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Insilico Analysis of the Potential Phytoestrogens of Selected Alkaloid from Soursop (*Annona muricata*) Leaves

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A r t i c l e   I n f o

A b s t r a c t

Estrogen is an important hormone in the female reproductive system, as well as other systems in men and women. The decrease of estrogen levels mainly occurs in the menopause phase. The decrease of estrogen is one indication of the presence of various diseases such as cardiovascular disease. Decreased levels of estrogen can be treated by administering exogenous estrogen such as Estradiol-17β. However, long-term administration of estradiol plays an important role in tumor cell growth. Phytoestrogens are plant-based polyphenolic compounds that have a structure and biological activity similar to the estrogen. Phytoestrogens can be derived from alkaloid compounds, one of which is found in the soursop (Annona muricata) leaves. The purpose of this study was to analysis the potential phytoestrogens of selected alkaloid in the A. muricata leaves. The analysis was carried out using the insilico approach using molecular docking methods, pharmacokinetic analysis and druglikeness. The molecular docking results showed that the ligands could bind to the receptors on the specific binding site with the lowest binding affinity value approaching the control ligands, namely the Anonaine compound of -8.6 kcal / mol, followed by Xylopine compound of -7.8 kcal / mol and Annonamine of -7.0 kcal / mol. The results of pharmacokinetic and druglikeness showed that all ligand compounds have a good ability to be absorbed in the gastrointestine and have a good bioavalaibility as an oral drug.

1. INTRODUCTION

Estrogen is a hormone that plays an important role in sexual development and reproduction, especially in women. Estrogen is produced in the ovary and has one of the main functions in regulating the estrous or menstrual cycle in women, therefore estrogen is very important for female reproduction. Estrogen is not only important for reproduction, but also in other systems such as the neuroendocrine, skeletal and immune system in men and women (Hamilton et al., 2014). The main mechanism of action of estrogen is through the expression of estrogen receptors (ER) (Couse et al., 1997), both mediated by ERα which is dominant in the liver, uterus, mammary glands, pituitary, hypothalamus, cervix, and vagina, and ERβ which is dominant in the ovaries, lungs and prostate. (Nilsson et al., 2001).

The decrease of estrogen levels caused by various factors, mainly menopause. Menopause occurs when a woman stops ovulating and her monthly periods (menstruation) stop. The absence of estrogen during the menopausal phase is an indicative of the cardiovascular disease, which is characterized by variations in the lipid profile and predominantly abdominal fat accumulation (Hemalatha et al., 2019). Estradiol (E2) is the one of exogenous estrogen that can be used for hormone therapy in the menopause phase (Fait, 2019). However, long-term administration of exogenous estradiol plays an important role in facilitating tumor development both of by estrogen receptor and non-receptor mechanisms (Yue et al., 2010).

Phytoestrogens are the polyphenolic and non-steroid compounds which have a structure and biological activity similar to estrogen in humans (Nikolić et al., 2017). Most of the phytoestrogens are found in flavonoids and isoflavones. The reports of the potential phytostrogens of alkaloid structure are extremely rare. A possible reason for being neglected in the exploration for estrogenity could be that they are generally considered toxic. However, the striking structural variations of a large number of natural alkaloids are the basis of a broad spectrum of quite diverse activities. In addition, several pharmacological studies have shown that some alkaloids belonging to different chemical subgroups show only low toxicity. Alkaloids had a potential as a source of new phytoesterogens (Djiogue et al., 2014).

Soursop (*Annona muricata*) is a plant that is widely used as a traditional medicine and contains a number of alkaloids, especially in the leaves. (Abdul Wahab et al., 2018). Based on this background, it is deemed necessary to study the potential of phytoestrogens derived from alkaloids of *A. muricata* leaves.

