Molecular docking of phytosterols in *Stenochlaena palustris* as anti-breast cancer

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ABSTRACT

Background: *Stenochlaena palustris*, also known as kelakai or lemidi, is frequently linked to anti-inflammatory, anti-bacterial, anti-fungal, and antioxidant properties. *S. palustris* phytosterols are suggested to suppress the progression of breast cancer.

Objective: The objective of this study is to evaluate the potential of phytosterols found in *S. palustris* to act as estrogen receptor (ER) inhibitors.

Methods: Phytosterols (α-tocopherol, β-sitosterol, campesterol, stigmasterol, fucosterol) were docked to estrogen receptor (PDB ID: 7KBS). Molecular docking parameters included Gibb's free energy and interactions between ligand and protein. ADMET properties were analyzed using pkCSM and SwissADME.

Results: α-Tocopherol showed the highest interaction with the ER with ΔG value -8. 9254 kcal/mol (the native ligand, raloxifene, had a G value of -12.052 kcal/mol). Leu387 (hydrogen bond); Phe404 (Phi-phi-T shaped), Leu391, Leu346, Trp383, Leu354, Ala350, Leu525, Leu349 (Alkyl) were among the residues by which α-tocopherol interacted with ER. α-Tocopherol has no hepatotoxicity and no skin sensitization.

Conclusion: By suppressing ERα, phytosterols from *S. palustris* may have potential anti-breast cancer activity and may be used to prevent estrogen-dependent human cancers like breast cancer.

Keywords: alpha-tocopherol, breast cancer, stenochlaena palustris, molecular docking, LD-50

Introduction

Stenochlaena palustris, also known as kelakai, is a typical plant of Borneo's wetland marshes. In Kalimantan, *S. palustris* is a fern that is frequently consumed by locals as a vegetable [1]. The extract of *S. palustris* contains flavonoid, steroid, and alkaloid [2]. Extract of *S. palustris* leaves possesses antioxidant properties [3], decreases oxidative stress in guinea pigs, and modulates the immune system [4]. As a supplement for breastfeeding mothers, it is believed that the leaves can boost and accelerate the production of breast milk [5].

Extract of *S. palustris* leaves contained numerous phytosterols, including α-tocopherol, campesterol, stigmasterol, β-sitosterol, and fucosterol [6]. β-sitosterol is potent anticancer agent against a variety of human cancer cells [7,8]. β-sitosterol and campesterol affect breast cancer cell proliferation and metastasis [9]. Campesterol controls mitochondrial activity, reactive oxygen species (ROS) production, and calcium concentrations [10]. Stigmasterol is one of the most abundant sterols in the plasma membranes of plant cells, and it plays an important role in cell antiproliferation [11]. Fucosterol possesses several biological activities, including anticancer properties [12].

Breast cancer is initiated by the interaction of cancer cells with numerous carcinogens and
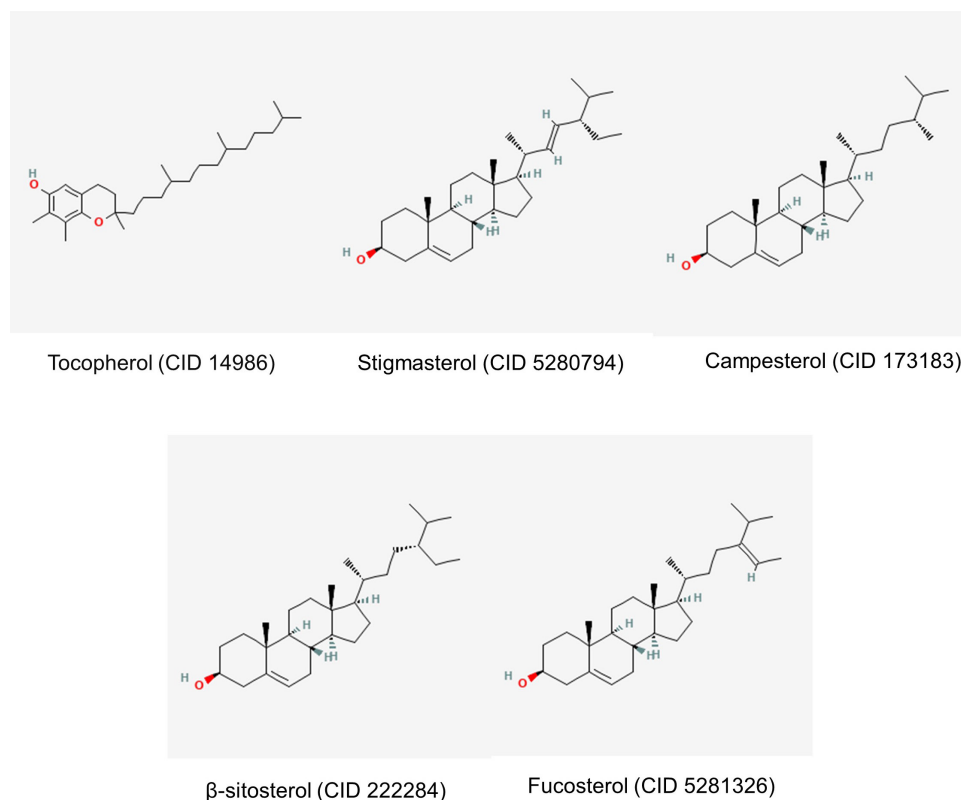
Tocopherol (CID 14986)   Stigmasterol (CID 5280794)   Campesterol (CID 173183)

