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In Silico Study of Potential Active Compounds in Guava Leaves (*Psidium guajava* L.) Targeting Beta Estrogen for Breast Cancer Therapy

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Abstract

Breast cancer is a disease that is the main cause of death, especially in women, where malignant cells form in breast tissue. Some breast cancers are sensitive to hormone estrogen. Overexpression that occurs from an estrogen receptor will increase excessive proliferation. ER-ß was chosen as a therapeutic target protein because it can inhibit the proliferation and invasion of breast cancer cells and trigger apoptosis. In this *in silico* study, a test was carried out on the activity of the active compound content of potential secondary metabolites in Guava plants (*Psidium guajava* L.) in the leaves aimed at the Estrogen Beta receptor (ER-ß) for breast cancer therapy. The goal is to obtain new compound candidates to help pharmacological therapy of breast cancer. This can be seen from the results of re-docking of natural ligands (Genistein) and docking of fifty test ligands which are potential secondary metabolite compounds contained in Guava plants (*Psidium guajava* L.) in the leaves. The compound that has the greatest potential to activate ER-ß as an antiproliferation of breast cancer cells is Clionasterol.

Keywords: *Breast cancer, Psidium guajava L., ER-ß Agonist, molecular docking, clionasterol.*

INTRODUCTION

Cancer is classified as a non-infectious disease, characterized by the rapid and uncontrolled proliferation of cells or tissues. This abnormal growth can disrupt metabolic processes and may spread to other cells and tissues throughout the body (Hero, 2021; Susmini and Supriyadi, 2021). Breast cancer is the most frequently diagnosed malignancy among women worldwide, accounting for approximately 22% of all new cancer cases in females, and is the second leading cause of cancer-related mortality after lung cancer (Hero, 2021).

Estrogen receptor beta (ER-β) is one of the two primary estrogen receptors, along with ER-α, and is encoded by the ESR2 gene. ER-β mainly acts as a tumor suppressor in breast cancer by binding estrogen and inhibiting cell proliferation, invasion, and migration, as well as promoting apoptosis, as shown in Lazennec, *et al*. (2001), where its

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overexpression in ER-negative MDA-MB-231 cells reduced motility and altered morphology. Therefore, ER-β is regarded as a promising therapeutic target for breast cancer treatment. This is particularly relevant in the development of selective agents that modulate ER-β without activating ER-α, whose overexpression is associated with increased cellular proliferation. Such selectivity is expected to reduce hormonal side effects and prevent resistance commonly observed with conventional anti-estrogen therapies (Ozyurt and Ozpolat, 2022).

Compounds derived from natural materials have the potential to be developed into drugs. In addition to having pharmacological activity, natural material compounds are also known to have intrinsic toxicity and low side effects (Wink, 2015). Guava leaves are one of the natural materials that contain compounds that have the potential as anticancer therapy materials. In a study conducted by Dwitiyanti in 2018, it was found that 70% ethanol extract of guava leaves had cytotoxic properties against T47D breast cancer cells with an LC_{50} value at 24-h incubation of 27.54 µg/ml in a very toxic range of 5-50 µg/ml. These results indicate the potential of guava leaves as a cytotoxic drug (Dwitiyanti, 2015).

The discovery of medicinal plants that have the potential as anticancer is not only as cytotoxic but also has the potential as selective ER-β agonists. This was obtained through an in silico approach, such as molecular docking and virtual screening, which are important in efforts to discover and develop more effective and selective anticancer drug candidates. *In silico* studies require validated procedures to identify possible interactions of ligand compounds with receptors. In addition, *in silico* studies are also useful in identifying natural compounds against target proteins (Hardianto, *et al*., 2019).

METHODS

Materials and Tools

This study was conducted using an HP EliteBook 840 G5 laptop equipped with an 8th-generation Intel® Core™ i7-vPro-8650U CPU @1.90GHz 2.11GHz, 32 GB of RAM, and a 64-bit operating system. The software and databases utilized included the RCSB Protein Data Bank for receptor structure retrieval, PubChem as the source of compound structures, ChemDraw Ultra 12.0 for chemical structure drawing, and Chem3D Pro 12.0 for energy minimization processes. Additionally, DUD-E was used as a source of active and decoy compounds, and PreADMET (https://preadmet.webservice.bmdrc.org/) was used as a web-based server for ADME and toxicity analysis. BIOVIA Discovery Studio for preparation and visualization, and AutoDock Tools version 1.5.6 for molecular docking simulations. The materials used in this study included the estrogen receptor beta protein with PDB ID 1QKM, the native ligand, and the test compounds, all of which were essential components for the computational analysis and simulation. The test compounds were derived from guava leaves (*Psidium guajava* L.), which served as the source of active constituents for this research (Nursanty, *et al*., 2023).

