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RESEARCH ARTICLE

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In Silico study of secondary metabolites of Borneo endemic plant *Shorea brunnescens* as an anti-cancer drug candidate

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Abstract

Borneo Island is one of the greenest islands in the world has thousands of endemic plants. Unfortunately, many of these plants are in endangered status. One of the endemic endangered plants from Borneo is *Shorea brunnescens* or locally named Selangan Batu Tinteng. Based on research, the bark of *Shorea brunnescens* contains the compound ε-viniferin, which is recorded in the literature as an anti-cancer compound, especially breast cancer. The anti-cancer potential of *Shorea brunnescens* could be a driving force for conservation and protection of the plant, which has never been done before. Therefore, further research is needed regarding the effectiveness and capacity of *Shorea brunnescens* as an anti-cancer medicinal material. This study aims to examine the potential success rate of the anti-cancer properties of *Shorea brunnescens* through molecular docking of the compound ε-viniferin against the proteotype of the main enzyme of breast cancer cells, Cytochrome P450. The method used was *in silico* modeling using PyRx and Discovery Studio software. First, screening of analog compounds through PubChem database (http://pubchem.ncbi.nlm.nih.gov/) based on Lipinski's rule, and obtained the three best analogs which will then be used as comparison ligands for docking simulation with Cytochrome P450 (CYP) receptors. The results obtained were that ε-viniferin in *Shorea brunnescens* as the test ligand had the smallest affinity compared to the comparison ligand, which was -9.2 kcal/mol. It was concluded that *Shorea brunnescens* is able to inhibit cancer cells very well, so it has the potential to be developed as an anti-cancer drug candidate.

Keywords: Breast Cancer; CYP450; ε-viniferin; Molecular Docking; *Shorea brunnescens*

Introduction

Cancer is a disease in which some body cells grow uncontrollably and can appear almost anywhere in the human body[1]. Breast cancer is one type of cancer in the list of the top five cancers in the world set by WHO in 2004 and is second in Indonesia as the most common cancer found in women[2]. Carcinogen compounds in cancer enter the body undergoing an activation process to become active metabolites that can cause mutations and result in the formation of cancer including breast cancer. The activation mechanism of this compound involves the cytochrome P-450 enzyme[3]. Inhibition of this enzyme can be one method to inhibit and even stop the development of breast cancer cells. There are many ways to treat breast cancer such as systemic therapy, hormonal therapy and radiotherapy. These therapies certainly have their own doses and side effects. Drugs have very strong side effects, for example in systemic therapies such as chemotherapy. This is because chemotherapy drugs not only attack cancer cells, but will also attack normal cells in the body[4]. The objective of this study is to investigate the potential anti-cancer properties of *Shorea brunnescens* by examining the binding affinity of *ε*-viniferin to the proteotype of the main enzyme of breast cancer cells Cytochrome P450, through molecular docking simulations. We hypothesize that *ε*-viniferin from *Shorea brunnescens* can inhibit the activity of Cytochrome P450 and therefore has potential as an anti-cancer drug candidate.

S*horea* is one of the plant genus that has the largest number of species from the *Dipterocarpaceae* family, which is more than 190 species[5]. Based on research conducted on *Shorea*, it shows that plants in this genus have the main chemical content in the form of resveratrol oligomer compounds. This compound has the ability to inhibit various biological activities including as antibacterial, antifungal, anticancer, anti-HIV, and antioxidant. Based on previous studies, some resveratrol oligomeric compounds can inhibit cytochrome P450 (CYP) enzyme proteins. One of the resveratrol oligomeric compounds known to inhibit cytochrome P450 (CYP) is *ε*-viniferin. Epsilon viniferin (*ε*-viniferin) compound is known to be contained in one of Borneo's endemic plants, *Shorea brunnescens*[6,7]. *Shorea brunnescens* is an endemic Borneo tree species distributed in North Borneo, East Borneo, and South Borneo and belongs to the meranti tree family of the *Dipterocarpaceae* family. The *ε*-viniferin compound present in the bark of this species makes it a potential candidate as a breast cancer drug[8]. Inhibitory ability of *ε*-viniferin in *Shorea brunnescens* against cytochrome P450 (CYP) enzymes will be tested in silico in this study. To the best of our knowledge, no previous studies have investigated the anti-cancer potential of *Shorea brunnescens*. This research aims to fill this gap in knowledge and contribute to the conservation of this endangered species.

