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Molecular docking of antioxidant activity of *bougainvillea spectabilis* wild bractea ethanol fraction with aryl hydro carbon inhibitors and vitamin C comparator

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ABSTRACT

Antioxidant compounds are increasingly being used in the health sector. In the health sector, antioxidant compounds also have a very important role. Antioxidant compounds have been scientifically proven to reduce the risk of chronic diseases, such as cancer, coronary heart disease, disorders such as redness, hyperpigmentation, wrinkles and premature aging. Antioxidant compound found in many plants, both bracteas, leaves and fruit, one of which is bracteas Bougainvillea spectabilis Willd. The ethanol fraction of bracteas Bougainvillea spectabillis Willd include: butil hydroxytoluene;ρ-Trimethylsilyloxyphenyl (tri.methylsilyloxy) trimethylsilylacrylate; 2,5 pyrrolidinedione, 1-methyl; Dimethylsulfoxonium formylmethylide; Formamide, N,N-dimethyl; Dimethyl sulfone and Cyclotetrasiloxane, octamethyl. Identified by the Liquid Chromatography - Mass Spectrometry method, then docked with Molegro Virtual Docker. Butyl hydroxytoluene has the same Root Mean Square Deviation value as vitamin C and smaller than the standard ligand BHF_401 (A), which can be seen from Rerank scores are - 76,0629, -76,1096, -105,0070. Hydrogen and electrostratic bonds in the ethanol fraction: Butyl hydroxytoluene with 3 amino acid residues including Tyr 334, Leu 292 and Leu 281, in the control Vitamin C with 9 amino acid residues Tyr 336, Leu 307, Val 308, As 306, Tyr 305, Gly 304, Gly 303, Phe 279 and His 275 with standard ligand BHF_401 (A) with 4 amino acid residues Val 316, Tyr 305, His 275 and Tyr 334. Butyl hydroxytoluenemore harmonious and stable and as a candidate for antioxidant drugs that have the same activity as vitamin C as a positive control.

Keywords: *Antioxidant*, Ethanol fraction, *Bougainvillea spectabilis* Wild, *Molecular docking*.

INTRODUCTION

Indonesia is a tropical country that is rich in sunshine throughout the year. Sunlight plays an important role for the life of all living things on earth. For humans, sunlight is useful for converting pro-vitamin D3 (7-dehydrocholesterol) into vitamin D for bone formation. But on the other hand, excessive exposure to sunlight can also cause unwanted effects such as premature aging, skin cancer and hyperpigmentation which can be prevented with antioxidants [1].

Antioxidants are needed by our body to overcome and prevent oxidative stress. [2] There are various original Indonesian natural ingredients that still contain a lot antioxidants with natural active ingredients in plants, namely polyphenolics compounds, carotenoids, vitamins, tocopherols, ascorbic acid, carotenoids, flavonoids and phenolic acids [3-4]. Antioxidants have pharmacological effects such as anticancer, antibacterial, anti-inflammatory, anti-aging and antiviral [5].

Antioxidants are very easily oxidized, so free radicals will oxidize antioxidants and protect other molecules from damage caused by oxidation by free radicals or reactive oxygen.[4] Antioxidants can suppress the formation and neutralize ROS, can slow down the oxidation process of free radicals by donating hydrogen atoms or protons to radical compounds so that they can inhibit the chain reaction of free radical formation and make radical compounds more stable and reduce oxidative stress damage.[6,7]

Currently, there are many sunscreen products with antioxidants on the market with various active ingredients and various mechanisms of action. The main principle of the mechanism of action of arylhydrocarbons as a regulator of metabolic enzymes is in the form of cytochrome P450 which functions in xenobiotic metabolic processes, has a role in regulating immunity, maintenance of stem cells undergoing cellular differentiation. Arylhydrocarbons can bind to several exogenous ligands such as *flavonoid*, *polyphenols* and *tanin*. The protective effect of flavonoids is by transferring electrons to free radicals, activating enzymatic antioxidants and inhibiting oxidases. Flavonoids can also function as antioxidants to capture free radicals, so that the melogenesis process is triggered by *Reactive Oxygen Species (*ROS) can be inhibited and neutralized. [8]

