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Cytotoxic effect of *Spatholobus littoralis* extract on breast cancer cells by in vitro and prediction of the mechanism of activity against estrogen receptors (ER-α and ER-β) by in silico

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ABSTRACT

Introduction: *Spatholobus littoralis* is widely used as an anticancer herbal medicine in Kalimantan, Indonesia. This study aims to determine the cytotoxic effect of *S. littoralis* on breast cancer cells in vitro and predict the mechanism of its activity on the estrogen receptors (ER) in silico.

Methods: Dry wood of *S. littoralis* was extracted using ethanol solvent by maceration and fractionated using *n*-hexane, chloroform, and ethyl acetate. The cytotoxic assay was evaluated using MTT reagent in T47D and 4T1 cells. Prediction of the interaction mechanism of phenolic compounds from the genus *Spatholobus* with ER-α and ER-β was carried out in silico.

Results: The results showed that the ethanolic extract of *S. littoralis* did not show a cytotoxic effect on T47D cells, but showed weak toxicity on 4T1 cells. Furthermore, *n*-hexane, chloroform, and ethyl acetate fractions of *S. littoralis* showed strong to moderate cytotoxic effects on T47D and 4T1 cells. In silico test results showed that 3'-4'-7-trihydroxy flavone was a phenolic compound with the highest binding energy compared to the ER native ligand Genistein in ER-α (-10.2 kcal/mol) and ER-β (-10.9 kcal/mol). The 3'-4'-7-trihydroxy flavone binding site in ER-α was bound to amino acid residues Arg394, Glu353, and Leu387, while in ER-β it was found at Arg346, Glu305, and Leu339.

Conclusion: These findings indicate that *S. littoralis* contains phenolic compounds that can inhibit the growth of breast cancer cells, so it may have the potential to be developed as a new drug for breast cancer.

Implication for health policy/practice/research/medical education:
The results of this research can be used as scientific evidence regarding the use of the *S. littoralis* plant in traditional medicine. This research data also increases the understanding of the molecular mechanisms of natural compounds from *S. littoralis* and can aid the search for new drugs to treat breast cancer.

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Introduction

Breast cancer is a disease in which breast cells grow abnormally out of control and form tumors. In 2022, 2.3 million new cases of breast cancer were diagnosed worldwide, surpassing lung cancer as the most common cancer. Breast cancer usually attacks breast tissue, especially in women, and only a few cases in men. Breast cancer accounts for 24.5% of all cancers in women (1). Currently, the cases of breast, prostate, and uterine corpus cancer are still showing a significant increase, although the death rate due to cancer is starting to decline (2). It shows that cancer treatment has made significant progress. Breast cancer treatment is usually based on removing tissue, killing cancer cells, and minimizing their effect on

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surrounding normal cells. However, in the early stages of metastasis, it is difficult to perform surgical procedures, while radiation treatment not only kills cancer cells in a particular area but also normal cancer cells around it. Most chemotherapy drugs, such as Taxol, 5-fluorouracil (5-FU), and Adriamycin target cell division, but on the other hand, the side effects of chemotherapy can cause severe diarrhea and hair loss in patients (3). Therefore, an effort to discover new compounds with high activity against cancer cells and fewer side effects is crucial.

Spatholobus littoralis Hassk is a plant from the genus *Spatholobus*, often found in tropical forests in Indonesia (4). The plant known as Bajakah root is widely used in traditional medicine in Kalimantan (5). Research has shown its pharmacological activities, such as anticancer (6), anti-inflammatory (7), antidiabetic (8), antioxidant (9,10), and antihepatotoxic (11). The chemical compounds contained in several plants of the genus *Spatholobus* are flavonoids and tannins (9), phenolics (10), and steroids (12). Research results on *S. suberectus* have shown that it contains flavones, flavanones, chalcones, isoflavans, and isoflavones (13,14). Some of these flavonoid compounds have anti-inflammatory and tyrosinase inhibitory activity (13), are cytotoxic to breast cancer cells (14), and are antioxidant and anticancer (15,16). Scientific proof of using plants from the genus *Spatholobus* as medicine is still being carried out.

Currently, the search for new drugs from natural ingredients is not only based on empirical data but also on computational methods to predict the pharmacokinetic and toxicological properties of drugs through an in-silico approach. This approach is also beneficial to understanding the interaction between compounds and molecular targets in which it is impossible to test all interactions experimentally (17). The estrogen receptor (ER) is essential for determining breast cancer diagnosis and therapeutic targets (18,19). Estrogen receptors alpha (ER-α) and beta (ER-β) are nucleic transcription factors that regulate many complex physiological processes in humans. ER-α is found mainly in the mammary glands, uterus, ovaries, bones, male reproductive organs, prostate, liver, and adipose tissue. In contrast, ER-β is found primarily in the prostate, bladder, ovaries, colon, adipose tissue, and immune system.

This study aims to determine the cytotoxic effect of *S. littoralis* wood ethanolic extract and its fractions in vitro and predict the mechanism of its activity on ERs in silico. In this study, we analysed the phytochemical content of *S. littoralis* wood ethanolic extract, *n*-hexane, chloroform, and ethyl acetate fractions both qualitatively and quantitatively. We also determined the half-maximal inhibitory concentration (IC_{50}) of *S. littoralis* wood ethanolic extract and various fractions towards breast cancer cell lines T-47D and 4T1 using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Then, we predicted the possible mechanism of anti-breast cancer activity of *S. littoralis* through the in-silico interaction of several compounds found in the genus *Spatholobus* to ER-α and ER-β.

Materials and Methods

Materials

The wood samples from the *S. littoralis* plant, ethanol, ascorbic acid, chloroform, *n*-hexane, ethyl acetate, distilled water, and gallic acid were used in this study. T-47D and 4T1 cell cultures were obtained from the Parasitology laboratory, Gadjah Mada University, Indonesia, and grown in Dulbecco's Modified Eagle's Medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco), 1% penicillin-streptomycin in temperature 37 °C, with 5% CO_2 flow. For MTT assay, MTT reagents ([3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide] (Sigma), dimethyl sulfoxide (DMSO), sodium dodecyl sulfate (SDS) 10%, and 0.01 N chloric acid were used.

