Jurnal Ilmiah Farmako Bahari
Journal Homepage : https://journal.uniga.ac.id/index.php/JFB

IN SILICO STUDY: SECONDARY METABOLITES FROM JAMBOLAN (*Syzygium cumini* L.) AS POTENTIAL BREAST CANCER TREATMENTS

Meilia Suherman, Effan Cahyati Junaedi*, Riska Prasetiawati, Ade Rena Purnamasari

Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Garut, Jl. Jati No.42B, Tarogong Kaler, West Java, 44151, Indonesia

*Corresponding author: Effan Cahyati Junaedi (effan@uniga.ac.id)

ARTICLE HISTORY

Received: 3 November 2023 | Revised: 12 July 2024 | Accepted: 23 July 2024

Abstract

Breast cancer ranks second in world cancer incidence rates in 2020, contributing to 2,261,419 new cases, or 11.7% of all new cancer cases globally. The search for cancer drugs that work selectively continues to be encouraged to obtain safe and effective therapy, particularly those derived from medicinal plants. Jambolan is a plant that can thrive in both subtropical and tropical climates, including Indonesia. Jambolan (*Syzygium cumini* L.) has 89% antioxidant activity and 69% cytotoxic activity against T47D cells. Pharmacophore modelling and molecular docking were used to study the binding of 117 active jambolan drugs to alpha and beta estrogen receptors. Rutin was found to be potentially selective for ER-β receptors, with a fit score of 53.13. Molecular docking to ER-β revealed that rutin has breast cancer activity with a free bond energy value of -10.5 kcal/mol and better conformation and affinity than native ligands (genistein). It also binds to essential amino acids as an anticancer breast at ARG 346 and GLU 305. Lipinski's rule of five prediction results and in silico ADMET prediction from rutin yielded results that met the candidate drug's parameters. Rutin is a potential therapeutic option for treating breast cancer by targeting the ER-β receptor.

Keywords: breast cancer, in silico study, Syzygium cumini, virtual screening

Introduction

Breast cancer is the most prevalent cause of mortality in women and one of the cancers with the highest prevalence. According to GLOBOCAN data, around 2.3 million women were diagnosed with breast cancer in 2020, outnumbering lung cancer diagnoses, and approximately 658,000 women died as a result of breast cancer.[1] Breast cancer has the highest frequency in Indonesia, with approximately 65,858 cases, while the death rate is second only to lung cancer, with approximately 22,430 fatalities attributable to breast cancer.[2] According to the Indonesian Ministry of Health, in 2022, over 70% of breast cancer is detected at an advanced stage, resulting in a high death rate, even though approximately 43% of the death rate can be decreased if patients do routine early detection and minimize cancer risk factors.[3] Breast cancer develops in epithelial cells in the ductus (85%) and lobules (15%) of glandular breast tissue. Breast

ISSN: 2087-0337
E-ISSN: 2715-9949

cancer usually causes no symptoms and spreads slowly. In situ, cancer can spread to the lymph nodes or other organs near the breast over time.[4]

Because of the numerous adverse effects of breast cancer treatments, including chemotherapy, which frequently fails because anticancer medications have poor selectivity, the therapy is currently being researched. Therefore, finding new anticancer potential is essential to overcoming the side effects of current cancer medications, including natural medicine.[5]

Jambolan (*Syzygium cumini* L.) is a plant commonly found and utilized as an alternative medicine because of its antioxidant, antidiabetic, cytotoxic, antibacterial, anti-inflammatory, and anticoagulant properties. 8-10 Several sections of the plant have been researched based on Syzygium cumini L. fruit extract, which suppresses the proliferation of breast cancer cells.[6] Fraction of the ethanol extract of *Syzygium cumini* L. leaves had 83% antioxidant activity and 69% cytotoxic activity in suppressing the proliferation of T47D breast cancer cells.[7] However, these studies have not identified active compounds that can act as an anti-breast cancer agent, so more in silico research is required to identify anti-breast cancer drugs against several target receptors, namely ER-α and ER-β, by determining whether the compounds found in jambolan have high affinity for the above receptors.

Method

Material

One hundred seventeen structures of secondary metabolites of jambolan were obtained from the site https://pubchem.ncbi.nlm.nih.gov. The PDB format of the breast cancer receptor, PDB ID 3ERT for ER-α and PDB ID 1QKM for ER-β receptor-ligand complex, both have resolutions of 1.90 Å and 1.80 Å, respectively, were obtained from the https://rcsb.org website. Hardware: M41TVI0 with Intel(R) Celeron(R) CPU N3050 @ 1.60GHz processing characteristics, 2.00GB RAM, and 250GB SSD with Windows 10 Pro 64-bit operating system, aided by an internet connection utilized to operate many apps and websites online. DESKTOP-0A91EGA is also associated with specs: Intel® Core(T) i5 CPU 750 @2.67GHz, RM 8.00 GB operating system Windows 10 Pro. Software: Discovery Studio Visualizer®, MarvinSketch Version 17.2.13®, Autodock Tools®, Autodock Vina®, KNApSAck (http://www.knapsackfamily.com/KNApSAcK/), PASS (Prediction of Activity Spectra for Substances) Online (http://way2drug.com/passonline/predict.php), Liganscout, PreADMET (http://preadmet.bmdrc.org/), PubChem (http://pubchem.ncbi.nlm.nih.gov), Lipinski's Rule of Five (http://www .scfbio-iitd.res.in/software/drugdesign/lipinski.jsp), and the Protein Data Bank (PDB) (https://www.rscb.org/).

