PALUWALA: Jurnal Ilmu Kesehatan
Special Issue (GICHS 2025) Pages 153-164
https://ejournal.airlangga.org/index.php/ghj
E-ISSN 3090-0239

Studi In Silico Senyawa Tanaman Asparagus officinalis terhadap Reseptor Estrogen Alfa (ER-α) sebagai Kandidat Antikanker Payudara

In Silico Study of Asparagus officinalis Plant Compounds Against Estrogen Receptor Alpha (ER-α) as a Candidate for Breast Anticancer

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Article info

Article history:
Received: 25 Agustus 2025
Revised: 1 September 2025
Accepted: 29 September 2025
Online: 29 September 2025

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Abstrak

Kanker payudara adalah jenis kanker yang paling umum dan penyebab utama kematian terkait kanker pada wanita di seluruh dunia. Keterbatasan terapi konvensional, seperti efek samping kemoterapi yang serius, mendorong pencarian pengobatan alternatif yang lebih aman dan efektif. Asparagus officinalis diketahui mengandung senyawa bioaktif dengan potensi sifat antikanker. Penelitian ini bertujuan untuk mengevaluasi potensi senyawa dari A. officinalis sebagai kandidat obat oral melalui analisis kelayakan farmasi berdasarkan Aturan Lipinski, profil farmakokinetik dan toksisitas, dan pemodelan interaksinya dengan reseptor estrogen alfa (ER-α) menggunakan docking molekuler. Dari 116 senyawa yang diidentifikasi, 64 memenuhi Aturan Lipinski, dan sebagian besar menunjukkan profil farmakokinetik dan toksisitas yang baik. Hasil docking molekuler mengungkapkan bahwa 19 senyawa memiliki afinitas pengikatan yang kuat (–7,09 hingga –9,59 kkal/mol). Katekin dan 3PGPC menunjukkan afinitas terkuat, mendekati afinitas ligan pembandingnya, 4-hidroksitamoksifen (–9,98 kkal/mol). Interaksi kedua senyawa ini dengan residu ER-α kunci dan tidak adanya ikatan hidrogen dengan residu His524 menunjukkan bahwa keduanya bertindak sebagai antagonis terhadap ER-α, yang dapat menghambat proliferasi sel kanker payudara. Temuan ini menunjukkan bahwa katekin dan 3PGPC berpotensi menjadi ligan alternatif untuk ER-α dan layak untuk diteliti lebih lanjut sebagai kandidat obat kanker payudara berbasis bahan alami.

Kata Kunci: *Kanker Payudara, Asparagus officinalis, Reseptor Estrogen Alfa (ER−α), Docking Molekuler*

Abstract

Breast cancer is the most prevalent type of cancer and the leading cause of cancer-related death in women worldwide. The limitations of conventional therapies, such as the serious side effects of chemotherapy, drive the search for safer and more effective alternative treatments. Asparagus officinalis is known to contain bioactive compounds with potential anticancer properties. This study aimed to evaluate the potential of compounds from A. officinalis as oral drug candidates through an analysis of pharmaceutical feasibility based on Lipinski's Rule, pharmacokinetic and toxicity profiles, and modeling of their interaction with the estrogen receptor alpha (ER-α) using molecular docking. From 116 identified compounds, 64 satisfied Lipinski's Rule, and most showed good pharmacokinetic and toxicity profiles. The molecular docking results revealed that 19 compounds had a strong binding affinity (–7.09 to –9.59 kcal/mol). Catechin and 3PGPC showed the strongest affinity, closely approaching that of the comparative ligand, 4-hydroxytamoxifen (–9.98 kcal/mol). The interaction of these two compounds with key ER-α residues and the absence of hydrogen bonding with the His524 residue indicate that they act as antagonists against ER-α, which can inhibit the proliferation of breast cancer cells. These findings suggest that catechin and 3PGPC have the potential to be alternative ligands for ER-α and are worthy of further investigation as candidates for natural-based breast cancer drugs.

Keywords: *Breast Cancer, Asparagus officinalis, Estrogen Receptor Alpha (ER−α), Molecular Docking*

BACKGROUND

Breast cancer (carcinoma mammae) is the most common type of cancer affecting women worldwide. According to the World Health Organization (WHO) report in 2020, it accounted for approximately 2.26 million new cases, with an annual mortality rate of 17%. In Indonesia, data from the Ministry of Health indicate that breast cancer has the highest incidence rate among women, reaching 41.8 per 100,000 population, with a mortality rate of 14.4 per 100,000 population (Ministry of Health, Republic of Indonesia (Ministry of Health, Republic of Indonesia, 2024). The high prevalence undrscores that breast cancer is a pressing public health issue that requires serious attention.

Breast cancer treatment generally involves local approaches such as surgery and radiation, as well as systemic therapies including chemotherapy, hormonal therapy, immunotherapy, and targeted therapy. However, the use of chemotherapy is often accompanied by significant side effects, including anemia, peripheral neuropathy, nausea, and immunosuppression, which adversely affect patients' quality of life (Kustanto et al., 2023). Therefore, the search for effective alternative treatments with minimal side effects has become an essential need in the development of cancer therapy.