2. MATERIALS AND METHODS

This research based on the insilico method which was conducted from April to June 2019 at the Laboratory of the Biology Study Program, Faculty of Science and Technology UIN Maulana Malik Ibrahim Malang.

a. Samples Preparation

Alkaloid derivatives of *A. muricata* used as ligan are: annonamine (PubChem CID: 56929881), anonaine (PubChem CID: 160597) and xylopine (PubChem CID: 160503). Estradiol 17-β (PubChem CID: 5757) used as a ligan control. Test and control ligands are downloaded in 3D format via PubChem Compound (https://www.ncbi.nlm.nih.gov/pccompound/). Ligand preparation was carried out by minimizing all active compound structures at PyRx 0.8. ERα structure as a reseptor downloaded from Protein Data Bank (RSCB) PDB ID: 1A52 (https://www.rscb.org). The water and drug molecules attached to the protein receptor structure are removed and the hydrogen atoms are eliminated.

b. Molecular Docking Analysis

Docking analysis was carried out using the Autodock Vina program in Pyrx software (Trott and Arthur, 2010) to find out the interaction between ligand and receptor. The result is binding affinity values in kcal / mol which will compared with the binding affinity value of control ligands as a basis for determine which alkaloids from the *A. murcata* leaves have an estrogenic effect with binding values close to the control ligands. Visualization of docking results was carried out using Pymol software, while interaction visualization in 3D using Discovery Studio software.
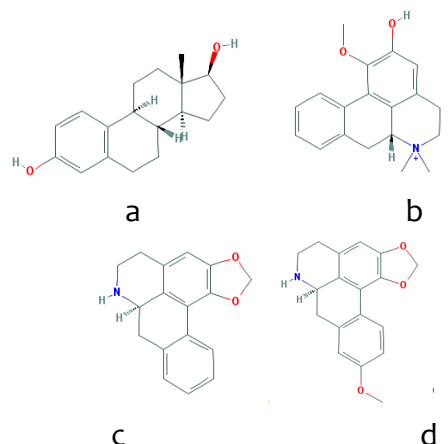
c. Pharmacokinetics analysis

Pharmacokinetic analysis was carried out through absorption, distribution, metabolism, and excretion (ADME) analysis of the ligands used in the study. ADME analysis was performed using SwissADME software (https://www.swissadme.ch)

3. **RESULTS**

R The ligands used in the study are annonamine, anonaine and xylopine which are the alkaloid compounds in *A. muricata* leaves (Abdul Wahab et al., 2018). The receptor used was an Estrogen Receptor alpha (ERα) (Figure 1). Estradiol-17β was used as a comparison ligand (control ligand). The ligand structure used in the study was shown in Figure 2.
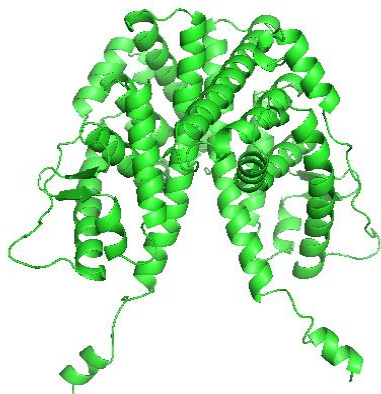

Figure 1. 3D strukcture of Estrogen Receptor Alpha (ERα)

Figure 2. The ligands   (a) Estradiol-17β, (b) Annonaine, (c) Anonaine, (d) Xylopine.

The results of molecular docking showed the presence of an alkaloid compound that is suitable for binding with ERα. The results showed that the anonaine compound had the smallest binding affinity value compared to other compounds (Figure 3). The smaller the binding energy, the better the stability of the bond between the ligand and the receptor because the bonds formed are stronger.
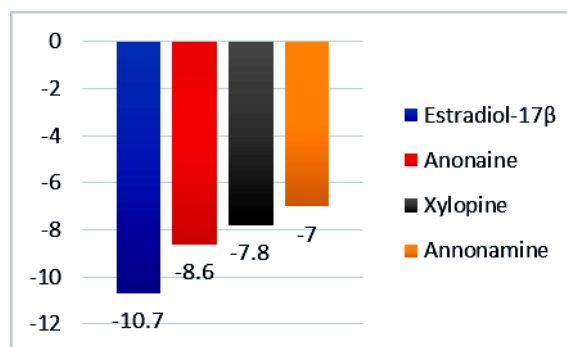

Figure 3.   Binding affinity values of alkaloid compound of A. muricata leaves with ERα

