In Silico Antiosteoporosis Activity of 96% Ethanol Extract of *Chrysophyllum cainito* L. Leaves

Burhan Ma'arif[1], Faisal Akhmal Muslikh[2*], Luqman Alfani Najib[1], Ria Ramadhani Dwi Atmaja[1], Meilina Ratna Dianti[1]

[1] Department of Pharmacy, Faculty of Health and Medicine, Maulana Malik Ibrahim State Islamic University of Malang, Malang, Indonesia
[2] Master Student of Pharmaceutical Science, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

Email: faisalakhmus16@gmail.com

Abstract

Estrogen deficiency causes various health problems in postmenopausal women, including osteoporosis. Phytoestrogens are emerging as potential estrogen alternatives with minimal side effects. This study aimed to predict the antiosteoporosis activity of the compounds from 96% ethanol extract of *Chyrsophyllum cainito* L. leaves, through in silico study on 3OLS protein, an X-ray protein of ERβ. In silico study was carried out on the compounds from metabolite profiling result of 96% ethanol extract of *C. cainito* leaves from previous studies. The structure of compounds resulted from metabolite profiling of 96% ethanol extract of *C. cainito* leaves was made using Avogadro 1.0.1 software, geometry optimization with Chemdraw molecular docking was carried out using PyRx 0.8 software, and Biovia Discovery Studio Visualizer 2021 software was used to visualize the structure of compounds against 3OLS proteins. The physicochemical characteristics of the compounds were analyzed using the SwissADME webtool. From the result, it was known that there were 7 compounds in the leaves of *C. cainito* which were suspected to be phytoestrogens that had ERβ agonist properties against 3OLS protein. It was said to be an ERβ agonist because the compound had similar parameters to 17β-estradiol in its interaction with the 3OLS protein, which had a pharmacophore distance of about 10,862, and bound to the amino acids His 475 and Glu 305 or Arg 346. The 96% ethanol extract of *C. cainito* leaves contained 7 compounds which were thought to be a phytoestrogen with an ERβ agonist that handled its antiosteoporosis activity.

Keywords: Antiosteoporosis; Chrysophyllum cainito L.; in silico; phytoestrogen

Introduction

The prevalence of osteoporosis increases with the increasing age in women. More than 30% of women aged 60-70 years suffer from osteoporosis and continue to increase at the age of 80 years to 70%. In 2050, it is estimated that there will be an increase to 5.2-11.5 million people with osteoporosis in elderly women from 2020 which is estimated at 5-11 million, elderly women are the age of women who experience the last phase of menopause called post-menopause

(Ramadani, 2010; Dimyati, 2017; Sipayung *et al*., 2017; Mustofa *et al*., 2019). Osteoporosis in postmenopausal women can be caused by estrogen deficiency (Villa *et al*., 2016).

Osteoporosis that occurs due to estrogen deficiency is generally treated with hormone replacement therapy (TSH) (Lee *et al*., 2013). Breast cancer and cardiovascular disorders can increase the risk if TSH is used in the long term, so safer alternative therapies are needed (Khalid and Krum 2016). Phytoestrogens are potential alternatives for estrogen deficiency with minimal side effects. Phytoestrogens are compounds derived from plants with functions and structures like estrogen so that they can replace the function of estrogen in maintaining homeostasis in the brain, both in relation to the estrogen receptor (ER-dependent) or not (ER-independent) (Cui *et al*., 2013). *Chyrsophyllum cainito* L. is a plant species that grows a lot in East Java, Indonesia (Das *et al*., 2010) and is thought to contain phytoestrogen compounds (Ma'arif *et al*., 2019). In vivo, 96% ethanol extract of *C. cainito* had antiosteoporosis activity by increasing the number of osteoblast cells in the trabecular vertebrae of male mice induced by dexamethasone. This is due to the presence of phytoestrogen compounds in *C. cainito* leaf extract (Ma'arif and aditama, 2019). The results of the preliminary study obtained as many as 29 compounds from the metabolite profiling of 96% ethanol extract of *C. cainito* leaves (Ma'arif *et al*., 2019).