One promising approach is the exploration of bioactive compounds from natural sources. Asparagus officinalis is a plant that has been traditionally used in medicine and is known to contain various active compounds, such as steroidal saponins, flavonoids, and polysaccharides, which possess cytotoxic and antitumor potential (Xu et al., 2021). Nevertheless, scientific exploration of the molecular mechanisms of A. officinalis active compounds, particularly against relevant targets such as estrogen receptor alpha (ER−α), remains limited.

In silico approaches, such as molecular docking and molecular dynamics, are effective methods for virtually screening potential drug candidates and evaluating ligand–receptor interaction profiles and complex stability under physiological conditions (Pinzi & Rastelli, 2019). ER−α is a key therapeutic target in luminal breast cancer, playing a crucial role in gene transcription regulation and cancer cell development (Ikhtiarudin et al., 2022). Tamoxifen, an ER−α antagonist, is widely used but is associated with serious side effects, including an increased risk of endometrial cancer and thromboembolism (Fauzi et al., 2024). Therefore, the search for safer natural compounds as alternative therapeutics is highly relevant.

Specific investigations into the molecular mechanisms of active compounds from Asparagus, particularly in targeting relevant receptors such as estrogen receptor alpha (ER−α) in the context of breast cancer, remain scarce. This lack of scientific exploration makes Asparagus officinalis an attractive subject for further research to explore its therapeutic potential as a safe and effective anticancer drug candidat

METHODS

Materials and Tools

The equipment used in this study consisted of hardware and software. The hardware utilized was an Acer A314-22-Athlon personal computer equipped with an AMD Athlon Silver 3050U processor with Radeon Graphics (2.30 GHz), 4.00 GB of Random Access Memory (RAM), and Windows 11 Home Single Language. The system operated on a 64-bit operating system with an x64- based processor. The software employed included AutoDock Tools, Discovery Studio 2024 Client, Gnina, KNApSAcK, pkCSM, Protein Data Bank, PubChem, and SwissADME.

The materials used in this study were compounds derived from Asparagus officinalis, obtained from the KNApSAcK database and downloaded from the PubChem website in .sdf format. The three-dimensional structure of estrogen receptor alpha (ER-α) was retrieved from the Protein Data Bank in .pdb format.

Preliminary Analysis and Compound Selection

Compounds from Asparagus officinalis were obtained from the KNApSAcK database and analyzed using SwissADME based on Lipinski's Rule of Five to assess their suitability as oral drug candidates. Compounds meeting the criteria were subsequently evaluated for their ADMET profiles using pkCSM, covering pharmacokinetic and toxicity parameters.

Receptor and Ligand Preparation

The receptor used in this study was estrogen receptor alpha (ER-α) with PDB ID 3ERT, downloaded from the Protein Data Bank in .pdb format. The receptor file was opened using AutoDock Tools and cleaned by removing water molecules and the native ligand using the "delete water" and "delete ligand" commands to obtain a pure receptor structure. The native ligand was also saved separately in .pdb format for docking validation purposes.

Meanwhile, the test compounds that met Lipinski's criteria were downloaded from PubChem in .sdf format, which contains structural and chemical bond information. Since the .sdf format is not directly compatible with GNINA, the files were converted into .pdb format using Discovery Studio. After conversion, the ligands were ready for the molecular docking process.

Grid Box Determination

The grid box was defined to focus the docking process on the active site (binding site) of the receptor. The grid box center was set based on the residues interacting with the natural ligand 4- hydroxytamoxifen (4-OHT) in the crystal structure of ER-α (PDB ID: 3ERT), identified through molecular visualization using Discovery Studio. The

grid box center coordinates and dimensions were configured in AutoDock Tools via the 'Grid' > 'Grid box' menu, adjusting the coverage to encompass these key residues so that docking was concentrated on the receptor's active site.

Docking Method Validation

Docking method validation was performed using a redocking approach with the natural ligand (4-hydroxytamoxifen) from the co-crystal structure of the receptor. The docking parameters were considered valid if the system was able to rebind the original ligand to its initial position with a Root Mean Square Deviation (RMSD) value of less than 2 Å. The success of this validation could also be influenced by the ligand size, whether large or small (Astuty & Komari, 2022).

Molecular Docking

The prepared estrogen receptor alpha and the test ligands were docked using Gnina. The protein file in .pdb format and the test ligands were uploaded via Google Colab. The docking process was executed according to the text format required by Gnina. The predefined grid box dimensions from AutoDock Tools were manually entered in the gridbox command in the Gnina script. The molecular docking results were presented in a table containing the free binding energy (ΔG) scores, with the most negative value selected as the best result.

Visualization of Molecular Docking Results

The molecular docking results from Gnina were visualized using Discovery Studio Visualizer to analyze ligand–receptor interactions in 2D. The receptor file was opened, and the ligand .pdb file was dragged onto the receptor display. Interactions were analyzed using the Show 2D Diagram feature, and bond distances were displayed using Ligand Interactions > Show Distance, which were then recorded in the visualization results table.

