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Etlingera elatior Compounds as Anticancer Agents of Breast Cancer Through Inhibition of Progesterone Receptor: An *In Silico* Study

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Abstract

Breast cancer is the leading cause of cancer-related death in women globally. Progesterone receptor (PR) is known as the prime example of receptors amenable to targeted breast cancer drug therapy. *Etlingera elatior* is an herbal plant that has been renowned to have anticancer effect. This study aimed to identify the potential compounds derived from *Etlingera elatior* as anticancer agents of PR in breast cancer using molecular docking method. This study used fifteen compounds from *Etlingera elatior* along with lonaprisan as the comparative drug. The PR was downloaded from RCSB, whereas compounds and lonaprisan were from Pubchem. The drug-likeness test based on Lipinski's rule of five was conducted using SwissADME. Toxicity analysis using admetSAR 2.0 was used to predict toxicological profile of the compounds. Compounds and lonaprisan were docked on PR using AutoDock tools 1.5.6 and AutoDock Vina 1.1.2. Molecular interactions were visualized by Discovery Studio v16. A total of nine compounds met the criteria as drugs based on drug-likeness and toxicity tests. All nine compounds except caffeic acid and vanillic acid had higher binding affinities on PR compared with lonaprisan. Ergosterol peroxide exhibited the highest binding affinity on PR with values of -9.8 kcal/mol. Moreover, ergosterol peroxide-PR interaction had thirteen hydrophobic bonds and a hydrogen bond with amino acid residues were found in the active site of PR. Most of the compounds found in *Etlingera elatior* have the potential to be anticancer agents of PR in breast cancer with ergosterol peroxide being the most potential compound. Further *in vitro* and *in vivo* research are needed.

Keywords: *breast cancer, ergosterol peroxide, etlingera elatior, progesterone receptor, in silico.*

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INTRODUCTION

Breast cancer is a growing global concern, with increasing incidence annually (Barrios, 2022). Breast cancer is the most common cancer in women and is the most common cancer that causes death (Anderson, *et al*., 2015; Feng, *et al*., 2018). According to the World Health Organization (WHO), breast cancer was diagnosed in 2,300,000 women and caused 685,000 deaths globally by 2020 (World Health Organization, 2021). In Indonesia, the number of new cases of breast cancer reached 68,858 cases with the number of deaths reaching more than 22,000 cases (Kemenkes RI, 2022). In breast cancer, progesterone receptor (PR) is crucial for cell proliferation (Dewi Harnis, *et al*., 2020). Previous studies reported that selective progesterone receptor modulators can inhibit PR, competing with progesterone and hindering cancer cell proliferation (Zarezade, *et al*., 2018). PR plays a crucial role in breast cancer growth with 54.6% of patients showing positive PR (Shah, *et al*., 2022). Another study conducted by Sohail, *et al*., reported that PR expression is found in 60%-70% of cases of invasive ductal carcinoma of breast cancer (Sohail, *et al*., 2020).

Hormone therapies like lonaprisan and tamoxifen have transformed hormone receptor-positive breast cancer treatment, reducing cancer-related deaths significantly (Tremont, *et al*., 2017). However, these commonly prescribed drugs pose a high risk of recurrence and severe side effects after 5 to 10 years (Adv, *et al*., 2018; Han, *et al*., 2018). Lonaprisan also has adverse effects, with a study reporting that 90% of 68 patients experienced side effects, including facial redness, breathlessness, nausea, weakness, headaches, constipation, vomiting, and reduced appetite (Jonat, *et al*., 2013). Moreover, serious adverse events also reported in a study by Jonat, *et al*., with three patients were endometrial hypertrophy, two patients were myocardial infarction, and two patients were ascites, subileus, and dyspnea (Jonat, *et al*., 2013). Tamoxifen also has various side effects with common side effects including hot flashes, menstrual irregularities, vaginal discharge, peripheral edema, high blood pressure, mood swings, pain, depressive symptoms, skin changes, nausea, vomiting, fatigue, joint pain, arthritis, lymphedema, and throat inflammation (Farrar and Jacobs, 2023). Hence, safe treatment is urgently needed in managing breast cancer (Wagenfeld, *et al*., 2016).