Compound Library Selection and Ligand Preparation

Jambolan compound was obtained from several library sources and was determined by searching the KNApSAcK online database at http://www.knapsackfamily.com/KNApSAcK/.

Screening Activity

The PASS Online prediction site was used to assess the anti-breast cancer activity of the jambolan compounds.

ISSN: 2087-0337
E-ISSN: 2715-9949

Geometry Optimization

The three-dimensional shape of the secondary metabolites of jambolan was achieved using geometric optimization with MarvinSketch and the semi-empirical AM1 method.

Lipinski's Rule of Five Testing

The ligands investigated in this study were the secondary metabolites of jambolan. After redrawing the ligands in ChemDraw® Ultra 12.0 and minimizing the energy in Chem3D® Pro 12.0, the files were saved in the pdb format. After the preparation, Lipinski's Rule of Five was used to determine the compound's physicochemical qualities.

Pre-ADMET Testing

The experiments were performed to investigate the initial characteristics of pharmacokinetics, such as absorption and distribution, and toxicity testing, such as drug mutagenic and carcinogenic properties. Testing uses a unique online tool at http:// preadme.bmdrc.kr. After drawing the test substance's structure, click the "Submit for Analysis" button. The obtained data is in pdb format.

Pharmacophore Modeling

Pharmacophore modelling is utilized to find and extract the potential interactions between ligand-receptor complexes. The Ligandscout program was used to conduct the testing. Target receptors from the PDB, standard compounds from the binding database, and active and decoy compounds from DUD-E must first be prepared. Following the discovery of the receptor's pharmacophore and validation of the pharmacophore model, the ROC curve—which displayed the hit molecule and the AUC value—was produced. Screening the test ligands after validation generated a list of hit compounds and pharmacophore fit scores.

Protein Selection and Preparation

Receptors were downloaded from the PDB website (https://www.rscb.org/) with PDB ID 3ERT for ER-α and PDB ID 1QKM for ER-β receptors. The receptor-ligand complex was separated and prepared using Autodock Vina® software before validation.

Molecular Docking

Redocking natural ligands on the ER-α and ER-β receptors served as a test for the docking technique. When doing molecular docking, previously established parameters are used. Canonical SMILES of the test drug were acquired from the Pubchem website (http://pubchem.ncbi.nlm.nih.gov). Autodock Vina® and Discovery Studio Visualizer software® were used for optimization, docking, and visualization. The enhanced 3D ligand structures were bound to the active sites of ER-α and ER-β. The binding energy (G) and interaction poses were computed from the results.

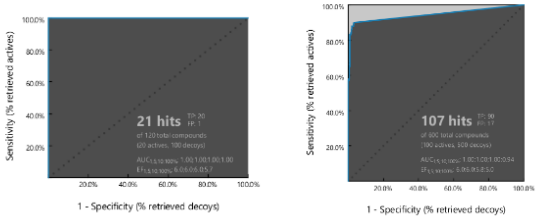
Molecular Dynamic

The open MM MD simulation stage entails building an MD folder with proteins and ligands in pdb format. The first step is to set up the program and connect Google Colab to Google Drive, which holds the folder used during the MD process, ensuring that all data is immediately saved. The next stage is to create a topology by applying the GAFF2 force field to the Ligand and the ff19sb force field to the protein, followed by solvation with the TIP3 model and a cubic-shaped box. Aside from that, neutralization was carried out by adding Na+ and Cl- ions, followed by minimization, equilibration, and production for 15 ns at 310K. RMSD, RMSF, and Radius of Gyration are the parameters utilized in the MD simulation.

ISSN: 2087-0337
E-ISSN: 2715-9949

Result

ER-α
AUC: 1.00
GHScore: 0.95



ER-β
AUC: 0.94
GH Score: 0.83

Figure 1. Pharmacophore validation results

Table 1. Pharmacophore Study Results Against ER-α

No	Compound	Matching Features	*Fit Score*
1.	Peonidin 3,5-diglicoside		60.78
2.	Cyanidin		60.66
3.	Coniferyl alcohol		59.82
4.	Syzygiresinol A		59.46
5.	Eugenol		54.88
6.	Rosmanol		54.32
7.	Kaempferol		53.10
8.	Quercetin		52.93
9.	Catechin		52.88
10.	Malvidin		52.76
11.	Delphinidin 3-gentiobisode		52.73
12.	Syzygiresinol B		52.47
13.	isoquercetin		52.42
14.	Isorhamnetin 3-rutinoside		52.19
15.	Chlorogenic acid		51.82
16.	Myricetin 3-robinobioside		51.51
17.	Petunidin		51.45
18.	Rutin		50.88
19.	Oleanolic acid		44.38