Previous reseach on cosmetics show that synthetic antioxidant ingredients have many side effects on the skin, for example, Butyl Hydroxy Anisol (BHA) and Butyl Hydroxy Toluene (BHT) in the long term and in large doses can cause irritation, burning, contact dermatitis, skin damage, even cultural cancer. [9] Therefore, it is necessary to develop safe antioxidant compounds by using antioxidants from nature, one of which is bracteas B*ougainvillea spectabilis* Willd. Based on previous research, it is said that the main ingredient is ethanol fraction of bracteas *Bougainvillea spectabilis* Willd seperti alkaloid, flavonoid, tannin, saponin, terpenoid. [10]

Drug development in the discovery of arylhydrocarbons inhibitors continues to increase. One of them is research on the compound content in the ethanol fraction of bracteas *Bougainvillea spectabilis* Willd. This could be a target for rational and effective drug design to seek arylhydrocarbons inhibitors as antioxidants in cosmetics. Currently, the molecular docking approach is widely used in modern drug design to help understand ligand-receptor interactions. Some literature shows that computational techniques can support and assist the design of compounds to obtain more potent inhibitors through the mechanism of ligand-receptor interaction.

Molecular docking is a key tool in computer-assisted molecular structural biology for the design of a drug ligand. The purpose of docking is to predict a model that binds ligands to proteins in a three-dimensional structure. [11] In this study, fast and flexible docking was used for the content of ethanol fraction compounds Bougainvillea spectabillis Willd by using Molegro Virtual Docker 2018. This research was conducted to understand the forms of interaction of the compounds in the ethanol fraction Bougainvillea spectabillis Willd against arylhydrocarbons enzymes compared to vitamin C compounds which have been proven as skin antioxidants.

MATERIALS AND METHODS

Design

The contents of the ethanol fraction compounds were identified by the LCMS method, then docked with Molegro Virtual Docker 2008 hardware and software (http://www.molegro.com) and (Sybilz) were used for molecular modeling with dual core processors. Molecular Structure and Structure Optimization of the main constituents of ethanol fraction Bougainvillea spectabilis Willd. The compounds contained in the ethanol fraction were drawn using the trial version of ChemBio Draw Ultra11.0 2008 (http://www.chemoffice.com) followed by its 3D shape.

The geometric isomer structure of the compound containing the ethanol fraction of bracteas *Bougainvillea spectabilis Willd* was further optimized using MM2 tools on Chembio 3D Ultra 11.0 trial version 2008. The protein structure of the arylhydrocarbons enzyme (PDB code 7VNH) was obtained from the Protein Data Bank.

Molecular docking

Molecular docking of compounds contained in bractea ethanol fraction *Bougainvillea spectabillis* Willd to the active site of the arylhydrocarbons enzyme was carried out using the Molegro Virtual Docker.5.0.0 docking modem and stored in the form of Sybil2 (http://www.molegro.com).This step is to look for a cavity with a detection algorithm to detect protein binding sites as potential areas on the active site to bind to drugs (ligands). The most stable form of the ligand was combined with MM3 to produce a conformational ligand pose consistent with the shape of the active site of the receptor protein (arylhydrocarbons enzyme). The energy-minimized pose candidate in the active site used a grid-based method to evaluate the protein-ligand energy interaction stored in Mol2. Docking was carried out with standard non-ligand settings on Molegro Virtual Docker with 5 cavities (volume 71.68). [12]

Assessment

The docked conformational forms were further printed using the different assessment functions available in [Sybil2]. The Molegro Virtual Docker algorithm uses an internal scoring function where the DockScore is used to select and again differentiate positions for each compound and estimate the energy of the interaction energy summing the ligand/protein and the internal energy of the ligand. Each purely minimized docking uses DockScore, molecular mechanics of the scoring functions used in this study such as Rerank Score, RMSD (Root Mean Standard Deviation) and Hydrogen Bond. [13]