Herbal treatments are popular in countries, especially in Indonesia. Herbal treatments are generally considered safe and effective (Sumarni, *et al*., 2019). However, they can have inherent toxicity, if they interact with other substances and lack quality control (Ardalan and Rafieian-Kopaei, 2013). *Etlingera elatior* is one of the native Indonesian herbal plants that has the potential to treat breast cancer. This plant has been reported to have various properties including antioxidant, anticancer, antiproliferative, antibacterial, and cytotoxic activity (Ghasemzadeh, *et al*., 2015; Nurlaili, *et al*., 2022). Its bioactive compounds are known to exert anticancer effects through various mechanisms, including inhibiting cell proliferation and clone formation, attenuating migration/invasion, inducing apoptosis, controlling the cell cycle, and suppressing β-catenin signalling (He, *et al*., 2018a; Marques, *et al*., 2013). Previous study had identified the phytochemical screening and anticancer activity of *Etlingera elatior* rhizome which had a cytotoxic effect against CEM-SS and MCF-7 cell lines and the ethanol extract of *Etlingera elatior* flowers against the MDA-MB-231 cell line, MCF-7 cells, and HeLa cells. However, there are no studies about the potential of *Etlingera elatior* compounds against PR in breast cancer currently (Wahyuni, *et al*., 2022). Therefore, this study aimed to identify potential compounds derived from *Etlingera elatior* which target PR in the management of breast cancer.

METHODS

Preparation of Receptor and Compounds

Through online screening using previous literatures, we used a total of fifteen screened compounds from *Etlingera elatior* which will be further mentioned as ligand (Wahyuni, *et al*., 2022) (Ghasemzadeh, *et al*., 2015). We used lonaprisan as a comparative drug. Compounds that have been proven to have a potential medicinal effect were selected. The structures of ligands and comparative drug were downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) whereas PR (PDB ID: 4OAR) as protein target was downloaded from Protein Data Bank (http://www.rcsb.org). The preparation of PR was performed by removing water molecules contained in the PR, adding polar hydrogen atoms, cleaning the target protein structure from natural ligands then saving its file in the pdbqt format (Madhavi Sastry, *et al*., 2013). The preparation of the compounds was carried out by changing the sdf format to pdbqt format using Discovery Studio and AutoDock software.

Lipinski and Toxicity Test

The Lipinski rule of five was used in this study to assess the drug-like properties of compounds. The molecular weight, number of hydrogen donors and acceptors, solubility, permeability, level of GI absorption, and number of Lipinski violations were performed using SwissADME, a free online website tool (http://www.swissadme.ch/) (Daina, Michielin and Zoete, 2017). AdmetSAR was then utilized to evaluate the toxicity of the compounds (http://lmmd.ecust.edu.cn/adm etsar2) (Yang, *et al*., 2019).

Docking Validation

Validation of the molecular docking method was done by redocking the PR receptor with native ligand ([(8S,11R,13S,14S,17R)-17-acetyl-11-[4-(dimethylamino)phenyl]-13-methyl-3-oxo-1,2,6,7,8,11,12,14,15,16-decahydrocyclopenta[a]phenanthren-17-yl] acetate) using AutoDock tools 1.5.6 software. In the redocking process, the root mean square deviation (RMSD) value was observed. The binding sites and the parameters of the native ligand-receptor are considered valid and can be used as parameters for other ligands if the RMSD value is ≤2Å. (Hassan, *et al*., 2017).

