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Integrative *In Vitro* and *In Silico* Evaluation of *Etlingera hemisphaerica* Reveals Potent Estrogen Receptor–Targeted Cytotoxicity Against MCF-7 Breast Cancer Cells

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ABSTRACT

Breast cancer remains a leading cause of cancer-related mortality worldwide, emphasizing the need for effective and affordable therapeutic agents derived from natural sources. *Etlingera hemisphaerica*, a member of the Zingiberaceae family, has received limited scientific attention despite its recognized ethnopharmacological relevance. This study aimed to investigate the anticancer potential of *E. hemisphaerica* through an integrated *in vitro* cytotoxicity and *in silico* molecular modeling approach. The cytotoxic activity of the ethanol extract of *E. hemisphaerica* was evaluated against estrogen receptor-positive ($ER\alpha^+$) MCF-7 human breast cancer cells using the MTT assay after 72 h of exposure. The extract exhibited a pronounced dose-dependent reduction in cell viability, yielding an exceptionally low IC_{50} value of 1.16 ppm, categorizing it as highly cytotoxic according to established screening criteria. Dose–response modeling using a four-parameter log-logistic regression confirmed a classical sigmoidal inhibition pattern, indicative of a specific and saturable biological effect. To elucidate potential molecular mechanisms, major flavonoid constituents were subjected to molecular docking analysis against estrogen receptor alpha (ERα). Several compounds, particularly quercetin, luteolin, isorhamnetin, and kaempferol, exhibited strong binding affinities ($\Delta G \leq -8.4$ kcal/mol), comparable to known ERα modulators. Drug-likeness and ADMET profiling further revealed that quercetin exhibits favorable pharmacokinetic properties, including high gastrointestinal absorption, non-*P*-glycoprotein substrate behavior, and full compliance with Lipinski's rule of five. Collectively, these findings indicate that *E. hemisphaerica* possesses potent cytotoxic activity against breast cancer cells, potentially mediated through modulation of ERα-related signaling pathways and mitochondrial-dependent mechanisms. This integrative study provides the first mechanistic insight into its anticancer potential and supports its promise as a valuable source of lead compounds for breast cancer drug discovery.

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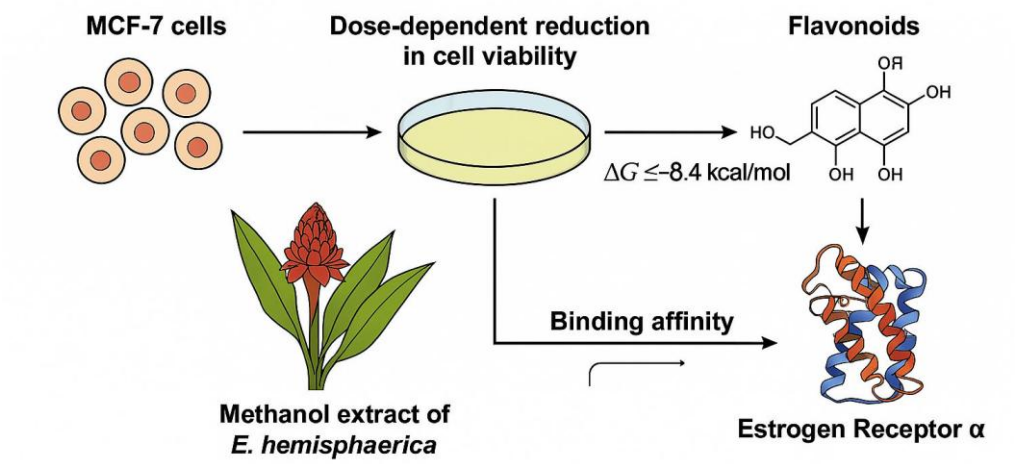
GRAPHICAL ABSTRACT



Introduction

Cancer continues to be a leading cause of morbidity and mortality globally, with over 10 million deaths recorded in 2020 [1,2]. While conventional treatments such as chemotherapy and radiation remain the primary therapeutic strategies, their side effects, limited efficacy in late-stage cancers, and high recurrence rates have prompted growing interest in complementary approaches, particularly plant-based therapies. Phytochemicals derived from medicinal plants offer a wide array of bioactive compounds, many of which demonstrate cytotoxic, antioxidant, and apoptosis-inducing properties that make them strong candidates for anti-cancer drug development [3].

One such plant that has garnered scientific attention in recent years is *Etlingera hemisphaerica*, a lesser-known member of the Zingiberaceae family [4]. Traditionally used in Indonesia and surrounding regions for culinary and medicinal purposes, this plant—commonly known as *forest honje*—is rich in secondary metabolites such as flavonoids, tannins, terpenoids, and phenolic acids [4]. These compounds are well-documented in the literature for their roles in modulating oxidative stress, inflammation, and abnormal cell proliferation, all of which are key processes in cancer development. Phytochemical analysis of *E. hemisphaerica* leaves revealed a high flavonoid content (67.85 mg QE/g) and strong antioxidant activity, particularly in ethanol extracts with an IC_{50} of 49.37 µg/mL suggesting potent free radical scavenging ability that could mitigate DNA damage and carcinogenesis [5]. Although *Etlingera elatior*, a close relative of *E. hemisphaerica*, has been more extensively studied for its anticancer effects, the presence of similar phytoconstituents in *E. hemisphaerica* strongly suggests comparable potential [6]. Furthermore, quercetin—one of the major flavonoids present in this species—has been shown in various studies to suppress tumor growth by inducing apoptosis and inhibiting angiogenesis and metastasis in several cancer cell lines. A comprehensive review by Koch *et al*. highlighted the genus *Etlingera* as a source of over 400 bioactive compounds, with many showing antimicrobial, cytotoxic, and antioxidant properties [7]. Notably, *E. hemisphaerica* was identified as one of the species with both ornamental and pharmacological value. In addition, *in vivo* studies have shown the protective effects of *E. hemisphaerica* extracts against mercury-induced toxicity in mice, further supporting its systemic safety and therapeutic potential [8]. These findings, taken together,