Physiological and phytochemical studies of endemic plants are expected to spur the preservation and conservation of these species due to consideration of their benefits to the wider community. Therefore, this research also carries a conservation mission for *Shorea brunnescens* which is classified as an endangered species with shrinking numbers and habitats[9]. Proof of the anti-cancer potential of *Shorea*

brunnescens is expected to spur conservation, replanting and multiplication of the number of individuals. This is based on the belief that plants that are functional and medicinal are more considered and preserved by the community and the ruling authority. The issue of conservation is of utmost importance, as *Shorea brunnescens* is an endangered species with shrinking numbers and habitats. The potential anti-cancer properties of this plant could serve as a driving force for its conservation and protection. This study has potential to contribute to the preservation of similar endemic flora in Borneo by the government and the community. The researcher found that there are compounds that can provide benefits as an effort to overcome the side effects of the previously mentioned therapies, which were obtained from the isolation of resveratrol oligomer compounds from the acetone extract of *Shorea brunnescens* stem bark, namely ε-viniferin. The isolation of this compound refers to the research by Haryoto *et al*. (2006), the previous study only provided a limited explanation of the compound content in *Shorea brunnescens*. Studies related to ε-viniferin as an anticancer compound are the results of research that has never been reported before. The results of this study may have broader implications for the development of natural anti-cancer drugs and the conservation of endemic plants[10].

Materials and methods

Materials

The materials used in this docking simulation are three-dimensional structures of the comparator ligands Doxorubicin (PubChem CID: 31703), Kaempferol (PubChem CID: 5280863), and Tamoxifen (PubChem CID: 2733526) as well as the test ligand Epsilon-Viniferin (PubChem CID: 5281728) which can be downloaded at https://pubchem.ncbi.nlm.nih.gov/. The selected macromolecule is Cytochrome P450 2C9 (PDB ID: 5XXI), downloadable at https://www.rcsb.org/.

Ligand Structure Preparation

The 3D structures of the bioactive compounds, namely Doxorubicin, Kaempferol, Tamoxifen, and Epsilon-Viniferin, were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The compounds were selected based on their reported bioactivity and potential for further study. The molecular structures were then prepared using the Discovery Studio software, which includes tools for structure visualization. The optimized structures were then saved in a folder with another *.pdb* format, according to the name of each compound, for further analysis and use in docking simulations or other molecular modeling studies. The resulting 3D structures are expected to be accurate and reliable, and will form the basis for further investigation of the bioactivity and potential therapeutic applications of these compounds[11].

Protein Structure Preparation

The protein structure of Cytochrome P450 2C9 was obtained from the Protein Data Bank (PDB) (https://www.rcsb.org), with PDB ID: 5XXI. The crystal structure was selected based on its high

resolution and relevance to the study of drug metabolism and toxicity. The structure was downloaded in *.pdb* format and imported into the Discovery Studio software for preparation and analysis. The preparation of the Cytochrome P450 2C9 molecule involved several steps to ensure the accuracy and reliability of the structure. First, any water molecules or other heteroatoms were removed from the structure, as these can interfere with docking simulations and other molecular modeling studies. Second, hydrogen atoms were added to the structure, as PDB files typically do not include complete hydrogen atoms. The addition of hydrogen atoms is important for accurately modeling the interactions between the protein and ligand molecules, as well as for calculating the electrostatic potential and other molecular properties. After the preparation of the Cytochrome P450 2C9 molecule, the structure was stored in a folder with other *.pdb* formats for further analysis and use in docking simulations or other molecular modeling studies. The resulting protein structure is expected to be accurate and reliable, and will form the basis for further investigation of the drug metabolism and toxicity of various compounds[12].

Determination of the Active Side of Cytochrome P450 2C9

To locate the active site of Cytochrome P450 2C9, where the flavonol group compound ligand binds, a comprehensive literature search was conducted to identify the critical amino acids and structural features that contribute to the stability and specificity of the active site. The active site was obtained from published literature. The identification and characterization of the active site is crucial for understanding the mechanisms of drug metabolism and toxicity and for the design of new drugs that can specifically target the protein.

