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MOLECULAR DOCKING STUDY ON ANTICANCER ACTIVITY OF ASTAXANTHIN

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ABSTRACT

Objective: Astaxanthin is a xanthophyll carotenoid naturally synthesized by a number of bacteria, microalgae, and yeast and one of the main natural sources is *Hematococcus pluvialis*. Astaxanthin is known to have high antioxidant activity and also as an anticancer. Molecular docking of astaxanthin compounds against 3 specific receptors: Procaspase 7, Protein Kinase B, and Vascular Endothelial Growth Factor Reseptor-2 (VEGFR2) aims to determine the mechanism of activity as an anticancer. These receptors were known to affect the growth and physiology of cancer. The results of the interaction of astaxanthin with these receptors will be compared with the native ligands that bind to each receptor in the binding pocket which is evaluated through the docking score and the bonding that formed between ligand and the receptor. **Materials and Methods:** The molecular docking process consists of preparation ligands and target receptors using software Pyrx, MgTool, Discovery Studio. The docking process was carried out using AutoDock Vina software and Discovery Studio Visualizer. The docking evaluation was done by comparing the binding affinity score between the native receptor ligands and astaxantin. **Results:** The evaluation of the docking score of the astaxanthin compound were -9.0 kcal/mol for procaspase 7; -.7.1 kcal/mol for PKB; and -.7.7 kcal/mol for the VEGFR2 receptor. **Conclusion:** The binding affinity value in the docking simulation of astaxanthin compound concluded that the activity as an anticancer was very dominant in the Procaspase 7 receptor inhibition mechanism.

KEYWORDS: Molecular docking, astaxanthin, anticancer, binding affinity, VEGFR2, procaspase 7, protein kinase B.

INTRODUCTION

Cancer is the leading cause of death in the world, which is caused by abnormal cell proliferation. This condition can interfere with body systems, organ function, and cause widespread cell death.[1] The trend of using natural compounds to treat cancer is due to their low toxicity and potential properties.[2] Astaxanthin is a secondary metabolite of the carotenoid class, which is shown to have very high antioxidant activity[3] and some scientists call it the "king of carotenoids". Astaxanthin is useful for strengthening the immune system,[4] preventing and treating various types of cancer,[5,6] antidiabetic,[7] anti-inflammatory,[8] skin-protective effects,[9] and cardiovaskularas.[10] Astaxanthin is widely distributed in the red pigment of shrimp, salmon, crab, asteroidean and especially in *Hematococcus pluvialis* (*H. pluvialis*) which is the main natural source. Astaxanthin does not cause adverse side effects, even at high doses.[11]

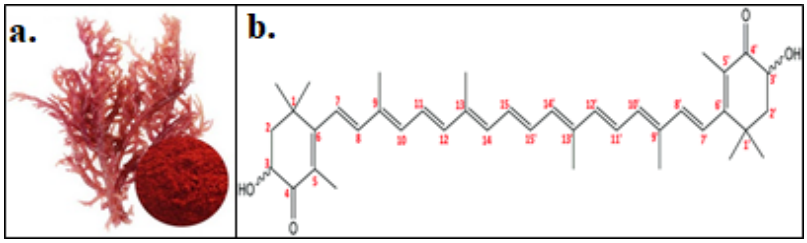

Figure 1: (a) *Hematococcus pluvialis;* (b) Astaxanthin Structure.

Conventional methods in the discovery of new drug candidates are expensive and time consuming. Therefore screening techniques through rational design of drug molecules based on binding of molecules to the active sites of the target receptors offer significant potential for identifying and developing anticancer molecules. One of the methods of computational chemistry is molecular docking. This method can screen a large number of molecules based on free binding energies and proposes structural hypotheses of how the molecules could inhibit the target effectively and efficiently.[12]

Determination of the anticancer activity of astaxanthin compounds through the molecular docking method approach using 3 target receptors that play an important role in cancer growth and physiology. The three receptors used in this study were procaspase 7 (PDB ID: 1K88), protein kinase B (PKB; PDB ID: 1GZN), and Vascular Endothelial Growth Factor R2's receptor kinase (VEGFR2; PDB ID: 1VR2).