underscore the potential relevance of *E. hemisphaerica* within integrative oncology, particularly in the context of hormone-dependent breast cancer [9]. By combining *in vitro* cytotoxicity profiling with high-resolution metabolomic annotation and estrogen receptor–focused molecular docking, this study provides a coherent mechanistic framework linking phytochemical composition to biological activity. Nevertheless, direct experimental evidence validating estrogen receptor–dependent mechanisms, downstream transcriptional effects, and pathway-level consequences remains limited for *E. hemisphaerica*. Moreover, in vivo pharmacokinetics, safety, and tumor-specific efficacy have not yet been established. Addressing these gaps through receptor-specific functional assays, metabolomic profiling, and animal models will be essential to translate these early mechanistic insights into a robust evaluation of its therapeutic relevance. Collectively, this work establishes a foundation for future investigations of *E. hemisphaerica* as a source of estrogen-modulating bioactive compounds with potential applications in breast cancer drug discovery.

Methods

Sampling and extraction process

E. hemisphaerica leaves were collected from the Gayo Highlands region, specifically in Atu Lintang Village, Central Aceh, Aceh Province, Indonesia (coordinates: 4°27′0″N 96°46′0″E) (Figure 1). The collected leaves were thoroughly washed and air-dried at room temperature. Once completely dried, the leaves were ground into a fine powder. A total of 100 g of the powdered material was subjected to maceration in 1,000 mL of ethanol for 72 hours at room temperature. During the extraction process, the mixture was stirred occasionally to enhance solvent penetration and extraction efficiency. After the maceration period, the mixture was filtered to remove plant debris, and the resulting filtrate was concentrated using a rotary evaporator to obtain the crude ethanol extract.

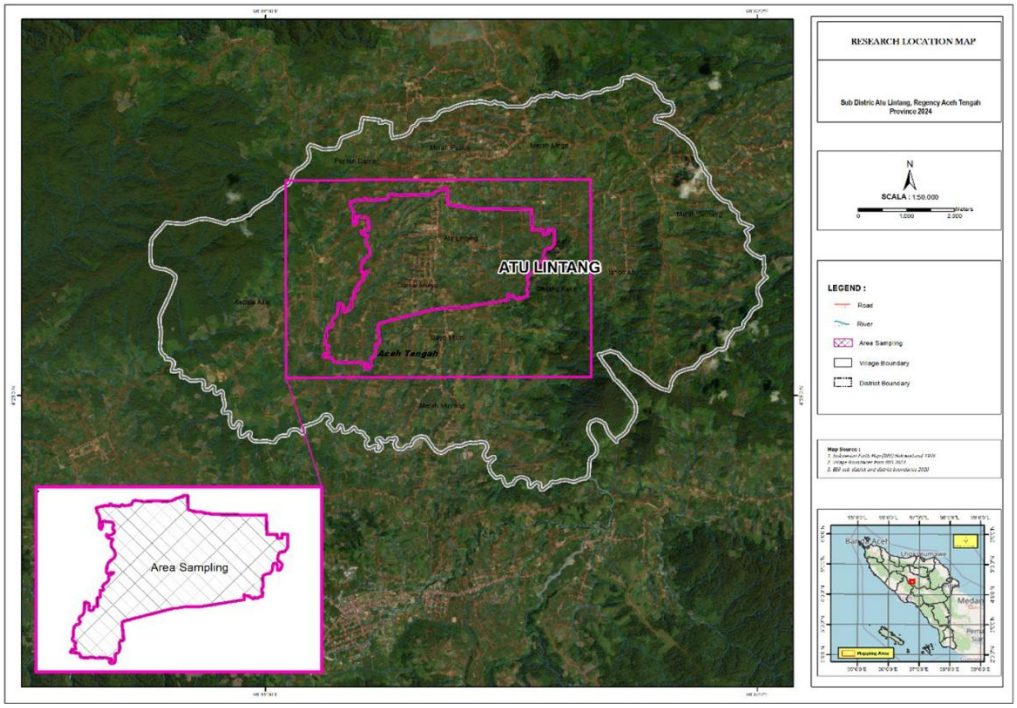


Figure 1. Map of the research location

LC-HRMS analysis

Phytochemical profiling of the ethanol extract of *E. hemisphaerica* leaves was carried out using liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS). Approximately 50 mg of dried extract was dissolved in 1 mL of LC-MS-grade ethanol, sonicated for 15 minutes, and filtered through a 0.22 µm syringe filter before analysis. Chromatographic separation was performed on a reverse-phase C18 column (*e.g.,* Waters Acquity UPLC BEH C18, 2.1 × 100 mm, 1.7 µm) using a gradient mobile phase of water (solvent A) and acetonitrile (solvent B), both containing 0.1% formic acid. The elution gradient progressed from 5% to 95% B over 15 minutes at a flow rate of 0.3 mL/min with a total run time of 20 minutes. High-resolution mass spectrometric detection was conducted using an Orbitrap-based instrument operating in both positive and negative electrospray ionization modes, with a scan range of m/z 100–1,000, resolution of 60,000 FWHM at m/z 200, capillary voltage of 3.5 kV (positive) and 2.8 kV (negative), and a source temperature of 320 °C. Sheath and auxiliary gases were set at 40 and 10 arbitrary units, respectively. Raw data were processed using Compound Discoverer or Xcalibur software, and compounds were tentatively identified by matching accurate mass (within 5 ppm), MS/MS fragmentation, and isotopic patterns against databases such as METLIN, mzCloud, and PubChem [10].

