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Research Paper

Bioactivity Study of *Russelia equisetiformis* Ethanol Extract as an MCF-7 Breast Cancer Cells Inhibitor with In vitro and In silico Approaches

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Abstract
Russelia equisetiformis has been used in traditional medicine, but its molecular mechanisms against breast cancer remain unexplored. This study aimed to investigate the cytotoxic potential of *R. equisetiformis* ethanol extract and elucidate its molecular interactions through a combined in vitro and in silico approach. Cytotoxicity evaluation using the Resazurin assay demonstrated the extract's activity against MCF-7 breast cancer cells with an IC_{50} value of 352.60 ± 0.23 μg/mL. To understand the underlying mechanism, the identified bioactive compounds that passed the druglikeness test were analyzed using molecular docking against the Progesterone Receptor (PDB ID: 2W8Y) and Estrogen Receptor-alpha (PDB ID: 3ERT). Docking analysis revealed that these compounds form binding interactions with target receptors, providing insight into their potential mechanism of action, albeit with lower affinity compared to standard drugs (tamoxifen and anastrozole). Importantly, ADMET prediction using ProTox 3.0 highlighted apigenin, kaempferol, isokaempferide, and kaempferol 3-*O*-rhamnoside as the most promising primary candidates, demonstrating inactivity against hepatotoxicity, carcinogenicity, and cytotoxicity. These findings provide initial scientific evidence for the potential anticancer properties of *R. equisetiformis* compounds, indicating that all four compounds are safe and viable bioactive candidates for further development as non-toxic therapeutic agents.

Keywords
Anti-Cancer, *Russelia equisetiformis*, Molecular Docking, MCF-7, Natural Products

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1. INTRODUCTION

Breast cancer is a disease caused by genetic changes resulting from a combination of several conditions. These risk factors include nutrition, environment, and heredity (Latief et al., 2023). Despite the development of therapeutic methods in the last decade, the number of deaths from breast cancer has increased annually, making it one of the leading causes of death in women (Barzegarparay et al., 2024; Bray et al., 2024). MCF-7 cells are the most representative cell model for estrogen receptor alpha (ERα-positive) and progesterone receptor (PgR-positive) breast cancer (Bajalovic et al., 2022; Konieczna and Rosińska, 2025; Kunc et al., 2021). PgR is often used as a positive prognostic marker of disease outcome (Faria et al., 2024). Meanwhile, ERα is a key endocrine regulatory protein in breast cancer (Elango et al., 2024). Conventional therapeutic modalities, such as surgery, radiotherapy, chemotherapy, and targeted molecular therapy, although effective in most cases, often cause significant side effects such as systemic toxicity, immunosuppression, gastrointestinal complications, and the emergence of drug resistance (Qamar, 2021). Therefore, due to their natural properties and minimal side effects, the use of herbal medicines made from natural ingredients is on the rise. Recent studies have extensively explored the bioactive compounds of plants. This includes analyzing their phytochemical profiles using LC-MS and assessing their specific pharmacological effects using a combination of laboratory and computational (in silico) methods (Pebriani et al., 2026; Susanti et al., 2023).

The fountain plant (*Russelia equisetiformis*) is often used as an ornamental plant due to its climbing green stems and attractive red tubular flowers (Riaz et al., 2012). The genus *Russelia* belongs to the family Plantaginaceae (Columba-Palomares et al., 2021). In its native Mexico (Ahmed et al., 2016), this plant is used as a complementary therapy for type 2 diabetes mellitus (Romero-Cerecero et al., 2009). Meanwhile, in other states, decoctions of this plant are used to treat kidney stones, diabetes, and leukemia (Gómez-Estrada et al., 2011; Oladeinde, 2005). Various extracts from this plant have been reported to have antioxidant, antimicrobial, anti-inflammatory, analgesic, and antimalarial properties (Awe et al., 2004; Kolawole and Kolawole, 2010; Ojurongbe et al., 2015; Riaz et al., 2012). Reports

on the alkaloid, flavonoid, tannin, saponin, and steroid content of this plant have also been published (Riaz et al., 2012). Furthermore, LC-ESI-MS/MS analysis of the methanol extract of *R. equisetiformis* showed the presence of phytochemicals such as catechin, syringic acid, gallic acid, caffeic acid, methyl protocatechuate, *p*-coumaric acid, 4-hydroxybenzoic acid, caftaric acid, and chlorogenic acid (Riaz et al., 2017). Aqueous extract from fresh leaves of *R. equisetiformis* showed the tentative identification of metabolite compounds in the form of russetinol, russelianoside A, syringic acid, protocatechuic acid methyl ester, methyl alpha-d-glucopyranoside, chrysoeriol 4′-*O*-beta-d-glucopyranoside, luteolin 7-*O*-glucoside, and phenylethanoids (Aldahasi et al., 2025). This is particularly important because certain flavonoid compounds, such as luteolin, *p*-coumaric acid, and apigenin, have been well documented to possess potent anticancer properties and have been reported to significantly inhibit the growth of MCF-7 breast cancer cells (Yang et al., 2022).

Although numerous reports have examined various plant extracts and flavonoid compounds for their anticancer activity using similar in vitro and in silico approaches, the specific bioactivity of *R. equisetiformis* and its main flavonoid constituents against breast cancer remains uncharacterized. No previous studies have evaluated the inhibitory potential of this plant against breast cancer cells. Therefore, the novelty of this study lies in the first comprehensive investigation of the ethanol extract of *R. equisetiformis* as an inhibitor of MCF-7 cells. This study aims to address the current research gap by integrating an in vitro resazurin-based cytotoxicity assay with in silico profiling of key compounds identified by LC-MS in the extracts. Through molecular docking of estrogen and progesterone receptors, this combined approach elucidates the specific bioactive compounds responsible for the extract's activity and its inhibitory mechanisms.

2. EXPERIMENTAL SECTION

2.1 Materials

R. equisetiformis was obtained from Batu, East Java, Indonesia. Ethanol 96% and dimethyl sulfoxide (DMSO) were obtained from Merck, Germany. The breast cancer cell line used was the MCF-7 cell line obtained from the Central Laboratory of Padjajaran University, Bandung, West Java, Indonesia. Penicillin-streptomycin antibiotics and Resazurin Sodium Salt reagent were obtained from Sigma Aldrich, Singapore. Roswell Park Memorial Institute (RPMI) cell media and Fetal Bovine Serum (FBS) were obtained from Gibco, USA. Cisplatin as a positive control was obtained from EDQM, France.