Table 2. Pharmacophore Study Results Against ER-β

No	Compound	Matching Features	*Fit Score*
1.	Chlorogenic acid		58.28
2.	Peonidin 3.5-diglicoside		58.10
3.	Naringin		57.73
4.	Syzygiresinol B		57.58
5.	Syzygiresinol A		56.49
6.	Isoquercetin		56.43
7.	Catechin		54.04
8.	Rutin		53.13
9.	Kaempferol		46.82
10.	Quercetin		46.62

ISSN: 2087-0337
E-ISSN: 2715-9949

Table 2. (Extension)

No	Compound	Matching Features	*Fit Score*
11.	Malvidin		46.43
12.	Petunidin		46.42
13.	Cyanidin		45.60
14.	Myricetin 3-robinobioside		44.66
15.	Oleanolic acid		44.41
16.	Isorhamnetin 3-rutinoside		44.29
17.	Delphinidin 3-gentiobioside		44.03
18.	Betulinic acid		43.88

Figure 2. Superimposition of the X-ray crystal structure (green) and docked conformation (pink) (left: 3ERT, right: 1QKM)

Table 3. Size of Grid Centre, Grid Box and RMSD Values from Docking Validation Results

PDB ID	Grid Center			Grid Box			RMSD
	X	Y	Z	X	Y	Z	
3ERT	30.282	-1.913	24.206	40	40	40	1.1055 Å
1QKM	22.438	8.003	113.538	40	40	40	0,2938 Å

Table 4. Results of Molecular Docking of ER-α and Analysis of Linked Amino Acid Residues

No.	Compound	ΔG (kcal/mol)	Hydrogen Interaction
1.	4-hidroxytamoxifen (native ligand)	-9.8	**ARG A:394, GLU A:353** (Conventional hydrogen)
2.	Rutin	-9.7	ASP A:351, THR A:347, CYS A:530, LEU A:536 (Conventional hydrogen) LYS A:529 (Carbon hydrogen)
3.	Delphinidin 3-gentiobioside	-9.5	MET A:522, LEU A:536, GLU A:380, ASP A:351 (Conventional hydrogen)
4.	Isorhamnetin 3-rutinoside	-9.4	ASP A:351, CYS A:530 (Conventional hydrogen) PRO A:535 (Carbon hydrogen)
5.	Oleanolic acid	-9.3	-
6.	Syzygiresinol B	-9	CYS A:530 (Conventional hydrogen)
7.	Myricetin-3-robinobioside	-8.9	CYS A:530, ASP A:351, LEU A:536 (Conventional hydrogen)

ISSN: 2087-0337
E-ISSN: 2715-9949

Table 4. (Extension)

No.	Compound	ΔG (kcal/mol)	Hydrogen Interaction
8.	Kaempferol	-8.8	**ARG A:394, GLU A:353** (Conventional hydrogen)
9.	Quercetin	-8.8	**ARG A:394, GLU A:353** (Conventional hydrogen)
10.	Cyanidin	-8.7	**GLU A:353**, LEU A:346, **ARG A:394**, LEU A:387, GLU A:419, GLY A:521, GLY A:420 (Conventional hydrogen)
11.	rosmanol	-8.7	LEU A:536, ASP A:351 (Conventional hydrogen)
12.	isoquercetin	-8.6	MET A:522, LEU A:536, VAL A:534 (Conventional hydrogen)
13.	Petunidin	-8.5	TRP A:393, **ARG A:394,** GLY A:390 (Conventional hydrogen) GLU A:353 (Carbon hydrogen)
14.	Syzygiresinol A	-8.5	TYR A:526 (Carbon hydrogen)
15.	Catechin	-8.4	LEU A:536, TRP A:383 (Conventional hydrogen)
16.	Chlorogenic acid	-8.4	GLY A:521 (Carbon hydrogen)
17.	Malvidin	-7.9	-
18.	Eugenol	-6	PRO A:325, **ARG A:394** (Conventional hydrogen) ILE A:326, GLY A:390 (Carbon hydrogen)
19.	coniferyl alcohol	-5.9	**ARG A:394** (Conventional hydrogen) PRO A:325 (Carbon hydrogen)

Table 5. Results of Molecular Docking of ER-β and analysis of linked amino acid residues

No.	Compound	ΔG (kcal/mol)	Hydrogen Interaction
1.	Rutin	-10.5	**GLU A:305**, GLU A:276, LYS A:401, **ARG A:346,** TYR A:397, HIS A:279, TRP A:345, HIS A:394 (Conventional hydrogen) GLU A:276, HIS A:394 (Carbon hydrogen)
2.	Genistein (native Ligand)	-10.4	LEU A:339, **GLU A:305, ARG A:346**, HIS A:475 (Conventional hydrogen)
3.	Myricetin-3-robinobioside	-10	PRO A:358, VAL A:280, **GLU A:305,** HIS A:279, TRP A:345 (Conventional hydrogen), ARG A:346 (Carbon hydrogen).