Molecular Docking

Molecular docking of the four bioactive compounds, Doxorubicin, Kaempferol, Tamoxifen, and Epsilon-Viniferin, with the Cytochrome P450 2C9 protein was performed using the Autodock Vina software with PyRx interface (GUI). This integrated program was used to predict the interaction and binding between the ligands and the proteins. The docking process involved several steps, including the preparation of the protein and ligand molecules, identification of potential binding sites on the protein, and prediction of the binding model of the ligand. The location and size of the grid box used for the docking simulation were determined based on previous studies and were set at X:30.5582, Y:16.0980, Z:23.7725, with dimensions X:17.3992, Y:19.4912, and Z:23.5291. The grid box was used to define the search space for potential binding sites on the protein surface. The ligand molecules were then docked onto the protein surface, and the resulting binding modes were evaluated based on their binding energy and RMSD (Root Mean Square Deviation) values[13].

The molecular docking purpose to identify the best possible binding modes of the ligand molecules with the Cytochrome P450 2C9 protein. The ligand molecule binds to the receptor and inhibits the function of the protein, which can lead to a therapeutic effect. The binding energy (kcal/mol) of the ligand-protein complex was calculated and evaluated to determine the strength of the interaction. The

parameters used in the molecular docking process with Autodock Vina included an RMSD cutoff of <2 Å and a binding energy (kcal/mol) of Cytochrome P450 2C9. The resulting docking models were analyzed and compared to evaluate the binding affinity and specificity of the bioactive compounds to the protein. The molecular docking process provides valuable insights into the mechanisms of ligand-protein interactions and can guide the design of new drugs with improved efficacy and safety profiles.

Analysis of Molecular Binding Result

The binding energy value/score obtained from molecular docking between the three comparative bioactive compounds with Cytochrome P450 2C9 is compared with the results/score of molecular docking binding energy between natural ligands, namely Epsilon-Viniferin with Cytochrome P450 2C9. If the binding energy results/score obtained from the three flavonol compounds are smaller/lower binding energy values than the binding energy of the natural ligand (Epsilon-Viniferin), then the compound can be concluded that the four flavonol compounds can compete to bind to the Cytochrome P450 2C9 molecule more potently. The best binding energy results/score obtained from molecular docking results are displayed in table form and then visualized the binding location and type of molecular bond in 3 dimensions (3D) with Biovia Discovery Studio Visualizer software[14].

Results

Ligand Screening

The study was conducted using Epsilon-viniferin analogs obtained from http://pubchem.ncbi.nlm.nih.gov/ (PubChem ID: 5281728). The screening results that have been carried out using the PubChem database, obtained the three best analogs which will then be used as comparison ligands for Cytochrome P450 (CYP) receptors. The selection of ligands used in tethering to target proteins is done by initial screening based on Lipinski's rule. Ligands are considered to have the potential to enter the cell membrane and be absorbed by the body if they meet Lipinski's rules with the criteria: (1) molecular weight <500 grams/mol, (2) number of hydrogen bond proton donor groups <5, (3) number of hydrogen bond proton acceptor groups <10, (4) logarithm value of partition coefficient in water and 1-octanol <5[15]. Based on **Table 1**, the compound contained in *Shorea brunnescens* and can be used as a drug candidate is Epsilon-viniferin.

Docking Simulation

The results of the docking simulation of the test ligand and the comparison ligand, obtained the binding energy value (ΔG) as can be seen on **Table 2** of the most stable Cytochrome P450 ligand-receptor interaction. Visualization of the ligand-receptor interaction shows the residues of the Cytochrome P450 receptor that play an important role in the binding site area (Figure binding site).

Overall interactions that occur with each test ligand and comparison ligand in the <5 Å area can be seen in the table (KLogP, BM, H-bond Acceptor, H-bond Donor).

Table 1. Predicted druglikeness of compounds contained in Shorea brunnescens.