2.2 Methods

2.2.1 Preparation and Extraction of Sample

Russelia equisetiformis samples were separated from their roots and then dried in the sun until dry. The dried stems (162 g) were ground and then macerated using 96% ethanol at a ratio of 1:20 (w/v). The maceration process was carried out for 24 hours at room temperature and repeated for three extraction cycles (Ramiizah et al., 2025) to ensure optimal recovery of secondary metabolites. The extract was concentrated using Rotavapor® R-180 (Buchi, Switzerland) after going through a filtration stage with a Buchner funnel and a vacuum pump. The evaporation process yielded 61 g of crude ethanol extract (REE), corresponding to an extraction yield of 37.65%. Since the product obtained was a crude extract consisting of a highly complex mixture of phytochemicals and not a single pure isolate, macroscopic and structural measurements using UV and IR spectroscopy were not performed. Instead, high-resolution LC-MS was chosen as a more reliable method. The evaporated extract was used for the MCF-7 cell viability test and LC-MS analysis.

2.2.2 MCF-7 Cell Viability Analysis

An antiproliferation method was used to evaluate the cytotoxicity of REE against MCF-7 breast cancer cells grown in RPMI liquid culture media containing 10% FBS and 50 μL/50 mL penicillin-streptomycin antibiotics. The test was carried out using the resazurin assay methodology. First, the cells were seeded into a 96-well plate at a density of 17000 cells/well and incubated for 24 hours at 37°C with 5% CO_2 gas until cell growth reached a minimum confluence of 70% (Mala et al., 2024). To prepare the test solutions, a stock solution was first made by dissolving 10 mg of the extract in 100 μL of 100% DMSO (yielding a concentration of 100,000 μg/mL). This stock was subsequently diluted with fresh media containing eight variations of sample concentrations (7.81, 15.63, 31.25, 62.5, 125, 250, 500, and 1000 μg/mL), cisplatin as a positive control, and DMSO as a negative control. The plate was then incubated again for 48 hours. All experimental treatments in this assay were performed in duplicate (n=2). Cell viability was then measured by adding Resazurin Sodium Salt reagent to each well. Metabolically viable cells will reduce resazurin (blue) to resorufin (pink). Absorbance was measured using a Multimode Reader (Tecan Infinite M200 PRO) at a wavelength of 570 nm with a reference wavelength of 600 nm (Manasathien and Indrapichate, 2017). The percentage of cell viability was calculated relative to the control, and the IC_{50} value was determined using the GraphPad Prism software (version 9; GraphPad, La Jolla, CA) (Nugraha et al., 2024) by analyzing the non-linear regression curve between the percentage of cell viability and the logarithm of the sample concentration. The results were expressed as the mean ± standard deviation (SD) of the two replicates.

2.2.3 LC-MS Analysis

The resulting crude extract consisted of a complex mixture of phytochemicals, and liquid chromatography-mass spectrometry (LC-MS) was chosen to comprehensively profile the various secondary metabolites present in the mixture (Barthwal and Mahar, 2024). Screening of bioactive compounds in the *R. equisetiformis* ethanol extract (REE) was conducted at the Laboratory of the University of Muhammadiyah Malang, Indonesia, by injecting 1 μL of the extract sample into a liquid chromatog-

raphy unit with C18 (2×150 mm, 3 μm) (Shimadzu Shim Pack FC-ODS, Shimadzu Corporation, Japan) as the stationary phase and eluent of ethanol: distilled water (90:10) as the mobile phase with isocratic mode. The eluent with a flow rate of 0.5 mL/min was run for 50 minutes in the column at 35°C. Separation of ionized compounds was achieved using positive Electrospray Ionization (ESI) at a source temperature of 100°C, with a capillary voltage of 3.0 kV and a cone voltage of 23.0 V to create a charged aerosol. Desolvation then occurred at 350°C with a gas flow rate of 60 mL/h. The ion masses were analyzed using a mass spectrometer. Fragmentation was performed using the Low Energy Collision Induced Dissociation (CID) method (5.0 V). The ion fragments were then captured by a detector that would map the different mass-to-charge (m/z) ratios to their abundance in the sample, producing a chromatogram.

LC-MS serves to identify compound content based on separation with LC, followed by compound identification with the MS detector. Compounds that are polar, ionic, have large molecular weights, are thermolabile, and difficult to evaporate, and are typically analyzed using LC-MS (Fiorelia et al., 2022). LC-MS data is used to provide information on the molecular weight, structure, identity, and quantity of certain sample components (Saibaba et al., 2016). Identification of the detected compounds was performed putatively by comparing the obtained mass spectra with the NIST Mass Spectrum Library (NIST 17) (Setiyanto et al., 2025), using the NIST Mass Spectrum Search Program version 2.3 and MS Interpreter version 3.4.5; pure reference standards were not used in this untargeted profile. As a selection criterion for further in silico studies, all identified compounds from the initial LC-MS screening were evaluated for their drug-likeness properties based on Lipinski's Rule of Five (Ro5). Only compounds that successfully met the Ro5 criteria were selected as viable candidates and proceeded to molecular docking simulations.

2.2.4 Druglikeness Lipinski Analysis

The compounds identified by LC-MS were predicted for their drug-like properties according to Lipinski's rule of five. Prediction of drug-like compounds was performed using the SwissADME web server (http://www.swissadme.ch/index.php) (Tukiran and Raihan, 2025). The use of an analog design module allows for the creation of a virtual chemical library accompanied by automated assessment of drug-likeness profiles. The standard used refers to Lipinski's Rule of Five, where ideal drug candidates have a molecular weight ≤ 500 Da, the number of H-bond donors ≤ 5, and H-bond acceptors ≤ 10, and a lipophilicity (LogP) of less than five (Lipinski et al., 2012). According to this rule, a molecule is said to have drug-like properties if it meets at least three of the four parameters for predicting oral permeability and absorption (Jayaram et al., 2012; Lipinski et al., 2012).

2.2.5 Molecular Docking in silico

Crystal structures of progesterone (PDB ID: 2W8Y) and estrogen alpha (PDB ID: 3ERT) receptors (Patel et al., 2021) obtained from the RCSB database (rscb.org) were prepared using BIOVIA Discovery Studio Visualizer (v24.1.0.23298) to obtain sterile proteins and their active sites. 3D structures of bioactive compounds identified in REE were used as ligands. Food and Drug Administration (FDA)-approved anticancer drugs in the form of anastrozole and tamoxifen were used as controls. For docking simulations, a grid box was specifically configured with dimensions of 20 × 20 × 20 Åto adequately cover the binding pocket. The docking coordinates were PgR (X= 11.212349; Y= 4.825880; Z= 7.048795) and then ERα (X= 27.447000; Y= -2.048820; Z= 26.412361). PyRx software (v0.8, 2011), integrating AutoDock Vina, was used to facilitate molecular docking simulations. Candidate compounds that exhibited stronger (more negative) binding affinities than the control drug were selected for further investigation using PyMOL (v3.1.6.1). Before screening the extracted compounds, the docking protocol was validated by re-docking the native ligand. The docking method was considered valid if the Root Mean Square Deviation (RMSD) value of the protein-ligand re-docking was ≤ 2Å (Amrulloh et al., 2023). Visualization of the interaction results was done in 2D and 3D using Discovery Studio and PyMOL.