RESULTS AND DISCUSSION

Preliminary Analysis and Compound Selection

The compound selection process was systematically conducted using the KNApSAcK Core System phytochemical database, which provides information on secondary metabolites from various plant species. The search focused on Asparagus officinalis, yielding more than 100 compounds reported to be derived from this plant. According Widhiastuti (2021), KNApSAcK is an integrated plant–metabolite database containing 101,500 species–metabolite relationships, encompassing 20,741 species and 50,048 metabolites. Each retrieved compound was exported in SMILES format for further analysis. Given the large number of compounds, an initial screening was performed based on physicochemical properties using Lipinski's Rule of Five to identify compounds with potential as oral drugs. The selected compounds were then further analyzed as candidate ligands for the Estrogen Receptor Alpha (ERα), a protein target known to play a crucial role in the progression of breast cancer with estrogen receptor alpha expression.

Lipinski's Rule of Five is a guideline used to predict the feasibility of a compound as an oral drug based on basic pharmacokinetic parameters, particularly related to absorption and permeation. According to this criterion, a compound is considered to have a high likelihood of good oral absorption if it meets the following requirements: it possesses no more than five hydrogen bond donors, no more than ten hydrogen bond acceptors, a molecular weight below 500 Daltons, and a log P value not greater than 5 (Fakih dkk., 2022). A total of 116 compounds were analyzed according to Lipinski's Rule of Five. Of these, 64 compounds fulfilled the requirements as potential oral drug candidates.

Table 1. Predicted Lipinski's Rule of Five Results

No	Compound	Lipinski's Rule of Five				Remarks
		MW (≤500 Da)	LogP (≤5)	H-Bond Donors (≤5)	H-Bond Acceptors (≤10)	
1	p-Coumaricacid	164.16	0.95	2	3	Pass
2	Asparagusicacidsyn-S-oxide	166.22	0.32	1	3	Pass
3	Asparagusicacid	150.22	1.03	1	2	Pass
4	Dihydroasparagusic acid	152.24	0.96	1	2	
5	S-Acetyl dihydroasparagusic acid	194.27	1.51	1	3	Pass
6	Caffeicacid	180.16	0.97	3	4	Pass
7	Luteolin	286.24	1.86	4	6	Pass
8	Taxifolin	304.25	0.71	5	7	Pass
9	Thiamine	265.35	-1.60	2	3	Pass
10	Catechin	290.27	1.33	5	6	Pass
11	Naringenin	272.25	1.75	3	5	Pass

12	Ascorbicacid	176.12	-0.31	4	6	Pass
13	Mevalonicacid	148.16	0.81	3	4	Pass
14	Linoleicacid	280.45	4.14	1	2	Pass
15	Pantothenicacid	219.23	0.95	4	5	Pass
16	Coumarin	146.14	1.75	0	2	Pass
17	4-Hydroxybenzaldehyd	122.12	0.99	1	2	Pass
18	Syringicacid	198.17	1.54	2	5	Pass
19	Vanillicacid	168.15	1.40	2	4	Pass
20	Vanillin	152.15	1.57	1	3	
21	Ferulicacid	194.18	1.62	2	4	Pass
22	Diosgenin	414.62	4.49	1	2	Pass
23	Sarsasapogenin	416.64	4.51	1	3	Pass
24	Yamogenin	414.62	4.43	1	3	Pass
25	Apigenin	270.24	1.89	3	5	Pass
26	Kaempferol	286.24	1.70	4	6	Pass
27	Quercetin	302.24	1.63	5	7	Pass
28	Naringeninchalcone	272.25	1.34	4	5	Pass
29	Dihydrokaempferol	288.25	1.58	4	6	Pass
30	Glycerol-3-phosphate	172.07	-0.45	4	6	Pass
31	DXP	214.11	0.09	4	7	Pass
32	2-C-Methyl-D-erythritol2,4-cyclodiphosphate	278.09	-0.16	4	9	Pass
33	2-C-Methyl-D-erythritol4-phosphate	216.13	-0.28	5	7	Pass
34	Adenosine	267.24	0.53	4	7	Pass
35	Syringaldehyde	182.17	1.66	1	4	Pass
36	Cinnamicacid	148.16	1.55	1	2	
37	Nonadienal	142.24	2.44	0	1	Pass
38	Palmiticacid amide	255.44	3.87	1	1	Pass
39	Glyceryl palmitate	330.50	3.93	2	4	Pass
40	Cytidine	243.22	0.44	4	6	Pass
41	Isopropylalcohol	60.10	1.38	1	1	Pass
42	2,3-Butanedione Diacetyl	86.09	1.19	0	2	Pass
43	DMAPP	246.09	-0.12	3	7	Pass
44	IPP	246.09	0.55	3	7	Pass
45	3-Hydroxycinnamic acid	164.16	1.14	2	3	Pass
46	2,6-Dimethylpyrazine	108.14	1.52	0	2	Pass
47	2-Heptenal	112.17	1.96	0	1	Pass
48	2-Methylpyrazine	94.11	1.17	0	2	Pass
49	Asparaptine	306.40	1.00	5	4	Pass
50	Blumenol C	210.31	2.54	1	2	Pass
51	Glycerophosphoglycerol	246.15	0.38	5	8	Pass
52	Phytosphingosine	317.51	3.84	4	4	
53	Asparenyol	280.32	3.38	1	3	Pass
54	1,2,3-Trithiane-5-carboxylic acid	182.28	1.24	1	2	Pass
55	3-Palmitoyl-sn- glycerol-1-phosphorylcholine	495.63	-0.47	1	7	Pass
56	Lysophosphatidylethanolamine C16:0	453.55	4.47	3	8	Pass
57	Decadienal	152.23	2.67	0	1	Pass
58	3-Methyl thiopropanal	104.17	1.10	0	1	Pass
59	Furancarboxaldehyde	96.08	1.03	0	2	Pass
60	2,3-Pentanedione	100.12	1.47	0	2	Pass
61	3-Penten-2-one	84.12	1.56	0	1	Pass
62	1-Palmitoyl-sn- glyceryl3-phosphate	410.48	3.41	3	7	Pass
63	Asparenyn	294.34	4.02	0	3	Pass
64	1-O-Feruloyl-3- O-p-coumaroylglycerol	414.41	3.51	3	8	Pass

