***In-silico* ANALYSIS OF VERNONIOSIDE D AND VERNONIOSIDE E FROM *Vernonia amygdalina* Delile. LEAVES AS INHIBITOR OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) AND MAMMALIAN TARGET OF RAPAMYCIN (mTOR)**

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ABSTRACT

Vernonia amygdalina Delile. Leaves contain cardiac glycosides, which are potential cardiogenic and anticancer. This study evaluated the activity of vernonioside D, and E inhibits the epidermal growth factor receptor (EGFR). The study of the mammalian target of rapamycin (mTOR) confirmed the activity of these glycosides. In silico docking using Autodock Vina PyRx 9.5 program and visualized by Ligplot 2.1. EGFR and mTOR structures were used as test receptors, binding pocket with the Protein Data Bank (PDB) code 1M17 and 3L16. To generate two and three dimensions of vernonioside D and E using the Marvin Sketch program. Both compounds and reference drugs (thienopyridine-2-yl) aminopyridine and erlotinib) inhibited EGFR and mTOR with docking score -10.4; -8.6; -6.9 and -6.3; -6.6; -9.2 respectively. Vernonioside D and E are more potent in inhibit EGFR compare to the reference drug.

Keywords: Vernonioside D, Vernonioside E, *In-silico*, EGFR, mTOR

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INTRODUCTION

Cancer can affect anyone, but this type of breast cancer only occurs in women.¹ The data states that breast cancer is ranked fifth as the type of cancer that causes the largest death cases in the world (522,000 deaths). In women, this type of cancer is a frightening threat (324,000 deaths, 14.3% of the total cases). Meanwhile, in strong and developed countries, breast cancer is second only to lung cancer-causing death (198,000 deaths, 15.4%).² The process of cellular survival of cancer cells is regulated by a variety of mechanisms, including EGFR and mTOR. EGFR is part of the HER transmembrane receptor. The inappropriate activity of the EGFR will have an impact on the formation of cancer cells.^{3,4} Inhibition of EGFR activity will weaken cancer cells so that it is beneficial for the cancer therapy process.⁵ Apart from EGFR, mTOR also has the same role in the process of cancer cell development, especially cell proliferation and growth. mTOR is in the PI3K / Akt / mTOR signaling pathway which is known to also play a role in the regulation of apoptosis, metastasis, and resistance of cancer cells to radiotherapy. In the last decade, the combination of PI3K/Akt/mTOR inhibitor with other therapy develop significant progress to overcome less effective treatment.⁶⁻⁷

The search for active compounds from plants to treat cancer continues. Active compounds such as cardiac glycosides are reported to inhibit cancer cell activity. Cardiac glycosides are steroid compounds with unsaturated α and β lactone rings. Cardiac glycosides are known as compounds that can affect cardiac activity through their influence on Na⁺ and K⁺ pumps.⁸⁻¹⁰ *Vernonia amygdalina* has many pharmacological

effects including antimalarial, antidiabetic, anti-cancer, hepatoprotection, nephroprotection, analgesic, antibacterial, antioxidant and also has an inotropic effect on the heart. *Vernonia amygdalina* has contained various secondary metabolites including sesquiterpene lactone (vernolide, vernodalol, vernoamygdalin, vernolepin), Flavonoid (luteolin, luteolin 7-O-beta-glucuronoside dan luteolin 7-O-glucoside), and also contained cardiac glycosides (vernionioside D and E).¹¹⁻¹³ Based on this explanation, the vernonioside D and E activity testing will be carried out against the EGFR and mTOR receptors using the *in silico method*.

Fig.-1: Chemical Structure of Vernonioside D and Vernonioside E

EXPERIMENTAL

Aspire Vivobook operated by Windows 7 Home Basic, Intel® Core™ i5 (3.4 GHz), 64-bit, hard disc drive 320 GB and RAM 4 GB DDR3 L were used to run the molecular docking process. In silico docking using Autodock Vina PyRx 9.5 program and visualized by Ligplot 2.1. The model of three dimensions of enzyme structure used in this research was epidermal growth factor receptor (EGFR) and mammalian target of rapamycin (mTOR) binding pocket with the Protein Data Bank (PDB) code 1M17 and 3L16., which were obtained through from <http://www.rcsb.org/pdb>. The Marvin sketch program generates the three-dimension conformation models of vernonioside D and E.¹⁴⁻¹⁵

RESULTS AND DISCUSSION

In-silico Analysis

Overexpression from EGFR and mTOR has long been found in the development of cancer cells, including breast cancer.¹⁶ EGFR is part of the tyrosine kinase receptor. EGFR has an important role in physiological terms. EGFR development in epithelial tissue development, homeostasis and tumor cell development.¹⁷ mTOR is an attractive protein used as a target for therapy in cancer. mTOR has distinctive functions such as convergence points for many growth stimuli and through downstream controlling cellular processes that contribute to cancer cell initiatives.¹⁸

Therefore, opposing EGFR and mTOR is very effective in finding therapies that inhibit the development of cancer cells. In this experiment the testing of the activity of natural compounds in silico against EGFR and mTOR. In silico docking between cardiac glycosides from *Vernonia amygdalina* Delile. Leaves (vernionioside D and E) into the 1M17 and 3L16 binding pocket result in the docking score in Tables-1 and 2.