2.2.6 Toxicity Prediction as a Drug Compound

Toxicity prediction was performed on the ethanol extract of *R. equisetiformis* using ProTox 3.0 (https://tox.charite.de/protox3/) (Banerjee et al., 2025). The toxicity parameters used in this study were hepatotoxicity, carcinogenicity, cytotoxicity, and predicted median lethal dose (LD_{50}) in mg/kg body weight (Kurniawidjaja et al., 2021).

3. RESULTS AND DISCUSSION

3.1 MCF-7 Cell Viability Analysis

The cytotoxicity of REE against MCF-7 breast cancer cells was evaluated using resazurin reagent to determine the concentration of 50% cell viability, expressed as IC_{50}. The test results were compared with the positive control cisplatin and the negative control dimethyl sulfoxide (DMSO). Cisplatin as a test comparator (Basu and Krishnamurthy, 2010), using a concentration of 12.95 μg/mL, was able to reduce cell viability by up to 46.82%. The dose-response curve illustrates the presence of cellular antiproliferative activity (Figure 1). The percentage of cell viability decreased significantly with increasing sample concentration. At a concentration of 500 μg/mL, MCF-7 cells showed more apoptotic cells than at a concentration of 250 μg/mL (Figure 2). Based on non-linear regression analysis, the IC_{50} value of REE was 352.60 ± 0.23 μg/mL.

The National Cancer Institute considers an extract to be highly active if it has an IC_{50} of 30 μg/mL (Sabu et al., 2023), while values below 100 μg/mL are still considered biologically significant for crude extracts (Kumi et al., 2026). Although the IC_{50} of REE falls into the weak category, this biological activity

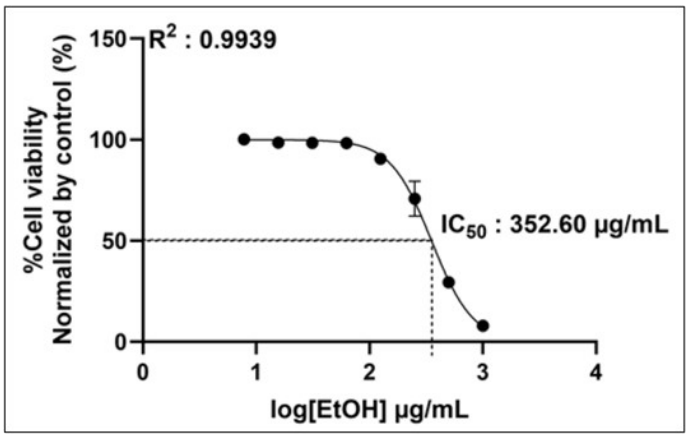


Figure 1. Dose-Response Curve Graph

remains highly relevant. The presence of interfering substances in crude extracts can interfere with the desired activity (Harris, 2002). Crude extracts contain a mixture of compounds, including active and inactive compounds (Sanjai et al., 2024), and the bioactive compound levels in these extracts are relatively low.

It is important to emphasize that the samples tested are highly complex components consisting of a wide variety of secondary metabolites, identified by LC-MS. Therefore, the weak in vitro activity of the bulk extract does not negate the potential of its individual constituents. This hypothesis is strongly supported by in silico findings, which show that specific pure flavonoids identified in the extracts have excellent binding affinity to breast cancer receptors. Thus, the crude extract serves as a valuable reservoir of potent anticancer agents that exhibit strong targeted activity upon individual evaluation, successfully bridging the gap between weak in vitro macroscopic results and strong in silico molecular interactions.

To provide a broader scientific insight into the observed cytotoxic activity, the IC_{50} values of the ethanolic extract of *R. equisetiformis* were compared with those of other previously reported plant extracts against MCF-7 breast cancer cells (Table 1). As shown in the table, the cytotoxicity of the crude extracts varied significantly depending on the plant species, ranging from highly active to relatively inactive. These data further strengthen the notion that bioactivity is highly dependent on the concentration of secondary metabolites in the complex, necessitating further in silico profiling to determine the active constituents.

3.2 LC-MS Analysis of Extract

Compounds in the *R. equisetiformis* ethanol extract (REE) were identified to result in 116 peaks, as shown in Figure 3. LC-MS profiling of REE identified 116 compounds consisting of flavonoids, glycosides, phenolics, steroids, betalains, terpenoids, and alkaloids, with the dominant compound being "kaempferol-3-(6"caffeoylglucoside)" at 2.43% at a retention time of 35,508. Chlorogenic acid (RT: 12.491 min, m/z: 354.31), *p*-coumaric acid (RT: 1.839 min, m/z: 164.16), gallic acid (RT: 3.042 min, m/z: 170.12), and caffeic acid (RT: 4.643 min, m/z: 180.16) were also identified in the methanol extract of this plant (Riaz et al., 2017).

3.3 Drug-likeness Lipinski Analysis

Lipinski's Rule of Five serves as a guide to evaluate the potential oral bioavailability of a compound, thus facilitating the identification of molecules that have drug-like characteristics (Zhang and Wilkinson, 2007). The results of the drug-likeness analysis of the total compounds showed that 78 compounds passed Lipinski's rule of five (Table 2).

3.4 Molecular Docking Analysis

Potential drug compounds were then analyzed using molecular docking. Molecular docking aims to model the interaction through a computational approach (in silico) of a compound as a ligand at the active site of a particular disease receptor (Damayanti et al., 2021). To determine the ability to inhibit MCF-7 cell growth, promising compounds must have inhibitory activity on both receptors (ERα and PgR) and work effectively together. Before evaluating the bioactive compounds from the extract, method validation was carried out through a re-docking process, resulting in excellent RMSD values of 0.848 Åfor PgR and 1.307 Åfor ERα (Table 3). The ligand images in Table 3 show green as the natural ligand, while orange indicates the ligand resulting from re-docking. A lower binding affinity value indicates a stronger interaction of the ligand with the receptor (Puspitasari et al., 2023).

Molecular docking simulations were performed on selected phytochemicals that successfully passed Lipinski's Rule of Five. Complete docking scores for all candidates are provided in the Table 4. To highlight the most promising candidates, the top 5 compounds that showed the strongest (most negative) binding affinity to ERα and PgR receptors, along with the control drug, are in bold. The PgR-tamoxifen complex has a binding affinity of −8.9 kcal/mol, and the ERα-anastrozole complex has a binding affinity of −8.0 kcal/mol. As shown in the summary results, five compounds exhibit lower binding affinities than the two control drug-receptor complexes synergistically: apigenin, luteolin, kaempferol, isokaempferide, and kaempferol 3-*O*-rhamnoside. These five compounds have the potential to act as inhibitory agents for breast cancer by inhibiting the activity of progesterone receptors and estrogen-alpha receptors.