ISSN: 2087-0337
E-ISSN: 2715-9949

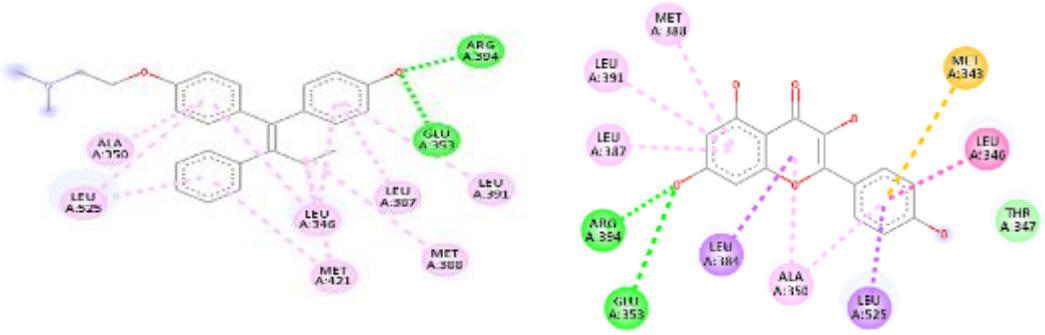
Table 5. (Extension)

No.	Compound	ΔG (kcal/mol)	Hydrogen Interaction
4.	Naringin	-10	VAL A:280, HIS A:279, TRP A:345 (Conventional hydrogen), HIS A:394, PRO A:358 (Carbon hydrogen)
5.	Isorhamnetin 3-rutinoside	-9.5	HIS A:279, VAL A:280, PRO A:358, **GLU A:305** (Conventional hydrogen) ARG A:346 (Carbon hydrogen)
6.	Delphinidin 3-gentiobioside	-9.5	PRO A:358, **ARG A:346,** VAL A:280, **GLU A:305,** TRP A:345 (Conventional hydrogen), HIS A:279 (Carbon hydrogen)
7.	Syzygiresinol B	-9.2	TYR A:397, LYS A:401, **ARG A:346, GLU A:305** (Conventional hydrogen), GLY A:342, TRP A:345 (Carbon hydrogen)
8.	Quercetin	-9.2	VAL A:280, HIS A:279, **GLU A:305,** TYR A:397, LYS A:401, GLU A:276 (Conventional hydrogen), PRO A:358 (Carbon hydrogen)
9.	Kaempferol	-9.1	HIS A:279, VAL A:280, LYS A:401, TYR A:397, GLU A:276 (Conventional hydrogen), PRO A:358 (Carbon hydrogen)
10.	Syzygiresinol A	-9.0	TYR A:397, LYS A:401, ARG A:346 (Conventional hydrogen), GLY A:342, TRP A:345 (Carbon hydrogen).
11.	Cyanidin	-8.9	HIS A:279, VAL A:280, GLU A:305, GLU A:276, LYS A:401 TYR A:397 (Conventional hydrogen) PRO A:358 (Carbon hydrogen)
12.	Isoquercetin	-8.4	LYS A:401, TYR A:397, GLU A:276, TRP A:345, HIS A:279 (Conventional hydrogen), HISA:279 (Carbon hydrogen dan Pi-donor hidrogen)
13.	Oleanolic acid	-8.4	TRP A:345 (carbon-hydrogen)
14.	Petunidin	-8.4	TRP A:345, VAL A:280, GLU A:305 (Conventional hydrogen), HIS A:279, VAL A:338 (Carbon hydrogen dan Pi-donor hidrogen)
15.	Catechin	-8.2	VAL A:280, PRO A:278, TYR A:397, GLU A:276, LYS A:401 (Conventional hydrogen), HIS A:279 (Carbon hydrogen)

ISSN: 2087-0337
E-ISSN: 2715-9949

Table 5. (Extension)

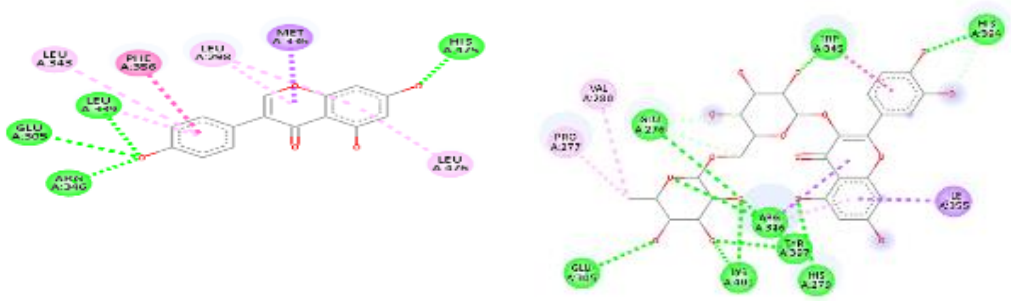
No.	Compound	ΔG (kcal/mol)	Hydrogen Interaction
16.	Peonidin 3.5-diglicoside	-7.9	TRP A:345, GLU A:276, TYR A:397, ARG A:346, VAL A:280 (Conventional hydrogen), PRO A:358, PRO A:277, HIS A:279, TRP A:345, ARG A:346 (Carbon hydrogen dan Pi-donor hidrogen)
17.	Chlorogenic acid	-7.8	LYS A:401, ARG A:346, VAL A:280, PRO A:277 (Conventional hydrogen) PRO A:358, HIS A:279 (Carbon hydrogen dan Pi-donor hydrogen)
18.	Malvidin	-7.5	GLU A:305, PRO A:278, LYS A:401 (Conventional hydrogen) GLU A:276 (Carbon hydrogen)
19.	Betulinic acid	-7.4	-