In drug discovery and development, the calculation of absorption, distribution, metabolism, and excretion (ADME), as well as the toxicity of a drug candidate, is essential to prevent potential pharmacokinetic issues. Table 2 presents the predicted results of absorption, distribution, metabolism, excretion, and toxicity. This process was carried out using the pkCSM platform. The SMILES data of the compounds that met Lipinski's rule were entered into the pkCSM website to predict their pharmacokinetic characteristics and toxicity profiles

Table 2. Pharmacokinetic and toxicity Analysis Results

NO	Test Compound	Parameter								
		Absorption		Distribution	Metabolism				Execration	Toxicity
		Caco 2 (log Papp in 10-6 cm/s)	HIA (%) Absorbed	VDss human	CY P2D6 substrate	CY P3A4 Substrate	CY P2D6 inhibitor	CY P3A4 inhibitor	Total Clearance (log ml/min/kg)	AMES Toxic ity
1	p-Coumaricacid	1.14	93.18	-0.60	No	No	No	No	0.69	No
2	Asparagusic acid syn-S-oxide	1.12	99.60	-0.74	No	No	No	No	0.44	Yes
3	Asparagusic acid	1.09	95.20	-0.69	No	No	No	No	0.37	No
4	Dihydroasparagusic acid	1.25	82.29	-1.08	No	No	No	No	0.41	No
5	S-Acetyl dihydroasparagusicacid	1.19	82.28	-1.19	No	No	No	No	0.43	No
6	Caffeicacid	0.08	59.00	-0.51	No	No	No	No	0.55	No
7	Luteolin	0.19	78.90	-0.11	No	No	No	Yes	0.55	Yes
8	Taxifolin	-0.45	69.86	0.56	No	No	No	No	-0.00	Yes
9	Thiamine	0.85	94.02	0.43	No	No	No	No	1.04	No
10	Catechin	-0.35	69.94	0.50	No	No	No	No	0.20	No
11	Naringenin	1.15	91.15	-0.06	No	No	No	No	0.05	Yes
12	Ascorbicacid	-0.39	39.71	-0.26	No	No	No	No	0.62	No
13	Mevalonicacid	0.45	62.97	-1.19	No	No	No	No	0.78	No
14	Linoleicacid	1.57	92.32	-0.58	No	Yes	No	No	1.93	No
15	Pantothenic acid	-0.46	32.58	-1.46	No	No	No	No	0.59	No
16	Coumarin	1.67	96.78	-0.06	No	No	No	No	0.96	Yes
17	4-Hydroxybenzalde hyde	1.38	85.87	-0.06	No	No	No	No	0.58	No
18	Syringicacid	1.26	73.23	-0.57	No	No	No	No	0.71	No
19	Vanillicacid	1.22	73.23	-0.70	No	No	No	No	0.68	No
20	Vanillin	1.19	89.86	0.14	No	No	No	No	0.63	No
21	Ferulicacid	0.02	94.1	-0.78	No	No	No	No	0.64	No
22	Diosgenin	1.30	96.36	0.46	No	Yes	No	No	0.32	No
23	Sarsasapogenin	1.31	97.28	0.21	No	Yes	No	No	0.32	No
24	Yamogenin	1.30	96.36	0.46	No	Yes	No	No	0.32	No
25	Apigenin	1.11	91.43	-0.19	No	No	No	No	0.59	No
26	Kaempferol	0.44	84.99	-0.01	No	No	No	Yes	0.59	Yes
27	Quercetin	0.28	74.9	0.11	No	No	No	No	0.55	No
28	Naringenin	0.40	71.47	-0.40	No	No	No	Yes	0.16	No