Visualization of the results and interactions of five potential compounds was performed to determine the position and type of bond between the ligand and the target macromolecule. Analysis of Figures 4 and 5 shows the contribution of various non-covalent forces, such as hydrogen bonds (interactions between hydrogen atoms with F, O, or N) (Widiastuti, 2019) and hydrophobic interactions in non-polar regions such as aromatic rings (Arwansyah et al., 2014). The stability of the complex is also supported by electrostatic forces based on charge differences (Lele et al., 2022) and halogen bonds. On the other hand, some interactions are categorized as unfavorable bonds

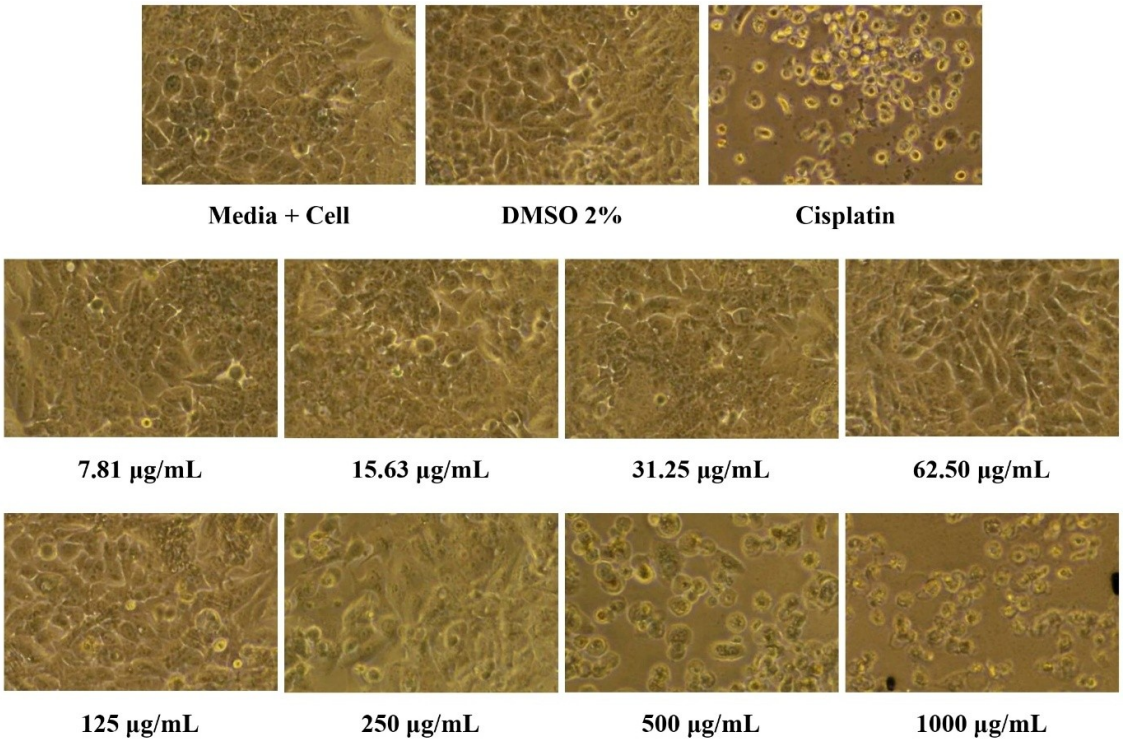


Figure 2. Morphological MCF-7 Cell

Table 1. Comparison of Cytotoxic Activity of Various Plant Extracts Against MCF-7 Cells

Plant Species	Extract Solvent	IC_{50} (μg/mL)	Reference
Ziziphus nummularia	Ethanol	38.27 ± 0.72	(Elya et al., 2025)
Russelia equisetiformis	Ethanol	352.60 ± 0.23	This Study
Dioscorea esculenta	n-Hexane : Acetone (7:3)	632.42	(Haryoto, 2019)
Plantago lanceolata	Ethanol 70%	674.20	(Alsaraf et al., 2019)
Annona tomentosa	Methanol	824.20	(Pinheiro et al., 2024)

