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The Bioactive Constituents of *Piper aduncum* L. as Estrogen Receptor-a Inhibitors: In Silico Studies

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Abstract

Wild betel (*Piper aduncum* L.) is a traditional medicinal plant with reported anticancer potential, particularly against breast cancer. Breast cancer remains a leading cause of cancer-related mortality worldwide, largely driven by estrogen receptor alpha (ERα)–mediated tumor growth. This study aimed to evaluate the potential of bioactive compounds from *P. aduncum* as ERα inhibitors using an in silico approach. Twenty compounds identified from P. aduncum were assessed for drug-likeness and ADMETox properties, followed by pharmacophore modeling and molecular docking against ERα. Phlorizin, linalool, and phloretin exhibited the highest pharmacophore fit scores, with the optimal model demonstrating strong predictive performance (AUC = 0.95). Among these compounds, phlorizin showed the strongest binding affinity for ERα (−10.38 kcal/mol), with a predicted inhibition constant of 24.77 μM, forming key interactions with GLU353, ALA350, and LEU525, comparable to those of the reference ligand 4-hydroxytamoxifen. Overall, these findings indicate that P. aduncum–derived compounds, particularly phlorizin, phloretin, and linalool, are promising ERα inhibitor candidates and merit further experimental validation for breast cancer therapy.

Keywords: Breast cancer, Luminal subtype, Molecular docking, Pharmacophore screening, Phloretin, *Piper aduncum* L.

Senyawa Bioaktif *Piper aduncum* L. sebagai Inhibitor Reseptor Estrogen-a: Studi *In Silico*

Abstrak

Sirih hutan (*Piper aduncum* L.) merupakan tanaman obat tradisional yang dilaporkan memiliki potensi aktivitas antikanker, khususnya terhadap kanker payudara. Kanker payudara masih menjadi salah satu penyebab utama kematian akibat kanker di seluruh dunia, yang sebagian besar dipicu oleh pertumbuhan tumor yang dimediasi oleh reseptor estrogen alfa (ERα). Penelitian ini bertujuan untuk mengevaluasi potensi senyawa bioaktif dari *P. aduncum* sebagai inhibitor ERα menggunakan pendekatan komputasi. Sebanyak 20 senyawa yang diidentifikasi dari P. aduncum dianalisis berdasarkan parameter drug-likeness dan ADMETox, kemudian dilanjutkan dengan pemodelan farmakofor dan penambatan molekuler terhadap ERα. Phlorizin, linalool, dan phloretin menunjukkan skor kecocokan farmakofor tertinggi, dengan model optimal memiliki performa prediktif yang sangat baik (AUC = 0,95). Di antara senyawa tersebut, phlorizin menunjukkan afinitas ikatan paling kuat terhadap ERα dengan energi ikatan sebesar −10,38 kcal/mol dan konstanta inhibisi sebesar 24,77 μM, serta membentuk interaksi kunci dengan residu GLU353, ALA350, dan LEU525 yang sebanding dengan ligan referensi 4-hidroksitamoksifen. Secara keseluruhan, hasil penelitian ini menunjukkan bahwa senyawa-senyawa dari P. aduncum, khususnya phlorizin, phloretin, dan linalool, berpotensi dikembangkan sebagai kandidat inhibitor ERα dan layak untuk divalidasi lebih lanjut melalui penelitian eksperimental sebagai terapi kanker payudara.

Kata Kunci: Kanker payudara, Penambatan molekuler, Phloretin, *Piper aduncum L.,* Skrining Farmakofor, Subtipe luminal

1. Introduction

Wild betel (*Piper aduncum* L.) is a member of the Piperaceae family, which includes over 2,000 species distributed across tropical and subtropical regions.[1] This plant has long been utilized in traditional medicine across Southeast Asia and South America due to its wide range of pharmacological activities, including antiemetic, digestive, insecticidal, antibacterial, antiviral, antioxidant, and anticancer properties.[2] Previous phytochemical investigations have revealed that *P. aduncum* contains diverse classes of bioactive compounds, including phenolics, flavonoids, terpenoids, and chalcones, which are known for their strong biological activities.[3] Furthermore, Bellatasie et al. (2024) reported that the ethanol extract of *P. aduncum* exhibited cytotoxic activity against T47D breast cancer cells, with an IC_{50} value of 171.2 µg/mL, indicating its potential as a source of natural anticancer agents.[3] Another study using HeLa cervical cancer cells demonstrated that *P. aduncum* exhibited superior antiproliferative activity compared to other species within the Piper genus, inducing DNA fragmentation as evidenced by the accumulation of cells in the sub-G1 phase.[4]