Apparatus

Analytical balance, Buchi Rotavapor R-114 evaporator, autoclave, biological safety cabinet, CO_2 incubator, inverted microscope, binocular microscope, hemocytometer, centrifuge, water bath, tube rack, aluminum foil, micropipette, microplate reader, glassware, as well as computer with Intel Xeon CPU specifications, 32 GB RAM, ten cores, 500 GB SSD were used. The software was AutoDock Tools 1.5.7, Pymol, Avogadro 2.0, LigPlot+2.2.8, and GIMP 2.0.

Preparation sample of *Spatholobus littoralis*

This research used *S. littoralis* wood samples obtained from traditional markets in Pontianak, Indonesia. Staff from the Faculty of Biology, Gadjah Mada University, Indonesia, identified the leaves and stem parts of the plant (Figure 1). The identification results showed that the plant was *S. littoralis* Hassk. Plant specimens were stored in the Herbarium with code HERB-SL-2023. A total of 3 kg of crushed *S. littoralis* wood was extracted with 96% ethanol by maceration. The extract was fractionated using solvents with increasing polarity, starting from *n*-hexane,

Figure 1. Wood and leaves of the *Spatholobus littoralis* plant.

chloroform, and ethyl acetate.

Phytochemical characterization of *Spatholobus littoralis*

The phytochemical characterization of each extract and fractions from *S. littoralis* wood was carried out qualitatively and quantitatively. Qualitative characterizations were performed using terpenoid and steroid tests with Salkowski reagent, alkaloid test with Wagner reagent, phenolic test with iron (III) chloride test, and foam test for saponins (20). Meanwhile, quantitative characterization was carried out by analyzing the total phenolic content (TPC) of extracts and fractions obtained from *S. littoralis* wood, which was determined using the Folin-Ciocalteu procedure (21). The sample was reacted with Folin-Ciocalteu reagent and sodium carbonate solution by heating at 50 °C for 10 minutes. The absorbance of each solution was measured at 725 nm. The total amount of phenolics was calculated as the gallic acid equivalent of the calibration curve. Gallic acid was used as a standard phenolic solution. Gallic acid was dissolved in ethanol at various concentrations, and absorbance was measured at the same wavelength. The calibration curve results obtained had a linear regression equation $y = 0.0043x + 0.0339$ ($R^2 = 0.993$). Each sample's TPC was expressed in mg gallic acid equivalents (GAE) per g sample.

Determination of the cytotoxic effect of *Spatholobus littoralis* on T47D and 4T1 Breast Cancer cell lines

To determine the cytotoxic effect *S. littoralis,* the T47D and 4T1 breast cancer cells were seeded in 96-well plates. Each well contained 2000 cells in Dulbecco's modified eagle's medium (DMEM, Gibco) supplemented with 10% FBS (Gibco) and 1% penicillin-streptomycin (Gibco). Cells were incubated in a 37 °C with 5% CO_2 flow incubator. The next day, the cells were treated with various concentrations of *S. littoralis* ethanolic extract, *n*-hexane, chloroform, and ethyl acetate fractions dissolved in a culture medium containing 0.05% DMSO. The final volume for each well was 100 µL and applied in triplicates. After 12 to 24 hours of incubation, 10 µL of MTT solution was added and the cells were incubated for an additional 4 hours in 37 °C with 5% CO_2 flow incubator. Then, 100 µL of formazan solution (10% SDS and 0.01 N HCl) was added and the plate was stirred on a shaker for five minutes. The plate was incubated at room temperature and protected from light for twelve to twenty-four hours. Three replications (n=3) of the data were collected for the absorbance, which was measured at a wavelength of 595 nm using a microplate reader (BioRad, USA). The linear regression equation from the graph showing the relationship between the average percentage (%) of cell viability and the log concentration was the basis for calculating the IC_{50} for each sample (22). The criteria used were as follows: IC_{50} <20 µg/mL (high cytotoxic activity), IC_{50}: 20-100 µg/mL (moderate cytotoxic activity), IC_{50}: 201-500 µg/mL (weak cytotoxic activity), IC_{50} >500 µg/mL (no cytotoxic activity) (23).

Prediction of activity of compounds in the genus Spatholobus against estrogen receptors (ER-α and ER-β)

Prediction of molecular interaction mechanisms using phenolic compounds reported from the genus *Spatholobus* was carried out in silico using the docking method. The structure of phenolic compounds in *Spatholobus* plants was obtained from the data on the KNApSAcK website (http://www.knapsackfamily.com/knapsack_core/top.php). The 3D structure was obtained from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) and stabilized using Avogadro 2.0 software. Next, the 3D structure of each compound was created in a pdb file using the Pymol software. Then, the AutodockTools-1.5.7 software was used to convert the pdp file to a pdbqt file.

The enzyme proteins used as receptors were carried out by downloading the ER-α (code: 1X7R) and ER-β (code: 1X7J), which were available in the Protein Data Bank (www.pdb organization). The protein was separated from the solvent and ligands or residues using Pymol software, which was then saved in the pdb extension. The docking process was carried out using AutoDockTools-1.5.7 software. Re-docking between receptors and natural ligands was carried out on a grid box, which could produce a root mean square deviation (RMSD) value <2 Å, showing the validity of the method (24). The test compound was attached to the receptor binding site following the grid box was used in validation. The results obtained from this docking process were in the form of compound or ligand binding affinity. This method was used to predict the mechanism of activity of chemical compounds that acted as ligands tethered to target receptors in the form of enzymes or proteins, which could be studied using computational approaches. The binding energy could be determined by the Gibbs free energy value (ΔG kcal/mol) (25). A Gibbs free energy value of less than zero (0) indicated that the bond between the compound and the target protein occurred spontaneously and was stable (26). To determine the interaction between the ligand and the active site on the receptor, the ligPlot+ 2.2.8 software was used and visualized using the GIMP 2.0 software and saved in jpg format (27).