Prediction of Physicochemical Properties

The active compounds in guava (*Psidium guajava* L.) were identified through a review of credible literature sources. The SMILES data of each compound were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and inputted into the SwissADME platform (http://www.swissadme.ch/index.php).Through SwissADME, physicochemical properties such as molecular weight, Log *P*, hydrogen bond donors,

and acceptors were predicted. The results were then evaluated using Lipinski's Rule of Five to determine the potential of each compound as an orally active drug (Daina, *et al*., 2017).

Prediction of Pharmacokinetic Properties and Toxicity

The pharmacokinetic (ADME) and toxicity profiles of active compounds were predicted using PreADMET based on parameters such as % HIA, CaCO2 permeability, % PPB, BBB penetration, Ames test, and rodent carcinogenicity. The 3D structures in .sdf format were retrieved from PubChem and inputted into PreADMET for analysis (Mardianingrum, *et al*., 2023).

Pharmacophore Screening

Active and decoy compound data from https://dude.docking.org/ were prepared using LigandScout and saved in .ldb format. Ten pharmacophore models were generated and validated using ROC curves, with the best model used to screen for potential hit compounds. The ROC curve graph is a curve reflecting the relationship between sensitivity and specificity (Luo, *et al*., 2021).

Docking Validation

Docking validation was performed using a redocking procedure with the native ligand (genistein) from the co-crystal structure of the receptor (PDB ID: 1QKM) (Sari, *et al*, 2020).

Molecular Docking

Protein structures were obtained from the RCSB PDB and prepared by removing native ligands with Biovia Discovery Studio and adding polar hydrogens and Kollman charges using AutoDock Tools. Test ligand SMILES were converted to 2D in ChemDraw, energy-minimized in Chem3D, and converted to 3D structures. Molecular docking was performed using AutoDock4 via command prompt. Results included binding energy, kI, cluster, and RMSD. Receptor–ligand interactions were visualized in 2D and 3D using Biovia Discovery Studio (Agu, *et al*., 2023).

Table 1. Lipinski rule of five (RO5) prediction results.

No	Name of Compound	Molecular Weight (<500 Da) (g/mol)	Log *P* (<5)	Hydrogen Bond		Result
				Donor (<5)	Acceptor (<10)	
1.	Quercetin	302.24	1.23	5	7	Acceptable
2.	Eucalyptol	154.14	2.58	0	1	Acceptable
3.	Pyrogallol	126.03	0.45	3	3	Acceptable
4.	α-Terpineol	154.14	2.58	0	1	Acceptable
5.	α-Copaene	204.19	4.84	0	0	Acceptable
6.	α-Muurolol	222.37	3.42	1	1	Acceptable
7.	Intermedeol	222.37	3.75	1	1	Acceptable
8.	Androstan-17-one, 3-ethyl-3-hydroxy-(5 alpha)	318.49	4.22	1	2	Acceptable
9.	Doconexent	328.49	6.55	1	2	Acceptable
10.	Isolongifolol	222.37	3.57	1	1	Acceptable
11.	β-Dihydroionone	194.31	3.28	0	1	Acceptable
12.	2-Hydroxy-2,4,4-trimethyl-3-[(1E)-3-methyl-1,3-butadienyl] cyclohexanone	222.32	2.77	1	2	Acceptable
13.	(-)-Epiglobulol	222.37	3.42	1	1	Acceptable
14.	Humulane-1,6-dien-3-ol	222.37	3.60	1	1	Acceptable
15.	Neophytadiene	278.52	7.07	0	0	Acceptable

Table 1. Lipinski rule of five (RO5) prediction results (continous).