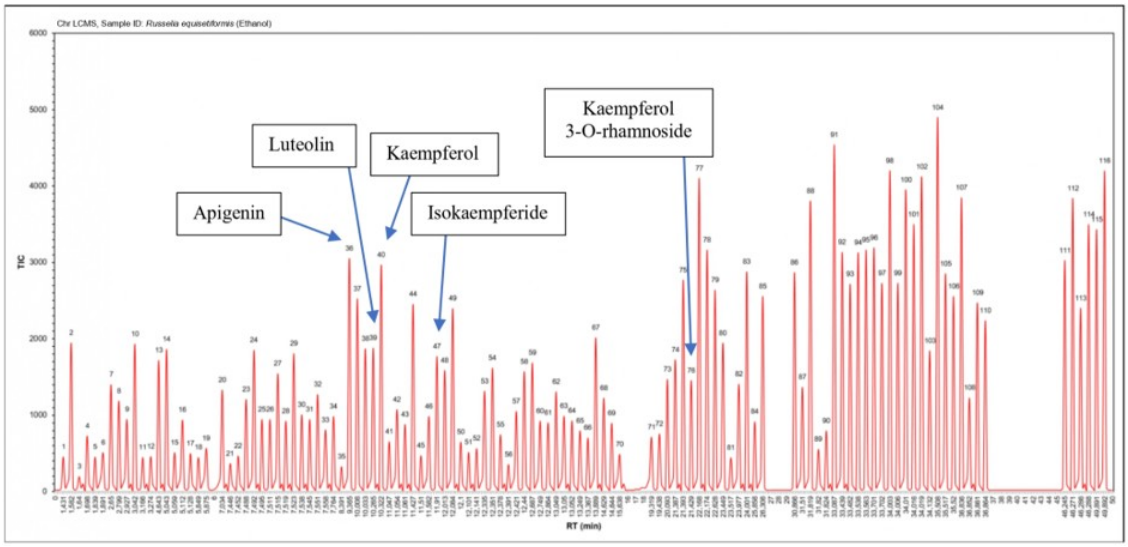


Figure 3. Chromatogram of *R. equisetiformis* Ethanol Extract

Table 2. Lipinski's Five Rules Analysis of *R. equisetiformis* Ethanol Extract

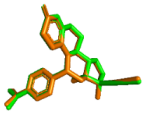
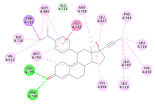
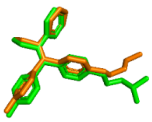
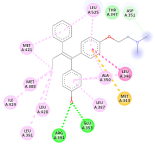
No.	Compound	MW <500 g/mol	HA ≤10	HD ≤5	logP <5	Violation Druglikeness
1	Cinnamaldehyde	132	1	0	2.3	0
2	Cinnamic Acid	148	2	1	2.6	0
3	Dopamine	153	3	4	-0.8	0
4	*p*-Methoxycinnamaldehyde	162	2	0	2.4	0
5	*p*-Coumaric Acid	164	3	2	1.4	0
6	3-Methoxytyramine	167	3	3	-0.3	0
7	Safrole	162	2	0	2.8	0
8	Vanillic Acid	168	4	2	1.3	0
9	Gallic Acid	170	5	4	0.7	0
10	L-Ascorbic Acid	176	6	4	-1.7	0
11	Coniferaldehyde	178	3	1	1.9	0
12	Caffeic Acid	180	4	3	1.2	0
13	Ferulic Acid	194	4	2	1.6	0
14	Leucodopachrome	195	5	4	-0.3	0
15	α-Amino-3,4-dioxo-1,5-cyclohexadiene-1-propanoic acid	195	5	3	-2.4	0
16	4-(2-Aminoethyl)-2,6-dimethoxyphenol	197	4	3	-0.3	0
17	Betalamic acid	211	6	3	0.2	0
18	Ocimarin	220	4	2	0.6	0
19	Sinapic acid	224	5	2	1.3	0
20	2,3-Dihydro-5,7-dihydroxy-2-(4-methoxyphenyl)-8-methyl-4H-1-benzopyran-4-one	300	5	2	3.1	0
21	3,5,7-Trihydroxy-2-(4-methoxyphenyl)-6-methyl-2,3-dihydrochromen-4-one	316	6	3	2.1	0
22	5,3'-Dihydroxy-2'-methoxy-6,7-methylenedioxyisoflavone	328	7	2	2.1	0
23	5,8-Dihydroxy-3-(3-hydroxy-2-methoxyphenyl)-7-methoxychromen-4-one	330	7	3	1.6	0
24	6,7-Dihydroxy-3-(3-hydroxy-2-methoxyphenyl)-5-methoxychromen-4-one	330	7	3	1.4	0
25	5,3'-Dihydroxy-7,8,2'-trimethoxyisoflavone	344	7	2	1.7	0
26	5-Hydroxy-3-(3-hydroxy-2-methoxyphenyl)-6,7-dimethoxychromen-4-one	344	7	2	1.9	0
27	8-Hydroxy-3-(3-hydroxy-2-methoxyphenyl)-5,7-dimethoxychromen-4-one	344	7	2	1.5	0
28	7-Hydroxy-3-(3-hydroxy-2-methoxyphenyl)-5,6-dimethoxychromen-4-one	344	7	2	1.6	0
29	6-Hydroxy-3-(3-hydroxy-2-methoxyphenyl)-5,7-dimethoxychromen-4-one	344	7	2	1.5	0
30	3-(3-Hydroxy-2-methoxyphenyl)-5,6,7-trimethoxychromen-4-one	358	7	1	1.8	0
31	5,3'-Dihydroxy-6,7,8,2'-tetramethoxyisoflavone	374	8	2	1.8	0
32	5,6-Dihydroxy-3-(3-hydroxy-2-methoxyphenyl)-7-methoxychromen-4-one	330	7	3	1.6	0
33	γ-Aminobutyric acid-betaxanthin	296	8	4	0.1	0
34	Tricin 4'-apioside	462	11	5	1.4	1
35	Apigenin	270	5	3	3.0	0
36	Acacetin	284	5	2	3.0	0
37	Genkwanin	284	5	2	2.8	0
38	Luteolin	286	6	4	2.2	0
39	Kaempferol	286	6	4	2.0	0
40	Taxifolin	304	7	5	0.9	0
41	Hesperetin	302	6	3	2.6	0
42	5-Hydroxy-7,4'-dimethoxyflavone	298	5	1	3.1	0
43	Quercetin	302	7	5	1.4	0
44	Indicaxanthin	308	8	3	-0.6	0
45	Nepetoidin A	314	6	4	2.1	0
46	Isokaempferide	300	6	3	2.7	0
47	Salvigenin	328	6	1	2.8	0
48	Zataroside B	328	7	5	1.0	0
49	Gonzalitosin I	328	6	1	2.8	0
50	Chrysoeriol	300	6	3	2.7	0
51	Cirsilineol	344	7	2	2.4	0
52	Nevadensin	344	7	2	2.6	0
53	5,6,4'-Trihydroxy-7,3'-dimethoxyflavone	330	7	3	2.2	0
54	Cyclo-DOPA-chrome-glucoside	340	8	5	-0.7	0
55	3-Methoxytyramine-betaxanthin	360	8	4	0.4	0
56	Chlorogenic Acid	354	9	6	1.0	1
57	Portulacaxanthin II	374	9	5	0.4	0
58	Mucroflavone B	360	8	3	2.0	0
59	Dopaxanthin	390	10	6	0.2	1
60	5,6-Dihydroxy-7,3',4'-Trimethoxyflavone	344	7	2	2.3	0
61	Betanidin	388	10	5	-0.3	0
62	Santin	344	7	2	2.7	0
63	Dopaxanthin quinone	388	10	4	0.4	0
64	Valine-betaxanthin	310	8	4	1.2	0
65	Squalene	410	0	0	11.2	0
66	3-Methoxyluteolin	316	7	4	2.1	0
67	Asparagine-betaxanthin	325	10	6	-0.9	1
68	(2S,4E)-4-[2-[(1S)-1-carboxy-2-phenylethyl]iminoethylidene]-2,3-dihydro-1H-pyridine-2,6-dicarboxylic acid	358	8	4	1.3	0
69	Stigmasterol	412	1	1	6.6	1
70	β-Amyrin	426	1	1	6.0	1
71	α-Amyrin	426	1	1	6.5	1
72	Apigenin 7-galactoside	432	10	6	1.1	1
73	Vitexin	432	10	7	1.0	1
74	Anthemoside	432	10	6	0.3	1
75	Kaempferol 3-*O*-rhamnoside	432	10	6	1.7	1
76	Tilianin	446	10	5	1.6	0
77	Betulinic Acid	456	3	2	4.2	0
78	6,7-dimethoxy-2-(4-methoxyphenyl)-5-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	490	11	4	1.4	1

Note: MW: Molecular Weight; HA: Hydrogen Bond Acceptors; HD: Hydrogen Bond Donor; logP: Lipophilicity

due to their destructive nature towards binding energy, which is usually caused by steric hindrance or similarity of charge polarity between adjacent atoms (Rambitan et al., 2021).

The visualization of five potential compounds resulting from molecular docking, shown in Figure 4, illustrates the interaction of PR receptors with ligand compounds. Apigenin forms the same hydrophobic bonds as tamoxifen at amino acid residues Leu A:718, Cys A:891, and Met A:759, which is the same as the native ligand. Luteolin interacts with the same amino acids as the native ligand at Met A:725 and Met A:759 and a hydrophobic bond at Cys A:891, which is similar to tamoxifen. Kaempferol and isokaempferide interact with the same amino acid residue as tamoxifen at Leu A:718 and bind to Met A:725, Met A:759, and Phe A:794, which are the same as the native ligand. Kaempferol 3-*O*-rhamnoside shares amino acid residues with tamoxifen at Leu A:718, Cys A:891, and Met A:909, as well as Gly A:722, Met A:725, Met A:759, and Tyr A:890, which are similar to the native ligand.