Compound	MW	mLOGP	Acceptor hidrogen	Donor hydrogen	Violation
Epsilon-viniferin	454.47 g/mol	2.86	6	5	Lipinski Yes; 0 violation
Laevifonol	628.58 g/mol	0.56	12	7	Lipinski No; 3 violations: MW>500; NorO>10; NHorOH>5
Isohopeaphenol	906.93 g/mol	3.84	12	10	Lipinski No; 3 violations: MW>500; NorO>10; NHorOH>5

The results of two-dimensional (2D) and three-dimensional (3D) visualization of the ligand and receptor which can be seen on **Figure 1,** tethering areas can only show hydrogen bonds and hydrophobic interactions while electrostatic interactions cannot be visualized with the software used. Electrostatic interactions also play a role in the stability of ligands to receptors. Electrostatic interactions are interactions between atoms caused by differences in their polarity. These interactions are weak and non-covalent interactions that are easily separated, but because of the large number of electrostatic interactions, they have a large contribution in the formation of protein conformation. Electrostatic interactions are in the form of salt bridges and van der walls. The amino acids or residues bound to the test ligands and comparator ligands can be seen in **Table 3.**

Table 2. Binding energy values of test ligands and comparator ligands against Cytochrome P450 enzyme

Ligand	Protein	Binding affinity (kcal/mol)
Epsilon-Viniferin	Cytochrome P450	Mode 0: -9.2
Doxorubicin		Mode 0: -8.7
Kaempferol		Mode 0: -8.2
Tamoxifen		Mode 0: -8.1

Hydrogen bonding involves the interaction of covalently bound hydrogen atoms with electronegative atoms such as flour (F), nitrogen (N), oxygen (O) Hydrophobic interactions play a role in determining the stability of ligands to androgen receptors. Hydrophobic interactions are interactions that avoid liquid environments and tend to cluster on the inside of the globular structure of the protein[16].

Interactions of each can be seen in **Figure 2**, which the formation of hydrophobic bonds minimizes the interaction of nonpolar residues with water.

Table 3. Amino acids bound of test ligands and comparator ligands against Cytochrome P450 enzymes.

Ligan	Bound Amino Acid
Epsilon-Viniferin	LEU:361, LEU:362, SER:478, ALA:477, ILE:205, PRO:367, PHE:114, ARG:108
Doxorubicin	ALA:477, GLU:300, SER:478. LEU:361, THR:301: ILE:205, SER:209, ARG:108, ASP: 293,
Kaempferol	THR:364, PRO:367, PHE:476, SER:365
Tamoxifen	ILE:205, ALA:477, PHE:476, LEU:366,

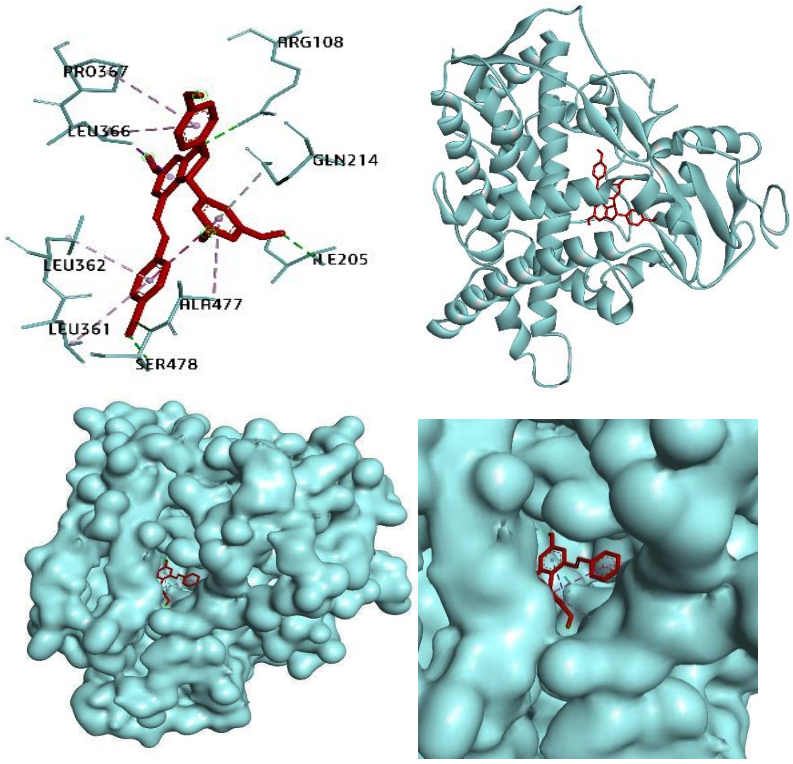

Figure 1. 3-dimensional visualization of epsilon-Viniferin and Cytochrome P450 interaction