No	Name of Compound	Molecular Weight (<500 Da) (g/mol)	Log *P* (<5)	Hydrogen Bond		Result
				Donor (<5)	Acceptor (<10)	
16.	Palmitic Acid	256.42	5.20	1	2	Acceptable
17.	Caryophyllene	204.19	5.34	0	0	Acceptable
18.	Aromadendrene	204.19	4.58	0	0	Acceptable
19.	Cadina-3,5-diene	204.19	5.15	0	0	Acceptable
20.	α-Humulene	204.19	4.37	0	0	Acceptable
21.	Alloaromadendrene	204.19	5.30	0	0	Acceptable
22.	γ-Muurolene	204.19	5.46	0	0	Acceptable
23.	β-Selinene	204.19	4.75	0	0	Acceptable
24.	α-Selinene	204.19	5.19	0	0	Acceptable
25.	α-Muurolene	204.19	6.18	0	0	Acceptable
26.	β-Bisabolene	204.19	6.25	0	0	Acceptable
27.	γ-Cadine	204.19	5.51	0	0	Acceptable
28.	trans-Calamenene	202.17	5.45	0	0	Acceptable
29	Naphthalene 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)	204.19	5.13	0	0	Acceptable
30.	Cyclohexane, 1,5-dimethyl-2,3-divinyl-	164.16	4.19	0	0	Acceptable
31.	Nerolidol	222.20	4.56	1	1	Acceptable
32.	Caryophyllene alcohol	222.20	4.47	1	1	Acceptable
33.	(-)-Globulol	222.20	4.74	1	1	Acceptable
34.	Viridiflorol	222.37	3.43	1	1	Acceptable
35.	(1R,3Z,7Z,11R)-1,5,5,8-tetramethyl-12-oxabicyclo[9.1.0] dodeca-3,7-diene	220.18	3.81	0	1	Acceptable
36.	1-Naphthalenol, decahydro-1,4a-dimethyl- 7-(1-methylethylidene)-,	222.20	4.31	1	1	Acceptable
37.	Epicubenol	222.20	4.61	1	1	Acceptable
38.	Caryophyllene oxide	220.18	4.53	0	1	Acceptable
39.	Cadinol	222.37	3.43	1	1	Acceptable
40.	Linoelaidic acid	280.45	5.45	1	2	Acceptable
41.	7-Tetradecenal (Z)	210.36	4.37	0	1	Acceptable
42.	α-tochopherol	430.71	8.29	1	2	Acceptable
43.	Clionasterol	414.71	7.24	1	1	Acceptable
44.	β-Longipinene	204.35	4.47	0	0	Acceptable
45.	Quinic acid	192.17	-1.63	5	6	Acceptable
46.	Apigenin-7-O-β-D-glucorono pyranoside	444.39	0.62	4	10	Not Acceptable
47.	Luteolin-7-O-glucuronide	462.36	-0.06	7	12	Not Acceptable
48.	Epigallocatechin (4β,8)-gallocatechin	610.52	0.67	11	14	Not Acceptable
49.	Naringenin4′-O-glucopyranoside	270.24	2.11	3	5	Acceptable
50.	Myricetin	318.24	0.79	6	8	Acceptable

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The results showed that the majority of compounds fulfilled the Lipinski criteria, indicating favorable drug-likeness properties. Specifically, most compounds exhibited molecular weights <500 Da, Log *P* values <5, hydrogen bond donors <5, and hydrogen bond acceptors <10. However, three compounds, namely Apigenin-7-O-β-D-glucuronopyranoside, Luteolin-7-O-glucuronide, and Epigallocatechin (4β,8)-gallocatechin did not comply with the Lipinski RO5 criteria. These compounds exceeded the acceptable limits in multiple parameters, particularly in molecular weight, hydrogen bond donors, and hydrogen bond acceptors. In addition, there are several compounds with acceptable status that do not meet one of the Lipinski parameters, such as Neophytadiene, α-tocopherol, and Clionasterol which show Log *P* values exceeding 5, which indicates high lipophilicity.

ADMETox Prediction Result

The ADMETox properties of the identified compounds are presented in Table 2, including Human Intestinal Absorption (HIA), Caco-2 permeability, Plasma Protein Binding (PPB), Blood-Brain Barrier (BBB) penetration, and toxicity parameters. Most compounds exhibited high absorption profiles with HIA values approaching 100% and favorable Caco-2 permeability indicating good intestinal absorption potential. In addition, most compounds exhibited high plasma protein binding and moderate to high BBB penetration. However, some compounds exhibited poor absorption characteristics. For example, Epigallocatechin (4β,8)-gallocatechin, Luteolin-7-O-glucuronide, and Apigenin-7-O-glucuronide exhibited low HIA values (≤35%) along with relatively low Caco-2 permeability indicating limited oral bioavailability. In terms of toxicity, a significant number of compounds were predicted to be mutagenic and exhibit carcinogenic potential in rats or mice. Compounds such as Eucalyptol, Doconexent, and Caryophyllene oxide exhibit mutagenic and carcinogenic properties. On the other hand, several compounds, including Nerolidol, α-Terpineol, and α-tochopherol, exhibit favorable ADMET profiles with high absorption and predicted lower toxicity.