Results

The ethanolic extract was evaporated to obtain a thick extract of 420 g. Fractionation of the ethanolic extract using *n*-hexane, chloroform, and ethyl acetate solvents, respectively, resulted in *n*-hexane (13.63 g), chloroform (104.26 g), and ethyl acetate (153.57 g) fractions. The extract and fractions were subjected to qualitative and quantitative phytochemical analysis. The qualitative phytochemical tests showed that *S. littoralis* wood extracts and fractions contained phenolic, flavonoid, and

Table 1. Total phenolic content and cytotoxic effect on T47D and 4T1 cancer cells of *Spatholobus littoralis* extract and its fractions

Sample of *S. littoralis*	TPC (mg GAE/g sample)	Cytotoxic activity			
		T47D		4T1	
		(IC_{50} µg/mL)	Cytotoxic activity criteria	(IC_{50} µg/mL)	Cytotoxic activity criteria
Total ethanolic extract	545.10 ± 9.81	1018.25	No cytotoxic	252.17	Weak cytotoxic
n-Hexan fraction	78.88 ± 1.31	110.71	Moderate	176.56	Moderate
Chloroform fraction	308.67 ± 2.52	75.47	Moderate	64.37	Moderate
Ethyl acetate fraction	909.91 ± 6.60	4.79	High cytotoxic	117.12	Moderate

TPC: Total phenolic content; GAE: Gallic acid equivalent; IC_{50}: Half-maximal inhibitory concentration.

triterpenoid compounds, except for the *n*-hexane fraction, which showed negative results for the phenolic and flavonoid tests. The total phenolic compound (TPC) from the extract and fractions, as well as the corresponding cytotoxic activity against T47D and T41 cells based on IC_{50} are described in Table 1.

Phenolic compounds found in the plants of the genus *Spatholobus* used as ligands in this study included dihydroquercetin (1), butin (2), (-)-epicatechin (3), eriodictyol (4), liquiritigenin (5), prunasin (6), afromosin (7), cajanin (8), formononetin (9), 3',4',7-trihydroxyflavone (10), licochalcone A (11), (+)-dihydrokaempferol (12), plathymenin (13), and 6-methoxyeriodictyol (14), with a structure like those in Figure 2.

Prediction of the molecular mechanism for the interaction of phenolic compounds from the genus *Spatholobus* on ER-α uses the 1X7R protein receptor model, while for ER-β uses the 1X7J protein model, all

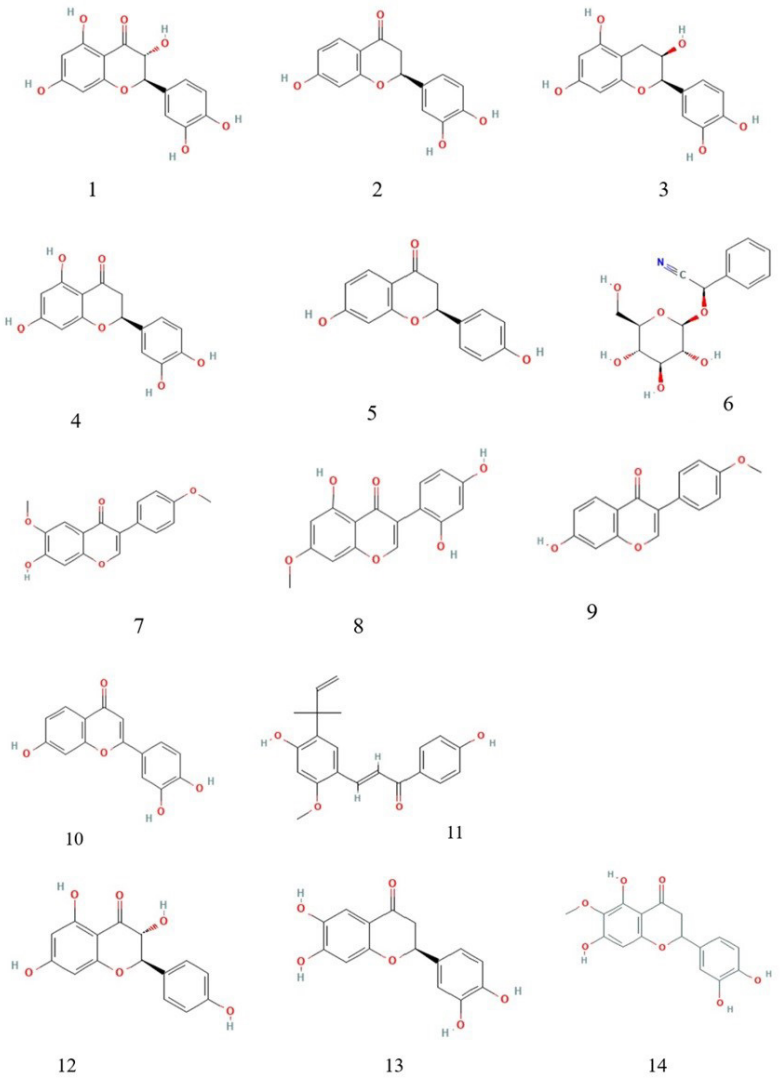

Figure 2. Structure of phenolic compounds from the genus *Spatholobus*.

of which were downloaded from the Protein Data Bank (https://www.pdb.org/). The native ligand found in the 1X7R and 1X7J receptor proteins was Genistein (C_{15} H_{10} O_5), a flavonoid compound with a structure similar to phenolic compounds from the genus *Spatholobus*. The method was validated in this research by redocking each receptor. Data validation of redocking for each receptor protein is presented in Table 2.

Tables 3 and 4 show the results of molecular docking analysis, including binding energy (ΔG kcal/mol), hydrogen bonds, and hydrophobic interactions with ER-α (1X7R) and ER-β (1X7J), respectively.

