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RESEARCH ARTICLE

Antioxidant activity of *Bougainvillea spectabilis* ethanol extracts as an aryl hydrocarbon inhibitor

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Abstract

Background: Antioxidants overcome ageing processes and skin tissue damage caused by free radicals. The flavonoid contents of *Bougainvillea spectabilis* Willd contain hydroxyl groups attached to the carbon of the aromatic rings which makes them capable of catching free radicals and absorbing UV-A and UV-B. **Objective:** This study aimed to investigate the *in silico* antioxidant activities of various compounds contained in *B. spectabilis* Willd ethanol extracts compared to control. **Method:** The identification was carried out using GCMS methods and Molegro Virtual Docker. The molecular mechanics of scoring functions used in this study were the Rerank score, Root Mean Standard Deviation, and H Bond. **Result:** *p*-Trimethylsilyloxyphenyl-(trimethylsilyloxy)trimethylsilylacrylate had a better bond than vitamin C used as the control and is smaller than BHF_401 (A) standard ligand, indicated by rerank scores of -92.6478, -76.0593, and -104.771. **Conclusion:** *p*-Trimethylsilyloxyphenyl-(trimethylsilyloxy)trimethylsilylacrylate had a better and more stable bond (rerank score: -92.6478) than the positive vitamin C control (76.0593).

Introduction

Sunlight benefits human beings, especially because UV light contains vitamin D3, which is important in bone formulation because of phosphate and calcium metabolism processes. On the other hand, excessive sunlight exposure may cause negative undesired effects, such as premature ageing, skin cancer, and hyperpigmentation, which can be prevented by using antioxidants (Danimayostu *et al.*, 2023). Antioxidants reduce carcinogenesis caused by oxidative stress by directly cleaning the reacting oxygen species (ROS) (Afroz *et al.*, 2019). Free radicals easily oxidise antioxidants and protect other molecules from damage caused by free radicals or ROS (Werdhasari, 2014). Free radical formation chain reaction stabilises radical compounds and reduces oxidative stress-induced damage (Kumamoto *et al.*, 1986; Kanaze *et al.*, 2009;).

Previous studies on cosmetics found that the use of synthetic antioxidants such as butylated

hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) over a long period and in high doses had adverse effects, such as skin irritation, burning sensations, contact dermatitis, skin damage, and even cancer (Zhu and Gao, 2008). For this reason, a safe antioxidant compound originating from natural ingredients need to be developed, one of which is *Bougainvillea spectabilis* Willd found ethanol extracts are composed of main substances of alkaloids, flavonoids, tannins, saponin, and terpenoids (Vukovic *et al.*, 2013).

Drug developments in the discovery of aryl hydrocarbon receptor inhibitors continue to grow. Among them are studies on the contents of *Bougainvillea spectabilis* Willd ethanol extract. This can be an effective and rational target for drug design to find aryl hydrocarbon inhibitors for cosmetics. The main principle in binding ligands to proteins should consider aryl hydrocarbon receptors more precisely and efficiently. The structure of ligands is optimised by

minimising energy to reach stable and flexible conformation. This optimisation phase is important because the two-dimensional structure of the ligands is energetically unstable. The docking was carried out by regulating ligand-receptor binding on a Molegro Virtual Docker with five cavities (volume 71.68) (Thomsen & Christensen, 2006).

Methods

Design

This study used ethanol extract obtained through manufacturing using the maceration method for five days. Exactly 265 g of flower powder was dissolved in 1 L of 96% ethanol solvent, vacuumed to extract the juice, and then evaporated at a temperature of 40C to produce ethanol extracts. The resulting ethanol extract was examined using a GC-MS tool so that several chemical compounds contained in the ethanol extract as ligands were obtained and then tested *in silico* using the Molegro Virtual Docker 2013 application. 6.0.0 (<https://www.molegro.com>) and (sybil2) with an Intel(R) Core TM i5-115G7 processor.

Scoring

The installed conformation forms were moulded using different scoring functions available at (Sybil2). Molegro Virtual Docker algorithms used internal scoring functions where DockScore was used to select and re-differentiate the positions of each compound and estimate the amount of interaction energy by summing ligands/proteins and internal energy of the

ligands. Every docking was purely minimised using DockScore. The molecular mechanics of scoring functions used in this study were the Rerank score, RMSD (Root Mean Standard Deviation), and H Bond (Zheng *et al.*, 2011).

