Pharmaciana
Vol.13, No.3, Nov 2023,Page. 268 – 282
ISSN: 2088 4559; e-ISSN: 2477 0256
DOI: 10.12928/pharmaciana.v13i3.26537

In silico study: secondary metabolites from malay apple (*Syzygium malaccense* (L.) Merr. & L.M. Perry) as potential breast cancer treatments

Riska Prasetiawati, Nawadhir Fauzan, Meilia Suherman*
Departemen of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Garut
Jl. Jati No.42B Garut, West Java, Indonesia

Submitted: 22-06-2023 ***Reviewed: 28-07-2023*** ***Accepted: 10-10-2023***

ABSTRACT

Breast cancer has the highest prevalence of all cancers. Breast cancer has overtaken lung cancer as the leading cause of global cancer incidence in 2020, accounting for 2,261,419 new cases, or 11.7% of all new cancer cases worldwide. Among the efforts that can be done are efforts to find breast cancer medications that are safe and selective for the treatment and prevention of cancer, particularly those derived from medicinal plants. The Malay apple (*Syzygium malaccense* (L.) Merr. & L.M. Perry) is one plant that has been extensively examined and proved to have an antiproliferative effect. The pharmacophore modelling, molecular docking, and molecular dynamic approach was conducted on 155 active compounds of Malay apple to alpha and beta estrogen receptors. According on the results of ER-α docking, numerous substances have binding free energy values less than 4-OHT yet are not bound to important amino acids, as the result, it is not continued to the next test. On other side, with a fit score of 45.81, rutin was potentially selective for ER-β receptors, molecular docking to ER-β obtained that rutin was predicted to have breast cancer activity with a free binding energy value of -10.6 kcal /mol with better conformation and affinity compared to native ligand (genistein), and bound to essential amino acids as anticancer breast at ARG 346, GLU 305, and molecular dynamics simulations show that the compound has good stability when binding to the receptor. In silico toxicity prediction from rutin showed outcomes that match the requirements for the candidate drug. However, because it does not match the ADME prediction and Lipinsky's rule of five, rutin must be optimalization to improve its pharmacokinetic and pharmacological profile before it can be further explored as a therapeutic option for the treatment of breast cancer that targets the ER- receptor.

Keywords: breast cancer, *Syzygium malaccense*, in silico study, virtual screening

Corresponding author:
Meilia Suherman
Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Garut
Jl. Jati No. 42 B Tarogong Kab. Garut, West Java, Indonesia
Email:meilia.suherman@uniga.ac.id

INTRODUCTION

Cancer is a disease that develops as a result of abnormal cell proliferation in body tissues. Cancer is a disease that kills many people in Indonesia and around the world. Breast cancer has the highest prevalence of all cancers. According to the Global Burden Cancer (Globocan) report, there were 65,858 new cases of breast cancer in 2020, representing 16.6% of the total 396,914 new cases of cancer in Indonesia (Globocan, 2020), whereas at the global level, breast cancer has become the leading cause of new cancer cases in 2020, accounting for 2.261,419 new cases, or 11.7% of the total number of new cancer cases worldwide (Sung et al., 2021).

Breast cancer therapy is still being developed due to the numerous side effects it produces, such as chemotherapy, which frequently leads to failure due to the low selectivity of anticancer medications (Moo et al., 2018). As a result, the identification of novel anticancer potential is critical in order to overcome this adverse effect of existing cancer medications, one of which is natural medicine.

Since ancient times, traditional herbal treatment has been regarded as an alternate strategy to treating and dealing with various disorders. Malay apple is one of the plants that has been actively researched in the search for new medication candidates. Malay apple (*Syzygium malaccense* (L.) Merr. & L.M. Perry) has high antioxidants activity, it has the potential to improve human health. Rabeta et al. (2013) discovered that Malay apple methanol extract had an antiproliferative impact on Michigan Cancer Foundation-7 (MCF-7) cells with 79% viability and IC_{50} = 632.3 g/mL (Rabeta et al., 2013). However, in vitro tests have not revealed an active anti-cancer compound in the breast, so further research is required.

Various new drug developments, including the *in silico* method, are being used to accelerate the discovery of anti-breast cancer drugs. Because they are supported by good computational techniques and also shorten the time in the drug discovery process, in silico methods for developing new drugs are growing rapidly (Ekins et al., 2007). Based on earlier research, the experiment was developed to look for anti-breast cancer candidates by studying active compounds in Malay apple utilizing in silico methodologies. The estrogen hormone is a major contributor to the occurrence of breast cancer in women. As a result, their receptor is becoming a focus in endocrine therapy (Ervina et al., 2021). To obtain comprehensive data, this study focuses on predicting metabolite compounds from Malay apple that have potential anticancer by targeting the estrogen receptor (ER-α and ER-β) using the Ligand Based Drug Design (LBDD) approach with pharmacophore modeling And Structure Based Drug Design (SBDD) with molecular docking and molecular dynamics.As a result, it is envisaged that the findings of this study will lead to the development of a lead compound for breast cancer therapy.

