Siphonaxanthin, A Functional Sea Grape'S Carotenoid Revealed Cholesterol Synthesis Inhibition; In Silico Study

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ABSTRACT

Sea grape is a nutritional macroalgae, contains high fiber, protein, vitamin, and mineral. Sea grape also contains several bioactive compounds and become functional food. Siphonaxantin was identified as a new carotenoid in sea grape extract, however the bioactivity has not been investigated yet. This study covered potential activity of siphonoxanthin as HMG CoA reductase inhibitor. Siphonoxanthin and fluvastatin structure were taken out from PubChem NCBI database, HMG CoA reductase protein structure was downloaded from Protein Data Bank (PDB). Compounds and protein were interacted using Molegro virtual docker version 5 and were visualized with Discovery studio version 21.1.1. Interestingly, the residue Asn755 was found on the siphonoxanthin bound to HMG CoA reductase at several residues that identified as binding pocket of Rosuvastatin and Atorvastatin. Compared to fluvastatin as a control, siphonoxanthin and Fluvastatin closed interaction with HMG CoA reductase protein. Based on the binding energy, siphonoxanthin performed a higher energy value than fluvastatin. Our study summarized that siphonoxanthin, a new carotenoid from Caulerpa racemosa inhibited cholesterol synthesis by blocking HMG CoA reductase. In vitro and in vivo are required for further investigation.

Keywords: *Caulerpa racemosa, hypercholesterolemia, molecular docking, siphonoxanthin.*

1. INTRODUCTION

Hypercholesterolemia is a metabolic disease that is the core for some metabolic disorders, including atherosclerosis, stroke, coronary heart diseases, and diabetes mellitus. Hypercholesterolemia began from a high fat diet and low physical activities. Low physical activities and a high fat diet promoted fat accumulation in adipose tissue and stimulated several risk metabolic disorders (1–4) . Besides that, low physical activity also increases the cholesterol synthesis.

Cholesterol synthesis was provided by the mevalonate pathway, which was regulated by 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA Reductase) enzyme (5–7). Inhibited HMG CoA reductase enzyme is an alternative therapeutic treatment for hypercholesterolemia. Reducing the activity of HMG CoA reductase caused decreasing the mevalonate accumulations, and indirectly repressed the cholesterol synthesis. Several synthetic drugs and herbal medicines were reported as HMG CoA reductase inhibitors (4–10).

Anthocyanins from black rice, cyanidin, peonidin, cyanidin – 3- O-glucoside, and peonidin – 3- O-glucoside were reduced total cholesterol in high fat diet mouse models (3,11,12) . Molecular docking also proved the cholesterol synthesis inhibition through blocking cJun-NH2 terminal kinase protein, toll-like receptor 4, and FabH protein (11–16) . Some ginger bioactive compounds also showed anti-dyslipidemia activity (17,18).

Phenolic compounds from coffee and cascara also showed anti-obesity (19–23) . *Caulerpa racemosa,* a sea grape, has reported high nutritional value and promoted some health benefits, such as antioxidant, anti-hyperlipidemia, and anti diabetes. *C. racemosa* also has high fibres, minerals, and vitamins. Besides that, *C. racemosa* extract contains several flavonoids, carotenoids, phenolic, and alkaloids compounds that proved a human health property (24–28).

One of the carotenoid compounds from *C. racemosa* is siphonaxantin (29) . Siphonaxantin was identified as new carotenoids and promoted anticancer, antiviral activities. However, the bioactivity of siphonoxanthin in cholesterol inhibition has not been explored yet. This study covered potential activity of siphonaxantin as HMG CoA reductase inhibitor in cholesterol synthesis mechanism.

2. MATERIAL AND METHODS

Ligand and protein retrieval

Siphonaxanthin (CID 11204185), a keto carotenoid of *C. racemosa, was* downloaded as a 3D structure from PubChem. Fluvastatin (CID 446155) was used as an inhibitor control. HMG CoA reductase protein was downloaded from Protein Data Bank with ID 1HWI (30).