Molecular docking analysis

A structure-based molecular docking approach was employed to evaluate the interactions between selected phytochemical constituents of *E. hemisphaerica* and estrogen receptor alpha (ERα). Ligand structures were retrieved from the PubChem database, energy minimized using Open Babel with the MMFF94 force field, and converted to PDBQT format with appropriate charge assignments. The three-dimensional structure of ERα was obtained from the RCSB Protein Data Bank and prepared by removing co-crystallized ligands and water molecules, followed by the addition of polar hydrogens and Kollman charges using AutoDock Tools. The ligand-binding site was defined based on the native ligand position within the ERα binding domain, and a grid box was centered to fully encompass the active site. Docking simulations were performed using AutoDock Vina with enhanced exhaustiveness, and the binding pose with the lowest predicted free energy (ΔG, kcal/mol) was selected for each ligand. Ligand–receptor interactions were subsequently analyzed using molecular visualization software to identify key hydrogen bonds and hydrophobic contacts [10].

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay procedure

MCF-7 human breast cancer cells (ATCC HTB-22) were cultured at a density of 5,000 cells per well in 100 µL of complete growth medium. The culture medium used was RPMI 1640 (Roswell Park Memorial Institute 1640), a nutrient-rich formulation commonly used for mammalian cell culture. It was supplemented with 5% fetal bovine serum (FBS) to provide growth factors, 100 U/mL penicillin, and 100 µg/mL streptomycin to prevent bacterial contamination. After 24 hours of incubation, when the cells reached approximately 50% confluency, the ethanol extract was added to the respective wells at various concentrations. Cytotoxicity was evaluated on the third day using the MTT assay. For this, 10 µL of MTT solution (5 mg/mL in phosphate-buffered saline) was added to each well, and the plate was incubated for 4 hours at 37 °C in a humidified atmosphere containing 5% CO_2. Following incubation, the resulting purple formazan crystals—formed by mitochondrial reduction of MTT in viable cells—were solubilized with ethanol. The absorbance was measured at 595 nm using a microplate

reader to determine cell viability, with lower absorbance indicating higher cytotoxicity.

Data analysis

The percentage of cell growth inhibition was calculated by comparing the absorbance of treated wells to that of untreated control wells. The mean % inhibition from three replicates at each concentration was used for further analysis. To determine the IC_{50} value—the concentration of extract that inhibits 50% of cell viability—a nonlinear regression model was fitted using the dose-response curve function from the *drc* package in R. A four-parameter log-logistic model (LL.4) was applied, which is commonly used for dose-response analysis in cytotoxicity studies [11]. The model estimates the minimum and maximum response levels, the IC_{50}, and the Hill slope of the curve. The IC_{50} value was derived using the ED function, with 95% confidence intervals. The dose-response curve was visualized using *ggplot2,* with the x-axis displayed on a logarithmic scale to reflect the nonlinear nature of the data [12]. Curve fitting quality and residual patterns were checked to validate the model assumptions.

Results and Discussion

Phytocompounds from the ethanolic extract of *E. hemisphaerica* were identified using LC-HRMS. The LC-HRMS analysis was conducted to characterize the non-volatile and thermally labile secondary metabolites present in the extract with high mass accuracy and sensitivity. This method enabled the detection of a broader range of phytochemicals, particularly polar and high-molecular-weight compounds that are not amenable to GC-MS. The LC-HRMS analysis identified a total of 110 compounds, classified into six major chemical classes: organic acids, amino acids/derivatives, flavonoids/phenolics, sugars/nucleotides, glycosides/others, and terpenoids/fatty compounds. The distribution of these classes provides insights into the biochemical composition and potential biological functions of the analyzed sample (Figure 2).

Organic acids were the most abundant class, accounting for 28 compounds (25.5%) of the total identified metabolites. This dominance suggests a highly active primary metabolism, possibly linked to the tricarboxylic acid (TCA) cycle, photorespiration, or plant defense responses, where organic acids play central roles as intermediates and signaling molecules. Three classes—amino acids/derivatives, flavonoids/phenolics, and sugars/nucleotides—each comprised 19 compounds (17.3%), reflecting a balance between primary and secondary metabolism. Amino acids and their derivatives are essential for protein biosynthesis, nitrogen transport, and serve as precursors for numerous secondary metabolites. The presence of flavonoids and phenolics highlights the plant's antioxidant defense system, as these compounds are known for their radical-scavenging activity, UV protection, and involvement in plant–microbe interactions [13]. The relatively high proportion of sugars and nucleotides points to robust energy metabolism, signal transduction, and nucleic acid turnover. The glycosides/others category represented 14 compounds (12.7%), a class that often includes glycosylated secondary metabolites, which enhance solubility, stability, and bioactivity. Lastly, terpenoids and fatty compounds, although the least abundant at 11 compounds (10%), are crucial for membrane structure, signaling, and stress adaptation, especially in lipid-rich tissues or specialized storage structures [14].