Novelty

From screening, several compounds with *in silico* antioxidant activity were obtained from *Bougainvillea spectabilis* Willd ethanol extract through GC-MS methods. The chemical contents of ethanol extract act as ligands that bind aryl hydrocarbon receptors. The bonds between ligands and drug receptors consist of hydrogen, steric, and electrostatic bonds.

Results

Table I shows the docking parameters of the ligands. This also includes the MolDock Scores, Rerank scores and the Hydrogen bond values. Figure 1 shows the chemical structures of the ethanol extracts of *Bougainvillea spectabilis* Willd, including the positive control structure. The formation of the exact hydrogen bond interactions of the aryl hydrocarbon receptors that bind to the main compounds of *Bougainvillea spectabilis* Willd ethanol extracts are shown in Figure 2. The main compounds include the five highest amino acid residues, namely Tyr 305, Phe 279, Gly 303, Asp 306 and Leu 307. Meanwhile, Figure 3 shows the organised positioning of the hydrogen bonds between the aryl hydrocarbon receptors and *Bougainvillea spectabilis* Willd ethanol extract contents.

Table I: The docking parameters of the ligands

Ligands/compounds	MolDock score	Rerank score	HBond
2-phenyl-4H benzo (h) chrome /BHF_401 (A) (standard ligand)	-123.707	-104.771	- 4.22333
<i>p</i> -Trimethylsilyloxyphenyl-(trimethylsilyloxy)trimethylsilylacrylate	-132.521	- 92.6478	- 0.16412
Vitamin C (control positive)	- 85.4436	- 76.0593	-13.1781
1,6-Octadien-3-ol,3,7-dimethyl	- 80.2863	- 70.3384	-2.93688
1 Heptanol,6-methyl	- 70.4765	- 58.5719	-2.88547
5-Hepten-2-one, 6-methyl	- 69.0572	- 58.3337	- 1.8645
3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl-, (R)-	- 62.5933	- 55.3509	- 2.5
Dimethylsulfoxonium formylmethylide	- 55.2239	- 46.8992	- 5.97048
L-Lactic acid	- 48.5215	- 44.2894	- 7.30632
Carbamite	- 47.9864	- 31.2267	- 5.97048
Formamide, N-N-dimethyl	- 41.3277	- 37.1401	- 4.14281
Formamide, N- methyl	- 41.6129	- 35.0292	- 6.31259