MATERIALS AND METHOD

Materials

Based on literature studies from LC-MS and GC MS data, 155 secondary metabolites from Malay apple were acquired, and the structures were retrieved from the website https://pubchem.ncbi.nlm.nih.gov. The breast cancer receptor (PDB ID 3ERT for ER-α and PDB ID 1QKM for ER-β receptor-ligand complex) were downloaded from the https://rcsb.org site in PDB format. **Hardware:** ASUS A456U notebook (operating system: Microsoft Windows 10 Pro and Ubuntu 17.04), 64-bit; Memory: 4GB; Processor: Intel Core i5-7200U, Personal Computer (operating system: Ubuntu 16.04 LTS, Xeon Processor, 16 GB Memory). **Software:** Discovery Studio Visualizer® Version 2021 freeware, MarvinSketch Version 17.2.13® freeware, Autodock Tools 1.5.6® freeware, Autodock Vina 1.1.2® freeware, Liganscout 4.4.5 30 days free trial. **Website:** KNApSAck (http://www.knapsackfamily.com/KNApSAcK/), PASS (Prediction of Activity Spectra for Substances) Online (http://way2drug.com/passonline/predict.php), 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/), Google Colabolatory (https://colab.research.google.com/?utm_source=scs-index), OpenMM (https://openmm.org/).

Method

Compound library selection and ligand preparation

The compound of Malay apple obtained through various library sources and based on search results using the KNApSAcK online database site (http://www.knapsackfamily.com/KNApSAcK/)

Screening activity

The Malay apple compound was examined for anti-breast cancer activity using the PASS Online prediction site.

Geometry optimization

The 3D molecular structure of secondary metabolites contained in Malay apple was carried out on geometric optimization using MarvinSketch with the semi-empirical AM1 method.

Lipinski's rule of five testing

The ligands or compounds tested in this investigation were Malay apple secondary metabolites obtained from pubbchem. Ligand preparation in Marvin Sketch by minimizing energy with MMFF94 and saving as mol2, then opening the mol2 file in Marvin and performing protonation at pH 7.4 and saving as PDB format. Following preparation, the compound's physicochemical properties were assessed by uploading the prepared ligand to Lipinski's Rule of Five site (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp).

Pre-ADMET testing

The tests conducted seek to examine the first characteristics of pharmacokinetics, such as absorption, and distribution, as well as toxicity testing, such as mutagenic and carcinogenic qualities of substances. Testing is done out using a specific program that is carried out online on the http://preadme.bmdrc.kr/site. After drawing the structure of the test substance, click submit for analysis. The collected results are data in.pdb format.

Pharmacophore modeling

Pharmacophore modeling was used to find and extract potential interactions between ligand-receptor complexes. Liganscout software was used to conduct the tests. The first stage entails synthesizing target receptors from the PDB, standard compounds from the binding data base, active compounds from DUD-E (Database of Useful Decoys Enhanced), and decoy compounds from DUD-E. The receptor's pharmacophore was then found, and the model of the pharmacophore was validated, providing the ROC (Receiver Operating Characteristic) curve, which revealed the hit molecule and the AUC (Area Under Curve) value. Another validation metric, the GH Score (Goodness of Hit Score), can also be used to identify a good hit score. After validation, the test ligands were screened to create a list of hit compounds and pharmacophore fit scores. The GH Score is calculated as follows:

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

D = Total *molecules in database*
A = Total *number of actives in database*
Ht = Total *Hits*
Ha = *Active Hits*

Protein selection and preparation

Receptors downloaded via PDB website (https://www.rscb.org/) with PDB ID 3ERT for ER-α and PDB ID 1QKM for ER-β receptor, receptor-ligand complex separated and prepared using Autodock

Tools® software, then validated and a scoring function to calculate the binding affinity using Autodock Vina®.

Molecular docking

The docking method was validated by redocking native ligands on the ER-α and ER-β receptors. Molecular docking is performed with previously validated parameters. Canonical SMILES of the test compound were obtained from the Pubchem website (http://pubchem.ncbi.nlm.nih.gov) then the optimization process was carried out, then the docking process was carried out using Autodock Tools 4.2.6® and visualization was carried out using the Discovery Studio Visualizer®. The 3D-ligand structures were then bound to the active sites of ER-α and ER-β. ΔG (change in Gibbs free energy) and interaction poses were calculated from the results.