Breast cancer remains the second leading cause of cancer-related mortality worldwide and continues to pose a major global health challenge, accounting for approximately 2.31 million new cases, predominantly affecting women, and the number is projected to rise to 26 million cases with 17 million deaths by 2030.[5] Among the molecular drivers of breast cancer, estrogen signaling plays a pivotal role, particularly through the estrogen receptor alpha (ERα), a key transcription factor involved in cell proliferation, differentiation, and tumor progression.[6] ERα is a nuclear receptor that mediates estrogen-dependent gene transcription. When activated by its ligand, 17β-estradiol (E2), ERα regulates the expression of genes involved in cell cycle progression, DNA synthesis, and survival pathways.[7] In luminal A and luminal B subtypes of breast cancer, ERα signaling serves as the principal driver of tumor growth and maintenance.[8] Persistent activation of ERα signaling contributes to uncontrolled cell proliferation, resistance to apoptosis, and endocrine therapy resistance, making ERα a critical molecular target in breast cancer management.[9] In addition to promoting breast cancer progression, the overexpression of ERα has also been implicated in other reproductive cancers, including ovarian, endometrial, and cervical cancers.[10] Despite the availability of chemotherapy and other adjuvant treatments for breast cancer, challenges such as drug resistance, toxicity, and limited selectivity remain significant obstacles in achieving effective and safe therapy.[11] In recent years, natural and nature-derived compounds targeting ERα have gained increasing attention due to their diverse structures, lower toxicity, and potential for multi-targeted action.[12] Given the central role of ERα in tumor development, the discovery of natural compounds capable of modulating its activity represents a promising avenue for therapeutic intervention and the development of more effective, natural-based anticancer agents.

In this study, we aimed to identify potential bioactive compounds from *P. aduncum* that probably inhibit ERα through subsequent in silico analysis. The research employed Lipinski's Rule of Five (RO5) to assess physicochemical properties and drug-likeness, and ADME/Tox prediction to evaluate pharmacokinetic properties. Additionally, pharmacophore modeling was used to assess structural compatibility, and molecular docking was employed to evaluate binding affinity at the ERα active site. The findings are expected to provide initial insights into the potential of *P. aduncum* as a source of ERα inhibitors, supporting the development of novel, effective, and selective, natural-based therapies for hormone-related cancers.

2. Methods

2.1. Tools

This research uses several software applications, including ChemDraw Ultra 12.0 and Chem3D Pro 12.0 for drawing structures and performing energy minimization of test and reference compounds, AutoDock Tools 1.5.6 for molecular modeling, BIOVIA

Discovery Studio 2025 for ligand and receptor preparation and result visualization, and LigandScout 4.4.5 for pharmacophore modeling. Meanwhile, the resources used include the Protein Data Bank (https://www.rcsb.org/) for receptor protein structures, PubChem (https://pubchem.ncbi.nlm.nih.gov/) for test and reference compound structures, DUD-E (https://dude.docking.org/targets) for the database of active compounds and decoys, Mcule Property Calculator (https://mcule.com/apps/property-calculator) for physicochemical property profiles, and PreADMET (https://preadmet.bmdrc.kr/) for predicting pharmacokinetic and toxicity characteristics based on Lipinski's Rule of Five.

The data used includes the three-dimensional structure of the ERα protein (PDB ID: 1SJ0) from the Protein Data Bank, as well as twenty compounds from wild betel leaves (Piper aduncum L.), as listed in Table 1, depicted in 3D format using ChemDraw Ultra 12.0 and Chem3D Pro 12.0. These compounds have been reported to exhibit various biological activities.[8]

2.2. Procedures

2.2.1. Lipinski's Rules of Five

The RO5 values are used as parameters in the testing of this compound. These parameters include a maximum LogP of 5 (high lipophilicity), a molecular weight of less than 500 Da, at most 10 hydrogen bond acceptors, and at most 5 hydrogen bond donors. The data were accessed through the website https://mcule.com/apps/property-calculator.

2.2.2. ADME Tox

The assessment of pharmacokinetic properties includes absorption, distribution, metabolism, excretion, and toxicity metrics such as human intestinal absorption (HIA), human colon adenocarcinoma cell lines (Caco-2) for the absorption profile, protein plasma binding (PPB), and blood-brain barrier (BBB) for the distribution profile, as well as mutagenic and carcinogenic evaluations for the toxicity profile. This information is sourced from the website https://preadmet.webservice.bmdrc.org/ through the input of SDF data.

