinin. Meanwhile, the prediction pathway of active compound xestoquinone in sea sponge (Xestospongia testudinaria) against PfPM-II is not yet known, because there is no interaction between xestoquinone from sea sponge (Xestospongia testudinaria) and PfPM-II. Lack of researchs and studies related to this receptor is the reason of the absence's of visualization in STITCH DB program. Even though the ADME test's score of xestoquinone is lower than artemisinin and more toxic than artemisinin, xestoquinone has fulfilled the Lipinski's criteria.

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