Molecular dynamics simulation

Molecular dynamics simulations were performed using open MM software on the complex between ER-β receptor and the ligand with the lowest binding energy based on molecular docking experiments. Begin by creating a protein topology and a ligand topology. The ff19SB force field was used to add the ligand topology, and the GAFF2 force field with the TIP3P water model was used to add the receptor topology then equilibration and production are carried out for 15 ns.

RESULT AND DISCUSSION

Screening compound

Based on literature investigations from related journals and screening for active compounds of *Syzygium malaccense* (L.) Merr. & L.M. Perry, 155 active compounds were discovered and employed in this study.

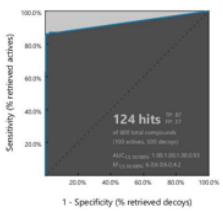
Screening of anti-breast cancer activity

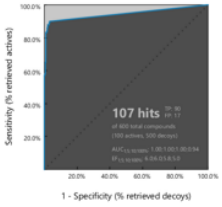
The active compound of Malay apple was screened for anti-breast cancer activity, and 80 out of 155 compounds were predicted to have anti-breast cancer activity. The goal of this screening is to make early predictions about the test substance as anti-breast cancer activity. The metrics employed in the PASS Online prediction findings include Activity, the value of Pa (Probability activity), and Pi (Probability inactivity), which indicate the test compound's likelihood or opportunity value in creating the expected biological activity.

Pharmacophore validation

Pharmacophore validation was performed using LigandScout 4.4.5 software to determine the suitability of the pharmacophore model for use in screening the test chemicals. A decent pharmacophore model can detect the majority of active drugs as well as a few decoy molecules. Ten pharmacophore models were created from a database of 200 chemicals and will be validated. Validation was performed using 100 active compound databases and 500 decoy compound databases obtained from the DUD-E site. The pharmacophore model is stated to be valid if the AUC-ROC value is greater than 0.7 or 70%; in other words, an AUC-ROC value near to one is considered good and is believed to be capable of distinguishing between active and decoy compounds. (Hamzah et al., 2014; Moussa et al., 2021; Suherman et al., 2020). The AUC-ROC results of active ligands in ER-α demonstrate that 124 hit compounds were acquired with an AUC value of 0.93 or 93% out of a total of 600 active and decoy compounds, whereas in ER-β, 107 of the total active and decoy compounds were 600 compounds. A good hit score can also be determined by the GH Score (Goodness of Hit Score), which is another validation metric. The GH Score calculation yielded a score of 0.81 or 81% for the active ligand ER-α, while it was 0.94 or 94% for ER- β. So it is said that pharmacophore modeling based on active ligands on ER-α and ER-β is legitimate and can be utilized to screen pharmacophore on test ligands (Figure 1**).**

ER-α
AUC : 0,93
GH Score : 0,81





ER-β
AUC : 0,94
GH Score : 0,94

Figure 1. Pharmacophore Validation Results

Pharmacophore modelling

The pharmacophore research of the test compounds against ER-α and ER-β yielded 27 and 25 hit compounds, respectively, as well as their pharmacophore fit-scores (Tables 1 and 2). Pharmacopore tests revealed that it had similar pharmacological action to the native ligand, pharmacophore fitscore number shows that the hit chemical has similar pharmacophore characteristics to the model, and hence it is predicted to have the same activity as the model/ligand that has been shown to be active and that it can be further evaluated in molecular docking simulations.

Table 1. Pharmacophore study results against ER-α

Compound	Pharmacophore-Fit Score
Cyanidin-3,5-O-diglucosid	63.97
Quercitrin	63.49
Peonidin 3,5-diglucosside	60.95
Pelargonidin-3-glucoside	60.74
Procyanidin B2	60.64
Peonidin-3-Glukosid	60.64
Procyanidin B1	60.58
Cyanidin-3-O-Glukosid	60.56
Cyanidin 3-glucoside	60.54
Procyanidin A2	60.37
2',4'-Dihydroxy-6'-Methoxy-3-Methyldihidrochalcone	56.26
Stercurensin	55.47
Kaempferol-3-glucoside	53.19
Isorhamnetin-3-glucoside	53.11
Isoquercitrin	53.04
(-)-Epicatechin	53.00
Quercetin	52.93
(+)-Catechin	52.88
Morin	52.71
Myricitrin	52.43
Chlorogenic Acid	51.98
Myricetin-3-(3"galloylrhamnoside)	51.95
Mearnsitrin	50.96
(-)-Epicatechin gallate	50.71
Desmanthin1	50.53
Rutin	50.24
Ursolic Acid	44.60