β-sitosterol (CID 222284)   Fucosterol (CID 5281326)

Figure 1. Phytosterols structure and its PubChem ID

the occurrence of aberrant DNA mutations. This mutation is produced by multiple circumstances, including exposure to estrogen, radiation exposure, an unhealthy lifestyle, nulliparity, menarche before the age of 12, late menopause, a family history of breast, endometrial, or ovarian cancer [13]. If these risk factors are not managed, the development of cancer cells will be accelerated, which will have a detrimental effect on the patient. Efforts to prevent and treat breast cancer can be made utilizing plant secondary metabolites. Phytosterols derived from the *S. palustris* are believed to have anticancer properties.

Phytosterols' anti-breast cancer activity can be evaluated *in silico* against estrogen receptor alpha (ERα). Since ER-α is primarily responsible for the onset and progression of breast cancer, viable methodologies are required to discover and synthesize novel therapeutic ligands that selectively bind to estrogen alpha receptors and block estrogen-dependent proliferative activity [14]. Typically, the ER complexed with genistein is the target of pharmacological action models [15]. In this molecular docking study, phytosterols were

docked to ER-α and used raloxifene as a native ligand. This study aims to examine *in silico* the potential of phytosterols contained in *S. palustris* as anti-breast cancer agents.

Methods
Ligand preparation

Structure of phytosterols commonly found in young leaves *S. palustris*: α-tocopherol, campesterol, stigmasterol, β-sitosterol, and fucosterol [6] were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) (Figure 1). Phytosterols and native ligand were prepared using the USCF Chimera1.14 program by minimizing its 3D structure. The minimized structures were saved in mol2 format.

Protein preparation

The 3D structure of the ERα protein (PDB ID: 7KBS) was obtained from the RCSB PDB database (http://www.rscb.org/) (Figure 2). This structure was ER-α ligand-binding domain (*Homo sapiens*) in complex with raloxifene. Protein was
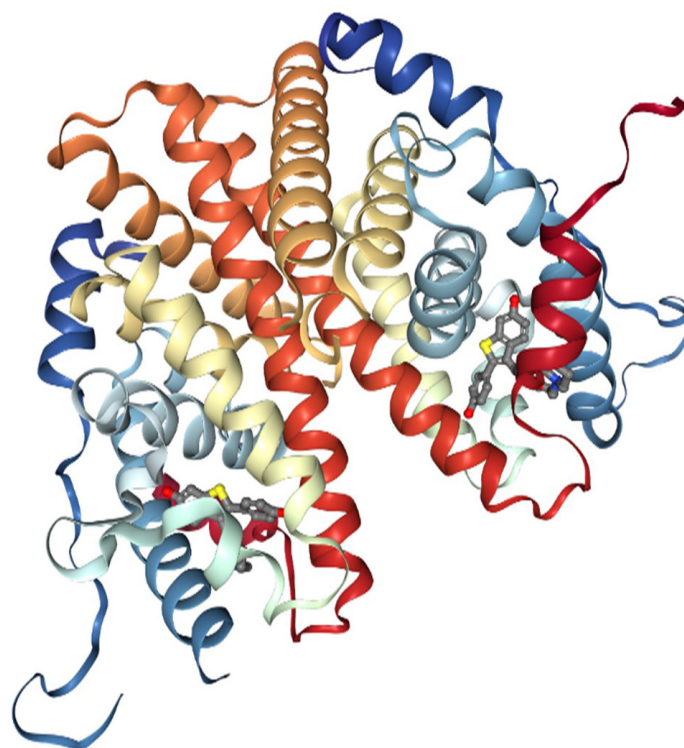
Figure 2. ER-α protein (PDB ID 7KBS)

deposited on 2020-10-02 and released on 2020-11-18. The protein determination method was X-Ray diffraction with a resolution of 1.83Å. The protein was prepared by selecting chain A and removing other ligands. The ready docking file was saved in *.pdb format. Protein was prepared using the USCF Chimera1.14 program [16].