Bioactivity test and pharmacokinetics prediction

The canonical SMILES of ligands were retrieved from PubChem NCBI database and were used for bioactivity and pharmacokinetics identification. Bioactivity test of ligand was carried out by Way2Drug online program, and pharmacokinetics prediction was conducted by PKCSM online tool (31).

Binding cavities identification and Docking simulation

The 3D structure of HMG CoA reductase was prepared by binding cavities prediction of the protein. van der Waals forces as the molecular surface was set as a parameter binding cavities prediction. Ligands and protein were docked using Molegro virtual Docker version 5.0 at specific sites with protein Grid box was X= 30.24; Y= -8.35; Z= 12.91 and Radius 15 (32).

Data analysis

Three–dimensional and two-dimensional complex structures of Fluvastatin – HMG CoA reductase were visualised by Discovery studio version 21.1.1 and PyMol 2.2. Ligand – protein complex interaction sites were analysed using Discovery studio version 21.1.1.

3. RESULT

Protein - ligands complexes of Siphonaxanthin and Fluvastatin with HMG CoA were presented on Figure 1 and table 1. Siphonaxanthin posed binding with HMG CoA through Ser626, Met659, Asn658, Ala654, Met655, Leu562, Asn755, Ala759, Thr758, Ala769, Thr758, Ile762, and Ala768 amino acid residues. The Ser626 and Asn755 bound to siphonaxanthin at hydrogen atom by hydrogen bond. Asn658 interacted with the oxygen atom of siphonaxantin. The π – alkyl (hydrophobic interaction) was observed on residues Ile762, Ala769, Ala759, Ala768, Met655, and Ala654. Three active sites of siphonaxantin, involved Leu562, Thr758, and Met659 were identified with unfavourable bonds. The type of interaction involved hydrophobic interaction, hydrogen bonds, and unfavourable bonds contributed to the binding energy and tight of interactions. varied interaction promoted lower binding energy and tighter interaction of ligands – protein complex.

Fluvastatin as a HMG CoA reductase inhibitor formed nine hydrogen bonds at Asn755, Glu559, Ser684, Lys691, Arg590, Lys735, Lys692, Asp690, and Ala751. The π – sigma was observed on the Leu853. Furthermore, Leu853, Leu857, and Ala856 also presented interaction with fluvastatin by π – alkyl. Compared to HMG CoA reductase - fluvastatin binding, the hydrogen bond of HMG CoA reductase - siphonaxantin was lower than HMG CoA reductase - fluvastatin. It affects the binding energy score, because more hydrogen bonds assumed could reduce binding energy score.