The results of the molecular docking were visualized using discovery studio software to found the binding of amino acid residues and then analyzed whether the ligand with the lowest energy also had the same bonded amino acids residue with the control ligand. This is important to know so we can predict the key of amino acids used to interact. The

results of visualization using discovery studio software showed that the selected alkaloid compounds of *A. muricata* leaves had some of the same binding residues as the control ligand (Estradiol-17β). Among the same binding residues indicated the type of alkyl bond at the Leu 220, Leu 86 and Ala 45 residues for Annonamine; Ile 119, Leu 79 and Ala 45 for Anonamine; LEu 220, Leu 79 and Ala 45 for Xylopine (Figure 4)
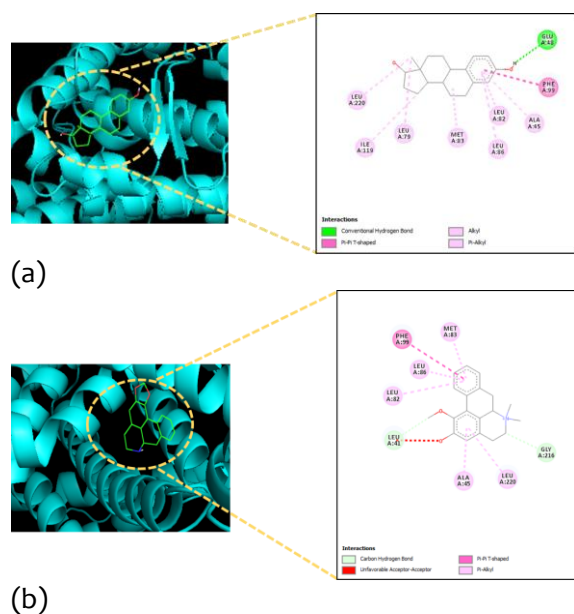

(a)

(b)

Figure 4. Molecular interaction analysis docking (a) Estradiol-17β (b) Anonamine

The results of pharmacokinetic analysis showed that the selected alkaloid compounds from *A. muricata* leaves had a good ability to be absorbed in the gastrointestinal tract indicated by the "High" category in the GI Absorption parameter. Meanwhile, the druglikeness results showed that the selected alkaloid compounds could be used as oral drugs (Table 1). The bioaviability score of 0.55 indicates that the active compound has a bioaviability potential of >10% (Zhu et al., 2011).

Tabel 1. Analysis result of swissADME

Active Compound	Pharmakokinetik		Druglikeness	
	GI Absorbtion	Log Kp (cm/s)	Lipinski	Bioavailability score
Annonamine	High	-5.80	Yes	0.55
Anonaine	High	-5.91	Yes	0.55
Xylopine	High	-6.11	Yes	0.55

4. DISCUSSION

The molecular docking used to analyze interactions between ligands and receptors. From the results of molecular docking, it can be seen the position of ligand binding at the receptor the energy of binding as binding affinity value. The docking process is carried out with Autodock vina software in the PyRx program (Trott and Arthur, 2010). The result of this process is a grid score, which the energy is used by the ligand to bind to a protein, commonly known as Gibbs energy/ bond energy (ΔG). This docking bond energy is the main parameter to determine the stability of the ligand and protein (Arwansyah et al., 2014).

Analysis of the docking method is scoring, which is calculating the strength of the ligand and receptor bonds expressed by the bond energy (ΔG). The more negative the ΔG value, the better the stability level between the ligand and the receptor, so the bonds formed will be stronger (Syahputra et al., 2014).

The results of molecular docking showed the interactions between ligands and proteins. The ligand has a binding site with the specific amino acids at the receptor (Adelin et al., 2013). This binding site affects the conformation and function of the receptor protein. The interaction between ligands and receptors will form hydrogen bonds and hydrophobic bonds (Arwansyah et al., 2014).

The alkyl bonds that are formed on ligand and receptor interactions are a type of hydrophobic bond (Lukman Chaubah et al., 2019). Hydrophobic bonds are a quite important type of bonding in the drugs design other than hydrogen bonds. The hydrophobic bond is one of the keys to the stability of

ligand and receptor interactions. The presence of water molecules in the hydrophobic bond region makes this area of interaction quite flexible and allows for increased binding affinity (Patil et al., 2010).

5. CONCLUSION

From the results of the study it can be concluded that anonamine is the most promising candidate as a phytoestrogen agent because it has a binding ability with a value similar to estradiol as a control. The results of this study provide basic information that can be considered before carrying out the in vivo test in the laboratory.

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