Molecular Docking of Selected Phytosterol from *Hydrilla verticillata* on Estrogen Receptors as Candidates of Anti-breast Cancer

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Abstract. *Hydrilla verticillata* is an aquatic plant that contains various secondary metabolites, such as triterpenoids and steroids. Several steroid compounds contained in *Hydrilla verticillata* are β-sitosterol, stigmasterol, fucosterol, campesterol, and cholesterol. Steroid compounds have the potential as candidates for breast cancer compounds. The important factor in inhibition and invasion of breast cancer cells are Estrogen receptors. The estrogen receptor complexed with genistein is often the target model for drug action. The docking of phytosterol compounds in *Hydrilla verticillata* to estrogen receptors has been carried out to test the docking energy associated with the affinity and type of binding between the ligand and the receptor. Five steroid compounds *Hydrilla verticillata* which have the potential to inhibit the growth of breast cancer cells as well as genistein as comparisons were taken from the PubChem database. Molecular docking of these compounds to estrogen-α and estrogen-β receptors was carried out using software such as RSCB Protein Data Bank, PyrX Virtual Screening Tool, and BIOVIA Discovery Studio Visualizer. The results showed that β-sitosterol, stigmasterol, fucosterol, campesterol, and cholesterol had binding affinities of −7.3, −8.1, −7.7, −8.1 and −7.6 kcal/mol towards 1X7R estrogen-α receptor and −6.8, −6.8, −7.5, −6.8 and −6.3 kcal/mol towards 1X7J estrogen-β receptor. While genistein as a control has a binding affinity of 7.7 kcal/mol towards 1X7R and 6.4 kcal/mol towards 1X7J. Some phytosterol compounds from *Hydriylla verticillata* have a better binding affinity than ginestein as a positive control, so it can be concluded that these compounds have the potential to anti-cancer.

Keywords: Phytosterol · Hydrilla verticillata · Estrogen receptor · anti-breast cancer

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1 Introduction

Cancer is one of the common health problems faced by the community. Cancer is caused by the uncontrolled excessive proliferation of normal cells in the formation of tissues in the body [1, 2]. In recent years, there has been an increase in the incidence of cancer-related to various factors including environmental factors, aging, infections, hormonal imbalances, and so on [3]. Cancer is the second leading cause of death after cardiovascular diseases [4]. Cases of death from cancer in 2015 reached 8.8 million from 14.1 million cancer cases [5].

One of the most common cancer causes of death is breast cancer. Breast cancer commonly occurs in women, more than 100 times compared to breast cancer in men [6]. Breast cancer also ranks as the first cause of death for women worldwide with a rate of 13.7% or 458,503 deaths from all deaths in women worldwide [7].

Some breast cancers are sensitive to hormones such as estrogen. The occurrence of overexpression of an estrogen receptor on tumor cells will increase the occurrence of excessive proliferation [8, 9]. The main function of the estrogen receptor here is as a DNA-binding transcription factor that regulates gene expression. The discovery of estrogen receptors, in addition to being used as a predictor and prognostic marker, can also be an efficient target for the treatment of hormone-dependent breast cancer with antiestrogens. [10].

Based on a review article by Bouker and Hilakivi-Clarke, it was reported that genistein has strong potential in the prevention and treatment of breast cancer due to its antiproliferative ability [11]. Genistein is associated with estrogen receptors, both estrogen receptor-β (ER-β) and estrogen receptor-α (ER-α). Both are distributed in different cells, regulating separate genes that cause different effects. As a therapeutic target, ER-α is an important factor for predicting the prognosis of breast cancer. While ER-β can inhibit the proliferation and invasion of breast cancer cells [12]. In the Protein Data Bank, genistein is a ligand for estrogen receptors, including ER-α (GDP ID: 2QA8 and 1X7R) and ER- (GDP ID: 1QKM and 1X7J) [13].

Hydrilla verticillata is an aquatic plant that contains various metabolites. The metabolite compounds in *H. verticillata* can provide many benefits. In the health sector, secondary metabolites in *H. verticillata* can be used, including antitumor and antibacterial [14, 15], anti-inflammatory and antibacterial [16], anti-malarial [17], and antioxidant [18, 19].

Bioactivity is strongly influenced by the active compounds. The steroid compounds of the n-hexane fraction of *H. verticillata* are β-sitosterol, stigmasterol, campesterol, fucosterol, and cholesterol [20]. The n-hexane fraction of *H. verticillata* has antioxidant activity with an EC_{50} value of 30.862 ppm [21]. The results of the separation using column chromatography of the n-hexane fraction *H. verticillata* obtained β-sitosterol, stigmasterol EC_{50} of 24.480 ppm [22], while the results of separation with KLP produced a single steroid isolate of -sitosterol with an EC_{50} of 5.109 ppm [23].