29	chalcone dihydrokaempferol	-0.42	77.22	0.22	No	No	No	No	0.05	Yes
30	Glycerol-3-phosphate	-0.45	45.33	-0.58	No	No	No	No	0.51	No
31	DXP	-0.43	34.21	0.15	No	No	No	No	0.40	No
32	2-C-Methyl-D-erythritol2,4-cyclodiphosphate	0.23	18.54	0.53	No	No	No	No	0.29	No
33	2-C-Methyl-D-erythritol4-phosphate	-0.51	28.93	0.03	No	No	No	No	0.54	No
34	Adenosine	-0.56	53.66	0.87	No	No	No	No	0.77	No
35	Syringaldehyde	1.18	89.97	0.07	No	No	No	No	0.65	No
36	Cinnamicacid	1.49	94.01	-0.58	No	No	No	No	0.80	No
37	Nonadienal	1.48	94.73	0.24	No	No	No	No	1.56	No
38	Palmiticacidamide	1.52	90.35	0.35	No	Yes	No	No	1.83	No
39	Glyceryl palmitate	0.42	90.49	-0.25	No	No	Yes	No	1.97	No
40	Cytidine	-0.12	40.61	-0.31	No	No	No	No	0.55	No
41	Isopropylalcohol	1.49	93.30	-0.13	No	No	No	No	0.64	No
42	2,3-Butanedione	1.59	100	-0.22	No	No	No	No	0.72	No
43	Dimethylallyl pyrophosphate	-0.35	34.43	0.16	No	No	No	No	0.22	No
44	IPP	-0.36	34.36	0.16	No	No	No	No	0.25	No
45	3-Hydroxycinnamic acid	1.14	93.57	-0.55	No	No	No	No	0.69	No
46	2,6-Dimethylpyrazine	1.72	100	-0.21	No	No	No	No	0.61	Yes
47	2-Heptenal	1.49	95.93	0.08	No	No	No	No	0.34	Yes
48	2-Methylpyrazine	1.79	100	-0.28	No	No	No	No	0.63	No
49	Asparaptine	-0.32	29.23	0.86	Yes	Yes	No	No	0.01	No
50	Blumenol C	1.38	94.62	0.11	No	No	No	No	1.25	No
51	Glycerophosphoglycerol	-0.09	36.29	-0.56	No	No	No	No	0.87	No
52	Phytosphingosine	0.41	93.85	-0.79	No	No	Yes	No	1.43	No
53	Asparenyol	1.22	94.92	-0.01	No	Yes	No	No	0.31	Yes
54	1,2,3-Trithiane-5-carboxylic acid	1.17	91.04	-0.74	No	No	No	No	0.25	No
55	3-Palmitoyl-sn- glycerol-1-phosphorylcholine	0.68	58.71	-0.14	No	Yes	No	Yes	1.03	No

56	Lysophosphatidyl ethanolamine C16:0	0.42	57.87	-0.21	No	No	No	No	1.06	No
57	Decadienal	1.49	95.40	0.23	No	No	No	No	0.42	No
58	3-Methyl thiopropanal	1.49	100	-0.07	No	No	No	No	0.36	No
59	Furancarboxaldeh yde	1.60	100	-0.15	No	No	No	No	0.59	Yes
60	2,3-Pentanedione	1.60	100	-0.18	No	No	No	No	0.41	No
61	3-Penten-2-one	1.5	100	-0.07	No	No	No	No	0.29	No
62	1-Palmitoyl-sn-glyceryl3-phosphate	0.74	46.789	-0.68	No	No	No	No	0.78	No
63	Asparenyn	1.25	96.851	0.01	No	Yes	No	No	0.39	No
64	1-O-Feruloyl-3-O-p-coumaroylglycerl	-0.03	68.27	-0.11	No	Yes	No	Yes	0.43	No

Based on pharmacokinetic and toxicity analysis of 64 compounds, the majority exhibited favorable pharmacokinetic profiles. In terms of absorption, 46 compounds demonstrated high human intestinal absorption (HIA), indicating good potential for oral bioavailability. Fifteen compounds showed moderate absorption, and only three exhibited low absorption. Regarding Caco-2 cell permeability, 35 compounds displayed high permeability (log Papp > 0.90), two moderate, and 27 low. For distribution, only seven compounds had a high volume of distribution (log VDss > 0.45), 28 were in the moderate range, and 29 were classified as low (log VDss < –0.15), suggesting that most compounds tend to remain within the bloodstream rather than extensively distributing into tissues. In terms of metabolism, the majority of compounds did not interact with the CYP2D6 or CYP3A4 enzymes. Only one compound was identified as a substrate and two as inhibitors of CYP2D6, while ten compounds were identified as substrates and five as inhibitors of CYP3A4. Regarding excretion, total clearance values ranged from –0.00 to 1.97 log (ml/min/kg), with 11 compounds classified as having slow excretion, 44 moderate, and nine rapid. The Ames toxicity test revealed that 53 compounds were non-mutagenic, whereas 11 compounds showed potential mutagenicity. These findings are essential for considering the safety and efficacy of candidate compounds in further drug development.

Receptor and Ligand Preparation

In this study, the crystal structure of ER-α bound to the active ligand 4-hydroxytamoxifen (PDB ID: 3ERT) was obtained from the Protein Data Bank. According to Kusuma (2024), the selection of the 3ERT crystal structure was based on its co-crystallization with 4-hydroxytamoxifen, an active metabolite of the drug tamoxifen that functions as a Selective Estrogen Receptor Modulator (SERM) targeting ER-α.