Table 2. Pharmacophore study results against ER-β

Compound	Pharmacophore-Fit Score
Chlorogenic Acid	57.94
Morin	57.40
(-)-Epicatechin gallate	57.08
Kaempferol-3-glucoside	57.03
Procyanidin B2	56.34
Isoquercitrin	55.91
Cyanidin-3,5-O-diglucosid	55.90
Pelargonidin-3-glucoside	54.37
Peonidin-3-Glukosid	54.18
(+)-Catechin	54.04
Cyanidin 3-glucoside	53.96
Isorhamnetin-3-glucoside	46.72
Quercetin	46.62
(-)-Epicatechin	46.61
Cyanidin-3-O-Glukosid	46.28
Procyanidin A2	46.22
Quercitrin	46.09
Procyanidin B1	46.01
Rutin	45.81
Peonidin 3,5-diglucosside	45.46
Myricitrin	44.54
Mearnsitrin	44.37
Ursolic Acid	44.15
Desmanthin 1	43.97
Myricetin-3-(3"galloylrhamnoside)	43.67

Docking validation

Docking parameter validation occurs prior to the docking process for the test ligands. The docking parameter is considered valid if it can re-docking the native ligand that has been withdrawn from the native ligand or the ligand complex to its original position with an RMSD value less than 2 Å (Astuty & Komari, 2022). The RMSD values obtained from re-docking native ligands are shown in Table 3. The RMSD values were declared valid and ready for use in molecular docking simulations of the test compounds. Figure 2 depicts the docking parameter validation findings.

a **b**

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

Table 3. Size of grid center, grid box and RMSD values from docking validation results

PDB ID	Grid Center			Grid Box (Å)			Exhaustiveness	RMSD
	X	Y	Z	X	Y	Z		
3ERT	30.282	-1.913	24.207	40	40	40	16	1.1162 Å
1QKM	22.438	8.003	113.538	40	40	40	16	0,2799 Å

Table 4. Results of molecular docking of ER-α and analysis of linked amino acid residues

Compound	ΔG (kcal/mol)	Conventional Hydrogen Interaction
Procyanidin A2	-11.166	CYS 530
Procyanidin B1	-10.393	LEU 525, CYS 530, ASP 351
Procyanidin B2	-10.207	MET 343, THR 347, CYS 530
Ursolic Acid	-10.103	-
Rutin	-10.057	VAL 534, LEU 536, THR 347, MET 522, CYS 530
Desmanthin1	-9.832	LEU 536, CYS 530, ASP 351, MET 522
Native Ligan (4OHT)	**-9.684**	**ARG 394, GLU 353**
Cyanidin-3,5-O-diglucosid	-9.579	LEU 536, MET 522, THR 347
Quercitrin	-9.510	THR 347
Myricetin-3-(3"galloylrhamnoside)	-9.486	CYS 530, THR 347, TRP 383, LEU 536
Mearnsitrin	-9.280	-
(-)-Epicatechin gallate	-9.176	THR 347, CYS 530, LEU 536
Peonidin 3,5-diglucosside	-9.157	GLU 380, TYR 537, LEU 536, THR 347
Kaempferol-3-glucoside	-8.985	VAL 534, CYS 530
Myricitrin	-8.980	THR 347
Quercetin	-8.840	**ARG 394, GLU 353**, LEU 387
Morin	-8.799	**ARG 394, GLU 353,** MET 343
Isoquercitrin	-8.744	VAL 534, MET 522, THR 347
(-)-Epicatechin	-8.713	GLU 353, LYS 449, TRP 393
Pelargonidin-3-glucoside	-8.701	CYS 530, VAL 534
Cyanidin-3-O-Glukosid	-8.514	CYS 530, VAL 534, LEU 536,
Peonidin-3-Glukosid	-8.495	ASP 351
Cyanidin 3-glucoside	-8.460	CYS 530, MET 522
Isorhamnetin-3-glucoside	-8.272	VAL 534, MET 522, THR 347
Chlorogenic Acid	-8.229	GLU 353, ASP 351
(+)-Catechin	-7.969	**ARG 394, GLU 353**, LEU 387, HIS 524
2',4'-Dihydroxy-6'-Methoxy-3-Methyldihidrochalcone	-7.710	-
Stercurensin	-7.649	-