Molecular docking

Molecular docking used the SwissDock web server (http://www.swissdock.ch/). The docking results were visualized using the Discovery Studio Visualizer Program (https://discover.3ds.com/discovery-studio-visualizer-download). The docking data obtained include Gibb's free energy (ΔG) and ligand interactions with amino acid residues in 3D and 2D visualization.

ADMET prediction analysis

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristic was performed using the pkCSM web server (http://biosig.unimelb.edu.au/pkcsm/prediction), bioavailability parameter and Lipinski rules were analyzed using the SwissADME (http://www.swissadme.ch/). Toxicity was predicted using pkCSM, which analyzed LD_{50}, hepatotoxicity, and skin sensitization.

Results
Molecular docking

Molecular docking used the SwissDock web server (http://www.swissdock.ch/) to predict possible molecular interactions between protein target and ligand. Docking was performed between the target protein and phytosterol ligands, which included α-tocopherol, stigmasterol, β-sitosterol, campesterol, and fucosterol as well as the native ligand raloxifene. The obtained results were energy data (ΔG) and the interaction between ligands and amino acid residues of protein. Molecular interactions between ligand and receptor are electrostatic interactions, hydrophobic interactions, and molecular bonding, contributing to the bond energy value (ΔG). Table 1 and Figure 3 show the docking of phytosterols and protein targets.

Table 1 shows that the interaction of α-tocopherol with estrogen receptor alpha (ER-α) has the highest affinity among the other compounds. The Gibbs
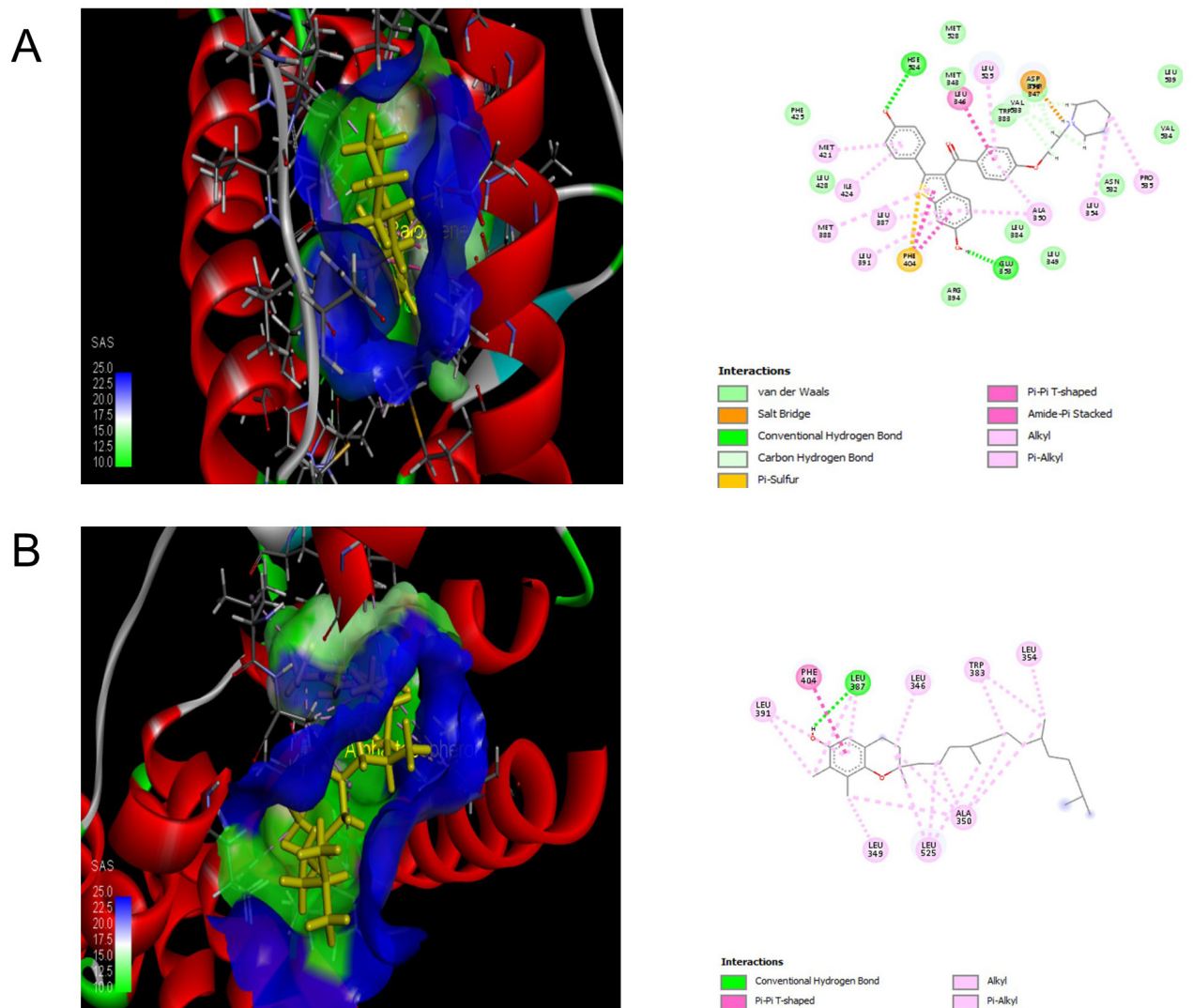
Figure 3. Interaction ligands and ER-α. A. Raloxifene (native ligand). B. α -tocopherol. Left panel in 3D, right panel in 2D visualization