Table-1: Docking Score between Vernonioside D and E in the Pocket of mTOR (3L16)

No	Compound	Binding Affinity (Kcal/mol)
1	Thienopyrimidin-2-yl-aminopyrimidines	-9.2
2	Vernonioside E	-6.6
3	Vernonioside D	-6.3

Table-1 showed docking score as a description mTOR inhibitory effect of Vernonioside D and E as natural compounds and Thienopyrimidin-2-yl-aminopyrimidines as comparison compound. Vernonioside D and E

have an affinity binding value of -6.6 Kcal/mol and -6.3 Kcal/mol, while Thienopyrimidin-2-yl-aminopyrimidines was -9.2 Kcal/mol. The result showed Thienopyrimidin-2-yl-aminopyrimidines has a better activity in mTOR inhibited. However, Vernonioides D and E also can inhibit mTOR.

Table-2: Docking Score between Vernonioides D and E in the Pocket of EGFR (IM17)

No.	Compound	Binding Affinity (Kcal/mol)
1	Erlotinib	-6.9
2	Vernonioides E	-8.6
3	Vernonioides D	-10.4

EGFR Inhibitory activity of Vernonioides D and E was carried out by *In-silico* test. The result in Table-2 showed Vernonioides D and E have an activity to inhibited EGFR with a docking score value of -10.4 Kcal/mol and -8.6 Kcal/mol. Erlotinib used as a comparison compound has an activity of -6.9 Kcal/mol docking score. Based on the result, Vernonioides D has a better activity to inhibited EGFR than Vernonioides E and Erlotinib. The lowest energy produced from the bond between the ligand and protein shows better inhibitory activity.¹⁹

The activity of the test material (ligands) binds to a receptor is influenced by many factors, one of which is the chemical bond formed between the test material (ligands) and amino acids at the receptor.¹⁴ The binding of amino acid residues to ligands was showed in Table-3.

Fig.-2: Three Dimensional Binding Form of (a) mTOR (control (red); Vernonioides E (green)); (b) EGFR (control (red); Vernonioides D (green))

Table-3: Interaction of Vernonioides D and E to Amino Residues of EGFR and mTOR

Ligands	Amino Acids Involved	
	Hydrogen-Binding Interaction	Hydrophobic Interaction
Thienopyrimidin-2-yl-aminopyrimidines	Ile879, Leu838, Ile831, Ile881, Ala805, Met804, Lys890, Trp812, Tyr867	Asp841
Vernonioides E	Lys802, Thr886, Val882, Trp812, Ala885, Met804, Met953, Ile963, Ser806, Asp950, Lys807, Lys808, Ile963, Ile831, Thr887	Asn951, Asp964, Lys833
Erlotinib	Glu783, Met742, Lys721, Leu764, Thr766, Thr830, Leu694, Phe699	Asp831, Met769
Vernonioides D	His781, Phe771, Glu780, Tyr777, Leu820, Gly772, Ile720, Leu764, Asp831, Met742, Val702, Cys773, Asp776, Leu694	Lys721, Ala719, Thr776, Glu738, Thr830

Table-3 showed hydrogen-bonding interaction and hydrophobic interaction of ligands and amino acids. Based on the result showed that ligands bond to different amino acids. Thienopyrimidin-2-yl-aminopyrimidines have a hydrogen bond interaction with Ile879, Leu838, Ile831, Ile881, Ala805, Met804, Lys890, Trp 812, Tyr867 and hydrophobic interaction with Asp841. Vernonioides E and Vernonioides D

have a hydrogen bond interaction and hydrophobic interaction with their respective amino acids Lys802, Thr886, Val882, Trp812, Ala885, Met804, Met953, Ile963, Ser806, Asp950, Lys807, Lys808, Ile963, Ile831, Thr887 and Asn951, Asp964, Lys833 for Vernonioid E and His781, Phe771, Glu780, Tyr777, Leu820, Gly772, Ile720, Leu764, Asp831, Met742, Val702, Cys773, Asp776, Leu694 and Lys721, Ala719, Thr776, Glu738, Thr830 for Vernonioid D. Hydrogen bond and hydrophobic interaction occurred on Erlotinib as Glu783, Met742, Lys721, Leu764, Thr766, Thr830, Leu694, Phe699 and Asp831, Met769. The results of visualization of Vernonioids D and Vernonioids E to EGFR and mTOR using Ligplot 2.1 can see in Fig.-2 Differences in affinity between test compounds to receptors may occur due to differences in bonding between each test compound with amino acids and the type of bond that occurs.²⁰

CONCLUSION

The results reveal that Vernonioid E and D are effective as anticancer through downregulation of EGFR and mTOR proteins.

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