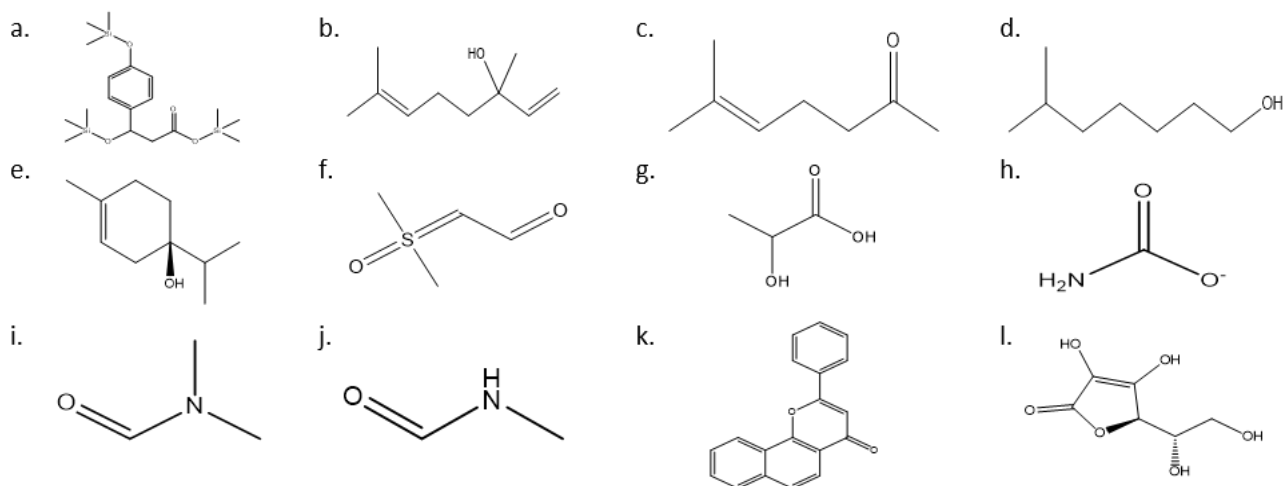


Figure 1: The chemical structure of the ethanol extract of *Bougainvillea spectabilis* Willd a. *p*-Trimethylsilyloxyphenyl-(trimethylsilyloxy)trimethylsilylacrylate, b. 1,6-Octadiene-3-ol,3,7-dimethyl, c. 5-Hepten-2-one 6-methyl, d. 1-Heptanol 6-methyl, e. 3-Cyclohexene-1-ol,4-methyl-1-(1-methylethyl)-, (R)-, f. Dimethylsulfoxonium formylmethylide, g. L-Lactidacid, h. Carbamate, i. N-N- dimethyl formamide, j. N-methyl formamide, k. 2-phenyl-4H-benzo(h)chromen-4-one, l. Vitamin C

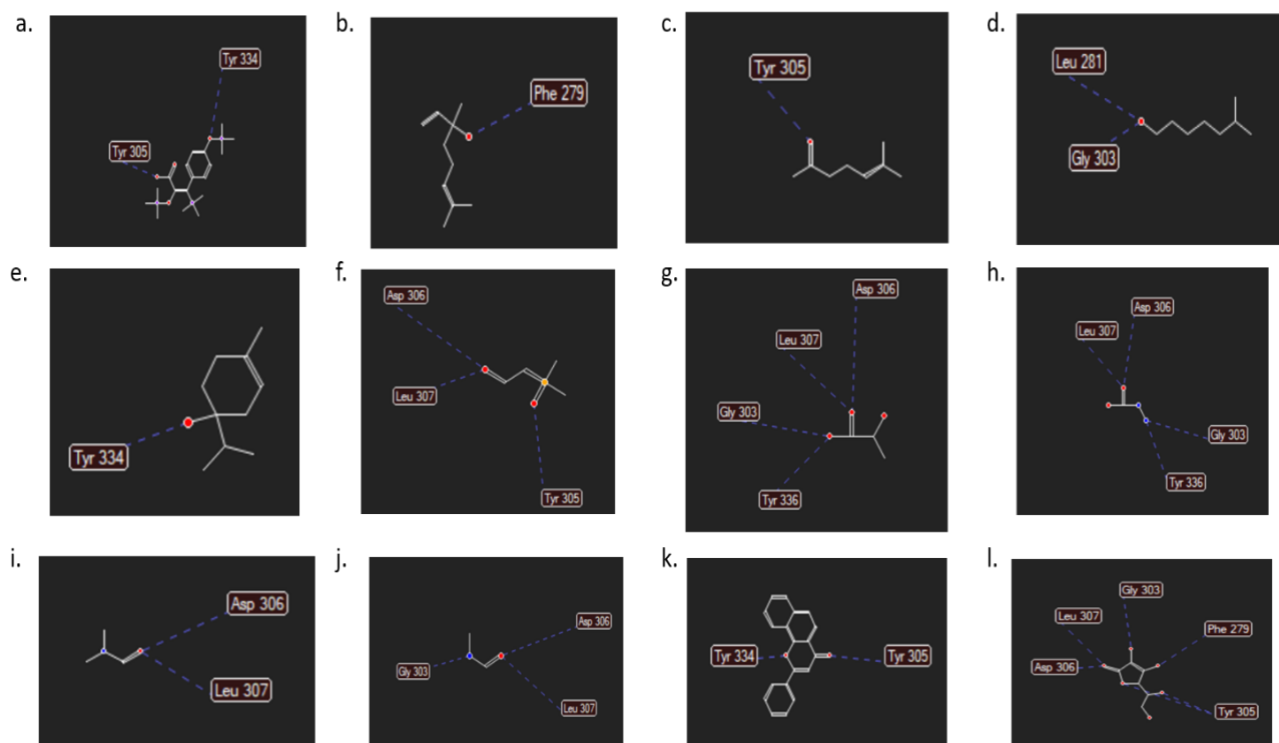


Figure 2: Hydrogen bond interactions of aryl hydrocarbon receptors that bind to the main compounds of *Bougainvillea spectabilis* Willd ethanol extract a. *p*-Trimethylsilyloxyphenyl-(trimethylsilyloxy)trimethylsilylacrylate, b. 1,6-Octadiene-3-ol,3,7-dimethyl, c. 5-Hepten-2-one 6-methyl, d. 1-Heptanol-6-methyl, e. 3-Cyclohexene-1-ol,4-methyl-1-(1-methylethyl)-, (R)-, f. Dimethylsulfoxonium formylmethylide, g. L-Lactic acid, h. Carbamate, i. N-N-dimethyl formamide, j. N-methyl formamide, k. 2-Phenyl-4H-benzo(h)chromen-4-one, l. Ascorbic acid/vitamin C.