Table 2. ADMETox prediction result.

No	Name of Compound	Absorption		Distribution		Toxicity		
		HIA (%)	Caco-2 (nm/sec)	PPB (%)	BBB	Mutagen	Carcinogen	
							Mice	Rat
1.	*Quercetin*	63.48	3.41	93.23	0.17	Mutagen	+	-
2.	*Eucalyptol*	100.00	21.89	100.00	1.46	Mutagen	+	+
3.	*Pyrogallol*	77.41	13.72	100.00	0.90	Mutagen	+	-
4.	*α-Terpineol*	100.00	50.80	23.41	5.11	Mutagen	-	-
5.	*α-Copaene*	100.00	23.63	100.00	11.14	Non-Mutagen	+	-
6.	*α-Muurolol*	100.00	55.40	100.00	9.21	Mutagen	-	-
7.	*Intermedeol*	100.00	55.68	86.27	9.24	Mutagen	-	+
8.	*Androstan-17-one, 3-ethyl-3-hydroxy-(5 alpha)*	95.62	33.27	100.00	6.61	Non-Mutagen	+	-
9.	*Doconexent*	97.87	32.32	98.82	7.43	Mutagen	+	+
10.	*Isolongifolol*	100.00	22.12	75.79	7.59	Mutagen	+	-
11.	*6-Dihydroionone*	100.00	50.81	87.59	2.09	Mutagen	-	-
12.	*2-Hydroxy-2,4,4-trimethyl-3-[(1E)-3-methyl-1,3-butadienyl] cyclohexanone*	95.17	40.17	85.44	2.15	Mutagen	+	+
13.	*(-)-Epiglobulol*	100.00	54.57	100.00	7.56	Non-Mutagen	+	-
14.	*Humulane-1,6-dien-3-ol*	100.00	52.77	100.00	10.25	Non-Mutagen	-	+
15.	*Neophytadine*	100.00	23.15	100.00	25.37	Non-Mutagen	+	-

Table 2. ADMETox prediction result.