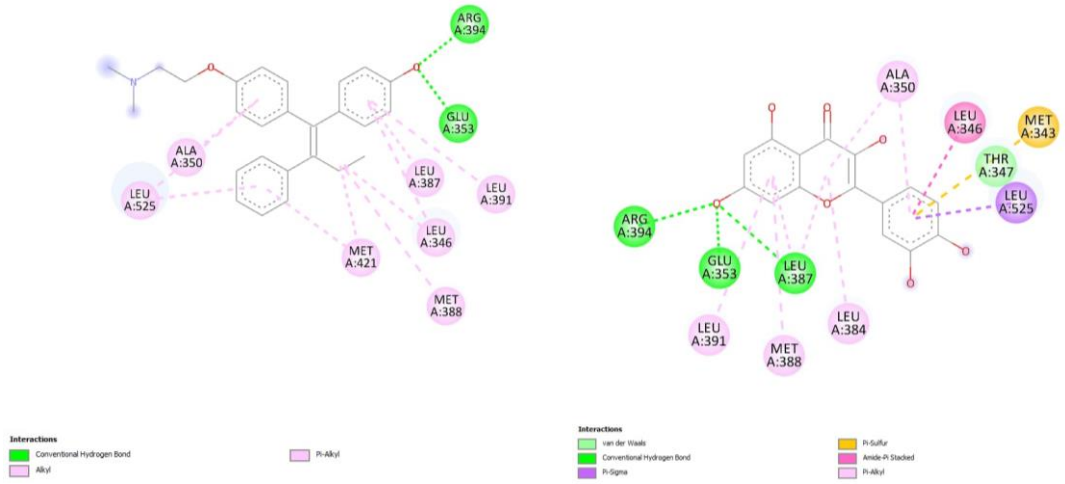
Molecular docking simulation

The docking of Malay apple compounds revealed that the 6 test compounds (Procyanidin A2, Procyanidin B1, Procyanidin B2, Ursolic Acid, Rutin, and Desmanthin1) had lower binding free energy than the native ligand (4OHT), although none of the complexes between Malay apple compounds and ER-α had interactions with essential amino acid residues. Quercetin is the compound that has the same amino acid interaction as the native ligand but does not have a lower binding free energy value than

the native ligand (Table 4 and Figure 3). In ER-β, rutin has a lower binding free energy (G) than the native ligand (Genistein). The molecular docking results between rutin and ER-β reveal the presence of hydrogen interactions residues surrounding the cavity at the catalytic site (**ARG 346** and **GLU 305**) (Table 5 and Figure 4).

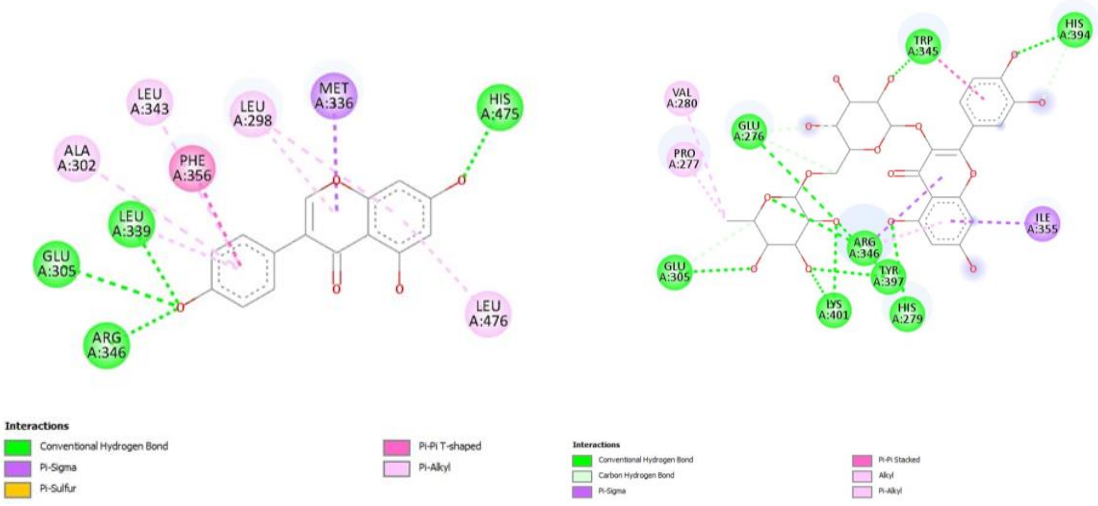
Table 5. Results of Molecular docking of ER-β and analysis of linked amino acid residues