The full-length procaspase-7 zymogen contains 303 amino acids as homodimers in the cytosol. The structure is made up of a centrally located 12-stranded ß-sheet with 10 helices surrounding it. Apoptosis is programmed cell death that involves the controlled dismantling of intracellular components while avoiding inflammation and damage to surrounding cells. This condition is initiated by the caspases family including procaspase 7.[13] Protein kinase B (PKB or Akt) has an important role in central regulation of metabolism, cell survival, motility, transcription, and cell cycle growth. PKB is regulated in 3-kinase phosphoinositide (PI) signaling, which is activated by autophosphorylation of tyrosine kinase receptors; (2) stimulation of G-protein-coupled receptors, or activation of integrin signalling.[14,15] VEGFR2 has important roles in physiological and pathological angiogenesis, including tumor angiogenesis.[16] In addition, VEGFR-2 functions in the primary regulation of antiapoptotic effects and maintenance of sinusoidal endothelial cell architecture (SEC).[17]

MATERIALS AND METHODS

Software and Tools

Chem Draw Ultra 12.0, AutoDock Vina 1.1.2, MGL tools, Discovery Studio Visualizer, Pyrx.

Ligand preparation

Astaxanthin and various ligands (positive control) were used as ligands for docking studies were listed in Table 1.

Table 1: Ligands used in the study.

No	Ligand	Molecular Formula	References
1	Astaxanthin	$C_{40}H_{52}O_4$	[5]
2	RGDS	$C_{15}H_{27}N_7O_8$	[13]
3	RPRTSSF	$C_{36}H_{59}N_{13}O_{11}$	[9]
4	Cilengitide	$C_{27}H_{40}N_8O_7$	[14]

Protein and Ligands preparation

The target receptors used for the study of astaxanthin activity as an anticancer can be obtained from the protein data bank (http://www.rcsb.org) were procaspase 7 (PDB ID: 1K88), protein kinase B (PDB ID: 1GZN), and receptor kinases R2 growth factor Endothelial Factor R2 (PDB ID: 1VR2). The receptor file is converted in PDBQT format. Meanwhile, the ligands file is obtained at the link Pubchem.ncbi (https://pubchem.ncbi.nlm.nih.gov/). File ligands were then minimized the energy and converted to PDBQT format.

Docking Studies and Evaluations

Astaxanthin and the native ligand as positive controls in PDBQT format simulated molecular docking with AutoDock Vina 1.1.2 software. The docking process is regulated with the receptors in a box spacing 1 Å. This will keep the receptors acting rigid so that the ligand will remain flexible to find the best position. The docking evaluation is obtained by comparing the binding affinity score (kcal/mol), and also evaluating the bonds formed with the amino acids on the active site of the binding pocket. The interaction between the target receptor and the ligand was visualized with the Discovery studio visualizer software. These interactions can explain the interactions between molecules, and the types of bonds that are formed.

RESULTS AND DISCUSSION

Ligand preparation

Astaxanthin structures and ligands are downloaded from the link https://pubchem.ncbi.nlm.nih.gov/. The physicochemical properties of ligands and the energy of the minimized structures are summarized in Table 2 and Figure 2.

Table 2: Physiochemical parameters of ligand.

No	Ligand	Molecular Weight (g/mol)	Hydrogen Bond Donor	Hydrogen Bond Acceptor	XLogP3-AA	Minimize Energy
1	Astaxanthin	596.8	2	4	10.3	2202.50
2	RGDS	433.42	9	10	-7.3	316.60
3	RPRTSSF	849.9	14	14	-7.7	1232.37
4	Cilengitide	588.65	7	8	-1.0	1216.28

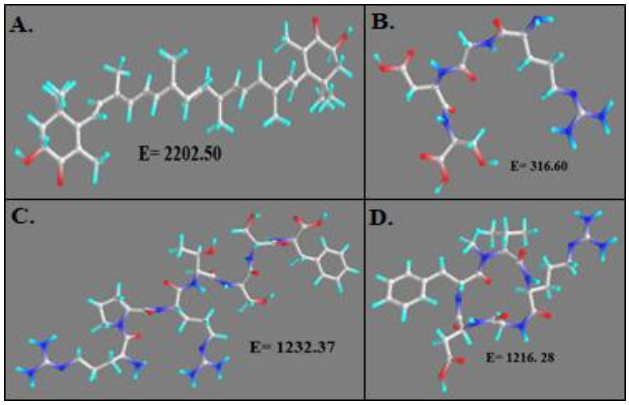

Figure 2: 3D ligand Structure and Energy minimized results. (A) Astaxanthin. (B). RGDS, (C) RPRTSSF, (D) Cilengitide.