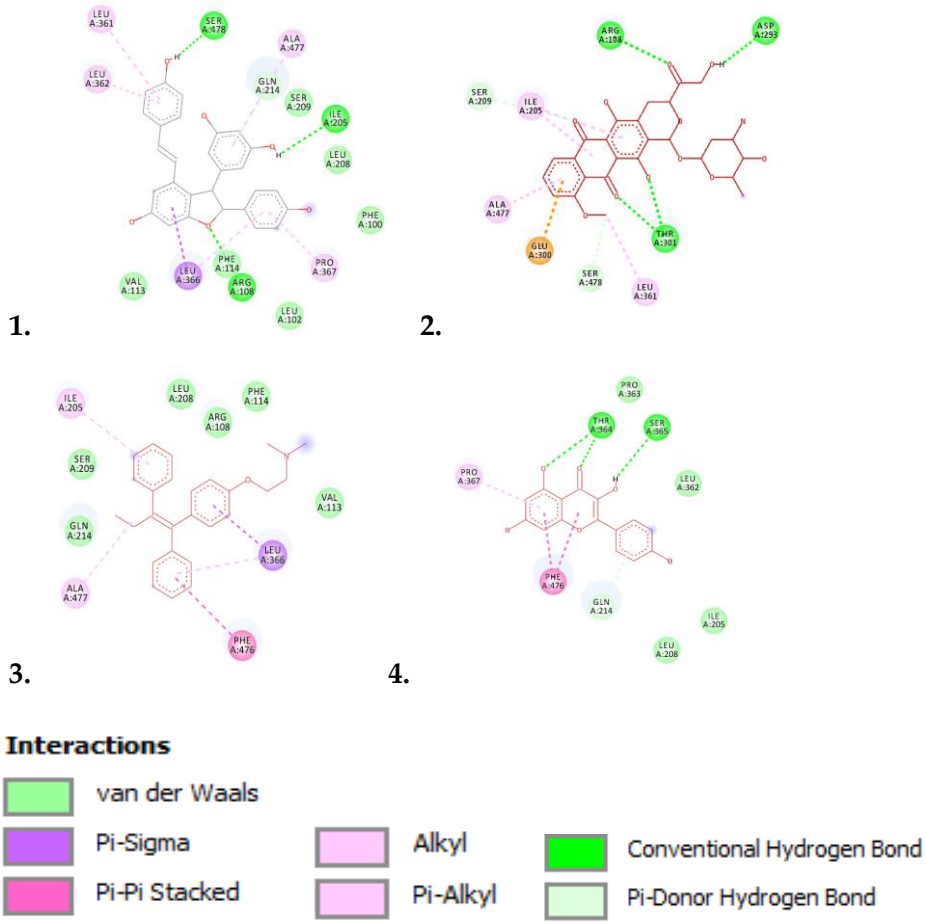

Figure 2. Hydrogen bond (green) and hydrophobic bond (orange, pink, and purple) interactions in Epsilon-viniferin (1), Doxorubicin (2), Tamoxifen (3), and Kaempferol (4).

Discussion

Cytochrome P450 is a large family of hemeprotein-type enzymes that function as oxidizing catalysts in the metabolic pathways of steroids, fatty acids, xenobiotics including drugs, toxins, and carcinogens. Various organic chemical reactions are accelerated by CYPs, such as monooxygenation, peroxidation, reduction, dealkylation, epoxidation, and dehalogenation reactions. These reactions are specifically aimed at converting substrate compounds into polar metabolites for excretion or processing by other enzymes in phase II metabolism into conjugate compound[17]. The three-dimensional 5 structure of Cytochrome P450 proteins can be determined by X-ray crystallography and Nuclear Magnetic Resonance (NMR) methods. Both methods can represent activity, stability, function, and provide atomic-level structural information of the protein in the unfolded state which is important in characterizing the protein folding process[18]. This study was conducted using Cytochrome P450 protein with PDB code 5XXI. The ligand-binding region is located at residues Ile-205, Gly-296, Phe-476, Thr-301, Ser-209, Leu-362.