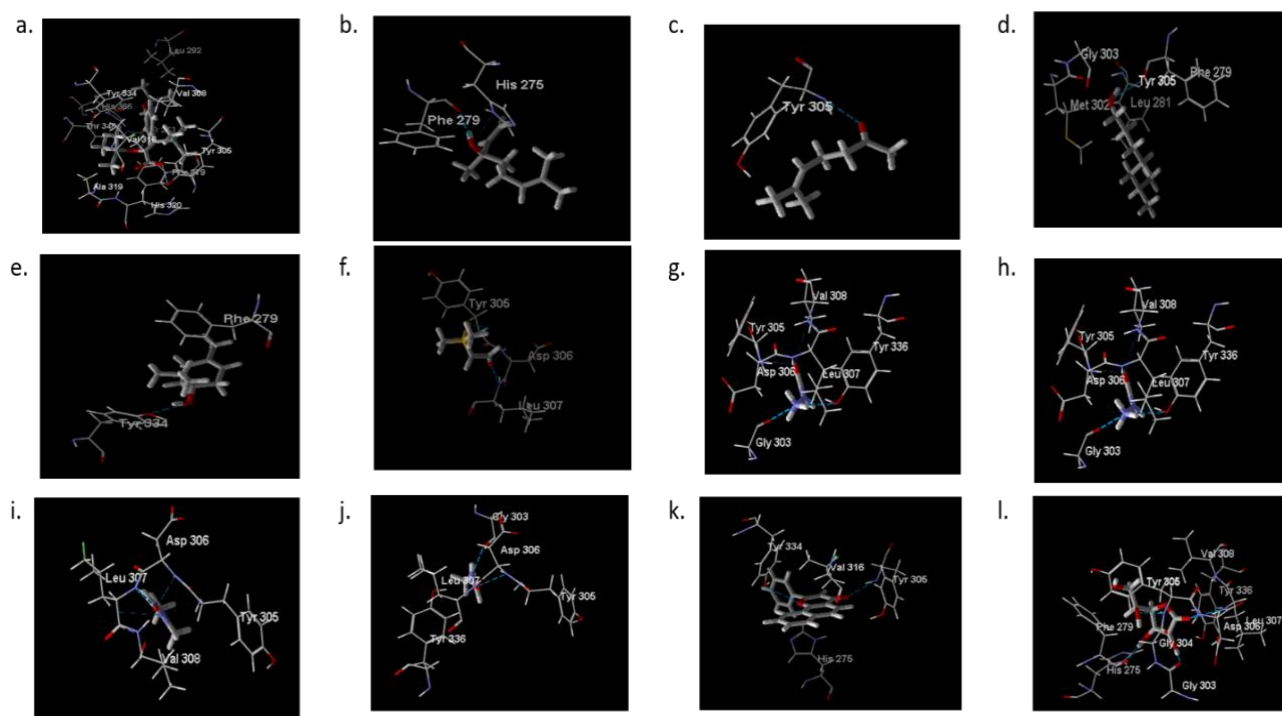


Figure 3: Organised position bond interactions (organised pose) between aryl hydrocarbon receptors that bind to the main compound of *Bougainvillea spectabilis* Willd ethanol extract. a. *p*-Trimethylsilyloxyphenyl-(trimethylsilyloxy)trimethylsilylacrylate, b. 1,6-Octadiene-3-ol,3,7-dimethyl, c. 5-Hepten-2 one 6-methyl, d. 1-Heptanol 6-methyl, e. 3-cyclohexene-1-ol,4-methyl-1-(1-methylethyl)-, (R)-, f. Dimethylsulfoxonium formylmethylide, g. L-Lactic acid, h. Carbamite, i. N-N- dimethyl formamide, j. N-methyl formamide, k. 2-phenyl-4H-benzo(h)chromen-4-one, l. Ascorbic acid.

The results of the docking process indicated that the highest rerank scores were 2-phenyl-4H benzo (h) chrome/ BHF_401 (A) (standard ligand) with rerank score of -104.771, and *p*-Trimethylsilyloxyphenyl-(trimethylsilyloxy)trimethylsilylacrylate ligand with rerank score of -92.6478 which were higher than that of vitamin c, the positive control, with a rerank score of -76.0593.