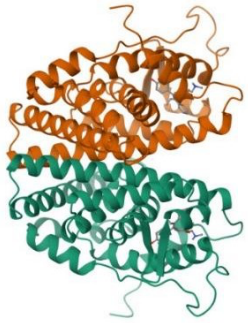

Figure 1. Structure of Estrogen Receptor Alpha (ER-α) (PDB ID: 3ERT)

The receptor preparation stage began by opening the .pdb file obtained from the Protein Data Bank using the File > Read Molecule command. The structure was then cleaned by removing water molecules (Edit > Delete Water) and the native ligand to obtain a purer protein structure. Water molecules and the native ligand were removed to prevent interference during the docking process and to reduce computational time (Herdini & Setyawati, 2023; Rachmania, 2019). Water molecules can form hydrogen bonds with the ligand, thereby complicating the simulation. In addition,

the native ligand bound to the active site could hinder the interaction of the test compounds (Ayuningrum, 2021). Therefore, the protein and the native ligand were separated using AutoDock Tools and saved again in .pdb format for use in the docking stage.

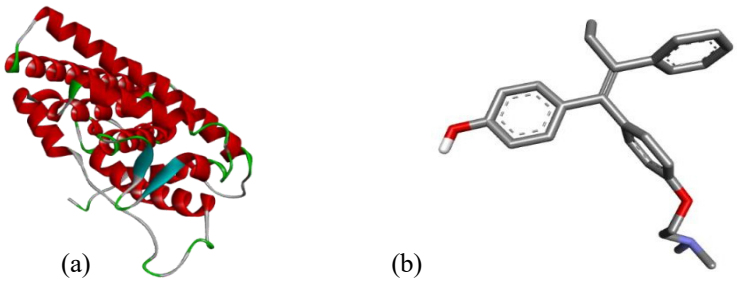

Figure 2. (a) Prepared protein and (b) prepared ligand

The test compounds that met Lipinski's criteria were downloaded from PubChem in .sdf format, which contains comprehensive molecular structural information. However, for docking simulations using Gnina, the .pdb format is required as it accurately represents the three-dimensional atomic coordinates. Since the .sdf format is generally not optimized for docking purposes, the files were converted to .pdb format using Discovery Studio.

Grid Box Determination

Determining the grid box is a crucial step in molecular docking as it defines the active site region of the protein where the ligand binds. In this study, a site-specific docking approach was employed by centering the grid box on the active site of the estrogen receptor alpha (PDB ID: 3ERT), based on the location of the native ligand 4-hydroxytamoxifen (4-OHT). This approach differs from blind docking, as it focuses the search on biologically relevant regions only. The active residues were identified through molecular visualization using Discovery Studio.
The center coordinates and grid box size were set using AutoDock Tools via the Grid > Grid Box menu. The grid box was adjusted to encompass all active residues, with parameters: center x = 29.626, center y = –0.543, center z = 29.985; size x = 24, size y = 18, size z = 30; and spacing = 1.00 Å.

Table 3. Grid Box Parameters

Receptor-Ligand	Grid Box Size						Spacing
	Center			Size			
	x	y	z	x	y	z	
EstrogenAlpha 4-OHT	29.626	-0.543	29.985	24	18	30	1.000

Docking Method Validation

The docking method was validated by redocking the native ligand, 4-hydroxytamoxifen, onto the estrogen receptor alpha (PDB ID: 3ERT) using the Gnina software. This step was performed to ensure the accuracy of the applied docking protocol. Validity was assessed based on the Root Mean Square Deviation (RMSD) value, with a threshold of less than 2.0 Å (Fauzi et al., 2024). The RMSD value reflects the agreement between the docked ligand conformation and the original conformation observed in the crystal structure (Nugroho & Fauzi, 2024; Puspitasari et al., 2024).

Table 4. Validation Results of Native Ligand Redocking

Receptor-Ligand	PDB Code	Binding Energy (kkal/mol	RMSD (Å)
Estrogen Alpha 4-OHT	3ERT	-9.89	1.026

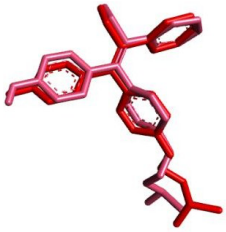

Figure 3. Visualization of native ligand and redocked ligand

The redocking results showed an RMSD value of 1.026 Å, indicating that the docking method used is valid and can be applied to test compounds from Asparagus officinalis. The visual overlay between the native ligand and the docked ligand demonstrated similar binding positions, further confirming the validity of the employed method.

Molecular Docking

Gnina is an advancement of AutoDock Vina that utilizes Convolutional Neural Network (CNN) technology to evaluate ligand poses based on 3D grid representations (Mcnutt et al., 2025). Gnina's advantages include automatic preparation features such as the addition of polar hydrogens, charge assignment, and removal of water molecules and native ligands. The docking process produces a binding affinity value (ΔG), which indicates the stability of the ligand–receptor interaction. The more negative the ΔG value, the stronger and more stable the binding formed (Puspita et al., 2022).