Protein preparation

The target receptor that has been downloaded from the protein data bank is in the form of a pdb file format, then converted into the pdbqt format with the Pyrx or OpenBabel GUI program. The target receptor structure can be seen in Figure 3.

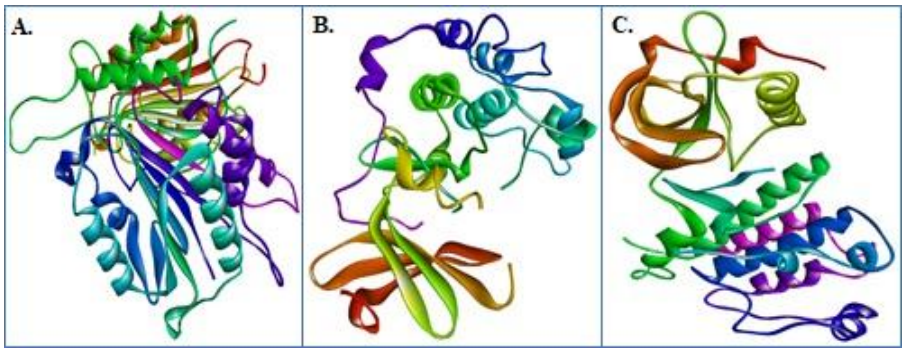

Figure 3: 3D structure of receptor, (A) Procaspase 7, (B) Protein Kinase B, (C) Vegfr 2.

Docking studies using autodock vina

Docking of luteolin into PDB structure of Procaspase 7 (PDB ID: 1K88)

The results of molecular docking simulations explained the similarity of bond interactions between the ligands RGDS and Astaxanthin against the procaspase 7 receptor targets on amino acids Arg 187, Val 86, His 144, Arg 87, Gln 184, Ala 186, Gly 228, Asn 88, Ser 231, Tyr 229, Tyr 230, Gly 89, Ser 239, and Lys 285. The interaction of the RGDS ligand and the procaspase 7 receptor has hydrogen bonds in the amino acids Asp 87, Asp 93, Arg 187, Arg 233, and Ser 239. While the astaxanthin ligand forms hydrogen bonds with the procaspase 7 receptor on the amino acid Gln 184.

Molecular docking evaluation with comparison the binding affinity score gave results that Astaxanthin has a value of -9.0 kcal/mol better than the RGDS ligand score of -6.9 kcal/mol. This provides information that astaxanthin compounds have anticancer activity through the Procaspase 7 receptor inhibition mechanism.

Docking of luteolin into PDB structure of protein kinase B (PKB; PDB ID: 1GZN)

The results of 2-D visualization of the RPRTSSF and Astaxanthin ligands against the PKB receptor target showed many similarities in the formation of bonds in amino acids Lys 277, Glu 200, Arg 274, Tyr 273, Thr 199, Phe 163, Gly 295. Interaction of RPRTSSF and PKB receptors has hydrogen bonds. the amino acids Thr 199, Val 198, Lys 191, while the astaxanthin ligand and the PKB receptor have hydrogen bonds at Lys 277.

The docking score of RPRTSSF -6.6 kcal / mol was weaker than the binding affinity of the astaxanthin ligand with PKB receptor -7.1 kcal / mol. It can be concluded that astaxanthin has a stronger interaction activity as an anticancer at PKB receptors.

Docking of luteolin into PDB structure of vascular endothelial growth factor R2's receptor kinase (VEGFR2; PDB ID: 1VR2)

The docking output data informed that the interaction of the cilengitide and astaxanthin ligands on the VEGFR2 receptor and VEGFR2 receptors were formed on the amino acids Lys 868, Phe 1047, and Glu 885. Whereas in astaxanthin the VEGFR2 receptor was on the amino acid Gly 846.