Table 3. Re-Docking Results

Receptor	PDB ID	Ligand ID	Ligand Docking Validation	RMSD Value ()	Binding Affinity (kcal/mol)	Amino Acid Interactions
Progesterone	2W8Y	486		0.848	-12.4	
Estrogen-α	3ERT	OHT		1.307	-9.8	

Note: Re-docking is considered valid if RMSD ≤ 2

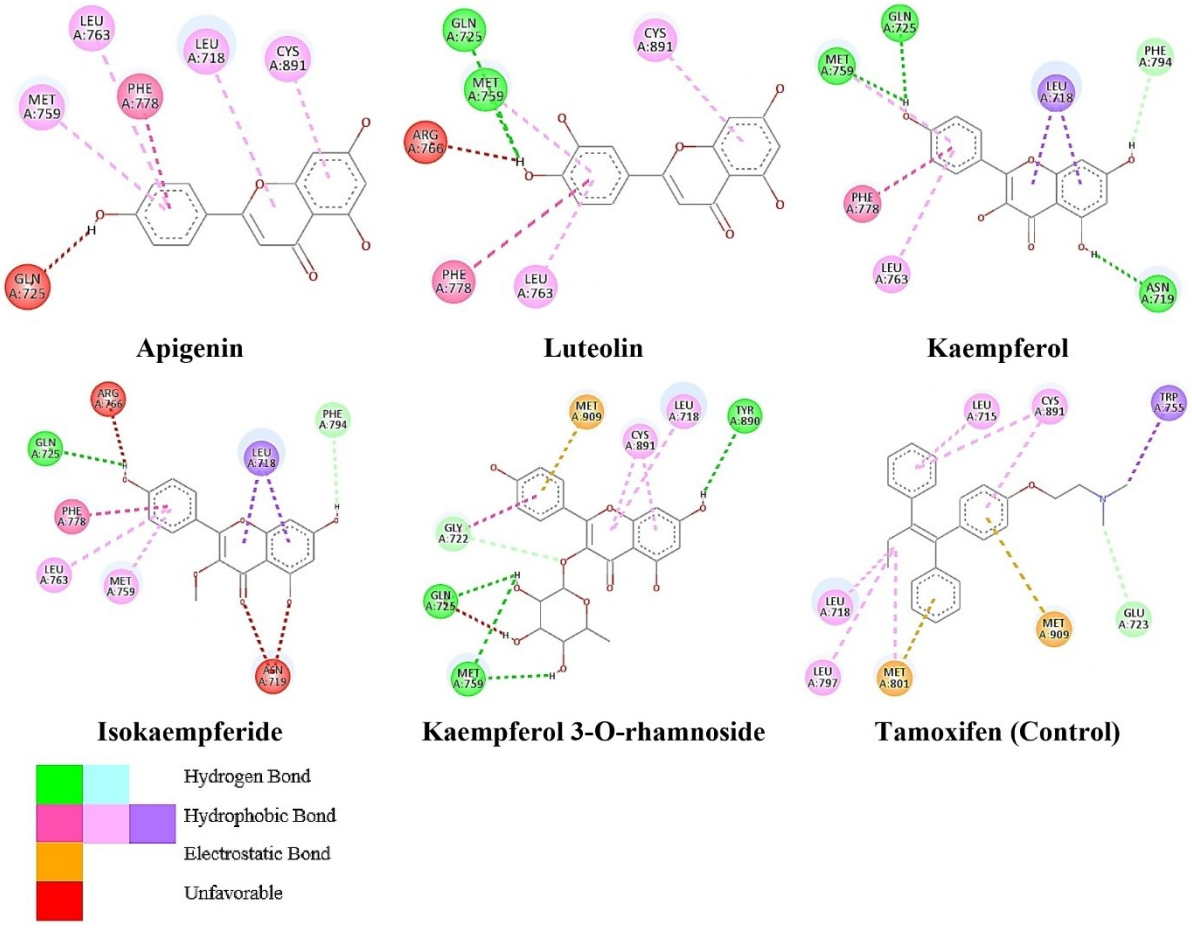


Figure 4. Visualization of PgR Complexes

In the ERα-ligand complex (Figure 5), apigenin, luteolin, and kaempferol bind to the same amino acid residues as anastrozole at Ala A:350 and Leu A:525, as well as to amino acids Leu A:346, Leu A:387, Met A:388, Met A:421, and Ile A:424, which are also bound by the native ligand. Luteolin also forms a hydrogen bond with Glu A:353, which is similar to the native ligand. Isokaempferide interacts with amino acid residues Met A:343, Ala A:350, and Leu A:525, as well as similar residues