Table 5. Molecular Docking Results of Target Receptor with Test Ligands

No	Ligand	Binding Energy (kkal/mol)	Hydrogen Bonds
1	Ligan alami 4 Hidroksitamoksifen	-9.89	Arg394,Glu353,Asp351
2	p-Coumaricacid	-6.28	Glu353
3	Asparagusicacidsyn-S-oxide	-4.73	Arg394,Lys449,Glu353
4	Asparagusicacid	-4.33	Arg394
5	Dihydroasparagusic acid	-4.15	Arg394, Leu346
6	S-Acetyldihydroasparagusicacid	-4.76	Arg394, Leu346
7	Caffeicacid	-6.70	Leu346
8	Luteolin	-9.03	Ala350,Leu387, Glu353,Gly521
9	Taxifolin	-9.23	Leu387,Thr347
10	Thiamine	-7.11	Arg394, Glu353
11	Catechin	-9.59	Glu353, Glu419, Gly521
12	Naringenin	-9.10	Ala350,Leu387, Glu353,Gly521
13	AscorbicAcid	-5.45	Glu353, Leu346
14	mevalonicacid	-5.23	Leu346,Leu387
15	Linoleicacid	-6.54	Arg394, Glu353
16	PantothenicAcid	-5.25	Glu353
17	Coumarin	-6.50	Arg394
18	4-Hydroxybenzaldehyde	-5.38	Lys449, Glu353, Gly390,Ile386
19	Syringicacid	-5.89	Arg394,Glu353,Glu353
20	Vanillicacid	-5.60	Arg394,Glu353,Glu353
21	Vanillin	-5.39	Leu346,Leu387
22	Ferulicacid	-6.35	Leu387,Gly521, Leu346
23	Diosgenin	-7.94	Asp351
24	Sarsasapogenin	-8.28	Asp351
25	Yamogenin	-8.25	Asp351
26	Apigenin	-9.07	Ala350,Leu387, Glu353,Gly521
27	Kaempferol	-8.99	Leu387,Thr347
28	Quercetin	-8.98	Leu387,Thr347
29	Naringeninchalcone	-8.45	Arg394,Glu353, Leu387, Gly521
30	(+)-Dihydrokaempferol	-9.16	Leu387,Thr347
31	Glycerol-3-phosphate	-4.13	Glu353, Leu387
32	DXP	-5.17	Leu346,Leu387
33	2-C-Methyl-D-erythritol2,4-cyclodiphosphate	-5.24	Leu525
34	2-C-Methyl-D-erythritol4-phosphate	-5.18	Glu353,Leu387, Leu346
35	Adenosine	-7.50	Glu353, Glu419
36	Syringaldehyde	-5.23	Arg394, Glu353
37	Cinnamicacid	-6.04	Arg394,Gly390,Trp393
38	Nonadienal	-4.78	Arg394
39	Palmiticacid amide	-6.06	Arg394, Leu387
40	Glyceryl palmitate	-5.87	Arg394,Glu353,Phe404
41	Cytidine	-6.89	Glu353,Leu387, Gly521

42	Isopropylalcohol	-3.09	Glu353
43	2,3-Butanedione Diacetyl	-3.68	Arg394
44	Dimethylallyl pyrophosphate	-5.16	-
45	Isopentenyl Pyrophosphate	-4.56	Thr347,Leu346
46	3-Hydroxycinnamic acid	-6.31	-
47	2,6-Dimethylpyrazine	-4.63	Glu353
48	2-Heptenal	-4.16	-
49	2-Methylpyrazine	-3.72	-
50	Asparaptine	-6.25	Leu387,Glu353, Leu346
51	Blumenol C	-7.09	Glu353, Gly521
52	Glycerophosphoglycerol	-5.07	Arg394, Glu353
53	Phytosphingosine	-6.06	Met522,Met522
54	Asparenyol	-4.47	-
55	1,2,3-Trithiane-5-carboxylicacid	-4.59	Arg394,Glu353,Glu353
56	3-Palmitoyl-sn-glycerol-1-phosphorylcholine	-9.27	Tyr526,Glu380
57	Lysophosphatidylethanolamine C16:0	-8.06	Thr347,Asp351, Thr347,Asp351
58	Decadienal	-5.15	Arg394
59	3-Methyl thiopropanal	-3.04	Arg394
60	Furancarboxaldehyde	-4.26	Lys449, Ile386
61	2,3-Pentanedione	-4.27	Arg394, Arg394
62	3-Penten-2-one	-4.17	Arg394
63	1-Palmitoyl-sn-glyceryl3-phosphate	-7.32	Met522
64	Asparenyn	-4.41	-
65	1-O-Feruloyl-3-O-p -coumaroylglycerol	-8.58	Thr347,Leu387, Met522, Met522

Based on Table 5, a total of 65 compounds from Asparagus officinalis were docked, each generating nine binding poses. The pose with the most negative binding affinity value was selected as the best. The docking results showed binding affinities ranging from –3.04 to –9.59 kcal/mol. Nineteen compounds exhibited high affinity (–7.09 to –9.59 kcal/mol), with the top two being catechin and 3-PGPC. Although their affinities did not surpass that of the reference ligand 4- hydroxytamoxifen (–9.89 kcal/mol), both compounds demonstrated strong and biologically relevant interactions. Variations in affinity values among compounds are influenced by the type and number of interactions formed, particularly hydrogen bonds, hydrophobic interactions, and van der Waals forces (Kusuma, 2024). The greater and stronger the interactions, the more stable the ligand–receptor complex formed.