Molecular Docking

Molecular docking simulation was carried out by arranging the docking parameters with AutoDock tools 1.5.6 and AutoDock Vina 1.1.2 software (Morris, *et al*., 2009), (Trott and Olson, 2009), (Eberhardt, *et al*., 2021). The docking simulation was done by arranging the docking parameters, which are the grid box size was 40x40x40, the grid box coordinate was (x=14.513, y=24.781, z=14.874) and 1.00 Å spacing on PR receptor. After the parameters were set, the simulation was performed which yielded ten conformation poses. The best binding affinities (the more negative ΔG value) were selected from a set of ten conformation poses after running the docking. The top three highest binding value compounds on PR were selected to be visualized their molecular interactions.

Docking Visualization

Visualization analyses were used to evaluate the ligand's binding sites and to observe how the ligands and protein targets formed chemical bonds. The visualization analyses were presented in two-dimensional (2D) using Discovery Studio program. Parameters assessed were amino acid residues, hydrogen bonds, hydrophobic bonds, and van der waals interactions.

RESULTS

Drug Likeness and Toxicity

Based on Table 1, all compounds used in this study have fulfilled Lipinski's Rule of Five.

Therefore, all compounds were considered as drug-like compounds and can be designed for oral delivery. In the meantime, based on the toxicity test in Table 2, all compounds in this study had a negative value on ames mutagenesis. Meanwhile, 1,7-bis(4-hydroxyphenyl)-2,4,6-heptatrienone, gallic acid, and sitostenone showed positive value in hepatotoxicity. Moreover, pinocembrin, sitosterol, and stigmasterol had categories III and IV in acute oral toxicity. Hence, only nine compounds were considered non-toxic compounds. Therefore, these nine compounds continued to be researched using molecular docking.

Molecular Docking

Using the established parameters, the validation was carried out by redocking the native ligand on receptors, which revealed a RMSD

Table 1. Lipinski's rule of five.

Compound	MW <500 (g/mol)	H-acceptor	H-donor	Log*P*	Violation
1,7-bis (4-hydroxyphenyl)-2,4,6-heptatrienone(Wahyuni, *et al.*, 2022) (Compound CID: 11277770)	292.33	3	2	2.98	0
16-hydroxylabda-8 (17),11,13-trien-15,16-olide (Wahyuni, *et al.*, 2022) (Compound CID: 146159916)	316.43	3	1	3.97	0
Caffeic acid (Ghasemzadeh, *et al.*, 2015) (Compound CID:689043)	180.16	4	3	0.7	0
Catechin (Wahyuni, *et al.*, 2022) (Compound CID:9064)	290.27	6	5	0.24	0
Demethoxy curcumin (Wahyuni, *et al.*, 2022) (Compound CID: 5469424)	338.35	5	3	1.8	0
Ergosterol peroxide (Wahyuni, *et al.*, 2022) (Compound CID: 5351516)	428.65	3	1	5.43	1
Gallic acid (Ghasemzadeh, *et al.*, 2015) (Compound CID: 370)	170.12	5	4	-0.16	0
Methyllinderatin (Wahyuni, *et al.*, 2022) (Compound CID: 42607684)	408.53	4	2	3.66	0
Pinocembrin (Wahyuni, *et al.*, 2022) (Compound CID: 68071)	256.25	4	2	1.27	0
Pinostrobin (Wahyuni, *et al.*, 2022) (Compound CID:73201)	270.28	4	1	1.52	0
Sitostenone (Wahyuni, *et al.*, 2022) (Compound CID:5484202)	384.64	1	0	6.23	1
Sitosterol (Wahyuni, *et al.*, 2022) (Compound CID:222284)	414.71	1	1	6.73	1
Stigmasterol (Wahyuni, *et al.*, 2022) (Compound CID:5280794)	412.69	1	1	6.62	1
Vanillic acid (Wahyuni, *et al.*, 2022) (Compound CID: 8468)	168.15	4	2	0.74	0
Yakuchinone A (Wahyuni, *et al.*, 2022) (Compound CID: 133145)	312.4	3	1	3.44	0

Table 2. Toxicity analysis.