In silico studies have several advantages such as the processing time is quite short, inexpensive, and can explain the mechanisms that may occur when a substance enters the body (Sliwoski *et al*., 2014; Wadood *et al*., 2013). This study aimed to analyze the results of the metabolite profiling of 96% ethanol extract of *C. cainito* leaves which act as antiosteoporosis through an in-silico study of 3OLS protein, which is an X-ray estrogen receptor beta (ERβ) protein. ERβ was chosen because it is distributed in bone cells, both osteoblasts and osteoclasts, and affects the bone remodeling process when it binds to 17β-estradiol in the body. 17β-Estradiol was found to have similarities with phytoestrogens (Widiyati, 2006). While the 3OLS protein was chosen because this protein has a classification as a receptor that binds 17β-estradiol as a native ligand, which is used as a positive control, and has good resolution. So, it can be predicted the type of phytoestrogen content that has anti-osteoporosis properties (Ma'arif *et al*., 2021).

Materials and Methods

The material used is a compound resulting from metabolite profiling of 96% ethanol extract of *C. cainito* leaves using ultra performance liquid chromatography - quadrupole time of flight - mass spectrometry (UPLC-QToF-MS/MS) from previous studies (Ma'arif *et al*., 2019), and protein X-ray ERβ (PDB ID: 3OLS) from the protein data bank (GDP) (https://www.rcsb.org).

The first step is to download the 3OLS protein and make preparations to separate the native ligand (17β-estradiol) from the 3OLS protein, using Biovia Discovery Studio Visualizer 2021. Preparation of the structure of the compound resulting from the metabolite profiling of 96% ethanol extract of *C. cainito* leaves was carried out using Avogadro 1.0.1 software, to draw 2D structures and optimized geometry to produce stable 3D structures using Chemdraw. Molecular docking of the compound structure was carried out using PyRx 0.8 software with the AutoDock Vina method, and the Biovia Discovery Studio Visualizer 2021 software was used to visualize the structure of the compound against the 3OLS protein, so about predict compounds that act as phytoestrogens with ERβ agonist properties based on their interactions with 3OLS proteins. Furthermore, the compounds predicted to be ERβ agonists were analyzed using the SwissADME web tool to determine their physicochemical properties.

Results and Discussion

The 96% ethanol extract of *C. cainito* leaves was analyzed by molecular docking using PyRx 0.8 software using the AutoDock Vina method as a docking simulator. From the native ligand redocking test on 3OLS protein, it was found that the root mean deviation (RMSD) <2. The RMSD value <2 indicates that the software for the molecular docking process is good and can be used for the docking of compounds in 3OLS proteins (Noviardi and Fachrurrazie, 2015; Pinto *et al*., 2019).

The next stage is the visualization of the bond between the native ligand and the compound to the 3OLS protein using the Biovia Discovery Visualizer 2021 software. From the visualization data, it can be seen that the compound is classified as an ERβ agonist if it has several parameters similar to the native ligand. These parameters are the pharmacophore distance which is like the native ligand of about 10,862, having one pharmacophore group that binds to the amino acid His 475, and another pharmacophore group that binds to the amino acid Glu 305 or Arg 346 (**Figure 1**). The more similar the amino acids bound by the compound in the 96% ethanol extract of *C. cainito* leaves similar to the native ligand, the more similar the types of interactions that occur (Ekins *et al*., 2007; Suhud, 2015).

From the analysis using Discovery Studio Visualizer 2021, a total of 7 compounds from 29 compounds of 96% ethanol extract of *C. cainito* leaves were found which were predicted to be phytoestrogens with ERβ agonist properties (**Table 1**).

(A)   (B)
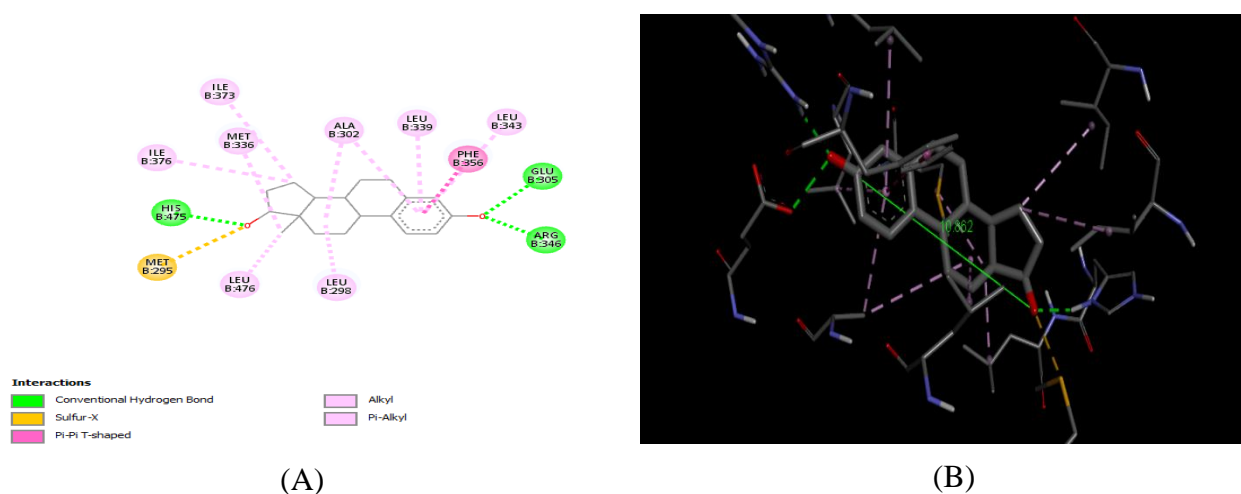
Figure 1. Visualization of *native ligand* (17β-estradiol) with 3OLS receptor: (A) 2D, (B) 3D