Table 4. Binding Affinity of REE Drug-Like Compounds with ER and PR

No.	Ligand Compound	2W8Y	3ERT
1	Cinnamaldehyde	-5.6	-5.5
2	Cinnamic Acid	-6.0	-5.8
3	Dopamine	-5.9	-5.6
4	*p*-Methoxycinnamaldehyde	-5.8	-5.7
5	*p*-Coumaric Acid	-6.5	-6.0
6	3-Methoxytyramine	-5.9	-5.3
7	Safrole	-6.1	-6.0
8	Vanillic Acid	-6.0	-5.5
9	Gallic Acid	-5.9	-5.5
10	L-Ascorbic Acid	-5.2	-5.1
11	Coniferaldehyde	-6.2	-5.7
12	Caffeic Acid	-6.6	-6.3
13	Ferulic Acid	-6.5	-6.3
14	Leucodopachrome	-6.5	-6.4
15	α-Amino-3,4-dioxo-1,5-cyclohexadiene-1-propanoic acid	-6.2	-6.3
16	4-(2-Aminoethyl)-2,6-dimethoxyphenol	-5.5	-5.4
17	Betalamic acid	-6.4	-6.3
18	Ocimarin	-7.0	-7.1
19	Sinapic acid	-6.6	-6.1
20	2,3-Dihydro-5,7-dihydroxy-2-(4-methoxyphenyl)-8-methyl-4H-1-benzopyran-4-one	-8.1	-8.2
21	3,5,7-Trihydroxy-2-(4-methoxyphenyl)-6-methyl-2,3-dihydrochromen-4-one	-7.5	-7.2
22	5,3'-Dihydroxy-2'-methoxy-6,7-methylenedioxyisoflavone	-8.6	-7.7
23	5,8-Dihydroxy-3-(3-hydroxy-2-methoxyphenyl)-7-methoxychromen-4-one	-8.1	-6.6
24	6,7-Dihydroxy-3-(3-hydroxy-2-methoxyphenyl)-5-methoxychromen-4-one	-8.3	-6.4
25	5,3'-Dihydroxy-7,8,2'-trimethoxyisoflavone	-7.7	-6.0
26	5-Hydroxy-3-(3-hydroxy-2-methoxyphenyl)-6,7-dimethoxychromen-4-one	-6.8	-6.0
27	8-Hydroxy-3-(3-hydroxy-2-methoxyphenyl)-5,7-dimethoxychromen-4-one	-7.8	-6.9
28	7-Hydroxy-3-(3-hydroxy-2-methoxyphenyl)-5,6-dimethoxychromen-4-one	-7.8	-6.0
29	6-Hydroxy-3-(3-hydroxy-2-methoxyphenyl)-5,7-dimethoxychromen-4-one	-7.6	-6.9
30	3-(3-Hydroxy-2-methoxyphenyl)-5,6,7-trimethoxychromen-4-one	-7.0	-6.3
31	5,3'-Dihydroxy-6,7,8,2'-tetramethoxyisoflavone	-6.6	-5.8
32	5,6-Dihydroxy-3-(3-hydroxy-2-methoxyphenyl)-7-methoxychromen-4-one	-7.8	-6.4
33	γ-Aminobutyric acid-betaxanthin	-7.1	-7.2
34	Tricin 4'-apioside	-1.8	-7.6
35	Apigenin	-9.2	-8.5
36	Acacetin	-7.8	-7.9
37	Genkwanin	-8.9	-7.5
38	Luteolin	-9.3	-8.3
39	Kaempferol	-9.0	-8.2
40	Taxifolin	-9.2	-7.9
41	Hesperetin	-7.8	-8.0
42	5-Hydroxy-7,4'-dimethoxyflavone	-6.8	-7.5
43	Quercetin	-9.3	-7.9
44	Indicaxanthin	-7.9	-7.2
45	Nepetoidin A	-8.7	-8.0
46	Isokaempferide	-8.9	-8.1
47	Salvigenin	-5.4	-7.1
48	Zataroside B	-7.8	-7.1
49	Gonzalitosin I	-6.6	-7.5
50	Chrysoeriol	-8.7	-7.8
51	Cirsilineol	-6.9	-7.3
52	Nevadensin	-6.1	-7.0
53	5,6,4'-Trihydroxy-7,3'-dimethoxyflavone	-7.9	-7.3
54	Cyclo-DOPA-chrome-glucoside	-7.8	-7.4
55	3-Methoxytyramine-betaxanthin	-7.6	-7.6
56	Chlorogenic Acid	-8.2	-7.7
57	Portulacaxanthin II	-8.7	-7.9
58	Mucroflavone B	-7.9	-7.1
59	Dopaxanthin	-8.4	-8.0
60	5,6-Dihydroxy-7,3',4'-Trimethoxyflavone	-6.4	-7.3
61	Betanidin	-5.4	-8.2
62	Santin	-6.4	-6.7
63	Dopaxanthin quinone	-8.2	-7.6
64	Valine-betaxanthin	-7.8	-7.0
65	Squalene	-8.3	-8.1
66	3-Methoxyluteolin	-8.8	-7.9
67	Asparagine-betaxanthin	-7.8	-7.1
68	(2S,4E)-4-[2-[(1S)-1-carboxy-2-phenylethyl]iminoethylidene]-2,3-dihydro-1H-pyridine-2,6-dicarboxylic acid	-8.2	-7.7
69	Stigmasterol	-4.0	-7.8
70	β-Amyrin	-1.3	-8.0
71	α-Amyrin	-6.6	-7.7
72	Apigenin 7-galactoside	-7.2	-7.6
73	Vitexin	-9.1	-7.1
74	Anthemoside	-6.5	-7.5
75	Kaempferol 3-*O*-rhamnoside	-9.6	-8.1
76	Tilianin	-5.3	-7.7
77	Betulinic Acid	-1.2	-7.9
78	6,7-dimethoxy-2-(4-methoxyphenyl)-5-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	-5.0	-8.3
79	Tamoxifen (Control)	-8.9	-
80	Anastrozole (Control)	-	-8.0

to the native ligand at Leu A:346, Glu A:353, Leu A:387, and Leu A:391. Kaempferol 3-*O*-rhamnoside interacts with the same amino acids as anastrozole at Ala A:350, Trp A:383, and Leu A:525 and forms a hydrogen bond at Asp A:351, which