No	Name of Compound	Absorption		Distribution		Toxicity		
		HIA (%)	Caco-2 (nm/sec)	PPB (%)	BBB	Mutagen	Carcinogen	
							Mice	Rat
16.	*Palmitic Acid*	98.29	26.07	100.00	8.21	Mutagen	+	-
17.	*Caryophyllene*	100.00	23.63	100.00	13.31	Mutagen	+	-
18.	*Aromadendrene*	100.00	23.49	100.00	11.36	Mutagen	+	-
19.	*Cadina-3,5-diene*	100.00	23.47	100.00	13.29	Mutagen	+	+
20.	*α-Humulene*	100.00	23.633	100.00	14.22	Non-Mutagen	+	+
21.	*Alloaromadendrene*	100.00	23.49	100.00	11.36	Mutagen	+	-
22.	*γ-Muurolene*	100.00	23.63	100.00	13.47	Mutagen	+	-
23.	*β-Selinene*	100.00	23.49	100.00	13.49	Mutagen	+	-
24.	*α-Selinene*	100.00	23.63	100.00	13.31	Mutagen	+	-
25.	*α-Muuolene*	100.00	23.63	100.00	13.29	Mutagen	+	+
26.	*β-Bisabolene*	100.00	23.40	100.00	15.06	Mutagen	+	-
27.	*γ-Cadine*	100.00	23.63	100.00	13.47	Mutagen	+	-
28.	*trans-Calamenene*	100.00	23.45	100.00	11.97	Mutagen	-	+
29.	*Naphthalene 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)*	100.00	23.64	100.00	13.29	Mutagen	+	+
30.	*Cyclohexane, 1,5-dimethyl-2,3-divinyl-*	100.00	23.19	98.96	9.91	Mutagen	+	-
31.	*Nerolidol*	100.00	26.61	100.00	13.98	Non-Mutagen	-	-
32.	*Caryophyllene alcohol*	100.00	46.24	100.00	9.40	Mutagen	-	+
33.	*(-)-Globulol*	100.00	54.57	100.00	7.56	Non-Mutagen	+	-
34.	*Viridiflorol*	100.00	54.57	100.00	7.56	Non-Mutagen	+	-
35.	*(1R,3Z,7Z,11R)-1,5,5,8-tetramethyl-12-oxabicyclo[9.1.0] dodeca-3,7-diene*	100.00	52.27	100.00	4.67	Mutagen	+	+
36.	*1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)-,*	100.00	55.65	85.61	9.61	Mutagen	-	+
37.	*Epicubenol*	100.00	55.40	100.00	9.57	Mutagen	-	+
38.	*Caryophyllene oxide*	100.00	56.34	90.84	3.75	Mutagen	+	+
39.	*Cadinol*	100.00	55.40	100.00	9.21	Mutagen	-	-
40.	*Linoelaidic acid*	98.37	28.08	100.00	7.31	Mutagen	+	+
41.	*7-Tetradecenal (Z)*	100.00	47.68	100.00	12.74	Non-Mutagen	+	-
42.	*α-tochopherol*	97.83	29.11	100.00	19.90	Non-Mutagen	-	-
43.	*Clionasterol*	100.00	52.37	100.00	19.883	Non-Mutagen	-	+
44.	*β-Longipinene*	100.00	23.49	100.00	11.18	Mutagen	+	-
45.	*Quinic acid*	20.28	8.27	6.59	0.42	Mutagen	+	-
46.	*Apigenin-7-O-β-D-glucorono pyranoside*	32.42	7.47	78.23	0.02	Mutagen	+	+
47.	*Luteolin-7-O-glucuronide*	15.78	4.84	77.45	0.02	Mutagen	+	+
48.	*Epigallocatechin (4β,8)-gallocatechin*	0.00	13.03	100.00	0.03	Non-Mutagen	+	-
49.	*Naringenin4'-O-glucopyranoside*	28.05	5.65	75.30	0.02	Mutagen	-	-
50.	*Myricetin*	40.96	0.99	96.78	0.11	Mutagen	+	-

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Pharmacophore Screening Results

The coordinate points and active pocket areas on the Estrogen Beta receptor (ER-β) in the grid box feature to determine the location of the natural ligand activity interacting with the receptor used. In Table 3, a grid box of positions is presented where the active side of the natural ligand (Genistein) binds to the selected target receptor. The grid box position is located at (x center: 22.333; y center: 8.098; z center: 113.228). This grid box will be used as a comparison during the docking process against the test compound ligand to determine the activity between the tested ligand and the selected receptor using the location of the active side activity of the natural ligand (Genistein).

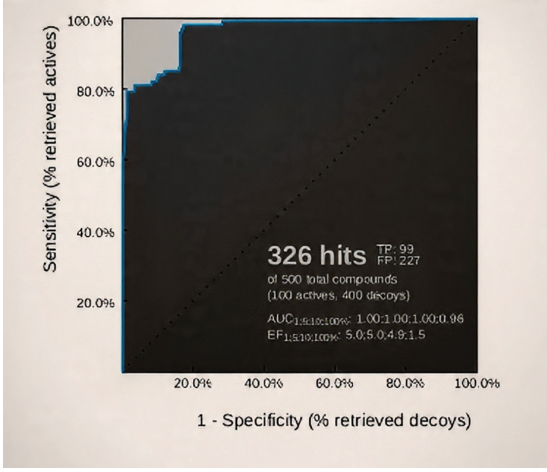


Figure 1. ROC curve of the eighth pharmacophore model.

Table 3. Estrogen Beta receptor (ER-ß) grid box size data.

Protein	PDB Code	Native Ligand	Grid Box Size					
			Centre X	Centre Y	Centre Z	Size		
						X	Y	Z
Estrogen Beta receptor (ER-β)	1QKM	Genistein	22.33	8.09	113.22	40	40	40

Docking Validation Results

Figure 2 shows the overlay between the natural ligand before docking (green) and after docking (blue). From the figure, it can be observed that the position and orientation of the ligand are still quite similar. This indicates that the docking process is able to reproduce the binding pose of the natural ligand in the active site.