Compound	Hepatotoxicity	Ames Mutagenesis	Acute Oral Toxicity
1,7-bis (4-hydroxyphenyl)-2,4,6-heptatrienone	0.6103	- 0.86	IV (0.5155)
16-hydroxylabda-8 (17),11,13-trien-15,16-olide	-0.6198	-0.88	III (0.4709)
Caffeic acid	- 0.6851	-0.91	IV (0.5588)
Catechin	- 0.7375	- 0.63	IV (0.6433)
Demethoxy curcumin	-0.9198	- 0.88	III (0.6250)
Ergosterol peroxide	-0.7	- 0.5828	III (0.3243)
Gallic acid	0.875	- 0.95	III (0.6904)
Methyllinderatin	- 0.6233	- 0.68	III (0.5562)
Sitostenone	0.5919	-0.8913	III (0.7154)
Vanillic acid	- 0.5125	- 0.86	III (0.4923)
Yakuchinone A	-0.6663	-0.58	III (0.6899)
Pinocembrin	-0.5875	- 0.58	II (0.3682)
Pinostrobin	-0.656	-0.5	III (0.5097)
Sitosterol -	0.6102	-0.9	I (0.4287)
Stigmasterol	5557	-0.8392	I (0.4287)

value of 0.96 on PR. Since the value is less than 2 Å, the docking method can be used to dock the test compounds. All nine compounds except caffeic acid, gallic acid, and vanillic acid showed higher binding energy on PR compared with lonaprisan-PR interaction (<-6.0 kcal/mol). Ergosterol peroxide showed the highest binding energy on PR with a value of -9.8 kcal/mol. The top three highest binding value compounds (ergosterol peroxide, methyllinderatin, yakuchinone A, and catechin) on PR were be visualized.

Visualization Analysis

The 2D visualization of molecular docking results are shown in Figure 1. The

Table 3. Molecular docking results.

Compound	Binding Energy (kcal/mol)
16-hydroxylabda-8 (17),11,13-trien-15,16-olide	-8
Caffeic acid	-6.1
Catechin	- 8.3
Demothoxy curcumin	- 7.7
Ergosterol peroxide	- 9.8
Methyllinderatin	- 8.4
Vanillic acid	- 6.6
Yakuchinone A	-8.3
Pinostrobin	-8.2
Lonaprisan	- 7.5

visualization analysis yielded that in the interaction with PR, ergosterol peroxide had thirteen hydrophobic interactions and a hydrogen bond along with six amino acid residues such as Leu718, Cys891(2), Tyr890, Leu797(2), Leu715, Phe794(2), Met801, Phe778, Met759(2), and Leu763. Methyllinderatin showed ten hydrophobic interactions, two hydrogen bond interactions, and a van der waals interaction along with amino acid residues such as Leu726, Trp755, Asn719, Leu715, Leu797, Leu718(2), Phe794, Leu887, Met756, Cys891(3). Moreover, catechin showed two hydrogen bond interactions and three hydrophobic interactions with amino acid residues Gln725, Arg766, Val698, and Pro696(2). Yakuchinone A showed two hydrogen bond interactions, five hydrophobic interactions, and a van der waals interaction with amino acid residues Leu758, Trp732, Pro696, Gly762, Pro780, Val698, Ile699, and Gln725. Besides, lonaprisan had seven hydrophobic interactions and three hydrogen bonds along with amino acid residues such as Arg724(2), Leu727(3), Lys731(3), Tyr700, and Ile699.