Flavonoids are a prominent class of secondary metabolites widely distributed across plant taxa and are particularly abundant in members of the Zingiberaceae family. In this study, LC-HRMS analysis of Etlingera species revealed the presence of 19 flavonoid and related phenolic compounds, reflecting the plant's rich phenylpropanoid biosynthetic capacity (Table 1).

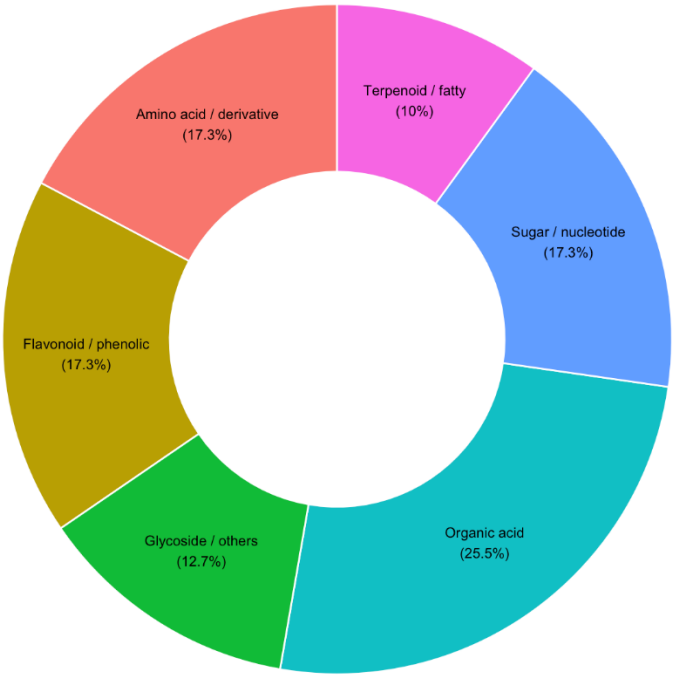


Figure 2. Overview of bioactive compounds from *E. hemisphaerica* extract based on LC-HRMS analysis

The identified compounds span several subclasses, including flavonols, flavones, glycosides, methoxylated flavonoids, and coumarins, many of which have been previously associated with strong antioxidant, anti-inflammatory, and antimicrobial activities [15,16]. The diverse flavonoid and coumarin derivatives identified in Etlingera species not only support the plant's biochemical richness, but also highlight its potential in therapeutic applications, particularly in cancer treatment. Among various malignancies, breast cancer remains a major global health concern and is the leading cause of cancer-related deaths among women [17]. In recent years, dietary polyphenols such as flavonoids have gained considerable attention for their chemopreventive and chemotherapeutic properties due to their ability to modulate multiple cancer-related signaling pathways.

Flavonols such as quercetin, kaempferol, and their glycosides, which were identified in the current LC-HRMS analysis of Etlingera, have shown substantial anticancer effects *in vitro* and *in vivo*. Quercetin has been reported to induce apoptosis, inhibit cell proliferation, and suppress metastasis in triple-negative breast cancer (TNBC) cells through mechanisms involving the PI3K/Akt, MAPK, and NF-κB signaling pathways [6]. It also enhances chemosensitivity when used in combination with standard chemotherapeutic agents. Similarly, kaempferol inhibits colorectal cancer cell growth action by suppressing angiogenesis, stimulating apoptosis, and causing cell cycle arrest [18]. Luteolin and apigenin, two flavones also detected in Etlingera, exert potent antiproliferative effects in cancer cells. Luteolin exerts anticancer effects in MCF-7 breast cancer cells by inhibiting proliferation and suppressing EGF-induced activation of p-STAT3, p-EGFR, p-Akt, and p-Erk1/2, while promoting Bax expression and reducing tumor growth and progestin-dependent VGF secretion [19]. Apigenin, on the other hand, has been shown to inhibit expression of vascular endothelial growth factor (VEGF) in human ovarian cancer cells [20].

Table 1. Flavonoid compounds detected by LC-HRMS of *E. hemisphaerica*

Compound	m/z	Retention time	Intensity	Class
Scutellarein 6,7,4'-trimethyl ether 5-glucoside	489.13998	6.783	3.42289E+15	Flavonoid
Scopolin	355.10294	7.51	2.96839E+15	Flavonoid
7,8-Dihydroxycoumarin	177.01933	7.511	6.29275E+15	Flavonoid
7-Hydroxycoumarine	163.03894	7.654	1.22698E+16	Flavonoid
Rutin	609.14594	9.49	1.49744E+16	Flavonoid
Vitexin	433.11279	9.672	2.11793E+16	Flavonoid
6,3'-Dihydroxy-4,4'-dimethoxy-5-methylaurone	327.08746	9.728	1.05569E+17	Flavonoid
Quercetin-3β-D-glucoside	463.08817	10.045	1.28171E+16	Flavonoid
Kaempferol	287.05487	11.318	2.30258E+15	Flavonoid
Trifolin	447.09323	11.32	1.17021E+16	Flavonoid
Rhoifolin	579.17053	11.57	2.65298E+15	Flavonoid
5,7,3',4',5'-Pentahydroxy-3,6,8-trimethoxyflavone	391.06693	11.886	9.97005E+14	Flavonoid
5,6-Dimethoxy- [2",3":7,8] furanoflavanone	325.10693	13.156	2.71723E+15	Flavonoid
Quercetin	301.0354	15.329	9.80638E+15	Flavonoid
Apigenin	269.04553	17.61	3.82498E+15	Flavonoid
Luteolin	285.04044	17.952	6.19233E+15	Flavonoid
Isorhamnetin	315.05115	18.276	9.29467E+14	Flavonoid
Linderoflavone A	357.06104	19.141	2.33627E+15	Flavonoid
Coumarin	145.02946	20.013	5.44192E+14	Flavonoid