RESULTS

Molecular modeling by docking compounds containing ethanol fraction *Bougainvillea spectabilis Willd* on the active site of the arylhydrocarbons enzymes is carried out through the program *Molegro Virtual Docker*. The results of molecular modeling (docking) in the form of a Dockscore are calculated from stable confirmations which indicate the binding affinity of the arylhydrocarbons enzyme inhibitor complexed with the ligands (compounds in the ethanol fraction *Bougainvillea spectabilis Willd* is bound to several amino acids, Including Gly 389, Val 391, Arg 321, Ser 329, Asp 306, Leu 307, Tyr 305, Gly 303 and Tyr 334. This can be seen in Figure 2 and Table2 namely the formation of hydrogen bonds between the arylhydrocarbons enzymes and content of bractea ethanol fraction *Bougainvillea spectabilis Willd* the more amino acids that are bound, especially in hydrogen bonds, the stronger the bond and the lower/stable energy score (Figure 1).[14]

Figure 1: Shows the chemical structure of the ethanol fraction of bracteas *Bougainvillea spectabilis* Willd, ligan and control positive a. *butil hydroxytoluene.* b. *ρ-Trimethylsilyloxyphenyl-(tri.methylsilyloxy)trimethylsilylacrylate*. c. 2, 5 *pyrrolidinedione, 1-methyl.* d. *Dimethylsulfoxonium formylmethylide.* e.*Formamide, N, N-dimethyl*. f.*Acetic acid.* g.*Dimethyl sulfone, h.Cyclotetrasiloxane, octamethyl.* i. *2-phenyl-4H benzo (h) chrome/ BHF_401 (A)* / standart ligan. j Vitamin C/ control positive

