***In-silico* ANALYSIS OF CARDIAC GLYCOSIDES FROM *Vernonia amygdalina* Delile. LEAVES AS CARDIOTONIC THROUGH INHIBITION OF Na⁺/K⁺ ATPase ION TRANSPORT**

**U.Harahap^{1,✉}, A. Dalimunthe¹, G. Haro², R.A. Syahputra¹, Widodo³,
D.H. Utomo³ and D. Satria⁴**

¹Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara,
Medan 20155, Indonesia

²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara,
Medan 20155, Indonesia

³Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang, Indonesia,
65145.

⁴Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara,
Medan 20155, Indonesia

✉Corresponding Author: urip@usu.ac.id

ABSTRACT

Vernonia amygdalina Delile. leaves are potential as cardiotoxic and anticancer cause of cardiac glycosides which contain in them. This study evaluated the mechanism of cardiac glycosides from *Vernonia amygdalina* Delile. leaves in inhibition of Na⁺/K⁺ ATPase with molecular docking. Docking was performed on the receptor file (PDB ID: 4HYT) using AutoDock Vina PyRx 9.5 program and visualized by Ligplot 2.1 dan PyMol 2.3.1. Two and three-dimension conformation models of compounds were generated by Chem Draw program. The docking score of Ouabain as the standard and Vernoniosides A2 were -10.7 Kcal/mol and -9.4 Kcal/mol. Vernoniosides A2 interacted with Phe316, Leu793, Phe783, Gly319, Ile800, Ile315, Glu116, Ala323, Leu795, Thr797, Asn122, Glu179, Asp884, Lys905, Asp121, Gln111, Glu117 and Asn122 residues.

Keywords: Inhibition, Na⁺/K⁺ ATPase, *In-silico*, Cardiac Glycosides, *Vernonia amygdalina*.

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INTRODUCTION

Cardiac glycoside is the presence of the ring lactone at carbon position 7 which consists of 5 carbon atoms called cardenolide commonly found in plants, whereas if it has 6 carbon atoms called bufanolide which is commonly found in animals.¹ Cardiac glycoside included digoxin is used for the treatment of congestive heart failure because it has a positive inotropic effect and also a diuretic effect and is known to inhibit cancer cell proliferation and block tumor growth.²⁻⁴

Vernonia amygdalina has antimalarial, anti-diabetic, anti-cancer, hepatoprotection, nephroprotection, analgesic, antibacterial, antioxidant and also has inotropes effect. *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 stigmastane p type of steroid (vernonioside A1, versonioside A2, versonioside A3, versonioside A4, versonioside B1, versonioside B2, versonioside B3, versonioside C, versonioside D, versonioside 3, vernoniamyoside A, vernoniamyoside B, vernoniamyoside C, vernoniamyoside D, vernoamyoside A, vernoamyoside B, vernoamyoside C, vernoamyoside D, dan jukka veramyoside A, veramyoside B, veramyoside C, veramyoside D, veramyoside E, veramyoside F, veramyoside G, veramyoside H, veramyoside I, veramyoside J) this compound has a structural similarity to cardiac glycoside.⁵⁻¹¹

Computational methods are being developed to predict the activity of compounds. In silico approaches contribute significantly to beginning pharmaceutical research and important in target discovery. The need for timely adaptation and application of in silico approaches in pharmaceutical research has been recognized

and is expected to improve further to get efficiency in drug discovery.¹²⁻¹³ This aimed of this study to assess the activity of cardiac glycosides from *Vernonia amygdalina* Delile. leaves in inhibition Na^+/K^+ -ATPase ion transport with the computational method.

EXPERIMENTAL

Material and Methods

Aspire Vivobook which is operated by Windows 7 Home Basic, Intel® Core™ i5 (3.4 GHz), 64-bit, hard disc drive 320 GB and RAM memory 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 dan PyMol 2.3.1. The model of the three dimensions of enzyme structure used in this research was Na^+/K^+ ATPase binding pocket with the Protein Data Bank (PDB) code 4HYT. It was obtained through <http://www.rcsb.org/pdb>. Two and three-dimension conformation models of compounds were generated by the Chem Draw program.¹²

RESULTS AND DISCUSSION

In-silico Analysis

In silico docking between cardiac glycosides from *Vernonia amygdalina* Delile. leaves into the 4HYT binding pocket result in the docking score in Table-1 and 2 are showed the results of docking score into 4HYT binding pocket. Figure-1 is showing the results of visualization of Ouabain and Vernoniosides A2 to Na^+/K^+ ATPase using Ligplot 2.1 dan PyMol 2.3.1.

Fig.-1: Three Dimension Binding of Ouabain and Vernoamyosides A with 4HYT

Table-1: The Binding Affinity of Ligand Standard and Compounds towards Na^+/K^+ -ATPase (4HYT)

No.	Ligand	Binding Affinity (Kcal/mol)
1	Ouabain	-10,7
2	Vernoniosides A2	-9,4
3	Vernoniosides A3	-9,4
4	Vernoniosides D	-9,4
5	Vernoamyosides A	-9,2
6	Vernoamyosides B	-9,2
7	Vernoniosides A1	-9,1
8	Vernoamyosides C	-8,9
9	Vernoniosides B3	-8,9
10	Vernoniosides D2	-8,9
11	Vernoamyosides D	-8,6
12	Vernoniosides B1	-8,6
13	Vernoniaamyosides C	-8,3
14	Vernoamyosides G	-8,2

15	Vernoniaamiosides D	-8,2
16	Vernoniaamiosides A	-8,1
17	Vernoniosides B2	-8
18	Vernoniosides E	-8
19	Vernoniaamiosides B	-7,6
20	Vernoniosides A4	-7,6

Vernonioside, vernoamyoside, veramyoside, vernoniamyoside are secondary metabolites found in African leaves, the structure of this compound is very similar to digoxin which is used as a standard in this study, which contains lactone groups in C-17 atoms, sugar groups, and steroid nuclei. But this compound is unique in that it has more than one ketone group compared to digoxin which has one ketone group, this ketone group is known to increase the inotropic effect, and this is related to Sembiring study states that ethanol extracts of African leaves have a higher inotropic effect than digoxin.^{8,14-18}

Table-2: Interaction Ligands and Protein through Amino Acids and the Bond Type

Ouabain	Vernoniosides A2	Bond Type
Arg880	-	Hydrophobic
Glu312	-	Hydrophobic
Phe316	Phe316	Hydrophobic
Phe783	Phe783	Hydrophobic
Leu793	Leu793	Hydrophobic
Ala323	Ala323	Hydrophobic
Val322	-	Hydrophobic
Leu125	-	Hydrophobic
Gly319	Gly319	Hydrophobic
Ile800	Ile800	Hydrophobic
Asp121	Asp121	Hydrophobic
Pro118	-	Hydrophobic
Ile315	Ile315	Hydrophobic
Glu116	Glu116	Hydrophobic
Gln111	Gln111	Hydrogen
Glu117	Glu117	Hydrogen
Asn122	Asn122	Hydrogen
-	Leu795	Hydrophobic
-	Thr797	Hydrophobic
-	Glu779	Hydrophobic
-	Asp884	Hydrogen
-	Lys905	Hydrogen

CONCLUSION

From the result, it can be recapitulated that vernoniosides A2 has higher activity on binding to Na⁺/K⁺-ATPase (4HYT) but lower than ouabain and this plant is the potential to develop as cardiotonic.

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