Table 1. Molecular docking of phytosterols and protein target

Protein	Compounds	ΔG (kcal/mol)	Residues
ER-α	Raloxifene (native ligand)	–12.052	Glu358 (hydrogen bond); Phe404, Asp357 (salt bridge); Phe425, Leu428, Arg394, Leu384, Leu349, Asp582, Val584, Leu589, Thr347, Val583, Trp383, Met343 (Van der Waals); Met421, Ile424, Met388, Leu387, Leu391, Ala350, Leu354, Pro585, Leu525 (alkyl); Leu346 (amida-phi)
	α-Tocopherol	–8.925	Leu387 (hydrogen bond); Phe404 (Phi-phi-T shaped), Leu391, Leu346, Trp383, Leu354, Ala350, Leu525, Leu349 (alkyl).
	β-sitosterol	–6.936	Gln441, Asn439, Met438, Arg503, Gln506, Gln502, Gln499 (Van der Waals); Ala493, Leu495 (alkyl)
	Campesterol	–6.857	Ala493 (alkyl)
	Stigmasterol	–6.996	Ser329, Asn407, Pro333, Tyr331, Glu330, Tyr328, Ile326, Arg352 (Van der Waals); Pro325, Leu327 (alkyl)
	Fucosterol	–7.279	Ala493, Leu435 (alkyl)

Table 2. Pharmacokinetic properties of phytosterols

Compounds	LD$_{50}$ (mol/kg)	Hepatotoxicity (Yes/No)	Skin sensitization (Yes/No)	Bioavability	Lipinski (Yes/No)
α-Tocopherol	2.21	No	No	0.55	Yes
Stigmasterol	2.54	No	No	0.55	Yes
Campesterol	2.08	No	No	0.55	Yes
β-Sitosterol	2.55	No	No	0.55	Yes
Fucosterol	2.55	No	No	0.55	Yes

free energy value (ΔG) of the interaction between α-tocopherol and raloxifene to ER-α was -8.925 kcal/mol and -12.052 kcal/mol, respectively.

The native ligand raloxifene binds to the ER-α binding site on 25 amino acid residues, namely Glu358 (hydrogen bond); Phe404, Asp357 (salt bridge); Phe425, Leu428, Arg394, Leu384, Leu349, Asp582, Val584, Leu589, Thr347, Val583, Trp383, Met343 (Van der Waals); Met421, Ile424, Met388, Leu387, Leu391, Ala350, Leu354, Pro585, Leu525 (alkyl); Leu346 (amida-phi) (Figure 2A). α-Tocopherol formed a complex with ER in nine residues in binding site: Leu387 (hydrogen bond); Phe404 (Phi-phi-T shaped), Leu391, Leu346, Trp383, Leu354, Ala350, Leu525, Leu349 (alkyl) (Figure 2B).

The value of ΔG interaction of stigmasterol, β-sitosterol, campesterol, and fucosterol with ER-α was higher than α-tocopherol. Analysis of the compound's interactions with proteins also revealed that stigmasterol, β-sitosterol, campesterol, and fucosterol have no interaction with the ER-α binding site.