2.2.3. Pharmacophore Screening

Pharmacophore screening was conducted to identify hit test compounds that resemble the pharmacophore and show potential biological activity. The receptor target (ER-α) with PDB ID 1SJ0, along with 100 active compounds and 400 decoy compounds, was downloaded from and prepared by converting the twenty test compound file format to .ldb. Pharmacophore modeling was then performed using LigandScout 4.4.5, with the active-compound database clustered into several groups, yielding 10 pharmacophore models.[9] Subsequently, pharmacophore validation was carried out using the 10 pharmacophore models, with an active compound and decoy database that had been classified by category. The pharmacophore model with the best performance on the ROC curve was selected and used to screen the test compound database to identify the most promising hit compounds.

2.2.4. Molecular Docking

The structure of the estrogen receptor alpha (ER-α) with PDB ID 1SJ0 was obtained from the Protein Data Bank of RCSB (https://www.rcsb.org) and then prepared in BIOVIA Discovery Studio 2025 by removing water molecules and separating the native ligand from the receptor. The test and reference ligands were constructed using the same application, and their energies were optimized using Chem3D Pro 12.0. The preparation of the ligand was continued using AutoDock, including the addition of hydrogen atoms, uniting the compound into a non-polar form, applying Gasteiger charges, and setting torsional elements. The receptor preparation was carried out by adding polar hydrogen atoms and applying Kollman charges, after the original ligand was removed using AutoDock 4.2.7.

Docking validation determines the position and size of the grid box in molecular docking simulations. This process includes redocking the native ligand onto the receptor using AutoDock, along with adjustments to the

grid position and dimensions. With a genetic algorithm parameter of 100, validation is considered successful if the RMSD value is < 2 Å and the binding energy is negative.[10] This study uses a grid box measuring 40 × 40 × 40 with a coordinate center (x = 30.885; y = –1.067; z = 23.464) and a point-to-point distance of 0.375 Å.

After validation was completed, the molecular modeling of the test ligand against the receptor was performed using the same method, employing the Lamarckian Genetic Algorithm and 100 parameters. The docking results were analyzed with AutoDock to obtain binding energy values and inhibition constants (Ki), and the results were visualized in 2D and 3D using BIOVIA Discovery Studio.[10]

3. Results

3.1. Lipinski's Rules of Five

According to the RO5 prediction results shown in Table 1, all compounds derived from *P. aduncum* comply with the drug similarity requirements essential for oral administration, except for quercetin-3-rutinoside and quercetin-3-rhamnoside. The

Table 1. Results of Lipinski's Rules of Five Prediction from Bioactive Constituents in *Piper Aduncum* L.

Compound	Molecular Weight (Da)	H-Acceptor Binding	H-Donor Binding	Log P	Lipinski Violations
Gallic acid	170.12	5	3	5.02	0
Catechin	290.27	6	1	1.55	0
Chlorogenic acid	354.31	9	1	-6.46	1
Epicatechin	290.27	6	1	1.55	0
Quercetin-3-rutinoside	610.52	16	1	-16.87	3
Quercetin-3-rhamnoside	448.38	11	1	4.89	2
Phlorizin	436.40	10	7	-2.02	2
Quercetin	302.24	7	5	1.99	0
Phloretin	274.27	5	4	2.32	0
Adunctin A	408.53	4	1	5.98	1
Adunctin B	406.51	4	1	5.68	1
Adunctin C	406.51	4	1	5.12	1
Piperaduncin A	490.54	7	3	5.51	1
Piperaduncin B	506.54	8	3	4.37	1
Dillapiole	506.54	8	3	4.37	1
Nerolidol	222.37	1	1	4.40	0
Spathulenol	220.35	1	1	3.38	0
Globulol	222.37	1	1	3.46	0
Rosifoliol	222.37	1	1	3.92	0
Linalool	154.25	1	1	2.67	0

compound quercetin-3-rutinoside fulfills the log P, hydrogen bond acceptor, and donor parameters, while quercetin-3-rhamnoside does not fulfill the hydrogen bond acceptor and donor parameters. Both compounds exhibit inadequate physicochemical properties and are not suitable for oral pharmaceuticals.

3.2. ADME Tox

ADME Tox analysis is listed in Table 2. Quercetin-3-rutinoside has a poor intestinal absorption (%HIA 2.861%), while other compounds range from moderate to good. Furthermore, Cathecin and Epicathecin had low intestinal permeability (Caco-2 <4), and five compounds have weak binding to plasma proteins (PPB < 90%). Only Adunctins group, Nerolidol, and Rosifoliol effectively crossed

Table 2. ADME Tox Prediction Results from Bioactive Constituents in *Piper Aduncum* L.