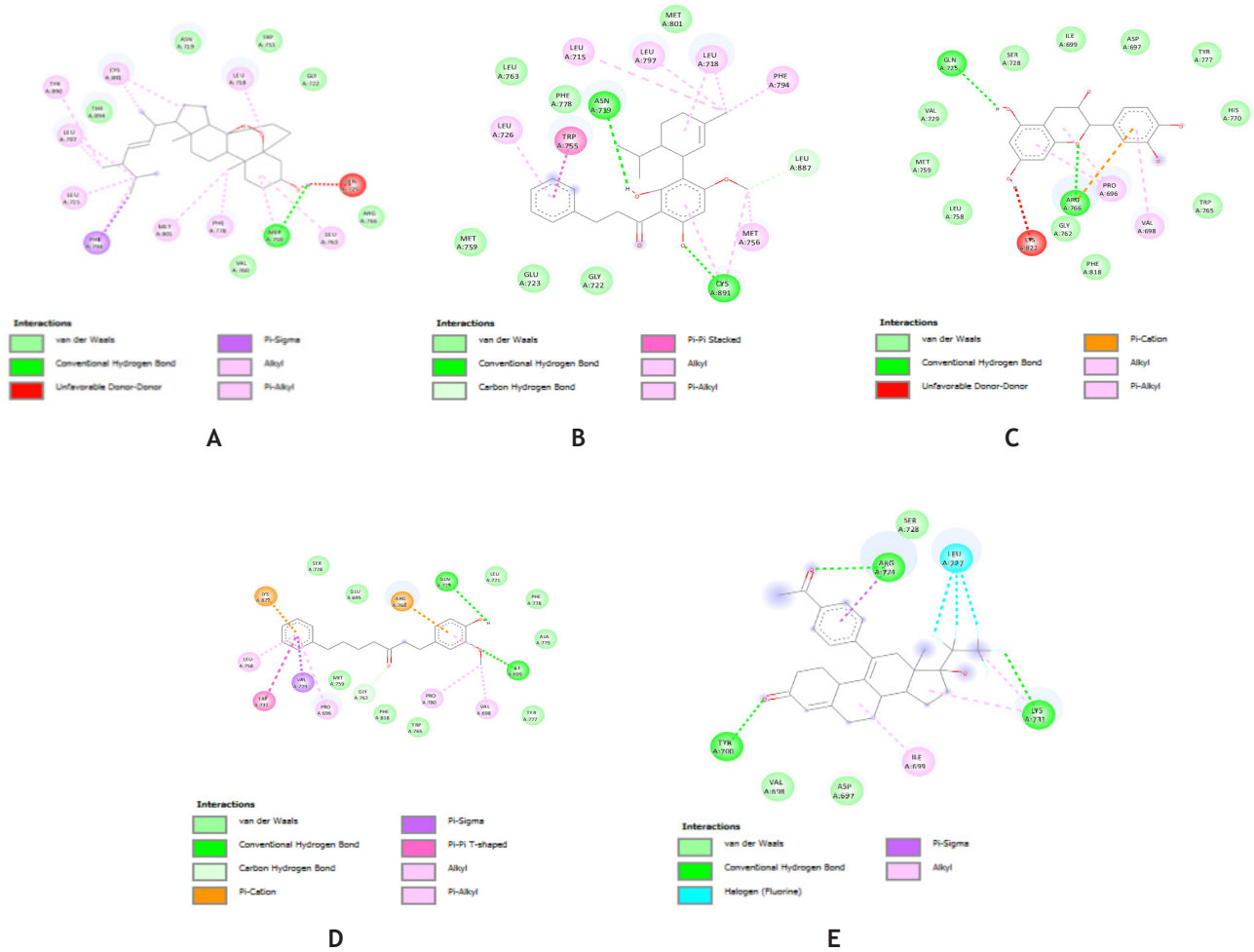


Figure 1. 2D visualization of the interaction between A. PR and Ergosterol peroxide; B. PR and Methyllinderatin; C PR and catechin; D. PR and Yakuchinone A; E. PR and Lonaprisan.