Furthermore, it can be depicted in the form of a 3D complex between the ligand and each receptor, the binding site, and the LigPlot+ 2.2.8 interaction of the most stable ligand, namely 3',4',7-trihydroxyflavone with ER-α (Figure 3) and with ER-β (Figure 4).

Discussion

In the drug discovery process, leads of molecules derived from natural products are considered promising since 60% of anticancer candidates in clinics are natural. In this study, we investigated *S. littoralis* anti-breast cancer potential through in vitro and silico approaches. For the cytotoxicity analysis, the T47D and 4T1 cell lines were chosen to represent two distinct types of breast cancer subtypes. The 4T1 model represented triple-negative breast cancer (TNBC) cells, while the T47D represented non-TNBC cells. The non-TNBC types of breast cancer, including cases with different receptors such as ER, progesterone receptor, and human epidermal growth factor receptor 2 (HER2), can be treated by using drugs that match their target receptors. In contrast, TNBC does not have a clear receptor target, making treatment more challenging because there is no specific focal point for

Table 2. Results of the validation of the redocking method

Receptor protein	PDB code	Ligand	Grid box coordinate	Grid box size	RMSD
ER-α	1X7R	Genistein	x = 42.521, y = 19.649, z =19.649	x= y=z = 30 Å	0.687 Å
ER-β	1X7J	Genistein	x = 9.166, y = -10.353, z = 38.398	x= y= z = 20 Å	0.414 Å

RMSD: Root means square deviation; ER-β: Estrogen receptor beta; ER-α: Estrogen receptor alpha.

Table 3. The binding energy, hydrogen bonds, and hydrophobic interactions of ligands with estrogen receptor-α (1X7R)

Ligands/ Compound	Binding energy (kcal/mol)	Hydrogen bond	Hydrophobic interaction
Genistein (native ligan)	-9.9	Arg394; His524	Glu353, Leu387, Phe404, Leu525, Met343, Leu346, Met421, Leu391, Ala350
Dihydroquercetin (1)	-9.6	Glu353, Leu346, His524	Leu387, Leu384, Leu525, Gly521, Met421, Ile424, Phe404, Leu349
Butin (2)	-9.5	Leu346	Leu349, Leu525, Ile424, His524, Gly521, Met421, Leu384, Glu353, Leu387, Phe404
Epicatechin (3)	-8.9	Glu353, His524, Leu387, Arg394	Phe404, Leu349, Leu346, Ala350, Gly521, Met421, Ile424, Met388, Leu384, Leu391
Eriodictyol (4)	-9.4	Glu353, Arg394, Gly521	Leu349, Phe404, Leu346, Met421, Ile424, His524, Leu525, Leu384, Met388, Leu387
Liquiritigenin (5)	-9.9	Arg394, His524	Glu353, Phe404, Leu346, Met421, Gly521, Leu384, Trp383, Leu525, Ala350, Leu387, Leu349
Prunasin (6)	-8.1	Leu346	Met421, Leu525, Leu540, Ala350, Thr347, Leu349, Glu353, Phe404, Leu391, Leu387, Met388, Leu384, Gly521, His524
Afrormosin (7)	-5.4	His524, Leu525	Glu353, Phe404, Leu387, Ile424, Met421, Gly521, Arg394, Leu349
Cajanin (8)	-7.5	Leu346, His524	Arg394, Glu353, Ala350, Met343, Met421, Leu525, Leu384, Leu387, Gly521, Met388, Leu391
Formononetin (9)	-7.6	His524	Arg394, Glu353, Leu387, Ala350, Met343, Leu525, Met421, Leu346, Phe404, Leu391
3'-4'-7-trihydroxy flavone (10)	-10.2	Arg394, Glu353	Leu391, Met388, Ala350, Leu387, Gly521, Ile424, Leu525, His524, Leu346, Thr347
Licochalcone-A (11)	1.8	Arg394, Phe404, His524	Glu353, Phe425, Met421, Ile424, Leu346, Met343, Gly521, Leu525, Trp383, Leu384, Leu387, Leu391, Leu349
Dihydrokaempferol (12)	-9.7	Arg394, Gly521	Leu349, Glu353, Leu387, Ala350, Leu346, Leu384, Leu525, Ile424, Met421, His524, Phe404
Plathymenin (13)	-9.7	Arg394, Glu353, Gly521, His524	Phe404, Leu387, Ala350, Leu384, Leu525, Met421, Leu349, Leu346, Trp383
6-Methoxyeriodictyol (14)	-6.7	Leu387, Glu353, His524	Phe404, Ala350, Leu349, Leu346, Gly521, Met343, Leu525, Met421, Leu384, Met388, Leu391, Arg394

Table 4. The binding energy, hydrogen bonds, and hydrophobic interactions of ligands with estrogen receptor-β (1X7J)

