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MOLECULAR DOCKING STUDY OF NUTMEG (Myristica Fragrans) CONSTITUENTS AS ANTI-SKIN CANCER AGENTS

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ABSTRACT

Molecular docking analysis was carried out to understand better the interaction between DHODH and inhibitor from nutmeg in this series. The nutmeg constituent binding orientations in the active site of DHODH was seen in a molecular docking analysis and helped design a potentially new inhibitor. This work aimed to study the molecular docking of nutmeg constituents with the DHODH inhibitor using a computer-aided drug design. Molecular docking using AutoDock 4.2 was done to explore the models of binding complexes. The 3D structure was derived using Discovery Studio to investigate the essential chemical interaction of complex structures. Dihydroguaiaretic acid was the most potent ligand having a docking score of -9.3 kcal/mol. This value was better than the standard drug 5-FU. The dihydroguaiaretic acid structure interacted with Tyr365 and Thr63 through a hydrogen bond similar to the native ligand. These results suggest that nutmeg seed could serve as the lead compound for potent DHODH inhibitors against skin.

Keywords: *DHODH, molecular docking, nutmeg, skin-cancer*

INTRODUCTION

Nutmeg (*Myristica fragrans*) extracted from the seed of nutmeg used for medicinal purposes [1]. Nutmeg oil is acquired from the seed of nutmeg fruit. The essential oil from nutmeg seeds has anticarcinogenic activity [2]. Studies on the nutmeg seed have reported extracts and many compounds with anti-cancer and antioxidants activities [3,4].

A flavonoid found in nutmeg shows that anti-cancer potent effects, mostly on UV-B, cause skin cancer, but molecular mechanisms and targets are open to questions [5]. Skin cancer in 2018 is the second biggest cancer cause of death worldwide, with new cases, approximately 9.88 million, and 1.08 million were monitored. Melanoma cancer rapidly increases than nonmelanoma cancer because of environ-mental changes. Skin cancer/melanoma is rising in current years due to exposure to UV radiation. Almost the aggressiveness of skin cancer caused by exposure to an excess of UV radiation from the sun [6].

Dihydroorotate dehydrogenase (DHODH), located in the internal mitochondrial membrane, plays a key function in the programming of UV-B induced energy. DHODH catalyzes the transformation of dihydroorotate in the fourth step of the six enzyme reactions of this pathway as the rate-limiting enzyme in the de novo pyrimidine synthesis pathway. DHODH up-regulation was crucial for sustaining higher activity in the radiated skin of the electron transport chain and for coordinating ATP generation [7]. Enhanced DHODH activity was involved as a biomarker for malignant tumours, including skin cancer. Ultraviolet radiation can induce DHODH expression transcriptionally by triggering STAT-3 [8]. DHODHODH inhibitors affect ATP depletion and endogenous ROS levels. A DHODH inhibitor proved an anti-tumour treatment effect in conjunction with fluorouracil [9]. DHODHODH inhibitors have great potential to be used for cancer treatment [9-13].

Melanoma skin cancer has a strong potency to grow to other parts of the body, and it becomes difficult to cure. The WHO reports that 132,000 cases of skin cancer happen on an annual basis. In 2016, approximately 76,380 skin cancer cases will be invading melanomas, with about 46,870 in males and 29,510 in women. There will be an annual increase of skin cancer impact by 4500 due to the 10% devastation of the ozone layer [14].

The invention of new bioactive compounds shows highly selective anti-cancer activity is of high order for cancer treatment. Possible anti-cancer activities of myristicin were predicted by computer-based molecular docking. This study includes molecular docking of myristicin from nutmeg against active centres of cancer-related DHODH [14]. New research is still required in finding new compounds for skin cancer. Finding a specific target drug is one of the most effective ways to inhibit skin cancer [15].

Molecular docking is a tool for predicting small molecules' binding orientation from drug candidates to their protein targets to predict the activity and affinity. Molecular docking plays a major role in the design of drugs. Autodock is one of the programs used to perform docking [16]. Several molecular docking works from phytochemical constituents for anti-cancer have been published elsewhere [17] [18] [19]. The molecular docking of nutmeg constituent toward DHODH for skin cancer has never been reported before.

The investigation of the binding site between the DHODH receptor and the selected structure from nutmeg was done. A docking score evaluation was also carried out for investigating the anti-cancer activity. This research examines the interaction binding site between nutmeg seed constituents and DHODH as anti-skin cancer agents.

METHODS

The ligands and receptor protein were prepared using Chimera 1.13.1 and Autodock 4.2.6 (The Scripps Research Institute, USA). Autodock Tools (ADT) 1.5.6 were used for the docking analysis. Computational visualization was performed using Discovery Studio (BIOVIA Discovery Studio 2019). The hardware used was Asus VivoBook Intel

Core i3-7020u, 4GB RAM, and 500GB hard disk using OS Windows 10.