DISCUSSION

Drug likeness tests using Lipinski's Rule of Five and toxicity tests were used to select the compounds. According to the Lipinski Rule of Five, the ideal drug molecule conforms to the requirements for physicochemical properties. The Lipinski rule of five predicts a substance's chemical similarity to a drug with a certain biological activity intended for oral administration (Ansori, *et al*., 2021). Lipinski's rules are as follows, molecular weight less than 500 Dalton, number of H-bond acceptors less than 10, number of H-bond donors less than 5, and Log*P* less than 5, with no more than two violations (Aamir, *et al*., 2018). Based on Lipinski's rule of five, all compounds had no more than 2 violations. Therefore, they were considered as a drug-like compound and can be used as oral preparations.

In assessing toxicity, we used three indicators which are hepatotoxicity, ames-mutagenesis, and acute oral toxicity, as shown in Table 3. An ames mutagenesis test determines whether a compound is mutagenic or not. In this study, all compounds yielded negative results, indicating that the compounds were non-mutagenic. Then, hepatotoxicity test can be used to determine whether a compound is hepatotoxic or not. All compounds except 1,7-bis(4-hydroxyphenyl)-2,4,6-heptatrienone, gallic acid, sitostenone, sitosterol, and stigmasterol produced negative results, indicating that they were not mutagenic. Moreover, Acute oral toxicity is classified into four categories based on whether the compound is toxic or not. Category I (LD_{50} 50 mg/kg) and category II (LD_{50} 500 mg/kg) were toxic, whereas category III (500 mg/kg LD_{50} 5000 mg/kg) and category IV (LD_{50}>5000 mg/kg) were non-toxic (Guan, *et al*., 2019; Nisha, *et al*., 2016). However, pinocembrin, sitosterol, and stigmasterol had oral toxicity in category I and II. Therefore, 1,7-bis(4-hydroxyphenyl)-2,4,6-heptatrienone, gallic acid, sitostenone, sitosterol, stigmasterol, and pinocembrin were excluded from docking due to toxicity. A previous study by Lachumy, *et al* showed that the flower extract of *Etlingera elatior* with LC_{50} value of 2.52 mg/ml (24 h) did not show significant toxicity to brine shrimp. This extract is not toxic to brine shrimp so it can be used as an antimicrobial agent in doses that were evaluated further *in vivo* (Lachumy, *et al*., 2010).

Our findings yielded that a total of nine compounds had high binding energies and seven compounds out of which had higher binding energies compared to lonaprisan. According to the docking result, ergosterol peroxide showed the lowest binding energy value. Binding energy (ΔG) is a parameter of ligand-protein conformational stability. The interaction between ligand and protein tends to be in the lowest energy state, causing the molecule to be in a stable state. As a result, the lower the ΔG value (the more negative ΔG value), the higher the binding affinity for the selected binding site of the receptor (Arwansyah, *et al*., 2014). Furthermore, Zafar, *et al.* stated that there is a linear relationship between the inhibition constant value (Ki) and the binding energy value. Thus, the value of binding energy can be used to predict a compound's stability to inhibit protein (Ismail, *et al*., 2019). In this study, it was found that all compounds had affinities to PR because those compounds had binding energy values of ≤-5.0 kcal/mol on PR. According to Jin, *et al*., the binding energy value threshold is -5.0 kcal/mol, so values less than -5.0 kcal/mol are considered to have high binding energy to receptor targets (Jin, *et al*., 2021). In this study there was the top three highest binding value on PR which were ergosterol peroxide, methyllinderatin, catechin, and yakuchinone a, so these compounds were selected to be analyzed their molecular interactions.