Compound	HIA (%)	Caco-2	PPB (%)	BBB	Mutagen	Carcinogenic	
						Mouse	Rat
Gallic acid	53.70	13.85	65.38	0.35	mutagen	-	+
Catechin	66.71	0.66	100.00	0.39	mutagen	-	-
Chlorogenic acid	20.43	18.72	41.96	0.03	mutagen	-	-
Epicatechin	66.71	0.66	100.00	0.39	mutagen	-	-
Quercetin-3-rutinoside	2.86	7.91	43.90	0.03	non-mutagen	-	-
Quercetin-3-rhamnoside	24.95	7.37	64.95	0.04	non-mutagen	-	-
Phlorizin	26.79	9.52	75.48	0.04	non-mutagen	-	
Quercetin	63.49	3.41	93.24	0.17	mutagen	-	+
Phloretin	78.98	18.10	100.00	0.73	mutagen	-	-
Adunctins A	96.26	46.87	100.00	6.18	non-mutagen	-	-
Adunctins B	96.26	50.94	97.87	3.87	non-mutagen	-	-
Adunctins C	96.26	49.64	98.41	4.54	non-mutagen	-	-
Piperaduncin A	93.22	21.53	95.25	1.84	non-mutagen	-	-
Piperaduncin B	93.05	21.76	89.37	0.22	non-mutagen	-	-
Dillapiole	98.89	57.24	89.09	1.72	non-mutagen	-	-
Nerolidol	100.00	26.61	100.00	13.98	non-mutagen	-	-
Spathulenol	100.00	54.42	82.89	6.97	mutagen	+	+
Globulol	100.00	54.57	100.00	7.57	non-mutagen	-	+
Rosifoliol	100.00	55.69	100.00	8.91	non-mutagen	-	-
Linalool	100.00	29.36	100.00	6.13	mutagen	-	-

the blood-brain barrier. Eight compounds were identified as mutagenic, including Gallic acid, Quercetin, and Spathulenol, also demonstrating carcinogenic potential in test animals.

3.3. Pharmacophore Screening

From the 10 generated pharmacophore models, model 10, with an AUC of 0.95, was selected to determine the pharmacophore features of the test ligand. The model was chosen over the others because its receiver operating characteristic (ROC) curve displayed the highest AUC. Moreover, based on the pharmacophore screening results, 14 hit compounds were identified that potentially have similar activity to the active compound, as shown in Table 3. The compound with the best fit score is phlorizin as shown in Figure 1.

3.4. Molecular Docking

The validation results show a clear overlay, with an RMSD of 0.92 Å. This outcome signifies that the validation procedure has fulfilled the acceptance criteria. The active compounds from Piper aduncum L. were then docked to the ER-α receptor to determine their conformations, binding energies, and inhibition constants. The docking analysis of the 20 compounds found in *P. aduncum* L. showed that all test compounds produced higher binding energy compared to the reference drug (4-Hydroxytamoxifen) with a value of -11.55 kcal/mol. Meanwhile, there were test compounds that produced inhibition constants smaller than the reference drug (4-Hydroxytamoxifen), which has a KI value of 3.44, including Catechin, Chlorogenic acid, Epicatechin, Quercetin-3-rutinoside, Quercetin, Nerolidol, and Spathulenol (Table 4). The amino acid residue interaction that most frequently appears in the test compounds is GLU353, which is also found in 4-hydroxytamoxifen and the native ligand.

4. Discussion

This study evaluated the potential of wild betel leaves (*P. aduncum* L.) as inhibitors of estrogen receptor alpha (ERα), a key driver of hormone-dependent breast cancer progression. An in silico approach was employed, integrating drug-likeness assessment, ADMETox profiling, pharmacophore modeling, and molecular docking to identify promising ERα-targeting compounds.

Drug-likeness analysis based on Lipinski's Rule of Five indicated that phloretin and linalool fully satisfied all criteria, suggesting favorable physicochemical properties for oral administration. Phloretin possesses suitable hydrogen bond donors and acceptors to support gastrointestinal absorption, while linalool's small and lipophilic structure

Table 3. Pharmacophore-Fit Score

Compound	Fit score
Rosifoliol	36.91
Nerolidol	37.38
Linalool	37.62
Spathulenol	36.02
Adunctin B	35.45
Phloretin	37.50
Adunctin C	35.43
Epicatechin	37.22
Catechin	37.22
Chlorogenic Acid	37.21
Phlorizin	46.21
Quercetin	37.18
Quercetin-3-rhamnoside	37.18
Quercetin-3-rutinoside	37.17