Ligands/Compound	Binding energy (kcal/mol)	Hydrogen bond	Hydrophobic interaction
Genistein (native ligand)	-10.8	Arg346; His475	Leu301, Leu298, Glu305, Ile373, Leu476, Gly472, Met295, Met479, Ile376, Phe356, Leu343, Leu339, Met340
Dihydroquercetin (1)	-7.2	His475; Leu298 Glu 305	Gly472, Ile373, Ile376, Phe356, Leu301, Leu339, Ala302, Met336, Leu476, Thr299, Met295
Butin (2)	-7.3	Leu339; Glu305 Arg346	Phe356, Leu301, Leu298, Ile373, Met295, Leu476, Ala302, Leu491, Met336, Met340, Leu343
Epicatechin (3)	-7.5	Glu305; Leu476 Gly472; His475	Ala302, Met295, Ile373, Phe356, Leu298, Met340, Leu343, Leu339, Leu301
Eriodictyol (4)	-6.7	His475	Met295, Ile373, Gly472, Ile376, Met336, Ala302, Glu305, Leu339, Leu301, Phe356, Leu298, Thr299, Leu476
Liquiritigenin (5)	-8.8	His475; Glu305	Met295, Gly472, Ile376, Ile373, Leu380, Leu298, Phe356, Leu339, Ala302, Leu301, Met336, Leu476
Prunasin (6)	-5.2	Glu305	Leu301, Leu339, Phe356, Ala302, Met340, Leu343, Gly472, Met336, Met295, Leu298, Ile373, Trp335, Leu476, Leu491
Afrormosin (7)	-1.5	-	Arg346, Phe356, Leu301, Glu305, Leu298, Gly472, Ile373, His475, Met479, Met295, Leu476, Met336, Leu343, Met340, Leu339,
Cajanin (8)	-7.9	Leu339, Glu305, Arg346	Met340, Phe356, Leu301, Leu298, Met479, Ile373, His475, Met295, Leu476, Met336, Ala302
Formononetin (9)	-7.3	His475	Arg346, Glu305, Leu301, Phe356, Leu298, Ile373, Met295, Met479, Leu476, Ala302, Leu339, Leu343
3'-4'-7-Trihydroxy flavone (10)	-10.9	Arg346, Glu305,	Phe356, Leu291, Thr299, Leu476, Ile373, His475, Gly472, Ile376, Leu301, Leu339, Ala302, Met340, Leu343
Licochalcone-A (11)	0.1	Leu339, Arg346, His475	Glu305, Met340, Met295, Met336, Leu298, Thr299, Leu476, Lys471, Ile376, Ile373, Gly472, Ala468, Leu343, Ala302
Dihydrokaempferol (12)	-8.4	His475, Glu305	Met479, Leu476, Met336, Ala302, Leu301, Leu339, Leu298, Phe356, Ile376, Gly472, Ile373, Met295
Plathymenin (13)	-7.2	Arg346, Leu339, Glu305, His475	Ala302, Phe356, Ile373, Met336, Met295, Gly472, Leu476, Leu298, Met340, Leu380, Leu343
6-Methoxyeriodictyol (14)	-6.4	Arg346, Glu305, Leu339	Phe356, Leu301, Met336, Ala302, Gly472, Leu476, His475, Met295, Ile373, Ile376, Met340, Leu343

intervention (28).

Several studies have shown that the plants of the genus *Spatholobus* contain various phenolic compounds (13,14). Correspondingly, in this study the *S. littoralis* also contained phenolic compounds. However, the structure of the phenolic compounds found in the *S. littoralis* plant has yet to be reported. Currently, in our Chemistry Education Laboratory, the process of isolating and identifying the structure of the wood of the *S. littoralis* plant is being carried out. Therefore, the docking method reported in the genus *Spatholobus* was obtained on the KNApSAcK website and only fourteen phenolic compounds were selected that have been reported in several references (13-15). We found that ethanolic extract of *S. littoralis* exhibited no cytotoxic effect in T47D cells with IC_{50} of 1018.25 µg/mL and only weak cytotoxicity in 4T1 cells with IC_{50} of 252.17 µg/mL. This finding is in line with a previous study that showed the IC_{50} of 988 µg/mL from ethanolic extract of *S. littoralis* against T47D cells (6). However, this finding can be reasoned that even though the TPC of the ethanolic extract of *S. littoralis* is relatively high (545.10 ± 9.81 mg GAE/g sample), the ethanolic extract still contains a mixture of polar and non-polar compounds that may be antagonistic. In contrast, *n*-hexane, chloroform, and ethyl acetate fractions from *S. littoralis* showed moderate to strong cytotoxicity on T47D cells. In T47D cells, the *n*-hexane, chloroform, and ethyl acetate fractions from *S. littoralis* showed IC_{50} values of 110.71; 75.47; and 4.79 µg/mL, respectively.

In 4T1 cells, the cytotoxic activity of *S. littoralis* has never been reported. In this study, a moderate effect was shown by the *n*-hexane, chloroform, and ethyl acetate fractions from *S. littoralis* with IC_{50} values of 176.56; 67.37; and 117.12 µg/mL, respectively. The results of this study supported previous studies, which have shown the cytotoxic and anticancer activities of several plants from the genus *Spatholobus* (6,10). Several plants from the genus *Spatholobus* have been widely used as part of crucial traditional medicine.

Spatholobus suberectus is a critical medicinal ingredient in traditional Chinese, Vietnamese, and Korean medicine to treat blood disorders, such as anemia, menstrual irregularities, and rheumatic diseases (29,30). Several compounds found in the plants of the genus *Spatholobus* showed antioxidant and anticancer activities (15). Furthermore, the *S. littoralis* plant showed cytotoxic

