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ANTICANCER ACTIVITY OF CHEBULIC ACID BY MOLECULAR DOCKING METHOD

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ABSTRACT

Objective: Chebulic acid, a phenolcarboxylic acid compound isolated from *Terminalia chebula*, has potent anti-oxidant and anticancer activity, which breaks the cross-links of proteins induced by advanced glycation end-products (AGEs) and inhibits the formation of AGEs. Molecular docking of Chebulic acid 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 value of the comparison between the interaction of chebulic acid with the native ligand receptor that binds to each receptor in the binding pocket becomes an evaluation of scoring docking and the bond formed between the 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 chebulix acid. **Results.** The evaluation of the docking score of the chebulic acid compound were -7.5 kcal/mol for procaspase 7; -6.0 kcal/mol for PKB; and -6.7 kcal/mol for the VEGFR2 receptor. **Conclusion:** The binding affinity value in the docking simulation of chebulic acid compound concluded that the activity as an anticancer was very dominant in the Procaspase 7 receptor inhibition mechanism.

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

INTRODUCTION

Cancer is an abnormal growth of cells in body which leads to death. These cells are born due to imbalance in cell proliferation mechanism. In 2018, WHO released new statistics on cancer incidence, mortality, and prevalence worldwide i.e., GLOBOCAN 2018 estimates for 28 types of cancer in which more prevalence of cervix and breast cancer. The trend of using natural compounds to treat cancer is due to their low toxicity and potential properties.[1, 2]

The ripe fruit of Terminalia chebula RETZIUS (T. chebula RETZ) (Combretsceae), which is widely found in India and Southeast Asia, has been widely used as a popular traditional medicine for homeostatic, antitussive, laxative, diuretic, and cardiotonic treatment. [3] Dried fruit of *Terminalia chebula* contains high quantities phenolic compounds consist of ellagic acid, gallic acid and chebulic acid. The fruit extract of *T. chebula* is having different biological properties like as a laxative and tonic agent, anticancer, antidiabetic, antimutagenic, antibacterial, antifungal and antiviral activities.[4-12] Terminalia chebula plant, and the Chebulic Acid Structure can be seen in Figure 1.

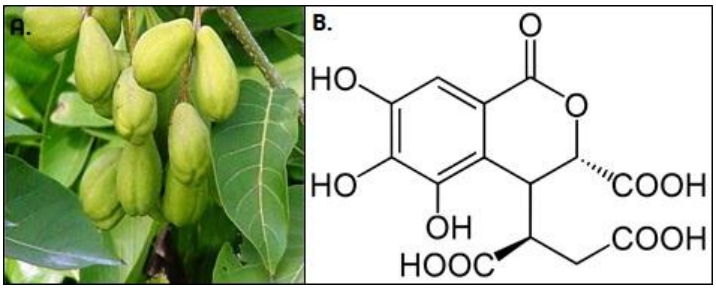

Figure 1. (a) *Terminalia chebula*; *(*b) Chebulic Acid Structure.

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.[13]

Determination of the anticancer activity of chebulic acid 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.[14] 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.[15,16] VEGFR2 has important roles in physiological and pathological angiogenesis, including tumor angiogenesis.[17] In addition, VEGFR-2 functions in the primary regulation of antiapoptotic effects and maintenance of sinusoidal endothelial cell architecture (SEC).[18]

MATERIALS AND METHODS

Software and Tools

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

Ligand Preparation

Chebulic acid 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	Chebulic acid	$C_{14}H_{12}O_{11}$	[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 chebulic acid 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

Chebulic acid 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

Chebulic acid 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	Chebulic acid	356.24	6	11	-0.8	366.78
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