The 3D structure of 20 ligands from nutmeg constituents was obtained using the Pubchem web database. The ligand was prepared to pdbqt format using Chimera 1.13.1. The 3D structure of DHODH was obtained from Protein Data Bank (www.rscb.org/pdb) using PDB ID (PDB entry: 2BXV) [20], resolution of 2.15 Å, organism *Homo sapiens*) in the database. The receptor protein was prepared using Chimera 1.13.1. All water molecules were removed, and hydrogen atoms were added.

The binding mode of the nutmeg seed active compound into the target of DHODH was investigated using molecular docking [21]. The pdbqt files for protein and ligands preparation and generating the grid box were completed using the graphical user interface program AutoDock Tools (ADT). AutoGrid was used for the preparation of the grid map using a grid box. The grid size was set to 38.88×44.27×42.81 XYZ points. The ligand was docked into the active sites of melanoma skin cancer (PDB ID: 2BXV).

Lamarckian genetic algorithm (LGA) was used to detect the most desirable ligand binding orientations. The interaction was produced, and the docking result of binding energy and residue were reported [22, 23]. The dock Score function was used to scoring all the dock ligands. Analyses were identified from the best pose.

RESULTS AND DISCUSSION

The application of molecular docking used in medicinal chemistry and drug discovery is predicted the interaction between two molecules. Docking is done between a small molecule drug and a large macromolecule receptor in drug discovery. Molecular mechanics apply for most docking program to calculate the binding energy [24]. Docking is useful for drug design by searching the optimized confirmation for the ligan-protein complex and their relative orientation. Minimum free energy in the overall system means the ligand is potent to select drug candidates [25]. There are three steps in molecular docking: pose prediction, virtual screening, and binding affinity calculation-Ligan conformation generated from the search algorithm and energy scoring function [26].

Molecular docking studies in this work were done using the AutoDock Tools (ADT) 1.5.6 and AutoDock 4.2.5.1 docking program. The docking program is used to explore the potential binding mode of the inhibitor from the nutmeg constituents. Nutmeg seeds essential oil have anticarcinogenic activity. The composition of nutmeg seed oil differ significantly based on their source. The essential oil of nutmeg seed contains sabinene (15-50%), a-pinene (10-22%), b-pinene (7-18%), myrcene (0,7-3%), 1.8-cineole (1,5-3,5%), a-phellandrene (0,3-6.2%), myristicin (0,5-13,5%), limonene (2,7-4,1%), eugenol (0,1-1%), safrole (0,1-3,2%), and terpinene-4-ol (0-11%) [27]. The potential chemopreventive agent in volatile oil is elemicin, and the most responsible for pharmacological effect in nutmeg is myristicin [28]. The main compounds in the aromatic essential oil are myristicin, elemicin, safrole (85-95%), and myristicin (4-12%) [29].

The algorithm of molecular docking had been originally tested by redocking ligands at the active receptor position for the reliability and reproductivity of the current docking algorithm, utilizing the root mean square deviation (RMSD) measurement. RMSD from redocking of 2XBV protein receptor was 1.733 Å. RMSD value less than 2.0 Å showing the algorithm validated compared to the crystallographic structure [30]. As can be seen from the comparison of our RMSD with the RMSD as mentioned above, this research could continue using our validated docking protocol [31]. The RMSD below 2.0 Å indicates the formation of good quality docking method [32].

Molecular docking has many benefits, including the system's capacity to check broad compound databases at low costs compared to experiments [33]. Molecular modelling is used to predict the compound's possible mode and determine the direction of the best pose from the optimizing structure [34]. Twenty compounds from nutmeg seed were docked to binding sites of the DHODH target. The calculated binding energy corresponds to the anti-cancer activity [35]. The docking score and interaction residue of the H-bond presented in Table 1. The best-docked ligand was the malignant compound. The binding energy of malignant with the DHODH is -10.4 kcal/mol.

Alpha-pinene performed low binding energy scores, respectively -6.0 kcal/mol. The low binding energy because the compound did not fit with the binding site of the DHODH receptor. The ΔG binding of 19 constituents from nutmeg was all greater than -7.0 kcal/mol, suggesting that these nutmeg constituents have the potential to be DHODH inhibitor. A stable complex that shows greater negative free binding energy indicates high inhibitor strength [36]. Table 1 indicated that the selected compounds from nutmeg showed good anti-cancer activity compared with the 5-fluorouracil, which is widely used in cancer treatment. According to the results from the molecular docking studies for DHODH, all nutmeg seed constituents performed significantly better scores when compared with 5-fluorouracil as drug standard [37] [38].