The identification of rutin, isorhamnetin, and trifolin adds further therapeutic promise. Rutin, a quercetin-based glycoside, exhibits potent anti-angiogenic and pro-apoptotic activities and has been demonstrated to suppress breast tumor progression in murine models [21]. *In vitro* studies further showed that rutin inhibits the proliferation of mouse breast cancer cells through modulation of the miR-129-1-3p/Ca^{2+} signaling axis. These findings highlight a regulatory role of rutin at the non-coding RNA level and provide a mechanistic basis supporting its potential development as an anticancer agent for breast tumor suppression [22]. Isorhamnetin, a methylated form of quercetin with better bioavailability, has been shown to strongly suppress breast cancer cell growth. It induces cancer cell death by increasing oxidative stress, which damages DNA and activates caspases, while also disrupting mitochondrial function [23]. At the same time, isorhamnetin slows cell division by regulating cyclins and cyclin-dependent kinases. Beyond its effects on tumor growth, it also reduces the ability of cancer cells to spread by lowering the expression of matrix metalloproteinases, VEGF, and key epithelial–mesenchymal transition markers, highlighting its potential to inhibit both tumor progression and metastasis [24]. Although trifolin (kaempferol-3-galactoside) is less studied in cancer, its aglycone

form (kaempferol) is well documented to modulate key regulators of cell survival and proliferation [18]. The detection of vitexin, a C-glycosylated flavone, is particularly significant. Unlike O-glycosylated flavonoids, vitexin contains a stable C–C glycosidic linkage at the C-8 position of its apigenin core, which confers greater resistance to metabolic degradation. Experimental studies have shown that vitexin regulates multiple cancer-related processes, including cell cycle control, apoptosis, autophagy, metastasis, angiogenesis, epigenetic regulation, and tumor glycolysis. These effects are mediated through the modulation of key oncogenic signaling pathways, notably PI3K/Akt/mTOR, NF-κB, and STAT3. Importantly, vitexin also shows promise as an adjuvant compound, with the potential to enhance the efficacy of existing therapies and help overcome drug resistance [25]. Scutellarein derivatives, including the 6,7,4′-trimethyl ether 5-glucoside identified in this study, are methylated flavones that have demonstrated cytotoxic effects against breast cancer cells by inhibiting topoisomerase activity and inducing apoptosis. In addition, scutellarin has been reported to suppress the progression of breast cancer stem cells, which are closely associated with tumor recurrence and therapeutic resistance [26]. Additionally, coumarins, such as umbelliferone (7-hydroxycoumarin) and daphnetin (7,8-dihydroxycoumarin), contribute to the chemopreventive potential of *Etlingera*. Coumarins have been found to induce cell cycle arrest, inhibit cytochrome P450 enzymes involved in procarcinogen activation, and promote oxidative stress-induced apoptosis in breast cancer cells [27]. Taken together, the flavonoid-rich profile of *Etlingera* suggests a multitargeted mechanism for potential breast cancer intervention. The combination of aglycones and glycosylated forms, hydroxylated and methoxylated derivatives, and rare flavonoid structures provides a unique chemotype that could be explored for synergistic anticancer activity, either as nutraceuticals or as lead compounds for drug development. This aligns with ethnopharmacological use of *Etlingera* spp. in Southeast Asian traditional medicine, where decoctions and extracts have been used for general health, inflammation, and cancer-like symptoms [28,29].

The molecular docking results (Table 2) demonstrate that flavonoid constituents of *E. elatior* exhibit moderate to strong binding affinities toward the ERα, with calculated binding free energies (ΔG) ranging from −9.0 to −5.9 kcal/mol. Overall, the data indicate a clear structure–affinity relationship, where aglycone flavonoids show stronger ERα interactions than their corresponding glycosylated derivatives and simple coumarins. The quercetin compound showed the strongest binding affinity (ΔG = −9.0 kcal/mol), suggesting a highly favorable interaction with the ERα ligand-binding domain. This result is consistent with the presence of multiple hydroxyl groups on the flavonol backbone, which facilitate hydrogen bonding with key amino acid residues within the ERα active site, while the planar aromatic system supports π–π stacking and hydrophobic interactions. Similarly, luteolin (−8.7 kcal/mol), isorhamnetin (−8.6 kcal/mol), and the highly substituted pentahydroxy-trimethoxyflavone (−8.6 kcal/mol) also showed strong affinities, reinforcing the importance of hydroxyl and methoxy substitutions in enhancing receptor binding. Other flavonoid aglycones, including linderoflavone A (−8.5 kcal/mol), kaempferol (−8.4 kcal/mol), and apigenin (−8.3 kcal/mol), exhibited comparably high binding energies, indicating that both flavonols and flavones can effectively interact with ERα. Minor differences in ΔG among these compounds likely reflect variations in substitution patterns that modulate hydrogen bonding capacity and steric compatibility within the receptor pocket. In contrast, glycosylated flavonoids such as quercetin-3-β-D-glucoside (−7.4 kcal/mol), vitexin (−7.3 kcal/mol), trifolin (−7.2 kcal/mol), rutin (−7.1 kcal/mol), scutellarein 6,7,4′-

