***In-silico* TOXICITY PREDICTION OF BIOACTIVE COMPOUNDS OF *Vernonia amygdalina* DELILE. AND DIGOXIN**

**R.A. Syahputra¹, U.Harahap^{1,*}, A. Dalimunthe¹, P. Nasution², G. Haro³,
Widodo⁴, D.H. Utomo⁴, and D. Satria²**

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

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

³Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara,
Medan-20155, Indonesia

⁴Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang-65145, Indonesia.
*E-mail: urip@usu.ac.id

ABSTRACT

Vernonia amygdalina Delile. is commonly found in a tropical area, it has an inotropic effect on the heart and has phytochemical constituents that are similar to digoxin such as vernonioside D and vernonioside A3. This study aimed to determine the prediction of toxicity level and value of the bond energy between digoxin, vernonioside D, and vernonioside A3 on the enzyme Na⁺/K⁺ATPase *In silico* method. Prediction of ADMET was determined by using the pkCSM website, Prediction of toxicity of chemical were determined by protox II website and docking analysis in this study was carried out using the AutoDock software with PDB code 4RET and visualization using polymol. The results showed that digoxin induces CYP3A4 receptors while vernonioside D and A3 did not induce CYP3A4 receptors. Prediction of LD₅₀ was digoxin 5 mg/kg (fatal), while vernonioside D has a prediction of LD₅₀ was 8000 mg/kg (non-toxic) and vernonioside A3 has a prediction of LD₅₀ was 8000 mg/kg (non-toxic). Digoxin, vernonioside D and vernonioside A3 inhibited hERG II. The binding energy value of Na⁺/K⁺ATPase to digoxin was obtained value -12 Kcal/mol, vernonioside D was obtained a value of -9 Kcal/mol while vernonioside A3 was obtained a value of -9.4 Kcal/mol. Based on the research, it can be concluded that vernonioside D and vernonioside A3 has a lower toxicity level compared to digoxin, while the affinity of digoxin for Na⁺/K⁺ATPase has higher compared to Vernonioside D and vernonioside A3.

Keywords: Toxicity, *In-silico*, Digoxin, Vernonioside D, Vernonioside A3.

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INTRODUCTION

Vernonia amygdalina Delile. is a plant that is commonly found in Africa and also in tropical regions such as Indonesia, which is a family of Asteraceae, this plant has been widely used traditionally for treatment, and it is known that *Vernonia amygdalina* extract can treat chimpanzees affected by malaria¹. *Vernonia amygdalina* has many pharmacological effects including antidiabetic, nephroprotective, antioxidant, antihyperlipidemic, immunological effects, anthelmintic, anti-obesity, hepatoprotection, and anti-inflammatory. based on the previous research reported that ethanol extract of *Vernonia amygdalina* is also known as a higher inotropic compared to digoxin in rats in the heart²⁻¹⁰. African leaves contain compounds such as saponins, flavonoids, lactone sesquiterpenes, and steroid glycosides such as vernonioside b1, vernonioside a2, vernonioside a3, vernonioside b1, vernonioside d, vernonioside e and vernonioside b2 and have steroid saponin vernonioside a3, vernonioside a3, vernonioside b1, vernonioside d, vernonioside e and vernonioside a2, vernonioside a2, vernonioside a3, vernonioside b1, vernonioside d, vernonioside e and vernonioside b2 and have steroid vernonioside, vernonioside a3, vernonioside b1, vernonioside d, vernonioside e2 and vernonioside b2, vernonioside a2, vernonioside a3,

vernonioside b1, versonioside d. vernoniamyoside a, vernoniamyoside b, vernoniamyoside c, vernoniamyoside d, and vernoamyoside d this structurally similar to digoxin¹¹⁻¹⁵. Digoxin is a cardiac glycoside isolated from the *Digitalis purpurea* foxglove plant. Cardiac glycosides are used as a treatment for positive inotropic congestive heart failure, atrial fibrillation, and diuretic. Lately known have anti-cancer activity¹⁶⁻¹⁸. This aimed of this study to determine the prediction of toxicity level and value of the bond energy between digoxin, versonioside D, versonioside A3 on the enzyme Na⁺/K⁺ATPase with *In silico* method.