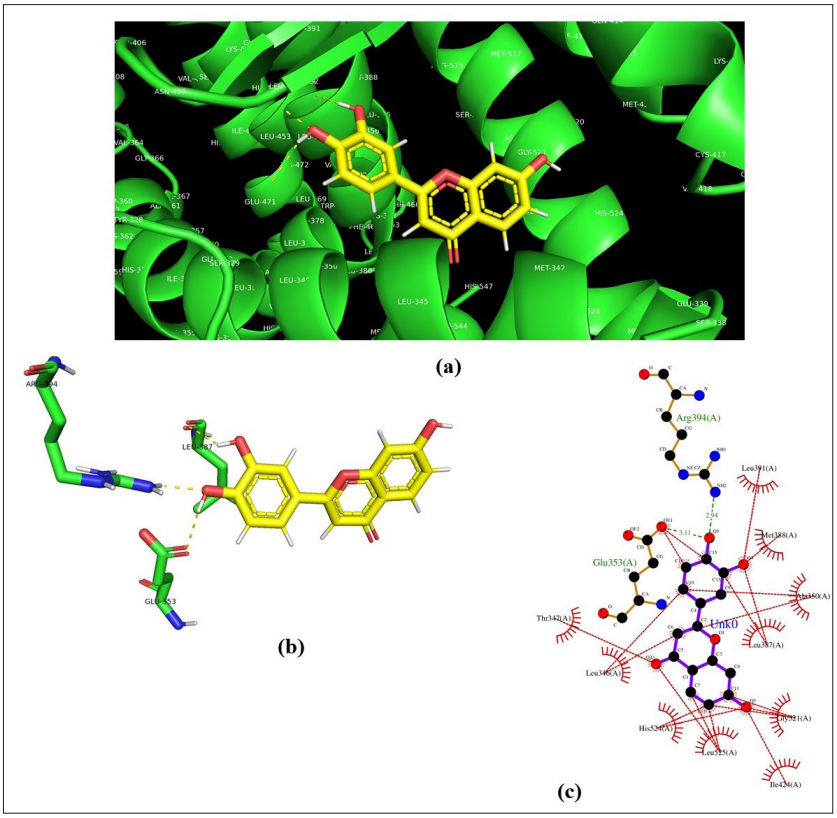

Figure 3. 3D ligand-receptor 1X7R structure (a), binding site (b), and ligand interactions with amino acid residues (c) at 3'-4'-7-trihydroxy flavone against ER-α (1X7R). Hydrogen bonds are shown by green lines and hydrophobic bonds are shown by red lines.

activity against breast cancer cells (6). Correspondingly, the results of this study showed that the *S. littoralis* plant contained active compounds that could inhibit the growth of T47D and 4T1 cancer cells. Among the all samples tested in this study for cytotoxicity, the ethyl acetate fraction contained the highest TPC. Indeed, several studies have also shown a relationship between phenolic levels, antioxidants, and anticancer activities (31,32). Phenolic compounds from plants generally have strong antioxidant properties and have a natural effect in preventing various diseases related to oxidative stress, such as cancer (33). Previous research showed that the compounds often found in the genus *Spatholobus* are phenolics, especially flavonoids (13-15).

Currently, docking methods are widely used to molecularly predict interactions between ligands and target receptors (34). Molecularly, the ER is considered necessary for determining diagnosis and therapeutic targets for breast cancer (18,19). Proof through molecular docking using ER-α and ER-β receptors shows that there are hydrogen bonds and hydrophobic interactions between the ligand and amino acid residues on the receptor, and several compounds have relatively stable binding energies. Various factors, including electrostatic forces, Van der Walls forces, hydrophobic bond interactions, hydrogen bonds, and the flexibility of the receptor structure, influence the binding affinity between the ligand and the receptor. The greater the number of hydrogen bonds and hydrophobic interactions formed between the ligand and receptor, the stronger the bond (35). Hydrogen bonds are formed between hydrogen atoms and atoms that have high electronegativity. The partial positive charge in a hydrogen bond comes from the H atom of the ligand. In contrast, the partial negative charge comes from highly electronegative atoms such as oxygen, nitrogen, and sulfur in the amino acid residues of the receptor, but the opposite can also occur. Hydrophobic interactions are interactions that occur between non-polar groups.

The results of method validation through redocking of natural ligands with their respective receptor proteins obtained an RMSD value of <2 Å, so it can be stated that the method used is valid (24). Next, the grid book configuration position was used to carry out docking using the ligands of 14 phenolic compounds from the genus *Spatholobus*. The docking method can be used to predict the mechanism of molecular interactions between phenolic compounds found in the genus *Spatholobus* to obtain binding energy and ligand interactions with receptor amino acid residues. Data on binding energy between compounds or ligands is expressed in Gibbs free energy (ΔG kcal/mol (25). These data indicate that 3',4',7-trihydroxyflavone has the most stable energy for

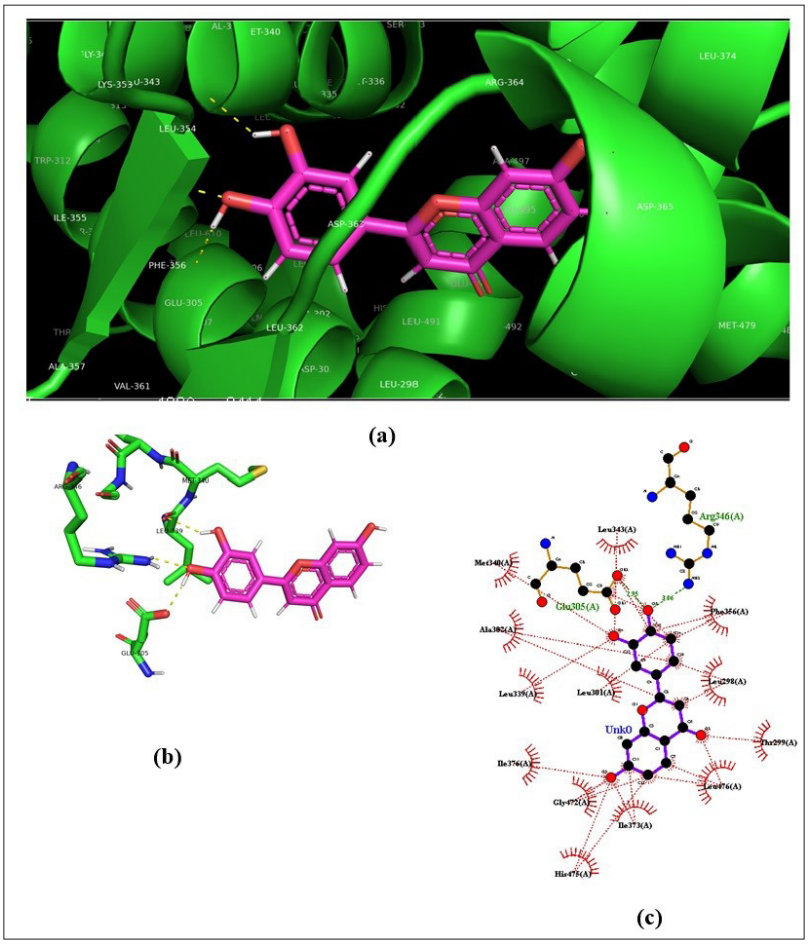

Figure 4. 3D ligand-receptor 1X7J structure (a), binding site (b), and ligand interactions with amino acid residues (c) at 3'-4'-7-trihydroxy flavone against ER-α (1X7R). Hydrogen bonds are shown by green lines and hydrophobic bonds are shown by red lines.

both ER-α and ER-β.