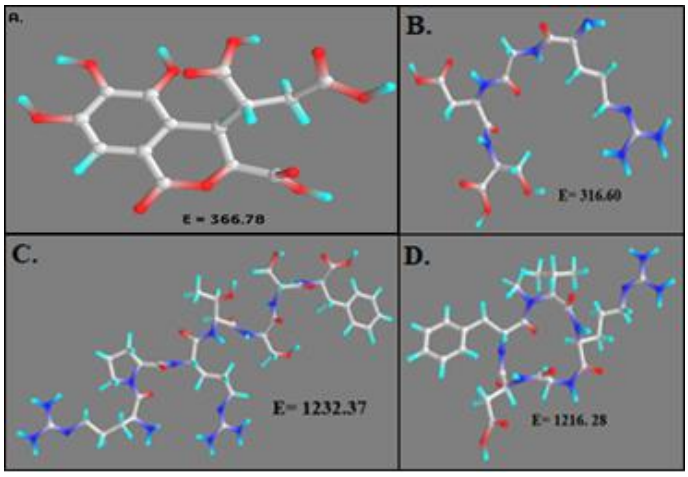

Figure 2. 3D ligand structure and energy minimized results. (A) Chebulic acid, (B). RGDS, (C) RPRTSSF, (D) Cilengitide.

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.

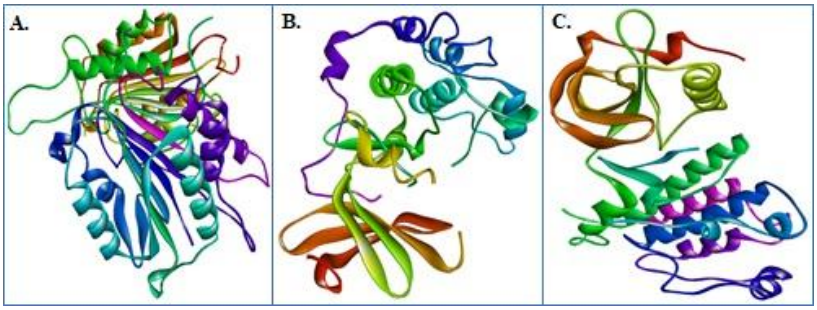

Figure 3: 3D structure of receptor, (A) Procaspase 7, (B) Protein Kinase B, (C) VEGFR2.

Docking Studies Using AutoDock Vina

Docking of Chebulic acid 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 Chebulic acid against the procaspase 7 receptor targets on amino acids Arg 87, Gln 184, His 144, Ser 239, Thr 90. 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 chebulic acid ligand forms hydrogen bonds with the procaspase 7 receptor on the amino acid Asn 88, Asn 85, Val 86, Arg 233, Arg 187.

Molecular docking evaluation with comparison the binding affinity score gave results that Chebulic acid has a value of -7.9 kcal/mol better than the RGDS ligand score of -6.9 kcal/mol. This provides information that chebulic acid compounds have anticancer activity through the Procaspase 7 receptor inhibition mechanism.

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

The results of 2-D visualization of the RPRTSSF and Chebulic acid ligands against the PKB receptor target showed many similarities in the formation of bonds in amino acids Arg 274, Asp 275, Asp 293, Val 272, Leu 183, Lys 181, Phe, 163, Thr 199, Tyr 273. Interaction of RPRTSSF and PKB receptors has hydrogen bonds. the amino acids Thr 199, Val 198, Lys 191, while the chebulic acid ligand and the PKB receptor have hydrogen bonds at Asp 293, Val 272, Lys 181, Tyr 273.

The docking score of RPRTSSF -6.6 kcal / mol was weaker than the binding affinity of the chebulic acid ligand with PKB receptor -6.0 kcal / mol. It can be concluded that chebulic acid has a stronger interaction activity as an anticancer at PKB receptors.

Docking of Chebulic acid 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 chebulic acid ligands on the VEGFR2 receptor and VEGFR2 receptors were formed on the same amino acids Ala 866, Cys 1045, Gly 841, Phe 918, Val 848. There were hydrogen bonds between chebulic acid the VEGFR2 receptor was on the amino acid Asn 923, Arg 1032, Cys 919, Leu 840s.