ER-α-4OHT
Docking score: -9.8
Hydrogen interaction: ARG 394, GLU 353

ER-α-Kaempferol
Docking score: -8.8
Hydrogen interaction: ARG 394, GLU 353

Figure 3. Results or ER-α docking with natural ligands and kaempferol



ER-β-Genistein
Docking score: -10.4
Hydrogen interaction: ARG 346. GLU 305, LEU 339, HIS 475

ER-β-Rutin
Docking score: -10.5
Hydrogen interaction: GLU 305, ARG 346, GLU 276, LYS 401, TYR 397, HIS A:279, TRP 345, HIS 394

Figure 4. Results of ER-β docking with natural ligands and rutin

ISSN: 2087-0337
E-ISSN: 2715-9949

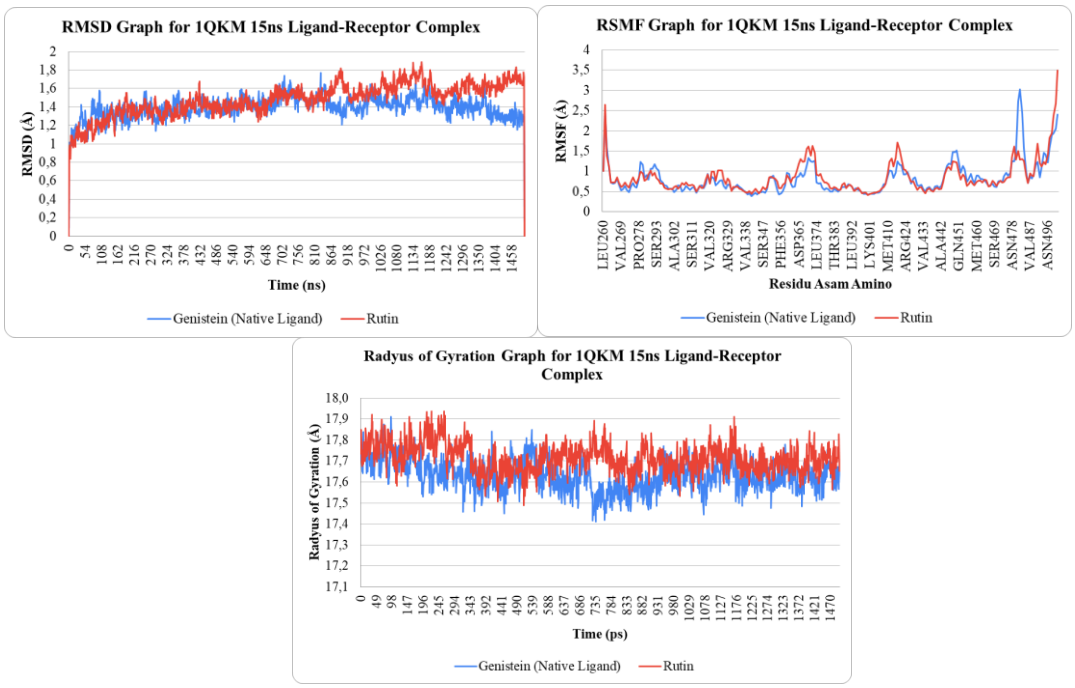


Figure 5. RMSD, RMSF, and radyus of gyration graph for 1qkm 15ns ligand receptor complex

ISSN: 2087-0337
E-ISSN: 2715-9949

Table 6. Results of Lipinski's Rule of Five Analysis, Pharmacokinetics and Toxicity Analysis

No.	Compound	Lipinski's Rule of Five Analysis				Pharmacokinetics and Toxicity Analysis				
						Absorption		Distribution		Toxicity
		MW (g/mol)	H Donor	H Acceptor	Log P	HIA (%)	CaCO-2 Cell (nm sec)	Plasma Protein Binding (%)	Ames Test	Carcinogenicity
1.	Catechin	290	5	6	1.5461	66.71**	0.66*	100.00**	+	-
2.	Chlorogenic acid	354	6	9	-0.6459	20.43**	18.72**	41.96*	+	+
3.	Conyferyl alcohol	180	2	3	1.4063	89.69***	21.8202**	42.72*	+	-
4.	Cyanidin	287	5	6	2.9089	72.51***	0.65*	100.00**	+	-
5.	Delphinidin 3-gentiobioside	627	12	17	-2.2782	0.77*	5.51**	69.801888*	-	+
6.	Eugenol	164	1	2	2.1293	96.77***	46.89**	100.00**	+	+
7.	Isoquercetin	464	8	12	-0.7306	11.78*	9.44**	59.16*	-	-
8.	Isorhamnetin 3-rutinoside	624	9	16	-1.5758	5.31*	8.05**	37.11*	-	-
9.	Kaempferol	286	4	6	2.3053	79.44***	9.58**	89.61*	+	-
10.	Malvidin	331	4	7	3.2205	83.10***	1.76*	89.657208*	+	-
11.	Myricetin 3-robinobioside	626	11	17	-2.1732	1.23*	6.58**	46.74*	-	+
12.	Oleanolic acid	456	2	3	7.2336	96.00***	21.89**	100.00**	-	+
13.	Peonidin 3.5-diglucoside	625	10	16	-3.3567	4.28*	3.84*	29.768936*	-	-