The docking score between DHODH and compound macilin F, macelignan, dihydroguaiaretic acid, and fragransin E1 was -10.0, -10.4, and 9.3 and –9.7, respectively. The order of activity was: macelignan > macilin F > fragransin E1 > dihydroguaiaretic acid. The docking score indicates the interaction affinity between the receptor and ligand by the optimized algorithm, speculating the scope of inhibitory activity. The evaluation index for fast preliminary steps and inhibitors is the key importance of the docking score.

The free binding free energy estimation is calculating from the docking score for the protein-ligand complex. The lowest energy score indicates the highest protein affinity with the best potent ligand means the best docking score [35]. The docking scores for each compound compared to the standard drug score. The result shows that the nutmeg constituents have better docking scores than standard drugs available in the market (5-fluorouracil [38]. The active site exhibit that several molecular interactions considered in control of the observed affinity. After checked the binding score, evaluation of docking score based on amino acid residue between ligand-receptor complex.

Table 1. Docking score of nutmeg constituents

No	Compound name	Docking score ΔG (kcal/mol)	Interacting residues (H-bond)	Interacting residue (non-hydrogen bond)	References
1	Dyclonin	-9.2	Asn145	Val143, Tyr356, Pro52, Ala55, **Ala59**	[39]
2	Dihydroguaiaretic acid	-9.3	**Tyr356, Thr63**	**Pro364,** Phe98, **Leu359, Ala59, Thr360, Ala55**	[39]
3	Eugenol	-7.0		Tyr356, Val143	[39]
4	Fragnasol A	-8.7	Lys255, Thr360	Tyr356, Val134, Val143	[39]
5	Fragnasol C	-8.6	Glin47	Met43, **Leu42, Ala59**, Leu58, **Pro364, Leu359,** Met43	[39]
6	Fragransin A2	-9.1		Phe62, Leu46, **Leu58, Ala59, Ala55**	[39]
7	Fragransin C2	-8.5	**Thr63**	Phe52, **Leu42, Ala59**, Tyr38, Pro364, Met43, Ley46	[39]
8	Fragransin E1	-9.7		Arg136, Val143, Tyr356, **Ala59, Ala55**, His56, Pro52, Val143	[39]
9	Fragransol B	-9.0	Gln47	Met43, **Pro364**, Tyr356, **Leu359**, Phe98	[39]
10	Guaiacin	-8.1	Tyr356	Ala96, Ala95	[39]
11	Isoeugenol	-7.4		Pro52, Val143,	[39]
12	Macelignan	-10.4	Ala96, Gln47	Tyr147, Val134, Tyr356, **Ala55, Thr360**, Val143, Phe62, **Leu359**, Phe98	[39]
13	Machilin F	-10.0		**Ala59, Pro364**, Leu46, Leu58	[39]
14	Methylisoeugenol	-7.7	Asb145	Val143	[39]
15	Myristic acid	-6.7	Ser305, Asn145	His56, Phe98, **Ala59**	[39]
16	Myristicanol A	-7.6		Met43, **Ala59**, Leu42, **Pro364, Tyr38**, Pro52	[39]
17	Myristicanol B	-8.0	Asn284, Lys255	Val143, **Ala55,** Ala34, Tyr147	[39]
18	Myristicin	-7.9	Ser305	Val143, Val134, Pro52	[39]
19	Safrole	-7.6	Asn145, Ser305	Val143, Tyr356, Val135, Pro52, **Leu42, Pro364, Ala59**	[39]
20	Alpha-pinene	-6.0		**Tyr38**, Pro69, Leu68, Leu58, Leu46, Phe62	[39]
21	Native ligand	-12.0	Tyr356, Thr63	Thr360, Ala55, Leu359, Ala59, Tyr38, Leu42, Pro364	[20]
22	5-fluorouracil	-6.4	Asn145, Asn217, Asn284, Asn212		

Interaction of nutmeg constituents and DHODH protein receptor can be seen in Table 1. Table 1 showed interacting residue hydrogen bond and non-hydrogen bond from 40 nutmeg constituent compared with native ligand and drug standard. Docking simu-lations colored 2D-binding mode represen-tation between DHODH and native ligand shown in Figure 1. Macelignan-DHODH complex and dihydroguaiaretic acid-DHODH complex binding mode shown in Figure 2 and 3, respectively. The binding interactions between DHODH and nutmeg constituents mainly involve conventional hydrogen bonds, Van der

Walls, pi-sigma, pi-alkyl, pi-pi stacked, and alkyl. Molecular docking reports showed that the compounds would interact in DHODH inhibitor. The binding between the bioactive compounds and DHODH was verified by molecular docking.