Molecular docking evaluation showed that the binding affinity score for VEGFR2 receptor and cilengitide ligand was -8.2 kcal/mol stronger than astaxanthin -7.7 kcal/mol. These results indicate that Astaxanthin compounds have anticancer activity through inhibition of the VEGFR2 receptor.

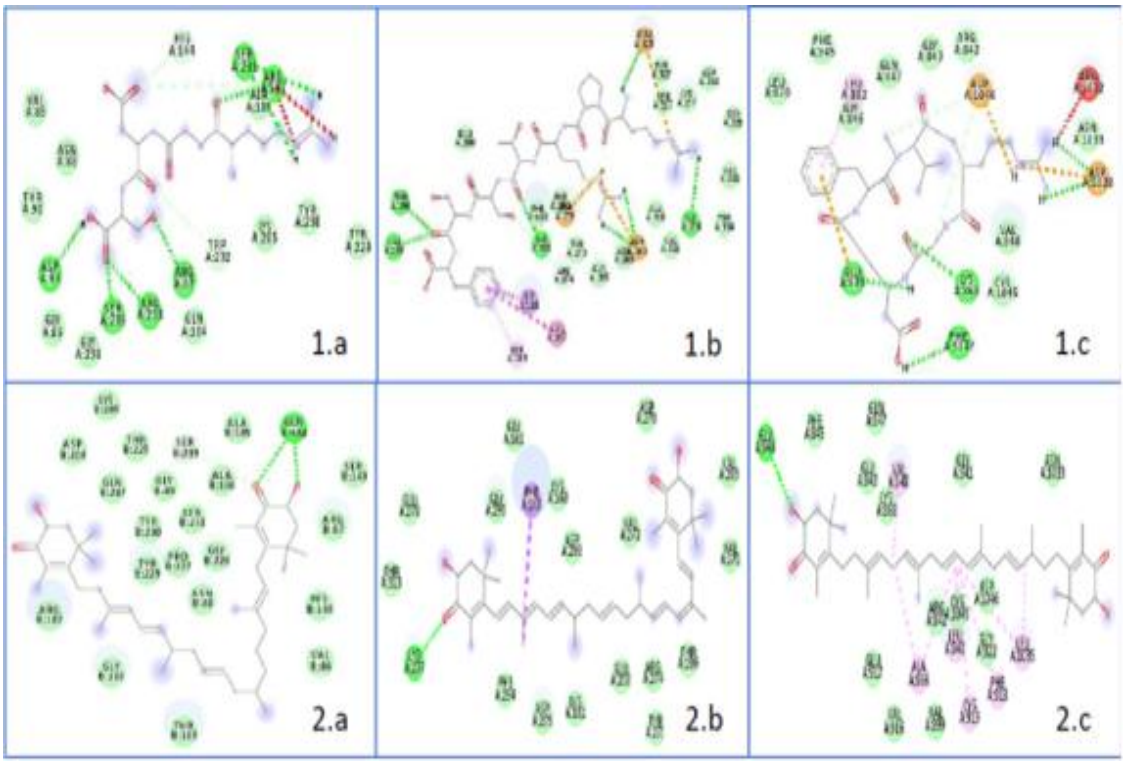

Figure 4: Interaction of ligands and target receptors. (1.a) RGDS bound to Procaspase 7, (1.b) RPRTSSF bound to PKB, (1.c) Cilengitide bound to VEGFR2, (2.a) Astaxanthin bound to Procaspase, (2.b) Astaxanthin bound to PKB, (2.c) Astaxanthin bound to VEGFR2.

Table 4: Comparative binding affinity of different ligands with receptors.

No.	Receptor	Ligand	Binding Affinity (kcal/mol)
1	Procaspase 7	RGDS Astaxanthin	-6.9 -9.0
2	Protein Kinase B	RPRTSSF Astaxanthin	-6.6 -7.1
3	Vascular Endothelial Growth Factor R 2	Cilengitide Astaxanthin	-8.2 -7.7

CONCLUSIONS

Molecular docking of Astaxanthin has the most potential binding affinity score as an anticancer agent agent in the inhibition mechanism of the procase 7 receptor.

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