Molecular docking evaluation showed that the binding affinity score for VEGFR2 receptor and cilengitide ligand was -8.2 kcal/mol stronger than chebulic acid -6.7 kcal/mol.

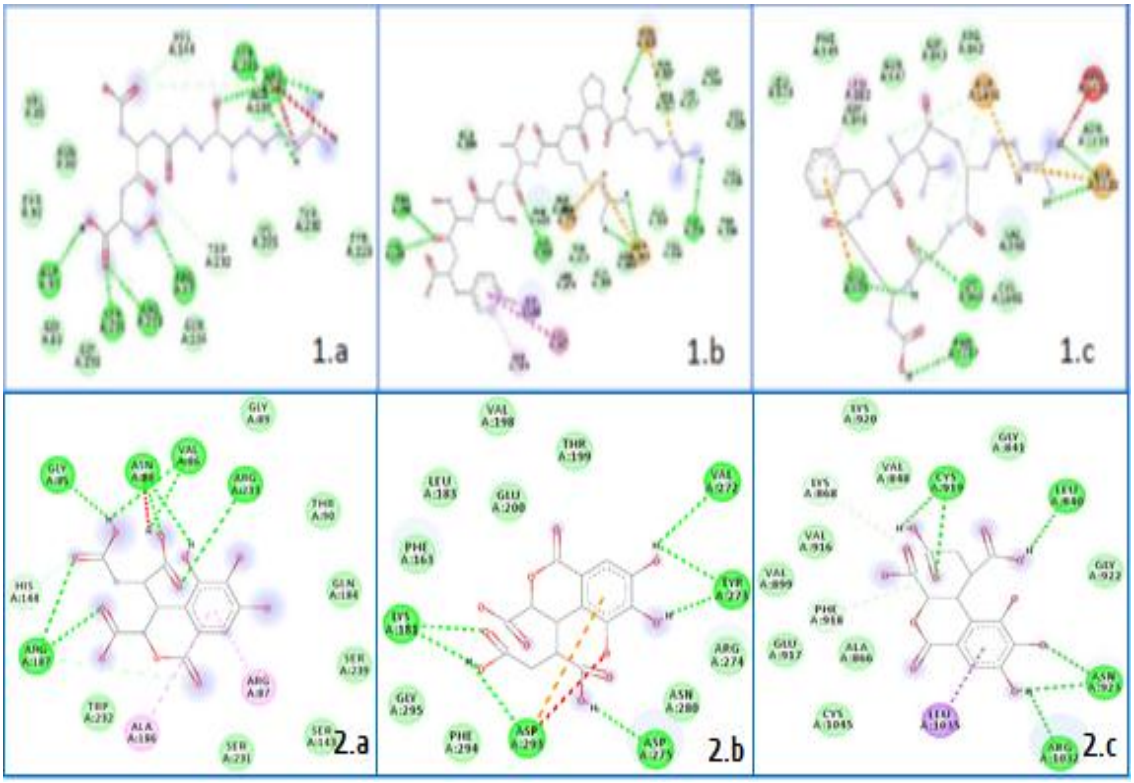

Figure 4: Interaction of ligands and target receptors. (1.a) RGDS bound to Procaspase 7, (1.b) RPRTSSF bound to PKB, (1.c) Cilengitide bound to VEGFR2, (2.a) Chebulic acid bound to Procaspase 7, (2.b) Chebulic acid bound to PKB, (2.c) Chebulic acid bound to VEGFR2.

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

No.	Receptor	Ligand	Binding Affinity (kcal/mol)
1	Procaspase 7	RGDS Chebulic acid	-6.9 -7.9
2	Protein Kinase B	RPRTSSF Chebulic acid	-6.6 -7.5
3	Vascular Endothelial Growth Factor R 2	Cilengitide Chebulic acid	-8.2 -6.7

CONCLUSIONS

Molecular docking of Chebulic acid 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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