Compound	ΔG (kcal/mol)	Conventional Hydrogen Interaction
Rutin	**-10,6**	**ARG 346, GLU 305,** LYS 401, GLU 276, TYR 397, HIS 279, TRP 345, HIS 394
Native Ligand (Genistein)	**-10,5**	**ARG 346, GLU 305,** LEU 339, HIS 475
Procyanidin B1	-10,4	TRP 345, LYS 401, PRO 358
Myricetin-3-(3"galloylrhamnoside)	-10,2	**ARG 346**, TRP 345, ASP 249, HIS 279, PHE 356, VAL 280, PRO 278
Procyanidin B2	-10,0	**ARG 346,** PRO 358, ASP 349, TRP 345, GLU 276
Procyanidin A2	-9,9	**GLU 305**, LYS 401
Myricitrin	-9,2	**GLU 305**, VAL 280, HIS 279, TRP 345
Quercitrin	-9,1	**GLU 305**, VAL 280, HIS 279, TRP 345
Morin	-9,0	**ARG 346, GLU 305**, VAL 280
Quercetin	-9,0	**GLU 305**, VAL 280, TRP 345
Mearnsitrin	-8,9	**GLU 305,** VAL 280, HIS 279, TRP 345
Desmanthin1	-8,6	**ARG 346,** TRP 345, HIS 394, ASP 349, ILE 348, HIS 350, PRO 277
(-)-Epicatechin	-8,5	**ARG, 346, GLU 305,** HIS 279
(-)-Epicatechin gallate	-8,5	**GLU 305**, VAL 280, **ARG 346,** TRP 345, GLU 276
Cyanidin-3,5-O-diglucosid	-8,5	TRP 345, PRO 277, HIS 279, PRO 278, VAL 280,
(+)-Catechin	-8,3	**ARG 346, GLU 305,** VAL 280,TRP 345, GLY 342
Isoquercitrin	-8,1	**GLU 305,** VAL 280, HIS 279, TRP 345
Cyanidin-3-O-Glukosid	-8,0	**GLU 305,** TRP 345, HIS 279
Isorhamnetin-3-glucoside	-8,0	VAL 280, PRO 278, TRP 345
Kaempferol-3-glucoside	-8,0	**GLU 305**, VAL 280, HIS 279, TRP 345
Peonidin 3,5-diglucosside	-8,0	VAL 280, PRO 277, TRP 345
Chlorogenic Acid	-7,7	TRP 345, GLU 276, VAL 280, HIS 279
Pelargonidin-3-glucoside	-7,7	**GLU 305**, VAL 280, TRP 345, HIS 279
Cyanidin 3-glucoside	-7,6	GLU 276, HIS 279, TRP 345
Peonidin-3-Glukosid	-7,6	**GLU 305**, TRP 345, HIS 279
Ursolic Acid	-7,6	-



a **b**

Figure 3. Result of ER-α docking with native ligans and Quercetin (a : complex Erα - 4OHT, b : complex Erα – Quercetin)



a **b**

Figure 4. Visualization result of ER-β docking with native ligans and rutin (a : complex Erβ - Genistein, b : complex erα – rutin)

Lipinski's rule of five analysis, pharmacokinetics and toxicity

The online portal http://scfbio-iitd.res.in is used to forecast the potential of the tested molecule to be employed as a medication candidate in oral dosage forms (Lipinsi's Rule of Five). This prediction was produced using Lipinski's Rule of Five, which indicates that the molecular weight (BM) should not exceed 500 Daltons since a high molecular weight will alter the concentration of substances absorbed on the surface of the intestinal epithelium. The rule also states that the partition coefficient (Log P) should not be greater than 5, because a higher partition coefficient indicates that a compound

has hydrophobic properties, which causes it to stay longer in the lipid bilayer membrane and cause the compound to become toxic, whereas a lower partition coefficient indicates that the compound is hydrophilic, which makes it difficult for the compound to penetrate the lipid bilayer membrane. Furthermore, hydrogen donor bonds must be fewer than 5 and bond acceptors must be no more than 10, because hydrogen donors and acceptors can absorb. The high number of hydrogen bonds influences the passive migration of molecules from the hydrophilic phase into the lipid bilayer membrane (Az-Zahra et al., 2022).