The ERβ agonist compounds were screened using the SwissADME web tool to identify the physicochemical properties of the compounds. After the analysis, it is known that all ERβ agonist compounds have the ability to penetrate cell membranes as indicated by a topological polar surface area (TPSA) value of < 140 A^2 (Chagas *et al*., 2018). Besides, compounds that are said to be agonists must meet Lipinski's rule of five, so that these compounds have biological activity for administration by the oral route (Daina *et al*., 2017) (**Table 1**).

Tabel 1. 3OLS agonist compounds from 96% ethanol extract of *C. cainito* leaves along with the results of pharmacokinetic and pharmacodynamic analysis

No	Compound	*Binding Affinity* (kkal/mol)	RMSD Average (Å)	Amino Acids	Pharmacophore Distance (Å)	TPSA (A^2)	Lipinski's role of five
Native Ligand							
1.	17β-estradiol	-10.5	0.000	His475 Glu305 Arg346	10.862	40.46	Yes
96% ethanol extract of *C. cainito* leaves							
1.	11-Aminoundecanoic acid	-5.5	2.350	His475 Glu305 Arg346	10.462	63.32	Yes
2.	Megalanthonine	-7.2	0.000	His475 Glu305	9.623	90.23	Yes
3.	Cetylamine	-5.8	0.000	His475 Glu305	9.973	26.02	Yes
4.	N-[4-Ethoxy-3-(1-pyrrolidinylsulfonyl)phenyl]-2-[4-(2-pyrimidinyl)-1-piperazinyl]acetamide	55.2	0.000	His475 Glu305	8.674	116.35	Yes
5.	Lauryldiethanolamine	-5.6	2.688	His475 Glu305	10.522	43.70	Yes
6.	N-[(2-Isopropoxyethyl)sulfonyl]glycyl-O,2-dimethylserine	-5.7	0.000	His475 Glu305	9.140	139.41	Yes
7.	Safingol	-4.6	2.764	His475 Glu305	10.021	66.48	Yes

Binding affinity aims to determine the energy stability of the complex binding of a compound with the receptor and the conformational stability of the compound with the receptor is indicated by the value of binding affinity. The smaller the binding affinity value, the smaller the energy required to bind (Siswandono, 1995; Kastritis, 2012; Harish *et al*., 2013). A large binding affinity value is predicted to be an agonist but has a less stable bond (Harish *et al*., 2013).

Compounds that have strong potential to become antiosteoporosis agents by meeting the 5 Lipinski legal parameters "Yes" and can penetrate the body's cell membranes consist of 7 compounds, namely 11-Aminoundecanoic acid; Megalanthonine; Cetylamine; N-[4-Ethoxy-3-(1-pyrrolidinylsulfonyl)phenyl]-2-[4-(2-pyrimidinyl)-1-piperazinel]acetamide; Lauryldiethanolamine; N-[(2-Isopropoxyethyl)sulfonyl]glycyl-O,2-dimethylserine, and Safingol. These compounds are predicted to be strong in binding to ERβ, so that they can increase the synergistic effect in the bone remodeling process, which plays a role in antiosteoporosis activity.

Conclusion

C. cainito leaves contain 7 compounds that are predicted to be phytoestrogens that play a role in antiosteoporosis activity because these 7 compounds have parameters like native ligand (17β-estradiol) which is an estrogen hormone produced in mammals and shows physicochemical properties that are acceptable to the body.

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