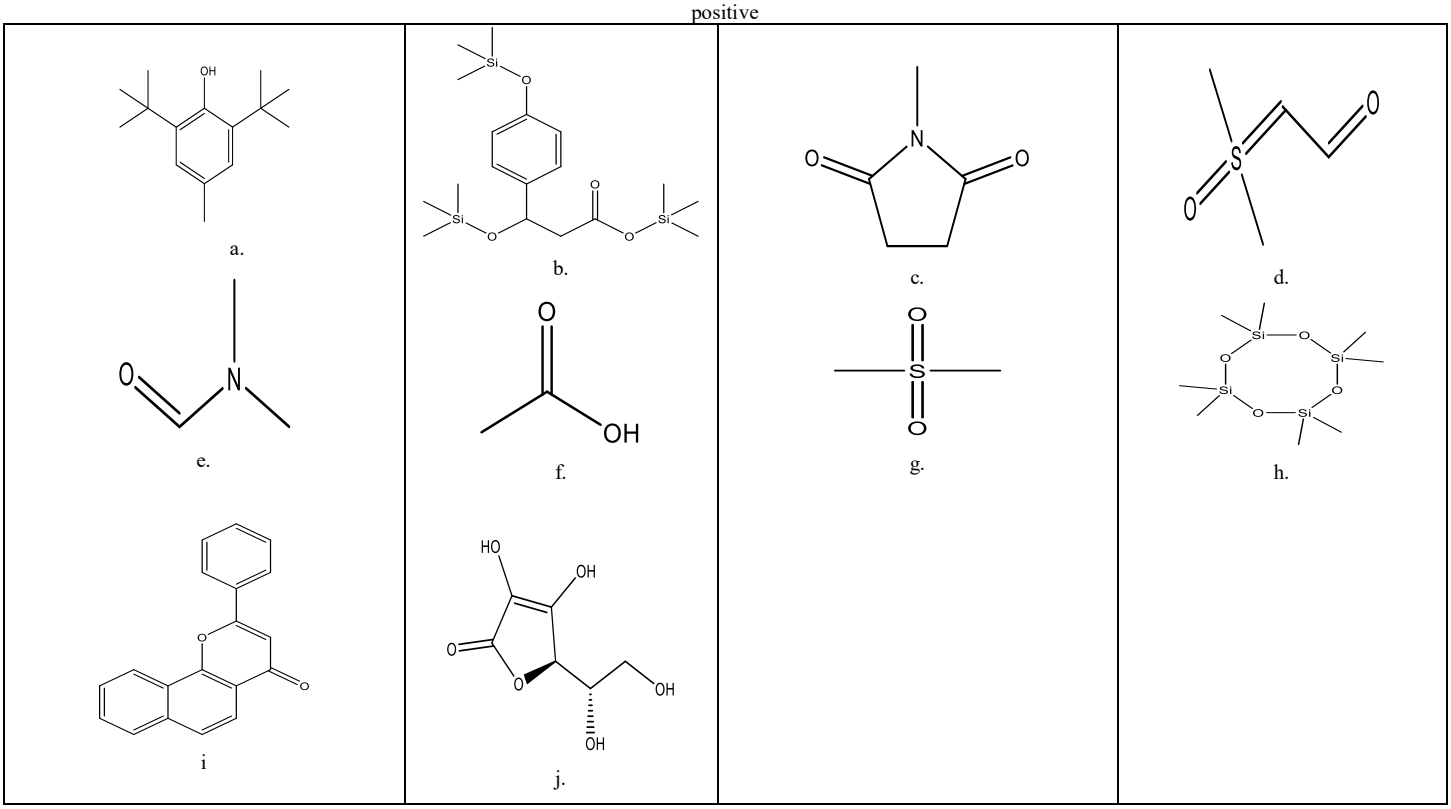
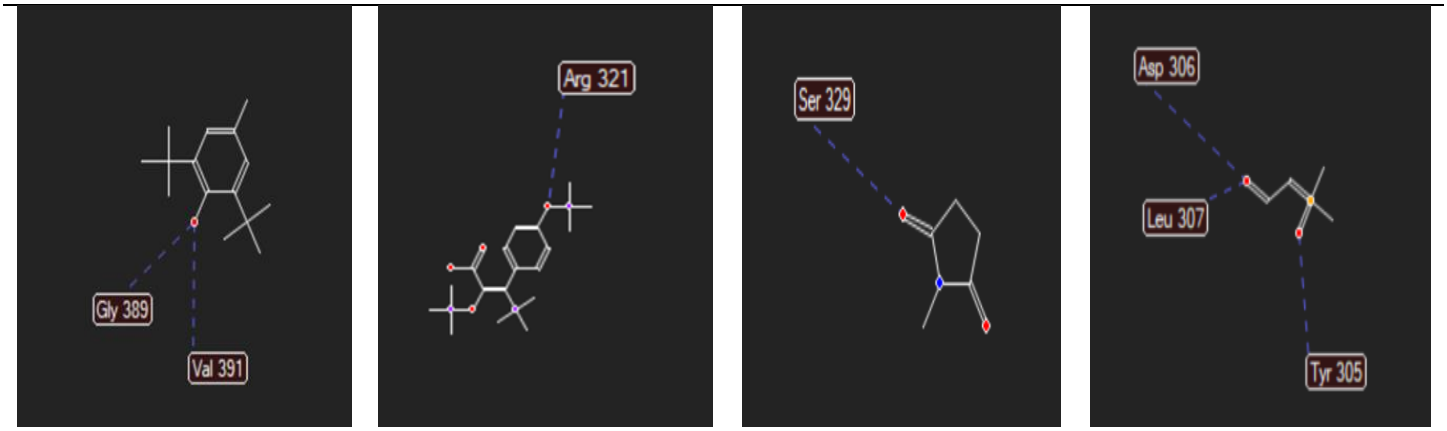