EXPERIMENTAL

Software

Asus core i3 64 bite, Chemdraw, pkCSM online tool, Protox online tool, AutoDock, dan Polymol.

Target and Template Selection

PDB code of Na⁺/K⁺ATPase: 4RET that was taken from Protein data bank (<https://www.rcsb.org/>) and smiles data of digoxin and versonioside D and A3 were taken from Pubchem compound (<https://PubChem.ncbi.nlm.nih.gov/>).

Computational Assay : Prediction of ADMET

In silico ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) using the SMILES series for Digoxin, Versonioside D, and Versonioside A3 isolates from African leaves. The properties of Digoxin and Versonioside isolates were determined using the pkCSM website (<http://biosig.unimelb.edu.au/pkcsml/>) and the Prediction of the Toxicity of Chemical was determined using Protox II website (http://tox.charite.de/protox_II/). Prediction of Toxicity Class of chemical divided into: Class 1: Fatal (LD50 ≤ 5), Class 2: Fatal (5 < LD50 ≤ 50), Class 3: Toxic (50 < LD50 ≤ 300), Class 4: Harmful (300 < LD50 ≤ 2000), Class 5: Maybe harmful (2000 < LD50 ≤ 5000), Class 6: non-toxic (LD50 > 5000).

In-silico Analysis of Inhibition Na⁺/K⁺-ATPase

Docking in this study was carried out using AutoDock software with PDB code (4RET). The affinity of the ligand with the target protein is shown in the resulting score. The lower the score the more stable the ligand-receptor bond and visualized in 2 D the bond using polymol.

RESULTS AND DISCUSSION

Prediction ADMET of Digoxin, Versonioside D, Versonioside A3 (pkCSM)

From the computational results obtained pharmacokinetic data of digoxin, versonioside D, and versonioside A3 below in Table-1.

Table-1: Data of pkCSM of Digoxin, Versonioside D, and Versonioside A3

Property	Model Name	Predicted Value of Digoxin	Predicted Value of Versonioside D	Predicted Value of Versonioside A3	Unit
Absorption	Water Solubility	-4.096	-3.215	-3.29	Numeric (log mol/L)
Distribution	VDss (human)	0.199	0.225	0.088	Numeric (log L/kg)
Metabolism	CYP3A4 Substrate	Yes	No	No	Categorical (Yes/No)
Excretion	Total Clearance	0.479	0.317	0.335	Numeric (log ml/min/kg)
Toxicity	hERG II Inhibitor	Yes	Yes	Yes	Categorical (Yes/No)

Table-1 showed that digoxin induces CYP3A4 receptors which can affect other drugs that are inhibiting the CYP3A4 enzyme and can cause toxicity such as Amiodarone, Atazanapir, Chloramphenicol,

Diltiazem, and others. Digoxin inhibits hERGII which is a gene that encodes the Na^+/K^+ ATPase protein sub-unit of alpha in the Potassium channel, the inhibitory effect of this potassium channel is needed to inhibit the exchange of Na which causes increased concentration of Na in the cell and is exchanged with Ca through the Na/Ca Exchanger channel which causes increased concentration Ca in cells and the inotropic activity to increase¹⁹⁻²⁰. Vernonioidide D and vernonioidide A3 did not induce CYP3A4 enzymes, this could indicate vernonioidide D and A3 are safer to use compared to digoxin. Vernonioidide D and vernonioidide A3 inhibit hERG II which has the same mechanism as digoxin to produce an inotropic effect²¹.

Prediction Rules of Five Lipinski

Table-2 showed that digoxin has a molecular weight of digoxin was 780.94g/mol greater than vernonioidide D was 664.78g/mol and vernonioidide A3 was 678.86g/mol so that all three compounds did not meet the Lipinski requirements, LogP all compounds meet the Lipinski requirements, the number of acceptors for digoxin was 14 is greater than that of vernonioidide D was 12 and vernonioidide A3 was 11 so that the three compounds did not meet Lipinski's requirements, and the number of donors for digoxin was 6, vernonioidide was D 7, and vernonioidide A3 was 6, the three compounds also did not meet Lipinski's requirements. Thus the three compounds are difficult to absorb and have low permeability, but when data is compared to the three compounds the vernonioidide compound is easier to absorb and higher permeability when compared to digoxin based on the results of the rules of five Lipinski²².