trimethyl ether 5-glucoside (−7.1 kcal/mol), and rhoifolin (−6.9 kcal/mol) showed reduced binding affinities. The presence of bulky sugar moieties likely introduces steric hindrance and reduces the ability of the flavonoid core to optimally fit within the ERα binding cavity, despite retaining some hydrogen-bonding interactions. Nevertheless, their ΔG values remain within a biologically relevant range, suggesting potential ERα modulation, albeit weaker than aglycone counterparts.

Coumarin derivatives exhibited the weakest interactions overall. Simple coumarin showed the lowest binding affinity (−5.9 kcal/mol), while hydroxylated and glycosylated coumarins such as 7,8-dihydroxycoumarin (−7.0 kcal/mol), 7-hydroxycoumarin (−6.7 kcal/mol), and scopolin (−6.6 kcal/mol) demonstrated only modest improvements. This trend highlights the limited interaction capacity of the coumarin scaffold with ERα compared to the more complex flavonoid structures. Collectively, these molecular docking findings suggest that *E. hemisphaerica* flavonoids—particularly aglycone flavonols and flavones—have a strong propensity to interact with ERα, supporting their potential role as natural estrogen receptor modulators. The superior binding performance of quercetin, luteolin, and related compounds provides a mechanistic basis for the estrogen-related bioactivities reported for *E. elatior* and justifies their prioritization for further *in vitro* and in vivo validation.

The drug-likeness and pharmacokinetic profiles of the selected compounds from *E. hemisphaerica*, quercetin and 5,7,3',4',5'-pentahydroxy-3,6,8-trimethoxyflavone, were evaluated using key physicochemical and ADMET-related parameters (Table 3).

Table 2. Binding free energy value of flavonoid compounds of *E. hemisphaerica*

Compound	ΔG (kcal/mol) to Erα receptor
Quercetin	−9.0
5,7,3',4',5'-Pentahydroxy-3,6,8-trimethoxyflavone	−8.6
Isorhamnetin	−8.6
Linderoflavone A	−8.5
Kaempferol	−8.4
Apigenin	−8.3
6,3'-Dihydroxy-4,4'-dimethoxy-5-methylaurone	−8.2
5,6-Dimethoxy-[2",3":7,8]furanoflavanone	−8.0
Luteolin	−8.7
Quercetin-3-β-D-glucoside	−7.4
Vitexin	−7.3
Trifolin	−7.2
Scutellarein 6,7,4'-trimethyl ether 5-glucoside	−7.1
Rutin	−7.1
7,8-Dihydroxycoumarin	−7.0
Rhoifolin	−6.9
7-Hydroxycoumarin	−6.7
Scopolin	−6.6
Coumarin	−5.9

Table 3. Druglike properties of selective compound from *E. hemisphaerica*

No.	Parameter	Quercetin	5,7,3',4',5'-Pentahydroxy-3,6,8-trimethoxyflavone
1	Molecular formula	$C_{15}H_{10}O_7$	$C_{18}H_{16}O_{10}$
2	Molecular weight	302.23 g/mol	392.31 g/mol
3	Number H-bond acceptors	7	10
4	Number H-bond donors	5	5
5	Log $P_{o/w}$	1.23	1.23
6	Gastrointestinal absorption	High	Low
7	Blood brain barrier	No	No
8	*P*-glycoprotein substrate	No	Yes
9	Bioavailability score	0.55	0.55
10	Meet Lipinski rules	Yes	Yes

Quercetin ($C_{15}H_{10}O_7$) possesses a relatively low molecular weight (302.23 g/mol), which is favorable for membrane permeability and oral drug development. Its balanced hydrogen-bonding capacity (7 acceptors and 5 donors) and moderate lipophilicity (Log P = 1.23) support adequate solubility while maintaining sufficient interaction potential with biological targets. Importantly, quercetin is predicted to have high gastrointestinal absorption, indicating a favorable oral uptake profile. The absence of blood–brain barrier (BBB) penetration suggests a reduced risk of central nervous system–related side effects, which is advantageous for compounds intended for peripheral targets. Additionally, quercetin is not predicted to be a *P*-glycoprotein (P-gp) substrate, implying a lower likelihood of efflux-mediated reduction in intracellular concentration and improved systemic exposure. In comparison, 5,7,3',4',5'-pentahydroxy-3,6,8-trimethoxyflavone ($C_{18}H_{16}O_{10}$) exhibits a higher molecular weight (392.31 g/mol) and increased polarity, as reflected by its greater number of hydrogen-bond acceptors (10) while maintaining the same number of donors (5). Although its Log P value (1.23) is identical to that of quercetin, 5,7,3',4',5'-pentahydroxy-3,6,8-trimethoxyflavone shows low gastrointestinal absorption, suggesting limited oral bioavailability. This reduced absorption is further supported by its classification as a *P*-glycoprotein substrate, indicating that active efflux mechanisms may restrict its intestinal uptake and intracellular accumulation. Similar to quercetin, garinimbine is not predicted to cross the BBB, which again favors peripheral selectivity.