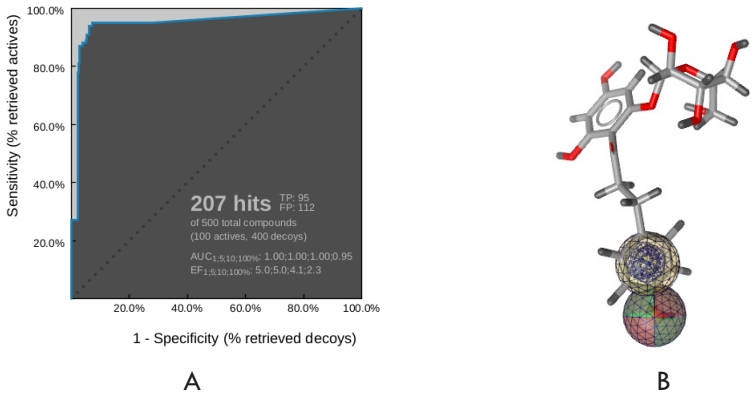


Figure 1. Pharmacophore screening results based on ROC curve and its pharmacophore visualization; (A) Pharmacophore screening validation curve (ROC curve model 10); (B) Phlorizin

facilitates passive membrane diffusion. These characteristics support their potential as lead compounds, although Lipinski's criteria alone are insufficient to predict clinical suitability, particularly when alternative routes of administration may be considered.[11]

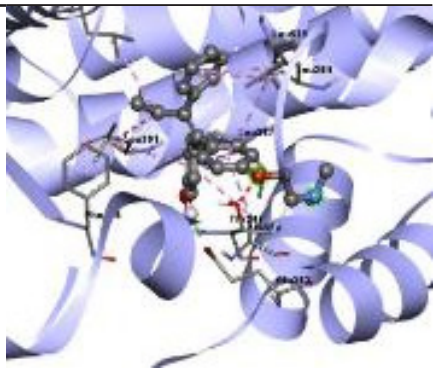
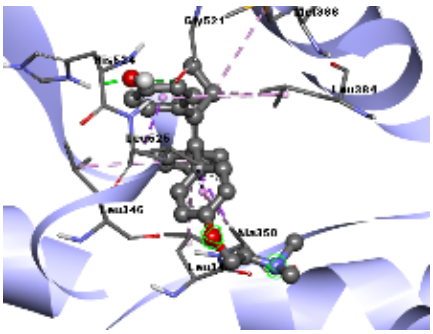
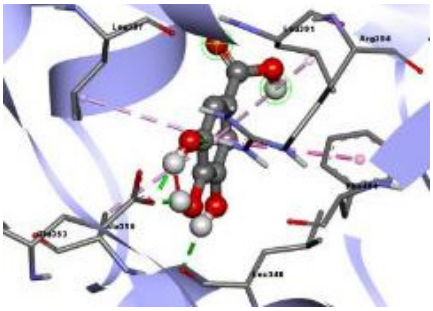
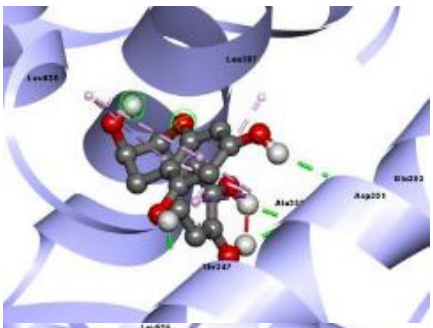
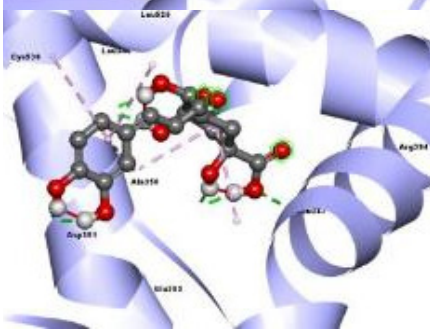
ADMETox profiling further refined candidate selection by highlighting pharmacokinetic and safety-related parameters relevant to breast cancer therapy. High intestinal absorption and adequate cell permeability are essential for systemic efficacy, while limited blood–brain barrier (BBB) penetration is preferred to minimize central nervous system side effects. Tamoxifen, the standard ER-positive breast cancer therapy, demonstrates effective ERα inhibition but exhibits BBB penetration, which has been associated with neurocognitive adverse effects during long-term treatment.[12,13] In this context, *Piper aduncum*–derived compounds with reduced BBB permeability are of interest. Phloretin demonstrated high intestinal absorption, strong plasma protein binding, moderate Caco-2 permeability, and low BBB penetration, indicating a potentially safer pharmacokinetic profile, although its predicted mutagenicity suggests that dose optimization and further safety evaluation are required.