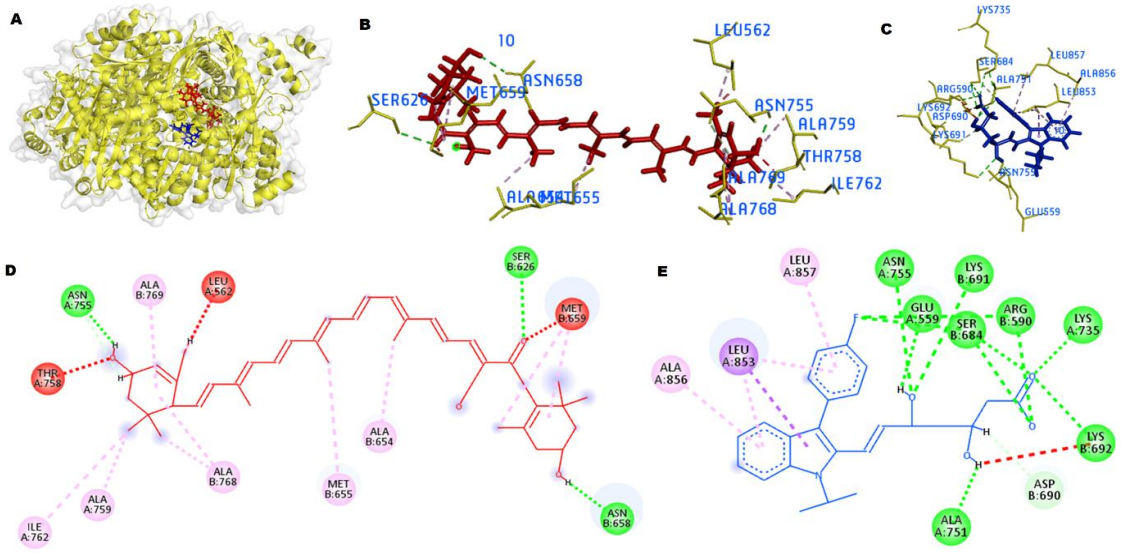

Figure 1. Three-dimensional and two-dimensional structures of ligands – HMG CoA reductase, A. superimposed of ligands – protein complex, B – C. Three-dimensional poses, D-E. Two – dimensional structure of the complex, yellow cartoon illustrated the HMG CoA reductase protein structure, the blue stick was Fluvastatin, and the red stick was Siphonaxanthin.

Table 1. Binding energy and binding sites of ligands – HMG CoA reductase complexes

Ligand	Binding Energy (kJ/mol)	Hydrogen Bond (Distance (A))	Hydrophobic (Distance (A))	Unfavorable
Siphonaxanthin	-390.8	Ser626 (2.7), Asn658 (2.4), Asn755 (1.8; 3.0),	Ala759 (4.3), Ala654 (3.9), Met659 (5.4, 3.1), Ala768 (5.1, 3.2), Ala769 (4.1), Ile762 (4.8), Leu562 (3.4), Met655 (4.6)	Thr758 (2.01), Leu562 (1.7), Met659 (2.0)
Fluvastatin	-449.8	Lys735 (2.4), Asn755 (2.6), Arg590 (3.1, 2.5), Ser684 (3.6, 3.2, 2.9), Lys691 (3.0), Lys692 (3.0), Ala751 (1.9), Glu559 (2.0), Ser684 (3.2), Asp690 (2.3)	Leu853 (3.1), Leu853 (4.9, 5.3), Ala856 (4.3), Leu857 (5.4)	Lys692 (2.7)

4. DISCUSSION

3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA Reductase) is an essential enzyme that contributes to the mevalonate pathway in cholesterol synthesis (4,9,10,33) . The HMG CoA reductase protein consists of 888 amino acid residues that are divided into three protein domains, membrane anchor domain, linker and catalytic domain. Membrane anchor domain of HMG CoA reductase was presented on the residue number 1 – 339, while linker was posed at 340 – 459, and catalytic domain at 460 – 888 (2,33). The molecular docking of this study presented interaction of siphonaxantin and fluvastatin at catalytic domain residues by hydrogen bonds and hydrophobic interaction. A previous study reported several residues that substrate – binding pocket of HMG CoA reductase, Glu559, Ser565, Val683, Ser684, Lys692, Asn755, Asp690, Lys691, and His866. Some amino acid residues of HMG CoA also identified as Rosuvastatin and Atorvastatin binding sites, Asp690, Lys691, Glu559, Ser565, Lys735, and Asn755 (7,9,34).

Interestingly, the residue Asn755 was found on the siphonaxantin – HMG CoA reductase binding pocket. The inhibition of HMG CoA reductase to siphonaxantin from *C. racemosa* is prospective to reduce the risk of obesity. A previous study found *Basella alba* leaf extract inhibit HMG CoA reductase and used for treating hypercholesterolemia (4) . Molecular docking and molecular dynamics approach performed three of 118 natural compounds potentially as HMG CoA inhibitor (6) . This study performed that siphonoxanthin from *Caulerpa racemosa* inhibited the HMG-CoA reductase at catalytic sites, indicating reduced or inactivated the activity of HMG-CoA reductase. Inactive HMG-CoA reductase repressed the mevalonate production and indirectly decreased cholesterol.

5. CONCLUSION

Siphonaxantin from *C. racemosa* able to inhibit HMG CoA reductase and might repressed cholesterol synthesis. It has the potential to reduce hypercholesterolemia risk.

6. ACKNOWLEDGEMENT

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