The HIA (Human Intestinal Absorption) and Caco2 value parameters are used to predict the pharmacokinetic absorption profile, while the distribution parameter is PPB (Plasma Protein Binding). HIA is a measure that describes absorption prediction in the human gut. If the HIA value is in the range 0-20%, it indicates a low level of absorption or poorly absorbed, HIA 20-70% indicates a sufficient amount of absorption, and HIA 70-100% indicates a high level of absorption or well absorbed (Sagitasa et al., 2021). Caco2 is an in vitro cell model that is used to predict drug absorption through the intestinal epithelium as measured by the permeability level (nm/sec), where a permeability value of <4 nm/sec indicates the compound is hydrophilic, 4-70 nm/sec indicates the permeability level of the compound which is moderate, which means the compound is neither too hydrophilic nor lipophilic, permeability >70 nm/sec indicates the compound is lipophilic (Suherman et al., 2020). The PPB (Protein Plasma Binding) value, which is the distribution level of chemical binding to proteins in plasma, is used to predict the pharmacokinetic distribution profile. A PPB value of 90% indicates that the molecule is poorly linked to plasma proteins, whereas a PPB value more than 90% suggests that the drug is highly bound to plasma proteins. The higher the compound's affinity for plasma proteins, the better the compound's distribution (Nusantoro & Fadlan, 2020). The Ames and carcinogenicity test parameters are used to predict toxicity. The Ames test is a method for determining the qualities of a test compound based on its mutagenic and carcinogenic capabilities, which are known due to the test compound's chemical structure. A positive Ames test result suggests that the substance is mutagenic and may be carcinogenic; therefore, a carcinogenicity prediction is performed to confirm (Hartanti et al., 2022). Table 6, shows the results of Lipinski's Rule of Five Analysis, Pharmacokinetics and Toxicity Analysis. ADMET prediction revealed that selected compounds (rutin) had poor absorption ability, was medium permeability, and was poorly bound to Plasma Binding Protein (PPB), requiring alteration of the preparation or carrier to reach the receptor. Toxicity testing reveals that it is not a mutagen or a carcinogen. Aside from that, Lipinski rule of five testing reveals that it does not match the requirements, therefore it cannot be converted into an oral dosage form due to the Lipinski rule of five factors, but the dosage form can be adjusted to reach the receptor.

Molecular dynamic simulation

Molecular Dynamic (MD) simulations were performed on the best docking compounds to determine the stability of the binding relationship between the test ligand and receptor under physiological settings (Chairunisa et al., 2023). The simulation was ran for 15ns using Open MM software and Google colab, which was linked to Google Drive.

The RMSD, RMSF, and RG values were produced and assessed based on the results of research conducted on the best compound obtained in the molecular docking simulation, namely the molecule Rutin on the ER-β receptor. The Rutin test ligand complex against the ER-β receptor had an average RMSD fluctuation value of 1.48 with the highest fluctuation of 1.89, while analysis of the RMSD value of the native ligand Genistein against the ER-β receptor resulted in an average RMSD fluctuation value of 1.39 with the highest fluctuation of 1.89. According to the results of the RMSD study, the test ligand Rutin and the native ligand Genistein have a stable conformation since the average RMSD value is 5, although the test ligand Rutin has a 0.09 higher average value than the native ligand Genistein (Elfita et al., 2023). According to the RMSF graph (shown in Figure 5), the results obtained for the Routine test ligand were amino acid residues that experienced high fluctuations, namely ASP 261, VAL 370, ALA 420, and MET 479, whereas high fluctuations occurred in the residues for the native ligand

Genistein. ASP 261, GLN 450, and LYS 482 are amino acids. High fluctuations suggest that a conformational change has occurred, causing the bond in the binding site to become unstable and inactive. According to the low fluctuation of amino acid residues, the amino acid residues that experienced low fluctuations in the Routine test ligand were MET 295, LEU 298, GLU 305, MET 336, LEU 339, and ARG 346, while low fluctuations occurred in acid amino LEU 298, GLU 305, MET 336, LEU 339, ARG 346, MET 295, and ILE 376 in the native ligand Genistein. Low fluctuations imply that the amino acid residues can bind stably and actively participate in the binding area. Both the test ligand complex and the native ligand can bind stably, although the native ligand Genistein is more stable than the conventional test ligand because low amino acid residues fluctuate more (Elfita et al., 2023). According to the RG value (shown in Figure 5), the test ligand Rutin and the native ligand Genistein had the same RG value at the start of the simulation. As the simulation progressed, there was an increase in fluctuation in the test ligand Routine, which indicated protein unfolding, but after that there was stability until the simulation ended, whereas there was a decrease in fluctuation in the native ligand Genistein, which indicated protein folding and lasted until the simulation ended. The RG analysis results demonstrate that both the Routin test ligand and the native ligand (Genistein) are stable during the simulation procedure, although the native ligand (Genistein) is more stable than the rutin. Figure 5 shows the RMSD, RMSF, and Radyus of Gyration Graph for 1QKM 15ns Ligand-Receptor Complex.