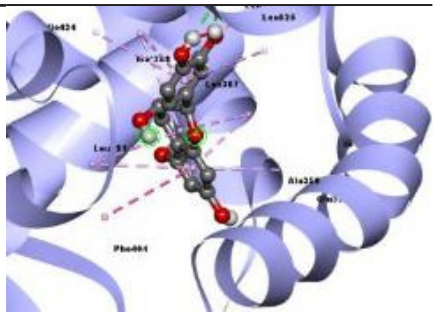
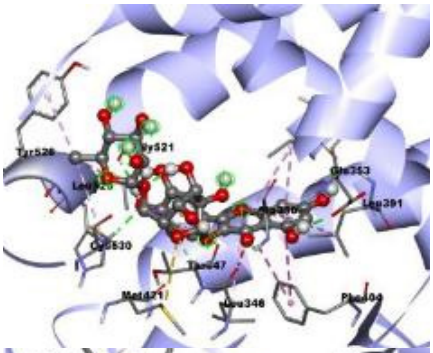
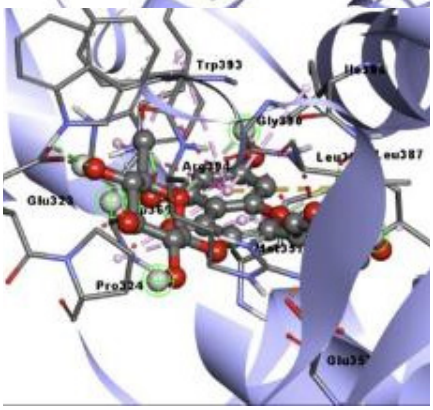
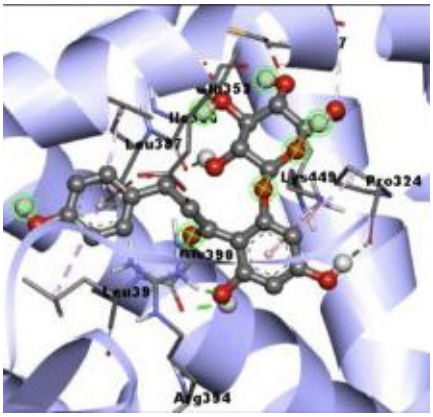
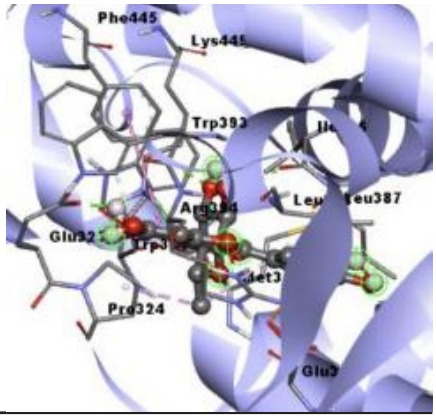
Pharmacophore modeling yielded a highly predictive model, indicating excellent capability to distinguish active ERα inhibitors from inactive compounds.[14,15,16] Among the screened constituents, phlorizin, phloretin, and linalool showed the highest fit scores, sharing key interaction features, including hydrophobic regions, hydrogen-bond donors and acceptors, and aromatic moieties, which are critical for ERα binding. Molecular docking analysis against the ERα crystal structure confirmed the relevance of these findings. The docking results revealed consistent involvement of GLU353 in 4-HT binding, a well-established critical within the estrogen-binding domain of ERα, in hydrogen bonding with both reference and test compounds.[17]