ISSN: 2087-0337
E-ISSN: 2715-9949

Table 6. (Extension)

No.	Compound	Lipinski's Rule of Five Analysis				Pharmacokinetics and Toxicity Analysis				
						Absorption	Distribution	Toxicity	Absorption	Distribution
		MW (g/mol)	H Donor	H Acceptor	Log P	HIA (%)	CaCO-2 Cell (nm sec)	Plasma Protein Binding (%)	Ames Test	Carcinogenicity
14.	Petunidin	31	5	7	2.72749	71.32***	0.97*	100.00**	+	-
15.	Quercetin	302	5	7	2.0109	63.49**	3.41*	93.24**	+	-
16.	Rutin	610	10	16	-1.8788	2.86*	7.91**	43.90*	-	-
17.	Syzygiresinol A	358	3	7	2.4138	88.43***	21.00**	80.78*	+	-
18.	Syzygiresinol B	360	5	8	1.8164	62.43**	20.84**	88.09*	+	-
19.	Betulinic acid	456	2	3	7.0895	96.00***	21.86**	100.00**	+	-
20.	Naringin	580	8	14	-1.1652	11.75*	7.89**	51.06*	-	-

Information

HIA (%):
70-100 is well absorbed
20-70 absorbed enough
<20 poorly adsorbed

CaCo-2 (nm sec):
>70 high permeability
4-70 medium permeability
<4 low permeability

PPB (%):
>90 tightly bound
<90 weakly bound

Toksisitas:
+ = Mutagen/carcinogen
- = Non-mutagen/Non-carcinogen

ISSN: 2087-0337
E-ISSN: 2715-9949

Discussion

Screening of Anti-Breast Cancer Activity

66 out of 117 compounds were anticipated to be successful when the active ingredient in jambolan was examined for its capacity to prevent breast cancer. The purpose of this screening is to offer preliminary estimates of the test substance's ability to prevent breast cancer. In the PASS Online prediction findings, the metrics showing the test compound's likelihood or opportunity value in inducing the expected biological activity are Activity, Pa (likelihood activity), and Pi (probability inactivity).

Pharmacophore Validation

The test chemicals could be screened using the pharmacophore model, and pharmacophore validation was performed using LigandScout 4.4.5 software. A well-constructed pharmacophore model can distinguish most active medications from a few decoys. Ten pharmacophore models have been created from a library of 200 chemicals; these will be verified. Five hundred decoy compound datasets and 100 active datasets that were downloaded from the DUD-E website were used for validation. If the AUC-ROC value is more significant than 0.7 or 70%, the pharmacophore model is considered valid; that is, an AUC-ROC value near one is considered good and is believed to be able to distinguish between active and decoy drugs. The AUC-ROC results of active ligands in ER-α reveal that 21 hit compounds were acquired with an AUC value of 1.00 or 100% out of 120 active and decoy compounds. In ER-β, 107 of the total active and decoy compounds had an AUC value of 0.94 or 94%. Another validation metric that might help identify a decent hit score is the GH Score (Godness of Hit Score). Another validation statistic that might help establish a strong hit score is the GH Score. The following computations generate the GH Score:

$$GH = [Ha4/HtA)(3A+Ht) \times (1-(Ht-Ha)/D-A)]$$

D = Total molecules in the database
A = Total number of activities in the database
Ht = Total Hits
Ha = Active Hits
The GH Score computation gave the active ligand ER-α a score of 0.95 (95%), while ER-β received a score of 0.83 (83%). Pharmacophore modelling using active ligands on ER-α and ER-β is a valid method for screening test ligands. (Figure 1)

Pharmacophore Modelling

By comparing the test drug with an already-existing active ligand, pharmacophore modelling seeks to determine which pharmacophore group is responsible for biological activity.[8] 19 and 18 hit compounds, respectively, coupled with their pharmacophore fit-scores were obtained from the pharmacophore research of the test compounds against ER-α and ER-β (Tables 1 and 2). Its pharmacological activity was comparable to that of the reference ligand, according to Pharmacopore investigations. Molecular docking simulations are a valuable tool for future investigation.