This result is supported by the RMSD value in Cluster RMSD 0, which is 1.78 Å. Since the RMSD value is less than 2 Å, it can be considered that the docking method used in this study is acceptable

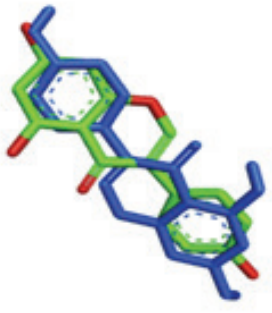


Figure 2. Overlay of natural ligand before (green) and after docking process (blue).

and has good accuracy. A low RMSD value means that there is only a small difference between the docked ligand and the original ligand structure. In addition, the similarity in structure suggests that the important interactions between the ligand and the active site are likely still maintained after docking. These interactions may include hydrogen bonds and hydrophobic interactions that play a role in stabilizing the ligand–protein complex.

Molecular Docking Results

Table 5 shows two- and three-dimensional visualization of the complex formed between the ligands Androstan-17-one, 3-ethyl-3-hydroxy-(5 alpha), Clionasterol, and 1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene) with the Beta Estrogen receptor (ER-β). In the Androstan-17-one, 3-ethyl-3-hydroxy-(5 alpha) compound of this complex forms one hydrogen bond with the amino acid residue, namely GLU A:305. Clionasterol compound and the Beta Estrogen receptor (ER-β). This complex forms one hydrogen bond from the amino acid residue, namely GLU A:305, nine van der Waals bonds, and 13 other amino acid bonds. The number of hydrogen bonds formed is less compared to the natural ligand complex which forms three hydrogen bonds. And finally for the 1-Naphthalenol complex, decahydro-1,4a-dimethyl-7-(1-methylethylidene) shows one hydrogen bond with the amino acid residue MET A:336, 4 Van der Waals interactions, and 11 other interactions with amino acid residues.

Table 4. Molecular docking simulation output.

No	Name of Compound	Cluster	Binding Energy (kkal/mol)	Ki (uM)	Interactions with Amino Acids		
					Hydrogen Bond	Van der Waals Bond	Others
1.	Genistein (Native ligand)	0.0	-9.61	89.72	Leu A:339 Leu A:475 His A:298	-	**Pi Sigma** Met A:336 **Pi Sulfur** Met A:340 **Pi-Pi T-Shaped** Phe A:356 **Pi-Alkyl** Ala A:302 Leu A:343 Leu A:476
2.	Androstan-17-one, 3-ethyl-3-hydroxy-(5 alpha)	0.0	-10.67	15.05	Glu A:305	Val A:487 Thr A:299 Leu A:476 Gly A:472 Arg A:346 Ile A:376 Met A:340 Leu A:343	**Pi-Alkyl/Alkyl** Ile A:373 Phe A:356 Leu A:298 His A:475 Met A:295 Met A:336 Ala A:302 Leu A:339 Leu A:301

Table 4. Molecular docking simulation output (continous).

No	Name of Compound	Cluster	Binding Energy (kkal/mol)	Ki (uM)	Interactions with Amino Acids		
					Hydrogen Bond	Van der Waals Bond	Others
3.	Clionasterol	0.0	-10.45	21.99	Glu A:305	Gly A:472 Phe A:377 Leu A:380 Arg A:346 Leu A:301 Trp A:335 Val A:487 Met A:479 Thr A:299	**Pi-Alkyl/Alkyl** Leu A:476 Met A:336 Val A:484 Met A:295 His A:475 Ile A:373 Ile A:376 Leu A:298 Phe A:356 Leu A:343 Met A:340 Leu A:339 Ala A:302
4.	1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)-	0.0	-9.69	423.24	Met A: 336	Arg A:346 Glu A:305 Trp A:335 Met A: 295	**Pi-Alkyl/Alkyl** Leu A:343 Leu A:301 Met A:340 Phe A:356 Leu A:339 Leu A:298 Leu A:380 Ile A: 376 Ala A:302 Ile A:373 Phe A:377

DISCUSSION

The results of Lipinski's rule of five predictions for 50 compounds are shown in Table 1. Among the 50 secondary metabolites found in guava leaves, 47 compounds met Lipinski's rule, while 3 compounds did not. The parameters of hydrogen bond donors and acceptors are crucial as they illustrate a compound's capability to create hydrogen bonds. Compounds with elevated hydrogen bonding typically exhibit reduced passive membrane permeability, which can hinder oral absorption. Furthermore, physicochemical characteristics like molecular weight and polarity are commonly utilized during the preliminary stages of drug-likeness evaluation (Coimbra, *et al*., 2021).