Despite these differences, both compounds share a comparable bioavailability score of 0.55, indicating moderate overall oral bioavailability potential. Importantly, both quercetin and 5,7,3',4',5'-pentahydroxy-3,6,8-trimethoxyflavone comply fully with Lipinski's rule of five, supporting their classification as drug-like molecules and justifying their consideration in further pharmacological development. Taken together, these results suggest that quercetin exhibits a more favorable pharmacokinetic profile than 5,7,3',4',5'-pentahydroxy-3,6,8-trimethoxyflavone, particularly in terms of gastrointestinal absorption and transporter interactions. While garinimbine remains drug-like and pharmacologically relevant, its lower predicted absorption and P-gp substrate status may limit its effectiveness as an orally administered agent without formulation or structural optimization. Consequently, quercetin emerges as the more promising lead compound from *E. hemisphaerica* for further experimental validation and development.

The cytotoxic potential of the ethanol extract from *E. hemisphaerica* was assessed against MCF-7 human breast cancer cells using the well-

established MTT assay. This assay evaluates cell viability based on the mitochondrial conversion of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into a purple formazan product, reflecting the metabolic activity of living cells. MCF-7 cells were exposed to a range of extract concentrations for a 72-hour incubation period. The results demonstrated a dose-dependent reduction in cell viability, indicative of the extract's cytotoxic efficacy. The concentration-response relationship was further analyzed by fitting the data to a four-parameter log-logistic model using the drm function, confirming a classic sigmoidal inhibition pattern (Figure 3). The calculated IC_{50} value of 1.16 ppm highlights the potent bioactivity of the ethanol extract, as compounds with IC_{50} values below 10 ppm are generally considered highly cytotoxic in *in vitro* screening models. This sharp and consistent decrease in viability suggests that the extract may be interfering with essential cellular pathways, such as apoptosis induction, mitochondrial disruption, or cell cycle arrest. The cytotoxic activity of the ethanol extract of *E. hemisphaerica* was evaluated against MCF-7 human breast cancer cells using the MTT assay, a gold-standard colorimetric method for assessing cell viability and proliferation. The MTT assay is based on the reduction of the tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) to insoluble purple formazan crystals by mitochondrial succinate dehydrogenase and related oxidoreductase enzymes in metabolically active cells [30]. Thus, the amount of formazan produced is directly proportional to the number of viable cells and their mitochondrial integrity. After 72 hours of exposure to increasing concentrations of the ethanol extract, MCF-7 cells exhibited a clear and concentration-dependent reduction in cell viability. At lower concentrations, a partial decline in metabolic activity was observed, whereas higher concentrations resulted in a marked suppression of cell viability, indicating a strong cytotoxic effect. This dose-dependent response suggests that the extract contains bioactive constituents capable of progressively disrupting cellular metabolic function and survival pathways, a hallmark of effective antiproliferative agents. The concentration–response relationship was quantitatively analyzed using a four-parameter log-logistic model implemented via the drm function, which is widely applied in pharmacological and toxicological studies to model sigmoidal dose–response curves [31]. The fitted curve (Figure 3) demonstrated a classic sigmoidal inhibition profile with a steep slope around the midpoint, indicating a specific and saturable biological response rather than nonspecific cytotoxicity. Such curve characteristics are typical of compounds that interact with defined molecular targets or signaling pathways involved in cancer cell survival. The calculated half-maximal inhibitory concentration (IC_{50}) of 1.16 ppm highlights the remarkable cytotoxic potency of the ethanol extract. From a mechanistic perspective, the pronounced reduction in MTT reduction suggests that the extract may impair mitochondrial function, which plays a central role in cancer cell metabolism and apoptosis regulation. In MCF-7 cells, mitochondrial dysfunction is closely linked to activation of the intrinsic apoptotic pathway, involving loss of mitochondrial membrane potential, cytochrome c release, and caspase cascade activation. Additionally, phytochemicals such as flavonoids and phenolic compounds commonly reported in *Etlingera* species are known to induce apoptosis, generate reactive oxygen species, and trigger cell cycle arrest in breast cancer cells [32]. Moreover, MCF-7 cells are estrogen receptor-positive ($ER\alpha^{+}$), and disruption of estrogen-mediated signaling pathways is a well-recognized strategy for inhibiting their proliferation [33].