Docking Validation

Validation of the docking parameters is performed before the test ligands are docked. Suppose the docking parameter can return the natural Ligand removed from the

ISSN: 2087-0337
E-ISSN: 2715-9949

ligand complex or the natural Ligand to its original location with an RMSD value of less than 2. In that case, it is considered to be valid.[9,10] The RMSD values derived by re-docking natural ligands are displayed in Table 3. The RMSD values were approved as legitimate and appropriate for the test compounds' molecular docking simulations. The docking parameter validation results are displayed in Figure 2.

Molecular Docking Simulation

With PDB ID 3ERT and 1QKM, the first molecular docking was performed on 19 test compounds against the Er receptor and 18 test compounds against Er-. The test chemical was discovered by pharmacophore screening and has a fit score of. All test compounds had affinity values, suggesting that they can bind to the receptor; nevertheless, compared to the comparator medication, 4-hydroxytamoxifen (4OHT), there is no single chemical with a lower affinity value. According to the study, substances with a lower affinity value than the comparative medication had higher activity.[11] The analytical results suggest that only one molecule may connect to the receptor's active site, namely kaempferol, which binds to ARG A:394 and GLU. A:353 via hydrogen bonding interactions.[12] (Figure 3). Rutin's binding free energy (G) in ER-β is lower compared to the native Ligand, genistein. In Figure 4, The hydrogen contact residues surrounding the catalytic site cavity are evident in the docking tests conducted between rutin and ERβ (ARG 346 and GLU 305).[13]

Lipinski's Rule of Five Analysis, Pharmacokinetics and Toxicity

Lipinski's rule of five is a drug-likeness prediction that looks at the capacity of medicines to penetrate membranes. These parameters include a molecular weight of 500 daltons, which indicates that the compound will pass through the membrane more efficiently, and a partition coefficient (Log-p) 5, which is related to hydrophobicity and hydrophilicity; the higher the Log-p value, the more hydrophobic it is, while the lower the Log-p value, the more hydrophilic it is. The higher the p, the more hydrophilic the chemical; the hydrogen binding acceptor is ten, and the hydrogen binding donor is 5. This relates to biological activity; the more potent the hydrogen bonds between the acceptor and donor, the more energy is required for absorption.[14,15] Pharmacokinetic and toxicity profiles are critical for determining absorption, distribution, and toxicity. Human Intestinal Absorption (HIA) and Caco-2 cells are the two forms of absorption process characteristics. The goal of HIA parameters is to predict drug absorption in the gut.

There are three types of HIA: 70-100% well absorbed, 20-70% moderately absorbed, and 0-20% low absorbed. Meanwhile, the Caco-2 cell characteristics are designed to assess permeability and measure drug passage across epithelial cells in the intestine. Caco-2 cell permeability is divided into three categories: >70 nm/sec is considered high, 4-70 nm/sec is considered medium, and 4 nm/sec is considered low.[16] Protein Plasma Binding (PPB) is one of the factors utilized in the distribution process. PPB is a protein in the plasma that binds to the medication, allowing it to be transported to the tissues. This metric calculates the plasma protein binding capability as a percentage of binding units. Compounds more than 90% bound to PPB are said to be firmly bound, whereas compounds that are 90% bound to PPB are weakly bound. Compounds highly linked to PPB suggest that the compound can be widely spread.[17] The Amest test and the carcinoma mouse are the measures used in toxicity screening. The amest test is used to determine the effects of mutagens on compounds. If a compound's amest test is positive, it has mutagenic qualities and may be a carcinogen; therefore, a carcinogen test is required to identify chemicals that can cause cancer.[9]

ISSN: 2087-0337
E-ISSN: 2715-9949

Table 6 shows the results of Lipinski's Rule of Five Analysis, Pharmacokinetics and Toxicity Analysis.

Molecular Dynamic Simulation

The analysis performed in molecular dynamics simulations is RMSD, RMSF, and Radius Of Gyration, where the analysis is performed to determine the stability of the ligand-receptor interaction. The system reached stability throughout the 15-ns MD simulation, as evidenced by the RMSD value of the genistein and routine complexes for the ER- receptor in the range of 2-5 Å.[11] Figure 5 demonstrates that the routine-ER-complex is more flexible than the genistein-ER-β complex due to a more excellent RMSD value, namely 1.4807 Å. The RMSF value describes the conformational changes in each amino acid residue that allow proteins to be more flexible. According to the complex analytical results of the compounds genistein-Er-β and rutin-Er-β, they exhibited low fluctuations at the same residue, namely ARG A:346 and GLU A:305. Low fluctuations suggest that residues bind stably to the active site.[11,18,19] The genistein and rutin complexes were subjected to a radius of gyration analyses. The predictions reveal that both compounds are stable because the range of RG values for each complex is not too wide, indicating that the two complexes are stable in binding. According to the average RG value, the rutin complex is no more stable than the genistein complex, which has an RG value of 17.7>17.6 Å.[11,20] Figure 5 shows the results of RMSD, RMSF and Radius Of Gyration.