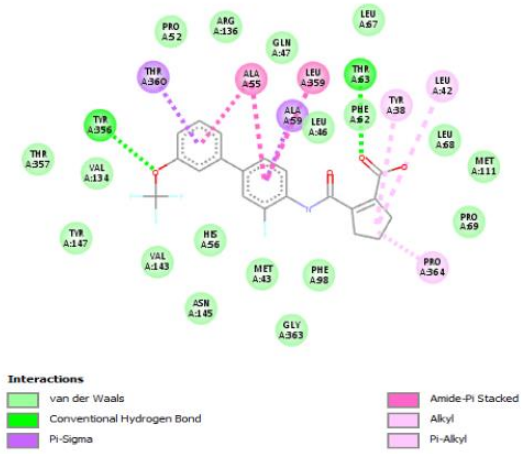

Figure 1. Two-dimensional interaction of native ligand and DHODH

There is no hydrogen bond in macilin F and fragransin E1. They interact only through alkyl and π-alkyl interaction. Native ligand-bound Tyr356 and Thr63 via hydrogen bond. The hydrogen bonds between Ala96, Gln47 residues and macelignan, the pi-pi stacked between Thr360 and macelignan, or pi-alkyl bonds between Ala55, Val143, Val134, Tyr356, Tyr147 and macelignan, not related to the native ligand interaction and DHODH. Two-dimensional interaction of malignant and DHODH could be seen in Figure 2.

The interaction of dihydroguaiaretic acid has two hydrogen bonds with amino acid, namely Thr63 and Tyr365, similar to the native ligand. Dihydroguaiaretic acid could strongly interact with Thr63 and Tyr635 via carbon-hydrogen. This association was powered by the forming of hydrogen interactions between the receptor and the compound. The π-sigma interaction found in Ala59 and Thr360. The π-alkyl interaction found in Pro364. The two-dimensional interaction of dihydroguaiaretic acid could be seen in Figure 3. Dihydro-guaiaretic acid has the best interaction in complex DHODH because of the amino acid residue similar with native ligand.

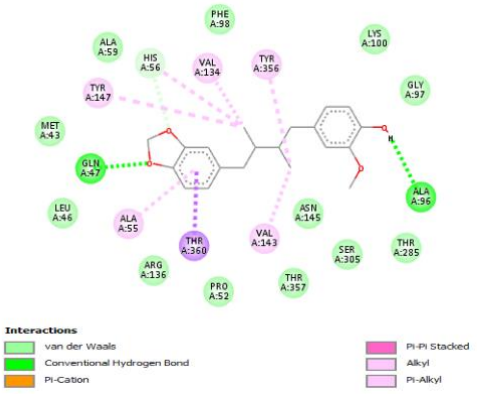

Figure 2. Two-dimensional interaction of macelignan and DHODH

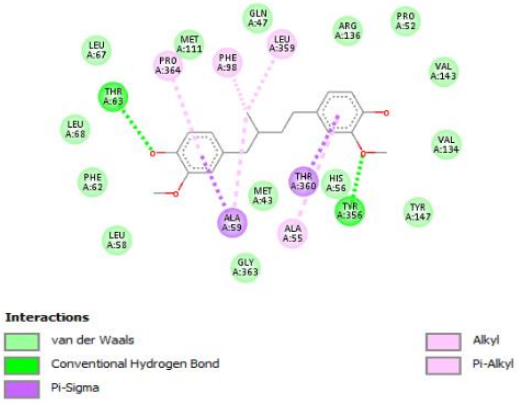

Figure 3. Two-dimensional interaction of dihydroguaiaretic acid

The molecular docking findings revealed a clear understanding and visual explanation of the nutmeg constituents and inhibitor binding mechanism. The same binding location and mode may be the same mechanism for inhibition [40]. However, the model established would be too early to be valid for compound anti-skin cancer without any experimental proof-the lower the energy, the stronger the

binding between ligand-receptor complex. Docking simulations of the binding position of nutmeg constituents in the hydrophobic pocket of DHODH indicates the inhibition mechanism on the oxidoreductase activity of DHODH. The combination mode and binding sites of DHODH and forty nutmeg compounds were studied by molecular simulation. Molecular docking is the most widely used approach for structure-based drug design and has many applications, such as binding energies and interaction [41]. The molecular docking method verified the good binding energy of 20 pairs of ligand-protein DHODH inhibitor complex.

CONCLUSION

The best interaction model of nutmeg constituents was dihydrogudiaretic acid with a binding energy score of -9.3 kcal/mol. The dihydroguaiaretic acid structure interacted with Tyr365 and Thr63 through a hydrogen bond similar to the native ligand. The result suggests that nutmeg seed could serve as the lead compound for potent DHODH inhibitor against skin cancer**.**

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