Based on the description above, to obtain compounds that are potential to have breast anticancer activity, an insilico study was carried out on steroid compounds from *Hydrilla verticillata* using estrogen-α receptors with PDB ID 1X7R and estrogen-β with PDB ID 1X7J. It is hoped that from this in silico test, steroid compounds have the potential as breast anticancer.

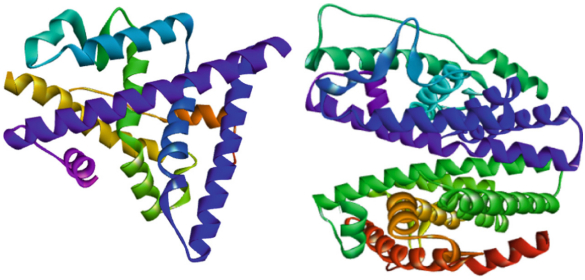


Fig. 1. The estrogen-α receptors and estrogen-β receptors (a), 1X7R (b), 1X7J

2 Methods

Five steroid compounds from *Hydrilla verticillata* and genistein compound as comparisons were taken from the PubChem database in SDF format. Both estrogen-α receptors and estrogen-β receptors (1X7R and 1X7J) were taken from the Protein Data Bank in PDB format. Molecular docking of these compounds to both estrogen-α and estrogen-β receptors (1X7R and 1X7J) was carried out using the PyrX Virtual Screening Tool software and to visualize the interaction between the receptor molecules and the bioactive compounds using the BIOVIA Discovery Studio Visualizer.

3 Results

3.1 Collection of Phytosterols Compounds

The five phytosterols compounds in this study were obtained from *Hydrilla verticillata.* These compounds belong to several chemical classes of steroids, as shown in Table 1. The structure of the five phytosterol compounds was obtained from the PubChem format in SMILE and SDF..

3.2 Collection of Estrogen Receptor

The estrogen-α receptors and estrogen-β receptors (1X7R and 1X7J) were taken from the Protein Data Bank. 3-D structures were visualized using the BIOVIA Discovery Studio Visualizer (Fig. 1).

3.3 The Results of Docking Between the Estrogen Receptor and Phytosterol Compounds

Docking simulations carried out using PyrX Virtual Screening Tool software between estrogen-α receptors 1X7R and estrogen-β receptors 1X7J and β-sitosterol, stigmasterol, fukosterol, campesterol, and cholesterol are shown in Table 2.

Table 1. The Phytosterol Compounds Identified from *Hydrilla verticillate*

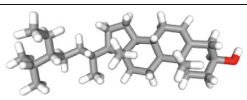
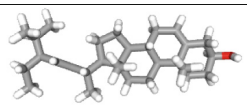
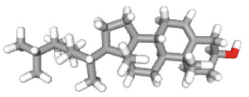
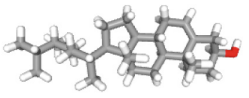
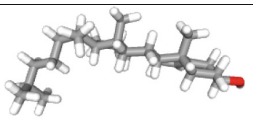
No.	Compounds	ID	Molecule Formula	SMILE	Structure
1.	β-sitosterol	PubChem CID: 222284	$C_{29}H_{50}O$ 414.7	CCC(CCC(C)C1CCC2C1(CCC3C2 CC=C4C3(CCC(C4)O)C)C)C(C)C	
2.	Stigmasterol	PubChem CID: 5280794	$C_{29}H_{48}O$ 412.7	CCC(C=CC(C)C1CCC2C1(CCC3C2 CC=C4C3(CCC(C4)O)C)C)C(C)C	
3.	Campesterol	PubChem CID: 173183	$C_{29}H_{48}O$ 400.7	CC(C)C(C)CCC(C)C1CCC2C1(CCC3 C2CC=C4C3(CCC(C4)O)C)	
4.	Fucosterol	PubChem CID: 5281328	$C_{29}H_{48}O$ 412.7	CC=C(CCC(C)C1CCC2C1(CCC3C2 CC=C4C3(CCC(C4)O)C)C)C(C)C	
5.	Kolesterol	PubChem CID: 5997	$C_{27}H_{46}O$ 386.7	CC(C)CCCC(C)C1CCC2C1(CCC3C2 CC=C4C3(CCC(C4)O)C)C	

Table 2. Docking result *Hydrilla verticillata* phytosterol with Estrogen Receptor 1X7R and 1X7J active site

No	**Compound Name**	**Binding Affinity (kcal/mol)**	
		1X7R	**1X7J**
1	β-sitosterol	−7.3	−6.8
	stigmasterol	−8.1	−6.8
	fukosterol	−7.7	−7.5
	campesterol	−8.1	−6.8
	cholesterol	−7.6	−6.3
2	Genistein	−7.6	−6.4

4 Discussion

Based on the analysis data from the docking of Hydrilla verticillata phytosterol compounds with estrogen-α 1X7R and estrogen-β 1X7J receptors (Table 2), it was found that stigmasterol, fukosterol, campesterol, and cholesterol had a lower binding affinity for estrogen-α receptors than ginestein, only -sitosterol which has a higher binding affinity. As for the estrogen-β receptor, only cholesterol has a higher binding affinity, stigmasterol, fukosterol, campesterol, and β-sitosterol have a lower binding affinity than genistein. Low binding affinity indicates stability and alignment in binding to estrogen-α and estrogen-β receptors compared to other compounds.