Previous *in silico* study stated that the catalytic dyad (active sites) of PR were Leu715, Leu718, Asn719, Leu721, Gly722, Gln725, Trp755, Met756, Met759, Val760, Leu763, Arg766, Phe778, Phe794, Leu797, Met801, Leu887,

Tyr890, Cys891, Thr894, Val903, Phe905, and Met909 (Mani, *et al*., 2021). Moreover, *in silico* study by Lenin, *et al.* reported that the compound biochanin on PR was well occupied with amino acid residues of Cys891, Met759, Phe794, and Gln752 (Lenin, *et al*., 2022). In line with previous study, our result showed that Leu718, Cys891, Tyr890, Leu797, and Phe794 were found in ergosterol peroxide-PR interaction. In Methyllinderatin-PR interaction showed amino acid residues that match with the active sites of PR such as Trp755, Asn719, Leu715, Leu797, Leu718(2), Phe794, Leu887, Met756, and Cys891(3). Moreover, Gln725 were shown in Catechin-PR and yakuchinone A-PR interaction. Thus, these compounds were in the active sites of PR. The binding area of proteins that are involved in amino acid residues and play a role in binding is known as the active site (Kulandaisamy, *et al*., 2017a). As a result of the compound's interaction with amino acid residues at the active site, the compound has the ability to inhibit protein target as a competitive inhibitor (Arwansyah, *et al*., 2014; Kulandaisamy, *et al*., 2017b).

In addition, hydrogen bond, hydrophobic, and van der waals interactions are involved in determining the value of binding energy. Hydrogen bond is the interaction of hydrogen atoms with electronegative atoms such as fluorine (F), nitrogen (N), and oxygen (O) (Trott and Olson, 2010). Meanwhile, hydrophobic interaction is an interaction that occurs between non-polar molecules such as pi-pi stacked, alkyl-alkyl, pi-alkyl, or pi-pi T-shaped (Benet, *et al*., 2016). According to previous study, both hydrogen and hydrophobic interactions can stabilize the compound at the active site of the protein, change the binding energy value, and increase the efficacy of the compounds when interacting with the protein (Pantsar and Poso, 2018). However, Macchiagodena, *et al*., stated that hydrophobic interactions contribute more to molecule bond strength than hydrogen bonds (Macchiagodena, *et al*., 2020). On the other hand, Glowacki, *et al*. stated that increasing the number of hydrophobic interactions at the active site of the protein can improve the compound's biological effect (Głowacki, *et al*., 2013). Van der Waals interactions are less strong than covalent and electrostatic bonds, but they nonetheless account for a significant portion of the total binding energies and are largely responsible for free energy shifts (Mohanty, *et al*., 2021). Based on this study, we can conclude that ergosterol peroxide, methyllinderatin, catechin, and yakuchinone A had both hydrophobic and hydrogen bond interactions on PR. Moreover, in methyllinderatin and yakuchinone A also had van der waals interactions. As a result, those interactions play roles in strengthening molecular bonds and increasing binding energy.

Previous study about secondary metabolites constituents and anticancer activities of *Etlingera elatior* (Jack) R.M.Sm grown in different locations of Malaysia reported that the flower extract of *Etlingera elatior* from Kelantan showed potent anticancer activity against the tumour cell lines MCF-7 and MDA-MB-231 with an IC_{50} of 173.1 and 196.2 µg/mL, respectively. Moreover, the extracts from Pahang (IC_{50}=204.5 and 246.2 µg/mL) and Johor samples (IC_{50}=277.1 and 296.7 µg/mL) (Ghasemzadeh, *et al*., 2015).

CONCLUSION

Nine selected compounds of *Etlingera elatior* had drug-likeness properties based on Lipinski's rule of five and were safe based on toxicity tests. All compounds except caffeic acid and vanillic acid had higher binding energy on PR compared with lonaprisan. Ergosterol peroxide had the best binding affinity on PR as indicated by the binding value of -9.8 kcal/mol and also had interactions with the active site of PR. All of the nine compounds have the potential to be

developed as anticancer agents of PR in breast cancer with ergosterol peroxide being the most potential compound. Further *in vitro* and *in vivo* studies including toxicity assay and optimal dosage of these compounds as PR inhibitors are needed before these compounds can be used clinically.

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