The molecular docking analysis performed in this study (Tables 2 and 3) showed the energy affinity data between the ligand and the receptor and the existence of hydrogen bonds and hydrophobic interactions between the ligand and the receptor. One of the phenolic compounds that had the highest binding energy to the ER-α (1X7R) and ER-β (1X7J) receptors was 3'-4'-7-trihydroxyflavone. This compound had the most stable binding to the ER-α and ER-β receptors compared to other phenolic compounds. We also depicted the interaction between ligand and receptor in Figures 3 and 4. The binding site of 3',4',7-trihydroxyflavone with ER-α is bound to amino acid residues Arg 394 and Glu 353, respectively using hydrogen bonds, and Leu 387 via hydrophobic interactions. Meanwhile, the ligand binding site on the ER-β receptor is bound to amino acid residues Arg 346 and Glu 305 using hydrogen bonds, as well as to Leu 339 via hydrophobic interactions. Thus, the compound 3',4',7-trihydroxyflavone is a potential compound to be developed as a breast cancer drug. A previous researcher suggested that this compound might be used as an adjuvant in various combinatorial therapies. It was the most common mechanism for enhancing the efficacy of chemotherapeutic drugs (36). Flavonoids are the main group of phytoestrogens that have been widely studied by experts. Several flavonoid compounds contain at least one chemical that mimics estrogen by varying mechanisms (37).

Conclusion

In conclusion, our results showed that the *S. littoralis* plant contained active compounds that could inhibit the growth of T47D and 4T1 cancer cells. The *n*-hexane, chloroform, and ethyl acetate fractions of *S. littoralis* have moderate to very active activity against T47D and 4T1 breast cancer cells. 3'-4'-7-trihydroxyflavone is a flavonoid compound that has the highest binding energy on both ER-α (1X7R) and ER-β (1X7J) receptors. The binding site for 3',4',7-trihydroxyflavone with ER-α is bound to amino acid residues Arg 394, Glu 353, and Leu 387, while the binding site for the ER-β receptor is bound to amino acid residues Arg 346, Glu 305, and Leu 339. The results of this study can complement previous information and support the potential for developing this plant as a new drug to cure breast cancer, although further research is still needed.

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Writing–original draft: Sri Atun.
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Conflict of interests

The authors declared no competing interests.

Ethical considerations

All authors observed ethical issues (including plagiarism, violations, falsification of data, falsification of double publication or submission, redundancy).

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References

1. World Health Organization (WHO). Breast Cancer. 2023. Available from: https://www.who.int/news-room/fact-sheets/detail/breast-cancer.
2. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin. 2023;73(1):17-48. doi: 10.3322/caac.21763.
3. Liu YP, Zheng CC, Huang YN, He ML, Xu WW, Li B. Molecular mechanisms of chemo- and radiotherapy resistance and the potential implications for cancer treatment. MedComm (2020). 2021;2(3):315-40. doi: 10.1002/mco2.55.
4. Ridder-Numan JW, Wiriadinata H. A revision of the genus *Spatholobus* (Leguminosae-Papilionoideae). Journal on Taxonomic Botany. 1985;10(2):107-270.
5. Istiqomah I, Safitri D. Pharmacological activities of *Spatholobus littoralis*. Infokes. 2021;11(2):463-9.
6. Aliviyanti RU, Sudibyo RS, Murwanti R. Efek sitotoksik beberapa akar bajakah kalimantan terhadap sel kanker payudara T47D. J Penelit Saintek. 2021;26(2):131-40. doi: 10.21831/jps.v26i2.41211.
7. Nastiti K, Nugraha DF. [Anti-inflammatory activity of bajakah wood extract (*Spatholobus littoralis* Hask)]. J Surya Medika. 2022;7(2):45-50. doi: 10.33084/jsm.v7i2.3202.
8. Arysanti RD, Wirjatmadi B, Winarni D. Effect of bajakah (*Spatholobus littoralis* Hassk.) extract on malondialdehyde serum of Wistar rats induced by streptozotocin. Jurnal Kesehatan Prima. 2022;16(2):143-8. doi: 10.32807/jkp.v16i2.837.
9. Fitriani F, Sampepana E, Saputra SH. [Characteristic of bajakah root plants (*Spatholobus littoralis* Hassk) from Loa Kulu, Kutai Kartanegara district]. Jurnal Riset Teknologi Industri. 2020;14(2):365-76. doi:10.26578/jrti.v14i2.6590.
10. Iskandar D, Widodo N, Warsito, Masruri, Rollando, Antang YP. Phenolic content, antioxidant, cytotoxic of fractions of *Spatholobus littoralis* Hassk from Kalimantan, Indonesia. J Hunan Univ Nat Sci. 2022;49(3):14-23. doi: 10.55463/issn.1674-2974.49.3.2.
11. Adhityasmara D, Ramonah D. Efek efek hepatoprotektor ekstrak etanol batang bajakah tampala (*Spatholobus Littoralis* Hassk) pada tikus yang diinduksi isoniazid. Jurnal Ilmiah Sains. 2022;22(1):40-51. doi: 10.35799/jis.v22i1.36293.
12. Astuti MD, Maulana A, Kuntowati EM. Isolasi steroid dari fraksi n-heksana batang kayu Bajakah tempala (*Spatholobus littoralis* Hassk.). Prosiding Seminar Nasional Kimia, Jurusan Kimia FMIPA Universitas Negeri Surabaya; 2014.
13. Liu RX, Xu YL, Ma LF, Ying YM, Zhan ZJ. A new flavanone from *Spatholobus suberectus* Dunn. J Chem Res. 2018;42(10):529-30. doi: 10.3184/174751918x15386515371813.
14. Peng F, Zhu H, Meng CW, Ren YR, Dai O, Xiong L. New isoflavanes from *Spatholobus suberectus* and their cytotoxicity against human breast cancer cell lines. Molecules. 2019;24(18):3218. doi: 10.3390/molecules24183218.
15. Li W, Liu J, Guan R, Chen J, Yang D, Zhao Z, et al. Chemical characterization of procyanidins from *Spatholobus suberectus* and their antioxidative and anticancer activities. J Funct Foods. 2015;12:468-77. doi: 10.1016/j.jff.2014.11.009.
16. Tejasari M, Respati T, Yuniarti L. Exploring anticancer potential in bajakah tampala by in silico virtual screening. KnE Life Sci. 2022;7(5):111-8. doi: 10.18502/kls.v7i5.12517.
17. Fang J, Liu C, Wang Q, Lin P, Cheng F. In silico polypharmacology of natural products. Brief Bioinform. 2018;19(6):1153-71. doi: 10.1093/bib/bbx045.
18. Paterni I, Granchi C, Katzenellenbogen JA, Minutolo F. Estrogen receptors alpha (ERα) and beta (ERβ): subtype-selective ligands and clinical potential. Steroids. 2014;90:13-29. doi: 10.1016/j.steroids.2014.06.012.
19. Zhou Y, Liu X. The role of estrogen receptor beta in breast cancer. Biomark Res. 2020;8:39. doi: 10.1186/s40364-020-00223-2.
20. Harborne AJ. Phytochemical Methods a Guide to Modern Techniques of Plant Analysis. 3rd ed. Springer; 1998.
21. Hagerman A, Mueller HI, Makkar AH, Mueller IH, Makar HP. Quantification of Tannins in Tree and Shrub Foliage: A Laboratory Manual. FAO, IAEA; 2000.
22. Rahmawati DR, Nurrochmad A, Jenie RI, Meiyanto E. The