Conclusion

As demonstrated by many virtual screens, the research results reveal that the ordinary chemical can be employed as a lead compound to develop into an anti-breast cancer agent. Molecular dynamics simulations show that the interaction of the rutin with the Er- receptor is stable but not more stable than the drug genistein and cannot be used as an oral preparation due to the Lipinski rule of five factors. Hence, it must be optimized for better activity and stability derived from genistein.

Acknowledgement

The authors thank The Ministry of Research and Technology and Higher Education of the Republic of Indonesia, LLDIKTI IV, and Universitas Garut for research support through Penelitian Dosen Pemula (PDP) year 2023.

Reference

1. The Global Cancer Observatory. Globocan 2020: breast. Vol. 419, International Agency for Research on Cancer. 2020.
2. World Health Organization. Cancer Indonesian country profile 2020. Indonesia; 2020.
3. Kementerian Kesehatan Republik Indonesia. Kanker payudara paling banyak di Indonesia, Kemenkes targetkan pemerataan layanan kesehatan. kemekes.go.id. 2022.
4. World Health Organization. Breast cancer. Who. int. 2020.
5. Sumayyah S, Salsabila N. Obat tradisional : antara khasiat dan efek sampingnya. Farmasetika.com (Online). 2017;2(5).
6. Aqil F, Jeyabalan J, Munagala R, Singh IP, Gupta RC. Prevention of hormonal breast cancer by dietary jamun. Mol Nutr Food Res. 2016;60(6).
7. Artanti N, Maryani F, Dewi RT, Handayani S, Dewijanti ID, Meilawati L, et al. In vitro antidiabetic, antioxidant and cytotoxic activities of syzygium cumini fractions

ISSN: 2087-0337
E-ISSN: 2715-9949

from leaves ethanol extract. Indones J Cancer Chemoprevention. 2019;10(1).
8. Dermawan D, Sumirtanurdin R, Dewantisari D. Molecular dynamics simulation estrogen receptor alpha against andrographolide as anti-breast cancer. Indones J Pharm Sci Technol. 2019;6(2).
9. Prasetiawati R, Suherman M, Permana B, Rahmawati R. Molecular docking study of anthocyanidin compounds against epidermal growth factor receptor (EGFR) as anti-lung cancer. Indones J Pharm Sci Technol. 2021;8(1).
10. Astuty P, Komari N. Kajian molecular docking Senyawa karwinaphthol B dari tanaman Bawang Dayak (Eleutherine palmifolia (L.) Merr) sebagai inhibitor enzyme glucokinase. J Nat Sci. 2022;2(1).
11. Elfita L, Apriadi A, Supandi S, Dianmurdedi S. Studi penambatan molekuler dan simulasi dinamika molekuler senyawa turunan furanokumarin terhadap Reseptor Estrogen alfa (ER-α) sebagai anti kanker payudara. J Sains Farm Klin. 2023;9(3).
12. Alamri A, Rauf A, Khalil AA, Alghamdi A, Alafnan A, Alshammari A, et al. In silico screening of marine compounds as an emerging and promising approach against estrogen receptor alpha-positive breast cancer. Biomed Res Int. 2021;2021.
13. Prasetiawati R, Permana B, Soni D, Agung SN. Molecular docking study of xanthone derivative compounds of Mangosteen Rind (Garcinia mangostana L.) to ER-α (estrogen receptor alfa) and ER-β (estrogen receptor beta) as anti-breast cancer. J Ilm Farm Bahari. 2018;10(1).
14. Suherman M, Prasetiawati R, Ramdani D. Virtual screening of Tamarind active compounds (Tamarindus indica l.) on selective inhibitor siklooksigenase-2. J Ilm Farm Bahari. 2020;11(2).
15. SCFBio. Lipinski's rule of five. scfbio-iitd.res.in. 2009.
16. Praceka MS, N. Yunita E, D. Semesta C, N. Putri R, N. Mikdar N, N. Sitinjak E, et al. Molecular docking and toxicity from Temulawak Rhizome (Curcuma xanthorrhiza Roxb.) against COX-2. Indones J Pharm Sci Technol. 2022;1(1).
17. Prasetiawati R, Zamri A, Barliana MI, Muchtaridi M. In silico predictive for modification of chalcone with pyrazole derivatives as a novel therapeutic compound for targeted breast cancer treatment. J Appl Pharm Sci. 2019;9(2):20–8.
18. Chairunisa F, Safithri M, Andrianto D, Kurniasih R, Irsal RAP. Molecular docking of Red Betel Leaf bioactive compounds (piper crocatum) as lipoxygenase inhibitor. Indones J Pharm Sci Technol. 2023;10(2).
19. Fakih TM, Wisnuwardhani HA, Dewi ML, Ramadhan DSF, Hidayat AF, Prayitno R. Simulasi dinamika molekuler senyawa asam ferulat dan turunannya dari kulit Buah Nanas (ananas comosus) sebagai inhibitor enzim tirosinase. J Sains Farm Klin. 2021;8(2).
20. Hashemzadeh H, Javadi H, Darvishi MH. Study of structural stability and formation mechanisms in DSPC and DPSM liposomes: a coarse-grained molecular dynamics simulation. Sci Rep. 2020;10(1).