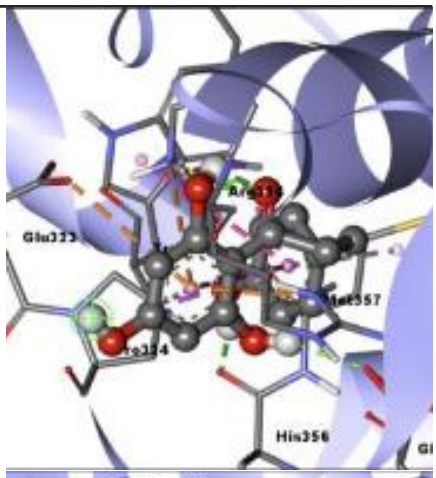
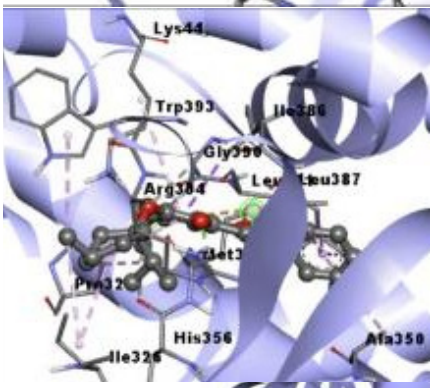
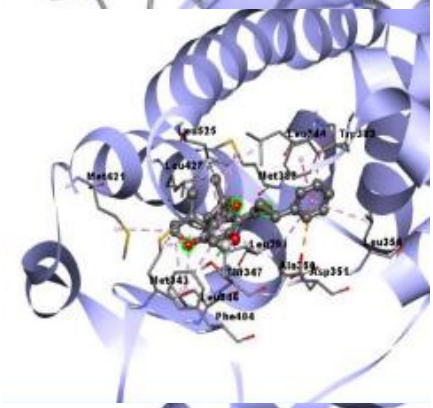
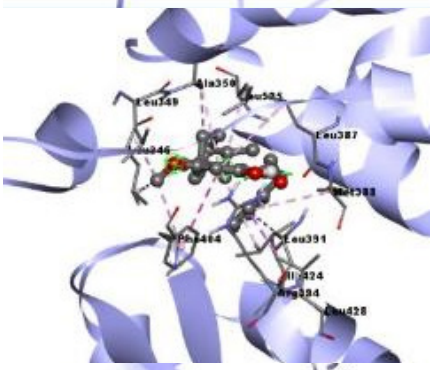
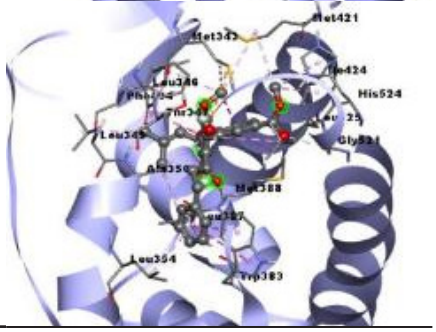
Among all screened compounds, phlorizin exhibited the strongest binding affinity (−10.38 kcal/mol) with a predicted inhibition constant of 24.77 μM. Although its binding energy was lower than that of the native ligand, phlorizin exhibited interaction patterns closely resembling those of 4-HT, including hydrogen bonding with GLU353 and hydrophobic interactions with ALA350 and LEU525, as reported for the receptor's catalytic activity.[18] These conserved interactions suggest that phlorizin may act as a competitive ERα antagonist with a binding mode similar to that of established anti-estrogen therapies.

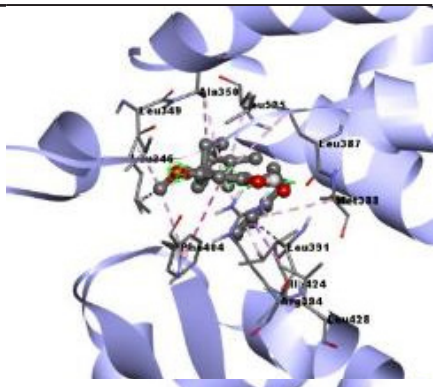
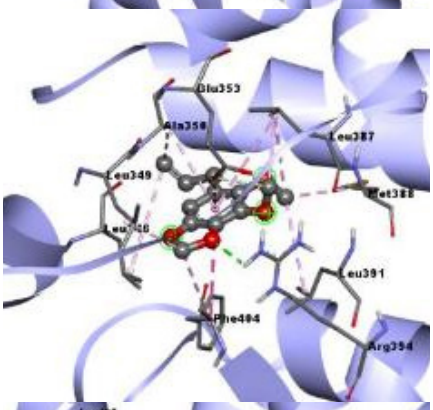
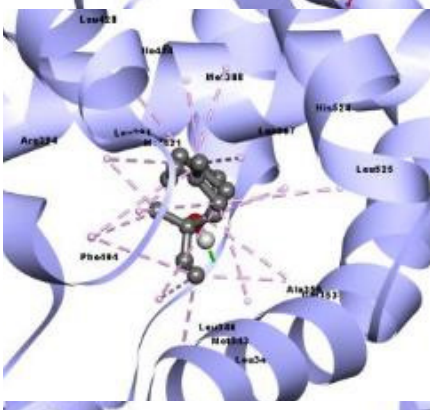
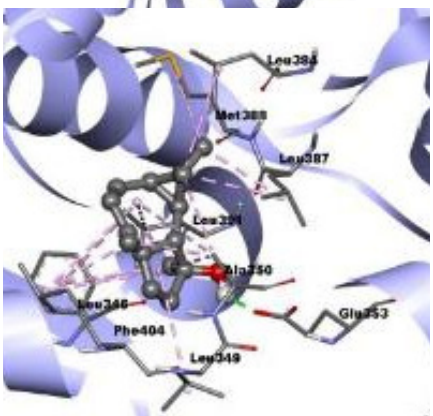
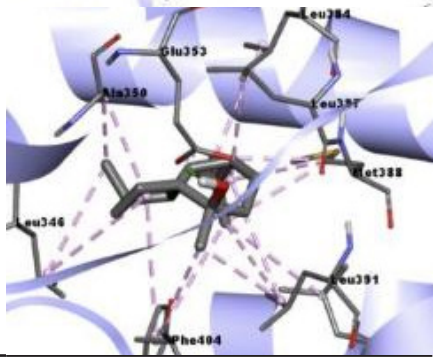
P. aduncum has gained increasing attention as a source of bioactive secondary metabolites with anticancer potential, particularly in hormone-related malignancies such as breast cancer. Extracts of *P. aduncum* have been reported to exhibit cytotoxic, antioxidant, and apoptosis-inducing activities in various cancer cell models, supporting their