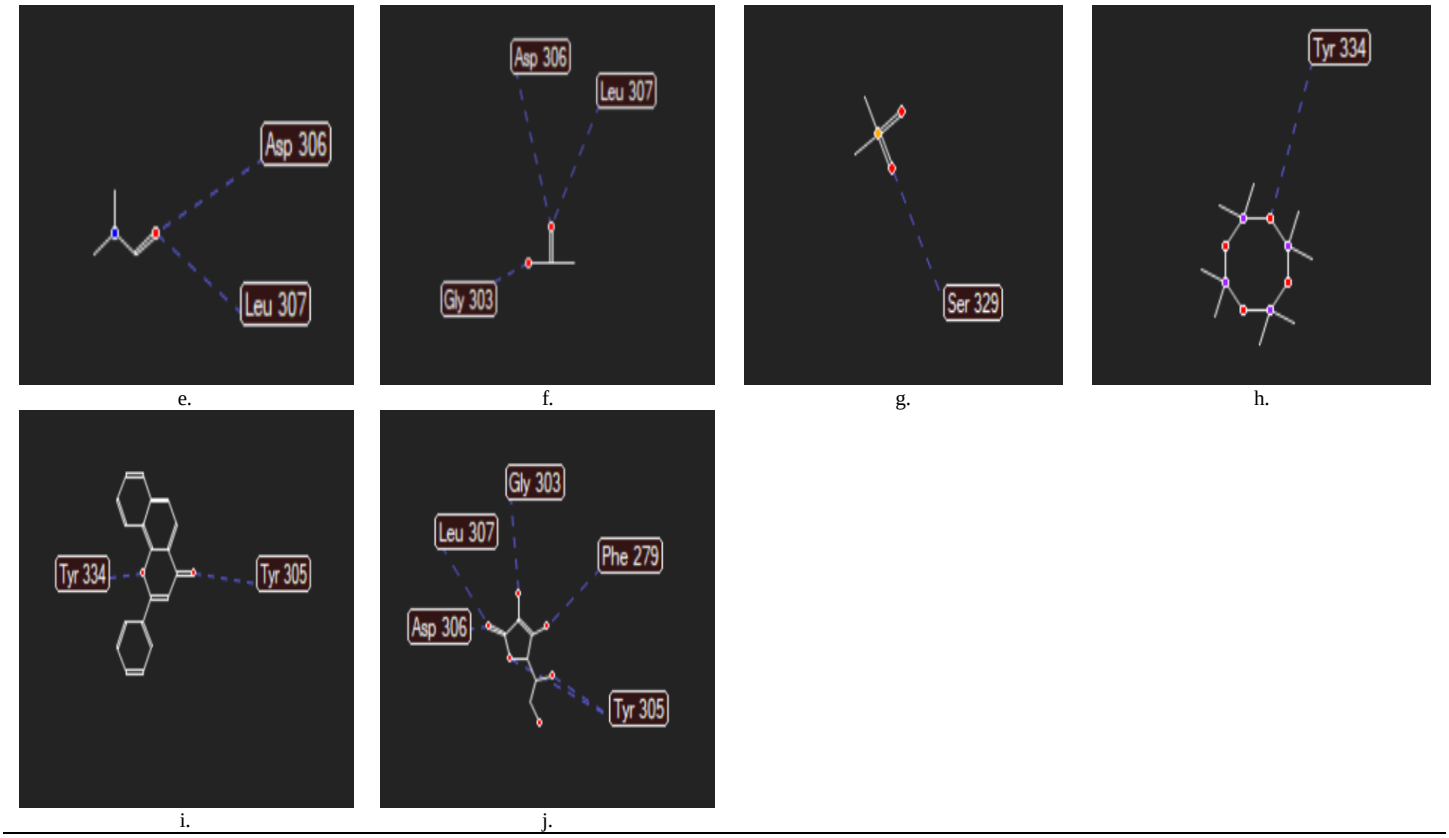