Table-2: Prediction of Rules of Five

No.	Parameter	Requierment	Digoxin	Vernonioidide D	Vernonioidide A3
1.	Molecular weight	<500	780.94	664.78	678.86
2.	LogP	≤5	2.21	0.48	2.54
3.	Acceptors	≤10	14	12	11
4.	Donors	≤5	6	7	6

Toxicity Prediction of Digoxin, Vernonioidide D, Vernonioidide A3

Table-3 showed that digoxin has fatal toxicity with LD₅₀ was 5 mg/kg body weight. The oral dose of digoxin is only 0.25 mg/kgBW so the use of digoxin is very limited and has a narrow therapeutic index²³. The vernonioidide D and vernonioidide A3 compounds have a prediction of LD₅₀ was 8000 mg/kg with the non-toxic category when compared with digoxin is categorized as fatal, vernonioidide D and vernonioidide A3 are far safer to use based on the data.

Table-3: Toxicity Prediction of Digoxin, Vernonioidide D, Vernonioidide A3

No.	Parameter	Digoxin	Vernonioidide D	Vernonioidide A3
1.	Predicted LD 50	5 mg/kg	8000 mg/kg	8000 mg/kg
2.	Predicted toxicity class	Class 1	Class 6	Class 6
3.	Average similarity	100%	78,94%	76,68%
4.	Prediction accuracy	100%	69,26%	69,26%

In silico Analysis of Inhibition Na^+/K^+ -ATPase

Table-4 showed that digoxin has an affinity binding value of -12 (Kcal/mol) while vernonioidide D was -9 (Kcal/mol) and vernonioidide A 3 was -9.4 (Kcal/mol). According to previous research, the bond energy value of bonding results *In silico* is the affinity of the bond between the test compound (ligand) and the bond bag. The lower the bond energy value of the bonding results *In silico*, the stronger the interaction between the test compound molecules (ligands) and the bond sac molecules. Digoxin has a stronger affinity for Na^+/K^+ ATPase compared to vernonioidide D and vernonioidide A3.

Table-4: Binding Affinity of Digoxin, Vernonioidide A3, Vernonioidide D

No.	Ligand	Binding Affinity (Kcal/mol)
1.	Digoksin	-12
2.	Vernonioidide A3	-9,4
3.	Vernonioidides D	-9

[illegible]

Fig.-2: Vernonioid A3

The Table-5 showed the hydrogen bonds of digoxin compounds with 4RET amino acids in several 5 bonds namely with glu312, arg886, asp885, asn122 and asp884 whereas with A3 vernonioside compounds a total of 4 bonds namely leu795, asp121, asn122, and thr797. The abundance of hydrogen amino acid digoxin bonds with 4RET compounds causes the bond energy to be lower than that of vernonioside A3 which causes digoxin activity to be higher compared to vernonioside A3. Table-5 and Table-6 show that the amino acid Asn122 (asparagine 122) is an amino acid that is bound to both compounds and the bond is a hydrogen bond that causes both compounds to inhibit receptors by competitive inhibitors.

Asam amino	Digoxin	Vernonioside A3
Phe783	Present	Present
Phe316	Present	Present
Glu116	Present	Present
Arg880	Present	Present
Phe786	Present	Present
Leu793	Present	Present
Asn122	Present	Present

Digoxin	Vernonioside A3
Glu312	Leu795
Arg886	Asp121
Asn122	Asn122
Asp885	Thr797
Asp884	

Based on the research, it can be concluded that vernonioside D and vernonioside A3 have a lower toxicity level compared to digoxin, while the affinity of digoxin for $\text{Na}^+/\text{K}^+\text{ATPase}$ are higher compared to Vernonioside D and Vernonioside A3

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