synergistic cytotoxic effect of pentagamavunon-1 (PGV-1) and curcumin correlates with the cell cycle arrest to induce mitotic catastrophe in 4T1 and T47D breast cancer cells. Indones Biomed J. 2023;15(5):318-27. doi: 10.18585/inabj.v15i5.2594.
23. Sajjadi SE, Ghanadian M, Haghighi M, Mouhebat L. Cytotoxic effect of *Cousinia verbascifolia* Bunge against OVCAR-3 and HT-29 cancer cells. J Herbmed Pharmacol. 2015;4(1):15-9.
24. Bagaria A, Jaravine V, Huang YJ, Montelione GT, Güntert P. Protein structure validation by generalized linear model root-mean-square deviation prediction. Protein Sci. 2012;21(2):229-38. doi: 10.1002/pro.2007.
25. Bajorath J. Computer-aided drug discovery. F1000Res. 2015;4. doi: 10.12688/f1000research.6653.1.
26. Hill AD, Reilly PJ. A Gibbs free energy correlation for automated docking of carbohydrates. J Comput Chem. 2008;29(7):1131-41. doi: 10.1002/jcc.20873.
27. Forli S, Huey R, Pique ME, Sanner MF, Goodsell DS, Olson AJ. Computational protein-ligand docking and virtual drug screening with the AutoDock suite. Nat Protoc. 2016;11(5):905-19. doi: 10.1038/nprot.2016.051.
28. Yu S, Kim T, Yoo KH, Kang K. The T47D cell line is an ideal experimental model to elucidate the progesterone-specific effects of a luminal A subtype of breast cancer. Biochem Biophys Res Commun. 2017;486(3):752-8. doi: 10.1016/j.bbrc.2017.03.114.
29. Huang X, Fei Q, Yu S, Liu S, Zhang L, Chen X, et al. A comprehensive review: botany, phytochemistry, traditional uses, pharmacology, and toxicology of *Spatholobus suberectus* vine stems. J Ethnopharmacol. 2023;312:116500. doi: 10.1016/j.jep.2023.116500.
30. Nguyen-Ngoc H, Vu-Van T, Pham-Ha-Thanh T, Le-Dang Q, Nguyen-Huu T. Ethnopharmacology, phytochemistry, and pharmacological activities of *Spatholobus suberectus* vine stem. Nat Prod Commun. 2022;17(12):1934578X221142724. doi: 10.1177/1934578X221142724.
31. Han M, Li G, Liu X, Li A, Mao P, Liu P, et al. Phenolic profile, antioxidant activity and anti-proliferative activity of crabapple fruits. Hortic Plant J. 2019;5(4):155-63. doi: 10.1016/j.hpj.2019.01.003.
32. Milella RA, De Rosso M, Gasparro M, Gigante I, Debiase G, Forleo LR, et al. Correlation between antioxidant and anticancer activity and phenolic profile of new Apulian table grape genotypes (*V. vinifera* L.). Front Plant Sci. 2022;13:1064023. doi: 10.3389/fpls.2022.1064023.
33. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010;15(10):7313-52. doi: 10.3390/molecules15107313.
34. Xue Q, Liu X, Russell P, Li J, Pan W, Fu J, et al. Evaluation of the binding performance of flavonoids to estrogen receptor alpha by AutoDock, AutoDock Vina and Surflex-Dock. Ecotoxicol Environ Saf. 2022;233:113323. doi: 10.1016/j.ecoenv.2022.113323.
35. Du X, Li Y, Xia YL, Ai SM, Liang J, Sang P, et al. Insights into protein-ligand interactions: mechanisms, models, and methods. Int J Mol Sci. 2016;17(2):144. doi: 10.3390/ijms17020144.
36. Nozhat Z, Heydarzadeh S, Memariani Z, Ahmadi A. Chemoprotective and chemosensitizing effects of apigenin on cancer therapy. Cancer Cell Int. 2021;21(1):574. doi: 10.1186/s12935-021-02282-3.
37. Kiyama R. Estrogenic flavonoids and their molecular mechanisms of action. J Nutr Biochem. 2023;114:109250. doi: 10.1016/j.jnutbio.2022.109250