Figure 2: Interaction of hydrogen bonds from arylhydrocarbons enzymes that bind to the main constituent compounds of the ethanol fraction of bracteas *Bougainvillea spectabilis* Willd a. *Butil hydroxytoluene.* b. ρ-Trimethylsilyloxyphenyl-(tri.methylsilyloxy)trimethylsilylacrylate. c. 2,5pyrrolidinedione, 1-methyl. d. Dimethylsulfoxonium formylmethylide. e. Formamide, N, N-dimethyl. f. Acetic acid. g. Dimethyl sulfone. h.Cyclotetrasiloxane, octamethyl. i. 2-phenyl-4H benzo (h) chrome/ BHF_401 (A) (standart ligan). J Vitamin C/ control positive.





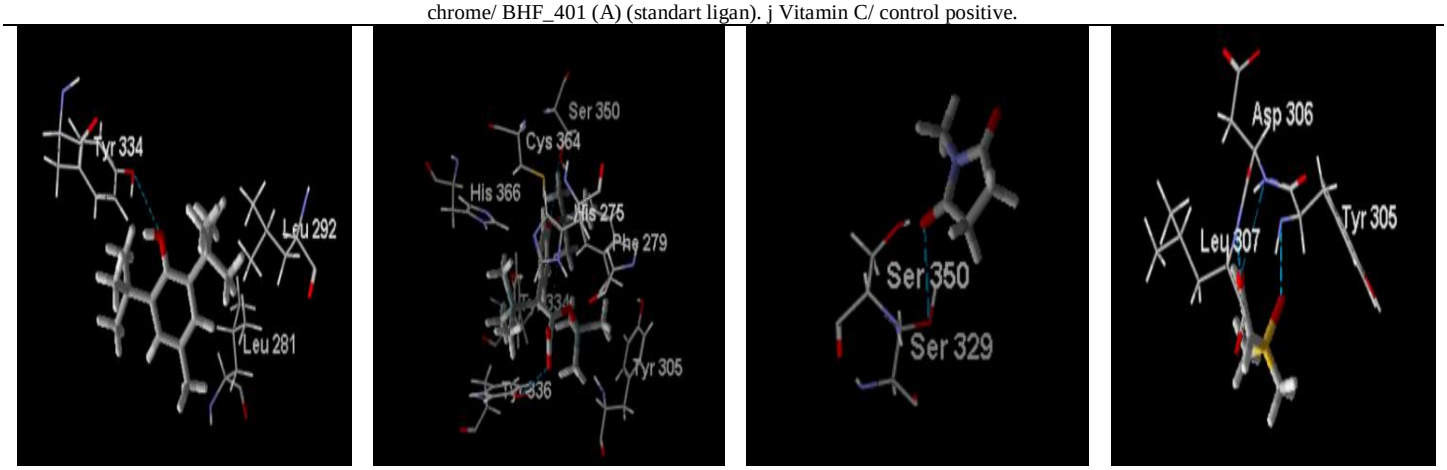
e. f. g. h.

i. j.

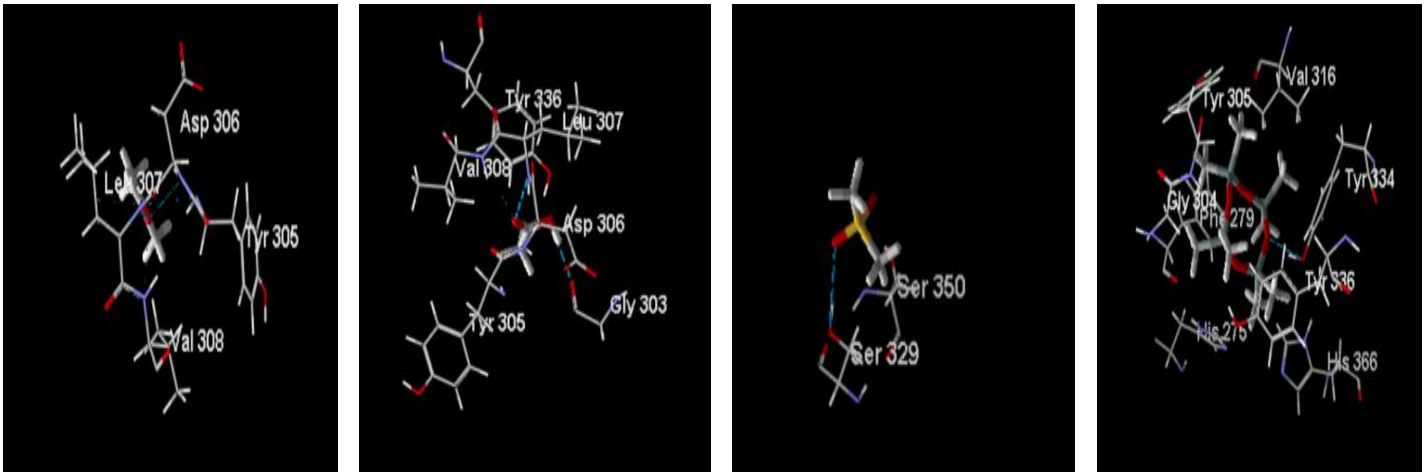
Table 1: Interaction of hydrogen bonds between ligands (the main constituents of ethanol fraction and arylhydrocarbons enzyme receptors