After evaluating the physicochemical properties, the 50 compounds were further analyzed based on their absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles. The ADMET prediction results are presented in

Table 5. Interactions of selected compound with estrogen receptor beta (ER-ß).

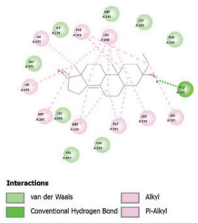
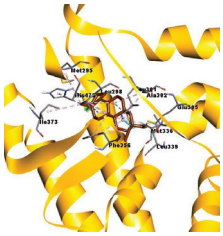
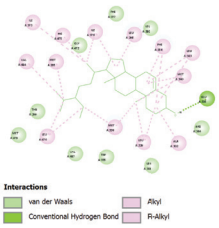
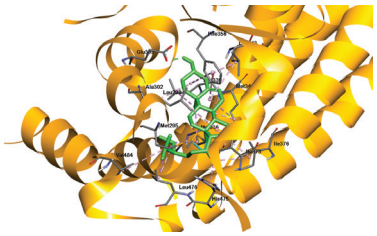
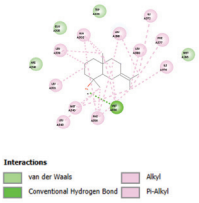
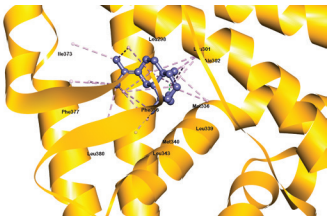
Compound	Dua-Dimensional Visualization	Three-Dimensional Visualization
Androstan-17-one,3 -ethyl-3-hydroxy-(5 alpha)		
Clionasterol		
1-Naphthalenol, decahydro-1,4a-dimethyl-7 -(1m ethylethylidene)		

Table 2. In this study, HIA was used to describe the potential absorption of compounds in the intestine. Caco-2 permeability was also evaluated because it is widely used to predict the ability of compounds to pass through intestinal epithelial cells (Rodrigues & Failla, 2021). BBB penetration was included to estimate whether the compounds could enter the brain (Wu, *et al*., 2023). Because breast cancer treatments do not primarily target the central nervous system, compounds with low BBB penetration are preferred. PPB was also analyzed to estimate the extent of compound binding to blood proteins, as this can affect drug distribution in the body (Wanat, 2020).

According to Table 2, toxicity data were also evaluated, including mutagenicity and carcinogenicity. The mutagenicity analysis showed that 13 compounds were predicted to be non-mutagenic. Meanwhile, the carcinogenicity prediction showed that 14 compounds were non-carcinogenic in rats and 21 compounds were non-carcinogenic in mice. Based on these results, it can be concluded that some test ligands may still have carcinogenic potential in experimental animals.

In pharmacophore modeling, ten models were generated, and the eighth model, as shown in Figure 1, was selected as the best model based on its ROC value (0.96), which was close to 1. The selected pharmacophore model consisted of key chemical features such as hydrogen bond donors, hydrogen bond acceptors, hydrophobic regions, and aromatic rings, which are important for molecular recognition and ligand–target interactions. Subsequently, this validated pharmacophore model was used for virtual screening of 50 secondary

metabolite compounds. The compound with the highest fit score was identified as humulane-1,6-dien-3-ol. However, pharmacophore screening was used only as an initial filtering step and was not sufficient as the sole criterion for compound selection. Therefore, further evaluation was carried out using molecular docking and ADMET analysis. Although humulane-1,6-dien-3-ol showed the highest pharmacophore fit score, it was not prioritized due to its less favorable docking performance and ADMET profile compared to the selected compounds.

Molecular docking was conducted to predict interactions between ligands and the Estrogen Receptor Beta (ER-β), following [3.1] pharmacophore screening. As an efficient and cost-effective *in silico* method, docking aids in identifying compounds with potential pharmacological activity, beginning with ligand and receptor preparation.

There are several parameters that need to be collected as data in the docking process, namely the Cluster RMSD of the natural and test ligand, Binding Energy, inhibition constant (Ki), and interactions with amino acids including hydrogen bonds, van der waals, and other types of bonds. In natural ligands, the RMSD value must be <2 Å because this value indicates that the natural ligand used has a similar structure to this protein and indicates the error of the calculation results is smaller. For the test ligand, the Cluster RMSD taken is when the test ligand has a Cluster RMSD 0 (Amrulloh, *et al*., 2023).