Nine active compounds of *Bougainvillea spectabilis* Willd showed various rerank scores, which fell below the score of vitamin C. The ligands were 1,6-Octadien-3-ol,3,7-dimethyl rerank score: -70.3384, 1-Heptanol,6-methyl rerank score: -58.5719, 5-Hepten-2-one, 6-methyl rerank score: -58.3337, 3-cyclohexen-1-ol, 4-methyl-1-(1-methyl ethyl)-,(R)- rerank score: -55.3509, dimethylsulfoxonium formylmethylide rerank score: -46.8992, L-Lactic acid rerank score: -44.2894, Carbamite rerank score: -31.2267, Formamide, N-N-dimethyl rerank score: -37.1401 and Formamide, N-methyl rerank score: -35.0292.

Discussion

The Molegro Virtual Docker method can be used to predict hydrogen bonds, electrostatic bonds, and hydrophobic bonds formed between compounds containing *Bougainvillea spectabilis* Willd ethanol extract and aryl hydrocarbon receptors, various amino acid residues, and vitamin C (positive control) as external ligands to obtain information about stable compounds with strong bonds and antioxidant effects namely *p*-trimethylsilyloxyphenyl(trimethylsilyloxy) trimethylsilylacrylate as a candidate of antioxidant drugs in the future.

The compound contents of the ethanol extracts of *B. spectabilis* Willd were the hydrogen-bonded ligand L-Lactic acid and Carbamite, and the most abundant amino acid residues were Phe 279, Gly 303, Asp 306, Leu 307, Tyr 336. The fewest hydrogen bonds and amino acid residues were the 1,6-Octadien-3-ol,3,7-dimethyl ligand with one bond, namely phe 279 and the 3-cyclohexen-1-ol,4-methyl-1-(1-methyl ethyl)-, (R)-ligand with one bond, namely Tyr 334. The more hydrogen bonds and amino acid residues, the stronger and more stable they are (Wen *et al.*, 2021).

The molecular modelling (docking) results in DockScore were measured from stable confirmations indicating the affinity of aryl hydrocarbon receptor inhibitors complexed with ligands. The main principle of the aryl hydrocarbon mechanism is to regulate metabolism enzymes in the form of P450 cytochromes, which function in xenobiotic metabolism and play a role in immunity regulation and maintenance of stem cells undergoing cellular differentiation, can bind with exogenous ligands such as flavonoids, polyphenols, and tannins. The protection effect of flavonoids is by transferring electrons to free radicals, activating enzymatic antioxidants, and inhibiting oxidase; they also function as antioxidants that catch free radicals so that melanogenesis processes induced by ROS can be inhibited and neutralised (Saewan & Jimtaisong, 2013). The compounds of *Bougainvillea spectabilis* Willd ethanol extract bind various amino acids, namely Tyr 305, Tyr 334, Phe 279, Leu 281, Gly 303, Asp 306, Leu 307, and Tyr 336.

The Rerank score reflects the energy required to form a bond with receptors. From this score, the activity of a compound can be predicted. The lower the rerank score, the more stable ligand-receptor bonds. From the results of the docking simulation, it can be predicted that *p*-Trimethylsilyloxyphenyl-(trimethylsilyloxy) trimethylsilylacrylate had higher activity than the positive control and the nine active compounds of *Bougainvillea spectabilis* Willd but a value lower than the standard ligand. Therefore, this compound is a prospective antioxidant therapy in the future.

Conclusion

p-Trimethylsilyloxyphenyl-(trimethylsilyloxy) trimethylsilylacrylate has a rerank score of -92.6478, which is higher than the rerank score of Vitamin C control (-76.0593) and lower than the rerank score of standard ligand 2-phenyl-4H benzo(h) chrome (-104.771). Based on this finding, it can be concluded that the organised bond between *p*-Trimethylsilyloxyphenyl(trimethylsilyloxy)trimethylsilylacrylate in *Bougainvillea spectabilis* Willd ethanol extract and aryl hydrocarbon receptors is more harmonious and stable than vitamin C with 11 amino residues, including Phe 279, Tyr 334, His 320, Tyr 348, Tyr 305, Leu 292, His 320, Ser 273, Val 308, Ala 319, and His 366 as alternative drug development in the future.

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