Nama Senyawa/ligand	Gly 389	Val 391	Arg 321	Ser 329	Asp 306	Leu 307	Tyr 305	Gly 303	Tyr 334
Butil hydroxytoluene	+	+	-	-	-	-	-	-	-
ρ-Trimethylsiloxyphenyl-(trimethylsilyloxy)trimethylsilylacrylate	-_	-	+	-	-	-	-	-	-
2,5 pyrrolidinedione, 1-methyl	-	-	-	+	-	-	-	-	-
Dimethylsulfoxonium formylmethylide	-	-	-	-	+	+	+	-	-
Formamide, N-N-dimethyl	-	-	-	-	+	+	-	-	-
Acetic acid	-	-	-	-	+	+	-	+	-
Dimethyl sulfone	-	-	-	+	-	-	-	-	-
Cyclotetrasiloxane, octamethyl	-	-	-	-	-	-	-	-	+
2-phenyl-4H benzo (h) chrome/ BHF_401(A) (ligand standart)	+	+	-	-	-	-	-	-	-
Vitamin C (control positive)	-	+	+	-	+	+	+	-	-

Figure 3: Interaction of bonds in organized positions (organized poses) between arylhydrocarbons enzymes that bind to the main compounds of the ethanol fraction Bougainvillea spectabilis Willd a. butil hydroxytoluene. b. ρ-Trimethylsilyl1`1`enyl-(tri.methylsilyloxy) trimethylsilylacrylate. c. 2, 5 pyrrolidinedione, 1-methyl. d. Dimethyl sulfoxonium formylmethylide. e.Formamide, N, N-dimethyl. f. Acetic acid. g.Dimethyl sulfone.h.Cyclotetrasiloxane, octamethyl. i. 2-phenyl-4H benzo (h) chrome/ BHF_401 (A) (standart ligan). j Vitamin C/ control positive.



a. b. c. d.



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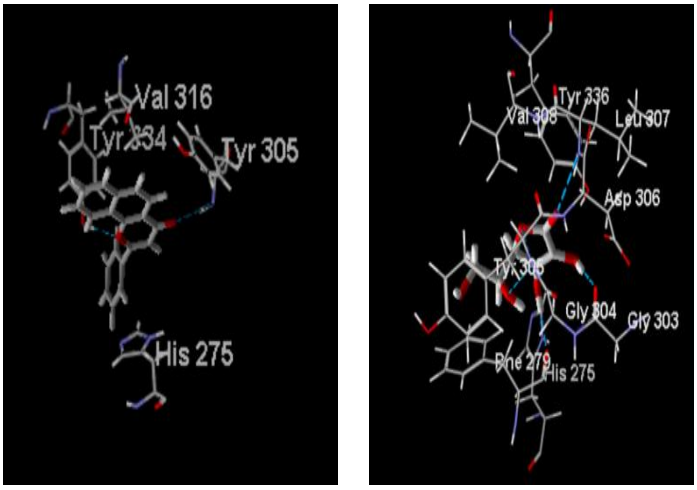

i. j.

Table 2: Parameters of docking Ligands (compounds in ethanol fraction of bracteas *Bougainvillea spectabilis* Willd on the active site of the arylhydrocarbons enzyme

Nama Senyawa/ligand	Moldock score	Rerank Score	H-Bond
2-phenyl-4H benzo (h) chrome /BHF_401 (A) (ligand standart)	-123,9690	-105,0070	- 4,29543
Vitamin C (control positive)	- 85,4414	- 76,1096	-13,1733
Butil hydroxytoluene	- 91,4690	- 76,0629	- 1,40741
ρ-Trimethylsiloxyphenyl-(trimethylsilyloxy)trimethylsilylacrylate	- 139,9410	- 74,3629	- 2,76319
2,5 pyrrolidinedione, 1-methyl	- 60,2398	- 52,8294	- 2,26814
Dimethylsulfoxonium formylmethylide	- 55,2651	- 46,9370	- 5,82504
Formamide, N-N-dimethyl	- 41,3910	- 37,1299	- 4,06958
Acetyc acid	- 41,5866	- 35,0870	- 6,40498
Dimethyl sulfone	- 37,6013	- 32,7519	- 1,69162
Cyclotetrasiloxane, octamethyl	- 88,9835	- 18,2322	- 2,5