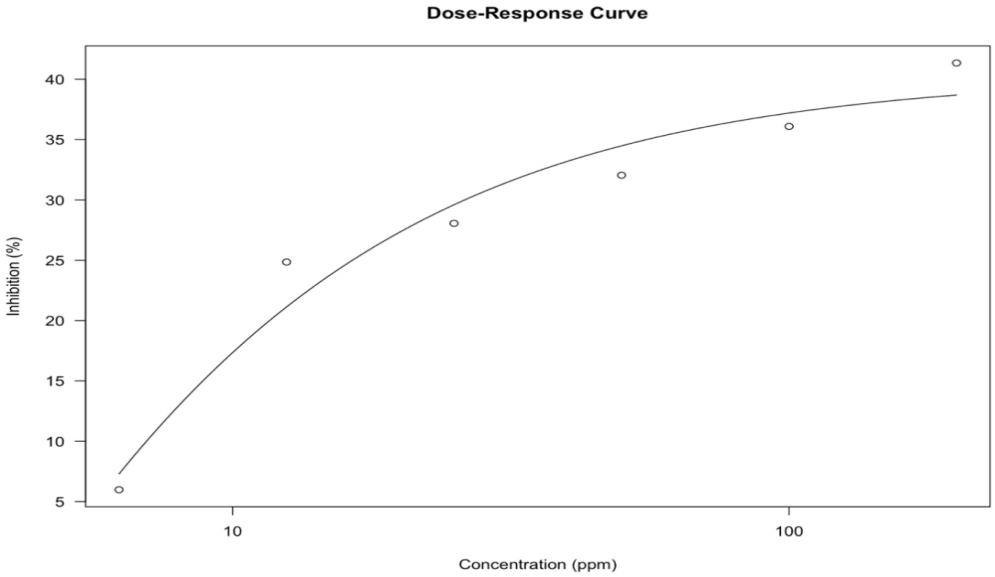


Figure 3. Dose-response curve of cytotoxic effect of ethanol extract on MCF-7 cells

Given earlier docking and drug-likeness results showing strong ERα interactions for flavonoid constituents, it is plausible that the observed cytotoxicity may also involve modulation of estrogen receptor signaling, leading to growth inhibition and programmed cell death. The ethanol extract of *E. hemisphaerica* exhibits potent, dose-dependent cytotoxic activity against MCF-7 breast cancer cells, characterized by a classical sigmoidal inhibition pattern and an exceptionally low IC_{50} value. These findings, supported by established cytotoxicity benchmarks and mechanistic cancer biology principles, strongly suggest that *E. hemisphaerica* is a promising source of anticancer agents. Further investigations, including apoptosis assays, cell cycle analysis, and isolation of active compounds, are warranted to elucidate the precise molecular mechanisms underlying its anticancer effects. These findings align with existing literature on the *Etlingera* genus, particularly *Etlingera elatior*, which has been widely studied for its phytochemical and pharmacological properties. Ethanol extracts of *E. elatior* leaves from Aceh Province, for example, revealed a diverse phytochemical composition with high levels of phenolic compounds (31.35 mg GAE/g), flavonoids (6.10 mg QE/g), and tannins (34.24 mg TAE/g) [34]. These constituents are strongly associated with antioxidant, anti-inflammatory, and anticancer activities. The same extract demonstrated a robust free radical scavenging effect in the DPPH assay, with an IC_{50} value of 19.37 µg/mL, further supporting its role in reducing oxidative stress—a key contributor to carcinogenesis.

This docking affinity pattern is in line with the phytochemical characteristics commonly reported in Etlingera species, including *E. hemisphaerica* and *E. elatior*, which are known to contain abundant flavonoid aglycones such as quercetin, kaempferol, luteolin, and several methoxylated flavones. These aglycones show stronger interaction tendencies toward breast cancer receptors, particularly ERα, compared to glycosylated derivatives, supporting the idea that their hydrophobic core and smaller molecular size allow better receptor accommodation and more stable binding. This may help explain why *E. hemisphaerica* exhibits strong cytotoxic effects against MCF-7 cells, as these core flavonoids are more prone to engage in molecular interactions

linked to anticancer mechanisms. Additionally, the well-documented antioxidant capacity of Etlingera species can further enhance anticancer pathways through redox modulation and apoptosis signaling. Taken together, these findings strengthen the biochemical connection between Etlingera phytochemistry and its potent activity on breast cancer cells.

Given the close taxonomic relationship between *E. elatior* and *E. hemisphaerica*, it is plausible that *E. hemisphaerica* harbors similar or even more potent bioactive compounds, which may be responsible for the observed cytotoxic effects. Preliminary phytochemical screenings of *E. hemisphaerica* have identified a high concentration of flavonoids, quercetin, and other polyphenolic compounds known to trigger apoptosis and suppress tumor growth through multiple mechanisms. Moreover, earlier studies have confirmed its strong antioxidant activity (IC_{50}: 49.37 µg/mL), which may work synergistically with cytotoxic pathways to enhance overall anticancer potential.

To advance these promising findings, further research should aim to isolate and characterize the specific phytoconstituents responsible for the cytotoxic effects, such as asarone, quercetin, or other alkaloids. Additionally, comparative cytotoxicity studies on non-cancerous cells are essential to confirm selectivity and safety profiles, ensuring that therapeutic benefits are not accompanied by undue toxicity to healthy tissues. Collectively, these results position *E. hemisphaerica* as a promising candidate for natural anticancer drug development, warranting deeper pharmacological investigation.

Conclusion

The ethanol extract of *Etlingera hemisphaerica* demonstrated notable cytotoxic activity against MCF-7 human breast cancer cells, with an IC_{50} value of 1.16 ppm. This finding suggests that the plant contains bioactive constituents with potential relevance to anticancer research. LC-HRMS profiling confirmed the presence of diverse phytochemicals, including flavonoids, polyphenols, and other secondary metabolites, which may collectively contribute to the observed activity. The dose-dependent inhibition and sigmoidal response curve further indicate specific interactions with cellular pathways, possibly involving apoptosis or mitochondrial disruption, although these mechanisms remain to be clarified. Importantly, while the results provide preliminary support for the traditional medicinal use of *E. hemisphaerica*, they should be interpreted with caution, as selectivity toward cancer cells versus normal cells has not yet been established. Taken together, these findings highlight the plant**.**

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