Stigmasterol, fukosterol, and campesterol have lower binding affinity than ginestein both on estrogen-α 1X7R and estrogen-β 1X7J receptors. Stigmasterol and campesterol have the lowest binding affinity for estrogen-α 1X7R, while fucosterol has the lowest binding affinity for estrogen- 1X7J receptors. This shows very good potential as a candidate for breast cancer compounds based on estrogen receptors.

The stigmasterol compound interacts with the estrogen receptor-α 1X7R via Pi-alkyl with His476 with a bond length of 4.83 Å, alkyl interaction with Leu479 (4.15 Å), Leu508 (4.66 Å), Leu511 (4.09 and 5.14 Å), Ile452 (4.48 Å) and Arg515 (3.88 and 4.45 Å) [Fig. 2]. Meanwhile, campesterol interacts with estrogen receptor-α 1X7R through Hydrogen bond with His476 with a bond length of 2.13 Å and Pi-alkyl 4.89 Å, alkyl interaction with Leu479 (4.19 Å), Leu508 (4.44 and 4.61 Å), Leu511 (4.84 and 5.14 Å), Arg515 (3.75 and 4.20 Å) and unfavorable acceptor-acceptor interaction with length 2.96 Å (Fig. 3).

Figure 4 shows the interaction of fucosterol with estrogen receptor-β 1X7J via Hydrogen bond with Leu495 with a bond length of 2.50 Å, alkyl interaction with Met473 (3.85 Å), Met473 (3.95 Å), Leu511 (420 Å), and Pi-alkyl with His467 (4.89 Å).

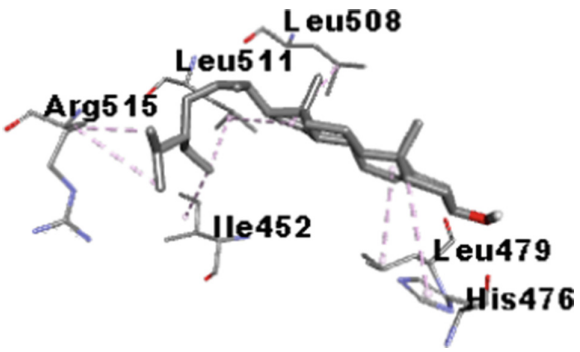


Fig. 2. Interaction of Stigmasterol with 1X7R

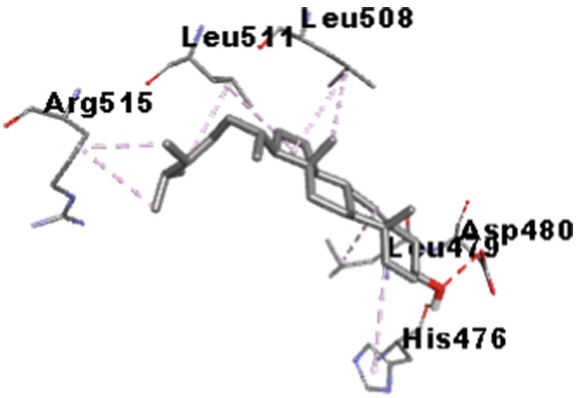


Fig. 3. Interaction of Campesterol with 1X7R

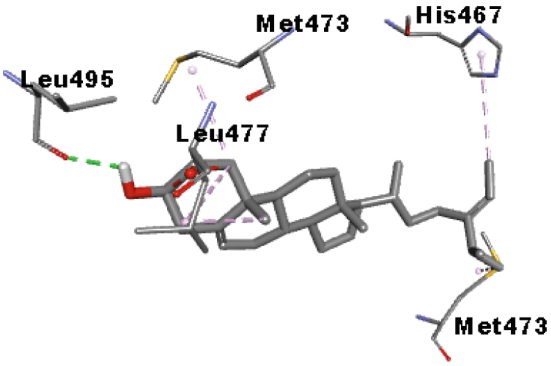


Fig. 4. Interaction of Fucosterol with 1X7J

5 Concussion

Based on the results of docking and analysis of interactions with estrogen-α and estrogen-β receptors and compared with genistein as a control, it can be concluded that phytosterol compounds contained in *Hydrilla verticillata* have potential as anticancer breasts based on the estrogen receptors.

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