DISCUSSION

The receptor structure used in this study is an arylhydrocarbons enzyme crystal with oxyreductase classification complexed with BHF_401 (A) substrate with a standard ligand inhibitor to provide information about the active site and the composition of the inhibitor binding space as well as the opportunity to use the protein enzyme in a functional conformation. *Butil hydroxytoluene* has the same rerank score value as the rerank score value for vitamin C, where as *ρ*-Trimethylsiloxypheny l(trimethylsilyloxy) trimethylsilylacrylate has a close rerank scorevitamin C control positive, mean while2,5 pyrrolidinedione, 1 methyl, Dimethylsulfoxonium formylmethylid, Acetyc acid, Dimethyl sulfone Cyclotetrasiloxane, octamethyl. The rerank score value is still below vitamin C and standard ligands, but has high antioxidant activity with a fairly high rerank score value (Table 2). Vitamin C has a bond of 5 amino acid residues including Val 391, Arg 321, Asp 306, Lue 307 and Tyr 305, while Acetic acid and Dimethylsulfoxonium formylmethylide each has 3 bonded amino acid residues Asp 306, Leu 307, Gly 303 and Asp 306, Leu 307, Tyr 305.

Standard ligands*BHF_401 (A)* has 2 binding amino acid residues Gly 389 and val 381, the same as the ligand *Butil hydroxytoluene* has 2 amino acid residues Gly 389 and val 391 and also a ligand *Formamide, N-N-dimethyl 2 Amino* acid residue bonds include Asp 306 and Leu 307, the more amino acid residue bonds the more stable the bond between the ligand and the receptor.

Docking method validation.

The validation stage is carried out to calibrate the docking

method compared between the native position of the ligand to the receptor that has been tested experimentally at the ligand binding site pocket. The basis used to provide a validity assessment is the RMSD value of less than 2, meaning that the position of the copy ligand after superimpose is closer to its position than that of the native ligand so that the method used will be more appropriate.[15] To ensure that the orientation of the ligand, the behavior in the ligand-receptor binding model and the position obtained from the docking study using the Molegro Virtual Docker program, the parameters of this docking method must first be validated by the structure of the receptor used, namely PDB code 7VNH, replicated 10 times each. Ligand BHF_401 (A) is code for a native ligand with arylhydrocarbon enzymes then the native ligands were fractioned and new ligands were added (the ethanol fraction compounds of interest *Bougainvillea spectabilis* Willd to the appropriate active site in binding to determine the ability of the new ligand to reproduce the orientation and position of the inhibitor on the crystal structure of the receptor in the cavity. The results of control docking show that with the program *Molegro Virtual Docker* the optimal orientation of the inhibitor determined in the native, namely BHF_401 (A) is located cavity 5 (volume 71.68) which is green in color and protein A (7VNH) in the arylhydrocarbons enzyme. Model interaction between the main ingredients of ethanol fraction *Bougainvillea spectabilis* Willd with arylhydrocarbons enzymes.

CONCLUSION

Butyl hydroxytoluene has the same RMSD value as vitamin C and smaller than the standard ligand BHF_401 (A), which can be seen from Rerank scores are - 76,0629, -76,1096, -105,0070. Hydrogen and electrostratic bonds in the ethanol fraction: Butyl hydroxytoluene with 3 amino acid residues including Tyr 334, Leu 292 and Leu 281, in the control Vitamin C with 9 amino acid residues Tyr 336, Leu 307, Val 308, As 306, Tyr 305, Gly 304, Gly 303, Phe 279 and His 275 with standard ligand BHF_401 (A) with 4 amino acid residues Val 316, Tyr 305, His 275 and Tyr 334 as an alternative for future drug development.

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Conflict of interests

Authors declears that thereis no conflicts of interests.

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