Catechin is a flavonoid widely found in plants, including Asparagus officinalis. Previous studies have shown that catechin exhibits antiproliferative activity against ER+ breast cancer cells through p53-mediated apoptosis pathways and downregulation of estrogen receptor expression (Xiang et al., 2016). An in silico study by Khudzaifi et al., (2024), also reported a binding affinity of –8.6 kcal/mol for catechin against ERα, supporting its biological potential as a natural anticancer agent. Meanwhile, the compound 3-PGPC lacks experimental data related to direct anticancer activity. Therefore, further studies, both in vitro and in vivo, are required to confirm its therapeutic potential, especially against breast cancer.

Visualization of Docking Results

Based on the binding affinity values, catechin and 3-PGPC were selected as the best test compounds due to their highest affinity toward the estrogen receptor alpha (ER-α). Subsequent molecular interaction analysis was performed to identify the types of bonds, key residues, and distances between interacting atoms. Both 2D and 3D visualizations were conducted using Discovery Studio to evaluate the involvement of important residues within the active site, such as Glu353, Arg394, Asp351, and Thr347.

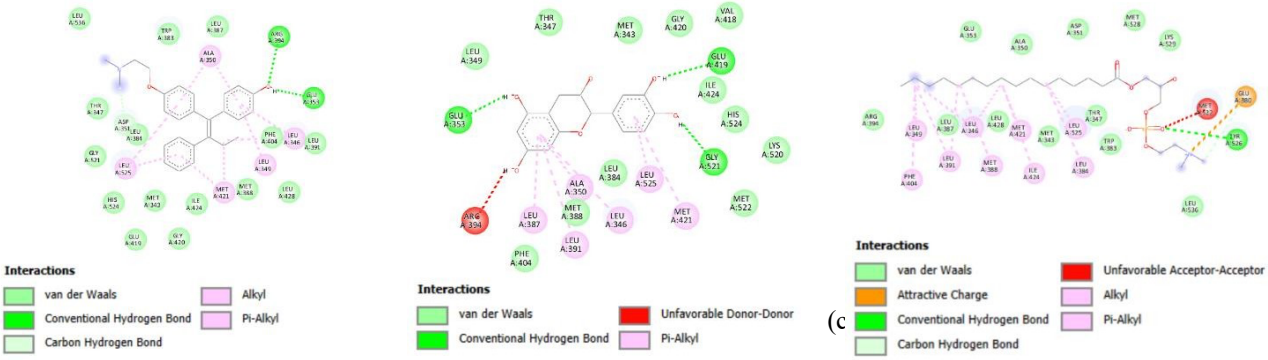

Figure 4. Visualization of interactions of (a) 4-OHT, (b) Catechin, and (c) 3-PGPC

Catechin forms hydrogen bonds with Glu353 and hydrophobic interactions with Thr347, whereas 3-PGPC exhibits electrostatic interactions with Glu353, as well as hydrophobic and hydrogen bonds with the other three key residues. This indicates that 3-PGPC better mimics the binding of the reference ligand (4-hydroxytamoxifen) in terms of involved residues. Catechin has the strongest hydrogen bond with a distance of 1.93 Å (Gly521), while the weakest bond is observed in 4-hydroxytamoxifen (Asp351, 3.68 Å).

Interaction with the His524 residue is known to determine agonist or antagonist activity. Both catechin and 3-PGPC do not form hydrogen bonds with His524, suggesting that they may possess antagonistic properties similar to tamoxifen, which can inhibit breast cancer cell proliferation. Both compounds exhibit high affinity and significant interactions with ER-α, and are derived from natural sources with potential minimal side effects

CONCLUSION

Of the 116 compounds identified from Asparagus officinalis, 64 met Lipinski's rule of five and demonstrated potential as oral drug candidates. The majority exhibited favorable ADMET profiles, including high absorption, good intestinal permeability, moderate distribution and elimination, and low interaction with CYP2D6 and CYP3A4. Most compounds were also non_mutagenic, making them suitable for further experimental investigation.

The molecular docking results revealed that 19 compounds had a strong binding affinity (– 7.09 to –9.59 kcal/mol). Catechin and 3PGPC showed the strongest affinity, closely approaching that of the comparative ligand, 4-hydroxytamoxifen (–9.98 kcal/mol). The interaction of these two compounds with key ER-α residues and the absence of hydrogen bonding with the His524 residue indicate that they act as antagonists against ER-α, which can inhibit the proliferation of breast cancer cells. These findings suggest that catechin and 3PGPC have the potential to be alternative ligands for ER-α and are worthy of further investigation as candidates for natural-based breast cancer drugs.

RECOMMENDATIONS

Based on the findings of this study, it is recommended to conduct further in vitro and in vivo validation of the active compounds that demonstrated strong affinity toward the estrogen receptor alpha, in order to experimentally confirm their potential biological activity.

AKNOWLEDGEMENTS

The authors would like to express their deepest gratitude to the Department of Pharmacy, Faculty of Sports and Health, Universitas Negeri Gorontalo, for providing facilities and guidance during the course of this research. Special thanks are extended to the academic supervisors for their valuable advice and constructive feedback in preparing and completing this study. The authors also sincerely appreciate all parties who contributed, directly or indirectly, to the successful completion of this research

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