The docking results are obtained in the form of .dlg file, which contains data for the relevant parameters mentioned earlier. The resulting molecular docking interactions can also be visualized in both two-dimensional and three-dimensional formats using BIOVIA Discovery Studio, allowing for the observation of predicted binding interactions between the test ligands and the selected receptor. Based on binding energy values, the top three compounds were selected and compared with the native ligand, as presented in Table 4. Table 4 shows the results of the validation method (redocking) of natural ligands, namely genistein and the test ligands contained in *Psidium guajava* L.

Each ligand shows a negative binding energy, which represents the strength of its binding to the receptor. The ligands androstan-17-one, 3-ethyl-3-hydroxy-(5 alpha), the ligand Clionasterol, and the ligand 1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene) have lower binding energy compared to the natural ligand, genistein. This means that the bonds formed between the three test ligands with the ER-β are more spontaneous and do not require much energy to bind compared to their natural ligands. In addition, the inhibition constant (Ki) of the ligands Androstan-17-one, 3-ethyl-3-hydroxy-(5 alpha) and the ligand Clionasterol are smaller than the natural ligands, indicating that these compounds are effective in inhibiting the receptor at low doses and indicating that the bond between the ligand and the ER-β receptor is stronger and more stable. Thus, both test ligands, Androstan-17-one, 3-ethyl-3-hydroxy-(5 alpha) and Clionasterol compounds have greater potential than natural ligands in interacting with ER-β receptors. However, the effectiveness of ligands in inhibiting ER-β receptor activity is not only determined by the bond energy and Ki value, but is also influenced by the type and number of molecular interactions formed between the ligand and the receptor. This interaction occurs on the active side of the receptor through various types of bonds, such as hydrogen bonds, hydrophobic interactions (Van der Waals forces), alkyl and bonds, and pi-alkyl bonds (Saputra, *et al*., 2024).

As a comparison, the complex formed between the natural ligand genistein and the ER-β receptor forms three hydrogen bonds, namely with amino acid residues Leu A: 339, Leu A: 475, and His A: 298. Hydrogen bonds are specific close-range interactions and reflect strong

electrostatic interactions. These three hydrogen bonds contribute to the strength of the natural ligand-receptor complex formed (Muchtaridi, *et al*., 2018).

Clionasterol has a unique structure as a phytosterol from the poriferast-5-ene group, with a β-hydroxy substituent at the C-3 position, which allows for stable hydrophobic interactions with receptors. Several previous studies have shown that phytosterol compounds, such as Clionasterol, are able to induce apoptosis and cell cycle arrest in cancer cells. Therefore, Clionasterol has the potential to be a candidate for anticancer agents, although its hydrogen bond interactions are not as strong as natural ligands such as genistein. As for other compounds, namely the ligand Androstan-17-one, 3-ethyl-3-hydroxy-(5 alpha) is most likely not a common guava metabolite because there is no strong evidence that this specific compound is a natural metabolite in guava plants. This compound can be detected in guava due to contaminants from other organisms detected in the GC-MS analysis results because plants do not produce Androstan compounds naturally and adrostan is an important and diverse group of steroid hormone molecular species (Van Eenoo and Delbeke, 2006; Alemany, 2022).

Based on the results of the binding energy and inhibition constant (Ki) values, the Clionasterol ligand shows a higher potential pharmacological activity in inhibiting the ER-β compared to natural ligands, making it a potential candidate for the most effective ligand. In addition, this ligand meets the RO5 criteria and shows good absorption ability, moderate permeability, high potential for penetration into the blood-brain barrier, and is non-mutagenic. Based on the results of pharmacophore screening, it shows that this ligand produces a match with the pharmacophore model built from the receptor. However, the predicted toxicity of this ligand is carcinogenic to mice, so this aspect needs to be considered further in compound development.

CONCLUSION

Based on Lipinski's Rule of Five, ADMET, and molecular docking of 50 compounds from guava leaves (*Psidium guajava* L.), clionasterol demonstrated the highest potential as an ER-β agonist. It showed a binding energy of −10.45 kcal/mol and an Ki of 21.99 μM, indicating stronger affinity than the natural ligand genistein, which exhibited a binding energy of −9.61 kcal/mol and Ki of 89.72 μM.

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