ADMET prediction analysis

Phytosterol compounds have LD$_{50}$ values between 2.08 and 2.55 mol/kg and are classified as level IV (quite toxic). Table 2 displays that none of the phytosterol samples are anticipated to be hepatotoxic or cause skin sensitivity.

Discussion

Estrogen has a vital role in the initiation and progression of breast malignancy. Consequently, estrogen receptor (ER) is a therapy target for breast cancer. Here, we observed that α-tocopherol inhibits the ER-α more effectively than any other phytosterol.

Raloxifene was selected because it is a native ligand for ER-α. In order for raloxifene to exert its effect, raloxifene must attach to the estrogen receptor. This binding stimulates (estrogen agonist impact) and blocks (estrogen antagonist effect) the estrogenic pathway in tissues expressing estrogen receptors. This receptor is expressed as the ER-α (activating effect) and ER-β isoforms (inhibitory effect). Therefore, the expression of these receptors modifies cellular and tissue estrogen responses. Raloxifene has a bioavailability of around 2% and an absorption rate of 60%. The onset of activity occurs eight weeks after administration, and the distribution is mostly protein-bound (more than 95%). The drug is metabolized in the liver and eliminated primarily in feces (over 93%) and urine (less than 0.2%) [17].

There are 26 amino acid residues at the ER-α binding site [18]. Interestingly, α-tocopherol forms a complex with ER in nine residues in the binding site: Leu387 (hydrogen bond), Phe404 (Phi-phi-T shaped), Leu391, Leu346, Trp383, Leu354, Ala350, Leu525, Leu349 (alkyl). This demonstrates that α-tocopherol binds to amino acid residues at the active site of the ER. The binding can influence the energy affinity value of the docking product. These results indicate that the α-tocopherol can fit into the ER-α active site with an ΔG value close to the affinity of the native ligand. The ability of α-tocopherol to inhibit the growth of breast cancer cells is by inducing apoptosis in specific mechanisms, such as binding to estrogen receptors [18].

α -Tocopherol is the primary form of vitamin E preferentially used by the human body to meet appropriate dietary requirements. Tocopherols react with the most reactive form of oxygen and protect unsaturated fatty acids from oxidation [19]. Vitamin E is a fat-soluble antioxidant that can protect the polyunsaturated fatty acids (PUFAs) in the membrane from oxidation, regulate the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and modulate signal transduction. Immunomodulatory effects of vitamin E have been observed in animal and human models under normal and disease conditions [20]. α -Tocopherol acts as an anticancer of the breast by binding to ROS produced during proliferation [21].

This binding residue of β-sitosterol, stigmasterol, campesterol, fucosterol with ER-α was not in binding site on ER-α, suggesting that these compounds are not an effective ER-α inhibitor. Possible role of action these compounds in breast cancer inhibition through other mechanisms, not the ER-α mechanism pathway. β-Sitosterol promotes its enrichment in altered cell membranes and significantly inhibits tumor cell growth. β-sitosterol is an effective agent that promotes apoptosis and that the incorporation of more phytosterols in the diet may serve as a preventive measure for breast cancer [22]. Stigmasterol is one of the major sterols in plasma membranes of plant cells and plays a role in cell proliferation and activation of plasma membrane H+-ATPase [14]. Campesterol suppressed cell proliferation, cell cycle progression, and cell aggregation in ovarian cancer cells. Campesterol also enhanced the anticancer effects of conventional anticancer agents. Campesterol can be used as a novel anticancer drug for human ovarian cancer [13].

Hepatotoxicity is a reaction caused by toxic metabolite or drug accumulation in the liver [23]. In this study, all phytosterol samples are predicted no hepatotoxic and do not cause skin sensitization. Skin sensitization is a side effect of dermally applied products and can cause allergies [24]. All phytosterol samples have a reasonably good bioavailability value of 0.55. A bioavailability value of more than 0.55 is good because the rate and relative amount of drug that reaches the circulation in the body is running well [25]. All phytosterol compounds comply with Lipinski's rules.

Conclusion

Based on a molecular docking study, phytosterols from *S. palustris* may have a potential anti-breast cancer activity by inhibiting ER-α and could be considered to prevent estrogen-dependent malignancies in humans, such as breast cancer.

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None.

Author contributions

Conceptualization, ES, NK; Methodology, LH, JS; Investigation, DM, SJ; Resources, NK; Writing – Original Draft, DM, ES; Review, NK; Supervision, NK.

Declaration of interest

The authors declare no conflict of interest.

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