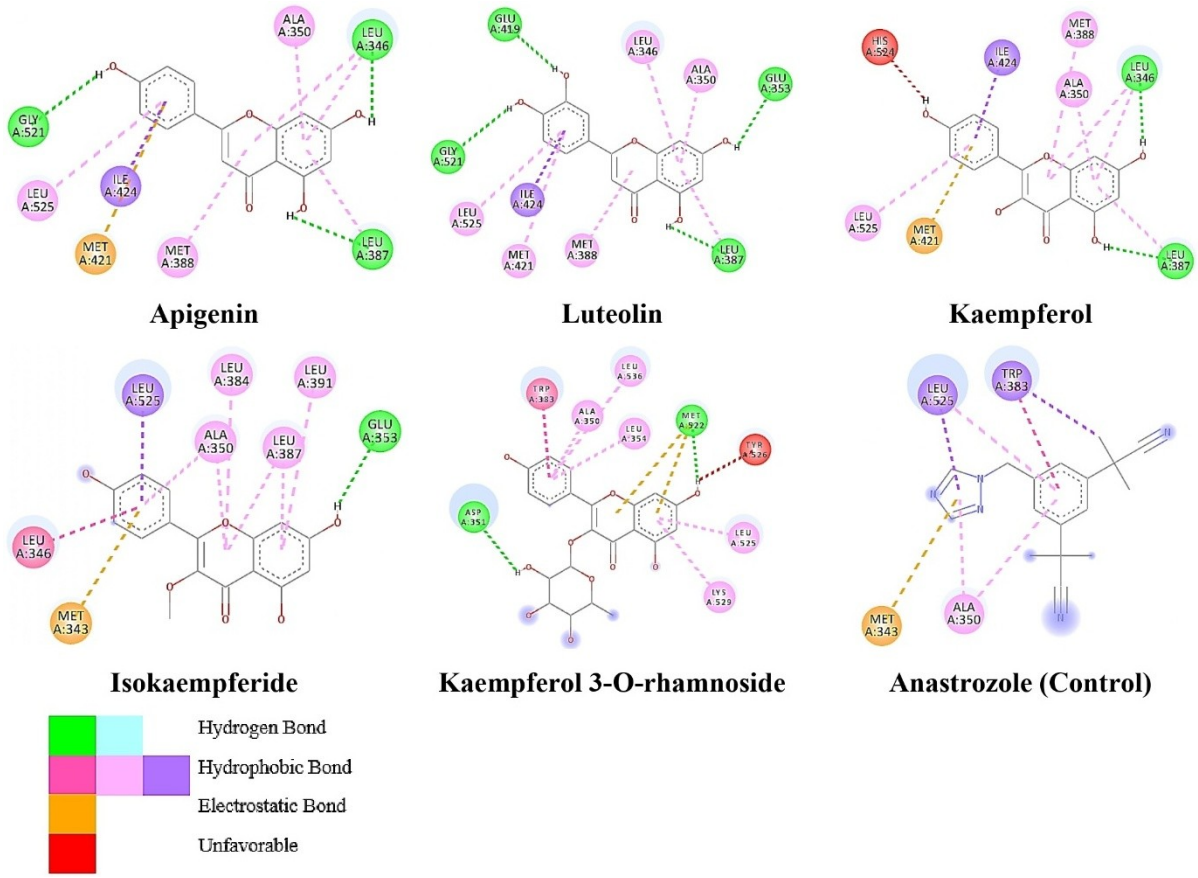


Figure 5. Visualization of ERα Complexes

Table 5. Toxicity Prediction

Potential Compound	LD_{50} (mg/kg)	Hepatotoxicity	Carcinogenicity	Cytotoxicity
Apigenin	2500	Inactive	Inactive	Inactive
Luteolin	3919	Inactive	Active	Inactive
Kaempferol	3919	Inactive	Inactive	Inactive
Isokaempferide	3919	Inactive	Inactive	Inactive
Kaempferol 3-*O*-rhamnoside	5000	Inactive	Inactive	Inactive

is also bound by the native ligand. Hydrogen bonds and hydrophobic interactions contribute significantly to stabilizing the interaction between ligand and receptor to maintain the binding conformation (Hurria et al., 2026).

This similarity supports validation that the potential compound has inhibitory activity, similar to that of a drug control (Nugroho et al., 2023). Control drugs work by occupying the active site of the receptor, therefore blocking the interaction between essential amino acid residues and specific ligands that play a role in the metabolic pathway. The similarity in the biological activity of a molecule and a drug compound correlates with the similarity of interactions with amino acid residues in the active site. This type of interaction optimizes the rigidity of the formed complex, which in turn lowers the binding free energy (ΔG). A stable complex composition maximizes favorable interactions while minimizing the formation of unfavorable bonds. These bonds can increase the stability of the complex formed by the receptor and ligand. The complex becomes more stable as more interactions are formed, leading to increased inhibitory activity (Sururi et al., 2023).

To highlight the significance of these results, the binding affinities obtained in this study were compared with previously reported molecular docking studies targeting the same receptor. Recent computational results of kaempferol reported a binding affinity of -8.4 kcal/mol to the ERα receptor (PDB ID: 3ERT) (Khudzaifi et al., 2024). Apigenin was also reported to bind to ERα with an energy of -8.13 kcal/mol (Hammami et al., 2025). In our study, the identified flavonoids exhibited even stronger binding affinities, ranging from -8.1 to -8.5 kcal/mol. Furthermore, a study evaluating flavonoid derivatives against

PgR (PDB ID: 2W8Y) determined that strong binding energies typically range from -8.30 to -12.37 kcal/mol (Widiyana et al., 2024). The top five candidates in this study (apigenin, luteolin, kaempferol, isokaempferide, and kaempferol 3-*O*-rhamnoside) align with previous studies, exhibiting binding energies between -8.9 and -9.6 kcal/mol for PgR. This analysis strongly confirms the potential of *R. equisetiformis* flavonoid compounds as highly competitive breast cancer receptor inhibitors.

Further validating these findings, the potent activity of these individual flavonoids is well supported by established anticancer mechanisms. Apigenin and kaempferol have previously been reported to induce apoptosis in MCF-7 cells by modulating the Bcl-2/Bax ratio and inhibiting the PI3K/Akt signaling pathway, which are essential for cancer cell survival (Lu et al., 2010; Yi et al., 2016). Similarly, luteolin is known to act as a competitive antagonist for the estrogen receptor (Puranik et al., 2019). Another study showed that kaempferol-3-*O*-rhamnoside dose-dependently inhibited proliferation in MCF-7 cells and promoted apoptosis through activation of the caspase signaling cascade, including caspase-9, caspase-3, and PARP (Diantini et al., 2012). Isokaempferide has also been reported to potently inhibit tumor cell lines (Costa-Lotufo et al., 2003). The fact that this specific compound was identified as the top-ranked ligand in the docking simulations strongly explains the molecular basis underlying the cytotoxic activity observed in the in vitro assay. The molecular-level synergy among these identified flavonoids provides a comprehensive explanation for the inhibitory effect of *R. equisetiformis* extract on breast cancer cells, thus bridging macroscopic laboratory test results with precise computational interactions.

3.5 Toxicity Prediction

The potential compounds were then analyzed for toxicity to support their profiles as anticancer drug candidates using the ProTox 3.0 web server to determine their toxic profiles (Banerjee et al., 2025). The safety evaluation of the compounds in this study included parameters of hepatotoxicity, carcinogenicity, cytotoxicity, and LD_{50} prediction. The level of liver damage due to exposure to the substance was measured through a hepatotoxicity profile (Nursanti et al., 2022). Meanwhile, the potential for triggering cancer due to abnormal cell proliferation was assessed through a carcinogenicity test (Nasyanka et al., 2023). In addition, the destructive effects of substances at the cellular level are identified through cytotoxicity parameters (Rock et al., 2024). As an indicator of acute toxicity, the LD_{50} value was used to predict the short-term lethal impact of a single dose of the compound (Kurniawidjaja et al., 2021). Based on data in Table 5, four of the five compounds showed promising profiles as drug candidates because they did not have hepatotoxic, carcinogenic, or cytotoxic properties, and were classified as LD_{50} Class 5. The four compounds, apigenin, kaempferol, isokaempferide, and kaempferol 3-*O*-rhamnoside, have a predicted LD_{50} in class 5. Meanwhile, compounds such as luteolin are predicted to have carcinogenic properties with a probability of 68%. These findings indicate the importance of optimizing the dose and method of administration to the target to minimize potential toxic effects (Hussein et al., 2025).

4. CONCLUSIONS

The results of the study indicate that *Russelia equisetiformis* has the potential to be an anti-breast cancer agent by inhibiting two receptors on MCF-7 cancer cells. In vitro testing of *Russelia equisetiformis* ethanol extract using the resazurin method demonstrated cytotoxic activity against MCF-7 cells with an IC_{50} of 352.60 ± 0.23 μg/mL. Molecular docking analysis of compounds that passed the drug-likeness test resulted in five potential compounds with strong binding affinity. Among these, the four compounds with the safest predicted toxicity as drug candidates are apigenin, kaempferol, isokaempferide, and kaempferol 3-*O*-rhamnoside. Further research is needed to provide stronger evidence regarding the potential of the *R. equisetiformis* plant as a candidate natural ingredient for medical use.

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