The crystallographic results on Cytochrome P450 with PDB code 5XXI include residues in the ligand-binding region which will then be used as a simulation of ligand docking against the Cytochrome P450 receptor. The docking method is done not only Cytochrome P450, but also required a ligand that will interact with the Cytochrome P450 protein. The structure of Epsilon-Viniferin that has gone through the initial screening process can be seen in the **Table 1**. Based on the criteria set by Lipinski, it is predicted that Epsilon Viniferin analog has a high bioavailability potential for the body. Bioavailability is the relative amount of drug that enters the systemic circulation after administration of the drug in a particular preparation, as well as the speed of increasing drug levels in the systemic circulation[19]. Provided that the violation exceeds 2 then the compound is a non-drug, which is a bat that can be absorbed and circulated in the body[20].

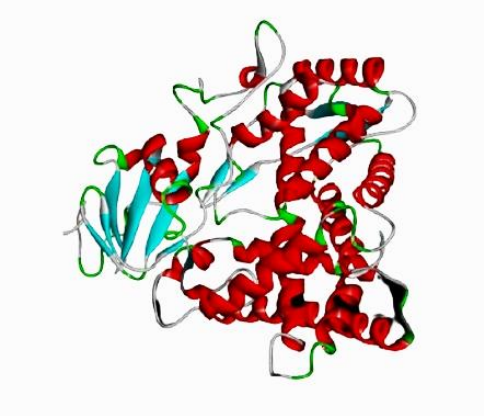

Figure 3. Cytochrome P450 protein

Based on the results written in **Table 3**, several similar amino acids were obtained including ALA: 477 for Epsilonviniferin, Doxorubicin, and Tamoxifen, SER: 478 for Epsilon-viniferin and Doxorubicin, ILE: 205 for Epsilon-viniferin, Doxorubicin, and Tamoxifen, PRO: 367 for Epsilon-viniferin, and Doxorubicin, ARG: 108 for Epsilon-viniferin and Doxorubicin, and PHE: 476 for Kaempferol and Tamoxifen. Protein binding sites are areas of protein binding to molecules and 8 ions (ligands) that will affect the conformation and function of the protein. The binding site area involves amino acid residues that play an important role in ligand binding. Interactions that occur between ligands and macromolecular amino acid residues are formed as hydrogen bonds, hydrophobic interactions and electrostatic interactions[21].

The difference in ΔG value is predicted because there are differences in ligand binding to amino acids in the Cytochrome P450 receptor so that the conformation can determine the most stable molecular geometry state. Based on **Table 2**, Epsilonviniferin has a lower binding energy (ΔG) value when

compared to other comparator ligands, which is -9.2 kcal/mol, so the test ligand in the form of Epsilon-viniferin has the potential as an inhibitor for Cytochrome P450 receptor activity in the treatment of breast cancer.

Conclusions

Based on the results of the research that has been done, it can be concluded that the test ligand epsilon viniferin has the smallest bond energy affinity, which is -9.2 kcal/mol compared to other comparator ligands on the Cytochrome P450 receptor (PDB code 5XXI). The results obtained are predictions by computational methods, therefore it is recommended to conduct further research in vitro/in vivo to study Epsilon-viniferin compounds as drug candidates in anti-breast cancer treatment. It is concluded that *Shorea brunnescens* is able to inhibit cancer cells very well so that it can be developed as an anti-cancer drug. Further in vitro/in vivo studies are needed to confirm the computational findings on the lowest binding affinity of Epsilon-viniferin with the Cytochrome P450 2C9 protein. Future research could focus on optimizing the structure of the bioactive compounds and exploring their mechanisms of action in broader contexts, such as other cancer cell lines or animal models. These findings could guide the development of new drugs for cancer treatment.

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Conflicts of Interest

There are not potential conflicts of interest.

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