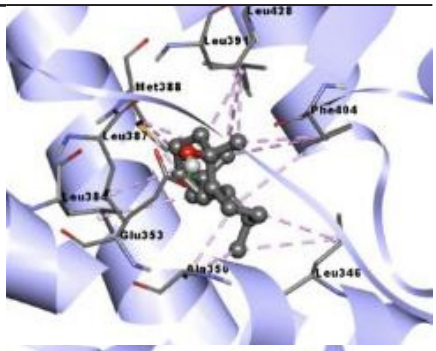
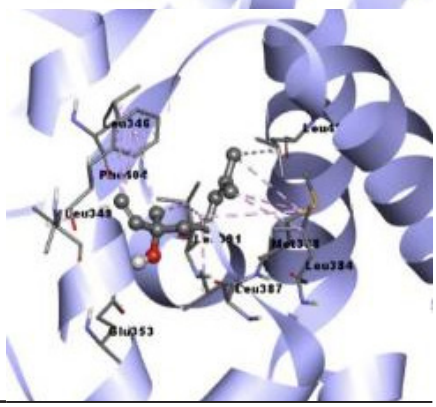
Table 4. Molecular Docking Results of Native Ligands and Test Compounds of Wild Betel Leaves (*Piper aduncum* L.) with ERα

Compound	Binding Energy (kcal/mol)	Inhibition Constant (Ki)	3D Visualization
4-Hydroxytamoxifen (Comparative drug)	-11.55	3.44	
Native ligand	-13.65	98.33	
Gallic Acid	-4.31	691.64	
Catechin	-7.67	2.39	
Chlorogenic Acid	-7.96	1.47	

Epicatechin	-7.91	1.58	
Quercetin-3-rutinoside	-8.03	1.29	
Quercetin-3-rhamnoside	-9.29	154.95	
Phlorizin	-10.38	24.77	
Quercetin	-8.00	1.37	

Phloretin	-6.46	18.49	
Adunctins A	-9.66	82.47	
Adunctin B	-9.89	56.13	
Adunctin C	-10.23	31.94	
Piperaduncin A	-10.25	30.62	

Piperaduncin B	-10.11	38.58	
Dillapiole	-5.94	44.47	
Nerolidol	-6.99	7.58	
Spathulenol	-7.41	3.72	
Globulol	-8.63	471.15	

Rosifoliol	-8.63	470.70	
Linalool	-5	470.70	

relevance in cancer chemoprevention and therapy.[19,20] In breast cancer, these biological effects are especially relevant because oxidative stress regulation and apoptosis induction are closely linked to estrogen receptor–mediated tumor progression. Among the identified constituents, phloretin, a naturally occurring dihydrochalcone, has demonstrated broad anticancer activity across multiple cancer types, including breast cancer, through mechanisms that include cell cycle arrest, apoptosis induction, inhibition of glucose transporters, and modulation of estrogen-related signaling pathways.[21-23] Recent computational and experimental studies suggest that phloretin can interact with estrogen receptor alpha (ERα), potentially disrupting estrogen-driven transcriptional activity and tumor growth.[24-26] Collectively, these findings support the rationale for further investigation of *Piper aduncum*–derived compounds, particularly phloretin, as promising candidates for the development of novel anti–estrogen receptor strategies in breast cancer management.

5. Conclusion

The in silico analysis of 20 active compounds from wild betel leaves (*Piper aduncum* L.) identified phlorizin, linalool, and phloretin as the most promising candidates. The optimal pharmacophore model showed high predictive performance. Phloretin and linalool met all RO5 criteria and demonstrated favorable ADMETox profiles, while phlorizin exhibited the strongest ERα binding affinity (−10.38 kcal/mol) and key interactions comparable to 4-hydroxytamoxifen. These findings support the potential of these compounds as ERα inhibitors for further development of breast cancer drugs.

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