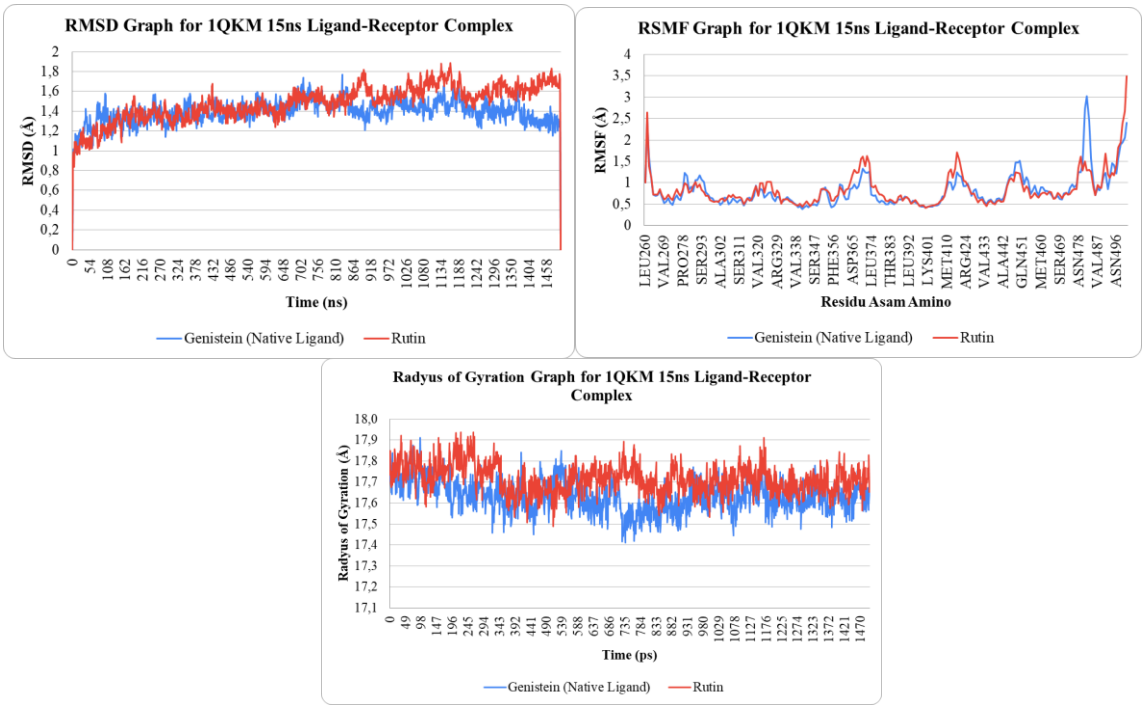


Figure 5. RMSD, RMSF, and radyus of gyration graph for 1QKM 15ns ligand-receptor complex

CONCLUSION

The results of in silico testing through pharmacophore modeling, molecular docking, molecular dynamic and toxicity prediction showed that rutin has the potential to be a therapeutic candidate for the treatment of breast cancer that targets the ER- β receptor. The pharmacophore study results show that the Rutin compound has a fitscore value of 45.81%, molecular docking simulations show a Gibbs

free energy (G) value of Rutin of -10.6 kcal/mol, which is lower than the comparison ligand, and molecular dynamics simulations show that the compound has good stability when binding to the receptor. However, because it does not match the ADME prediction and Lipinsky's rule of five, rutin must be optimalization to improve its pharmacokinetic and pharmacological profile before it can be further explored as a therapeutic option for the treatment of breast cancer that targets the ER- receptor.

ACKNOWLEDGEMENT

The author would like to thank to the Universitas Garut for supporting financially through the 2023 Faculty Grant scheme.

REFERENCES

Astuty, P., & Komari, N. (2022). Kajian molecular docking senyawa karwinaphthol B dari tanaman bawang Dayak (Eleutherine palmifolia (L.) Merr) sebagai inhibitor enzim glukokinase. *Jurnal Natural Scientiae*, *2*(1). https://doi.org/10.20527/jns.v2i1.5412

Az-Zahra, F., Afidika, J., Diamantha, S. D. A., Rahmani, A. E., Fatimah, S., Aulifa, D. L., Elaine, A. A., & Sitinjak, B. D. P. (2022). In silico study of betel leaves compound (Piper betle L.) as acetylcholinesterase (AChE) enzyme inhibitor in alzheimer disease. *Indonesian Journal of Biological Pharmacy*, *2*(2), 44–58. https://doi.org/10.24198/ijbp.v2i2.40462

Chairunisa, F., Safithri, M., Andrianto, D., Kurniasih, R., & Irsal, R. A. P. (2023). Molecular docking of red betel leaf bioactive compounds (Piper crocatum) as lipoxygenase inhibitor. *Indonesian Journal of Pharmaceutical Science and Technology*, *10*(2), 90–103. https://doi.org/10.24198/ijpst.v10i2.38934

Ekins, S., Mestres, J., & Testa, B. (2007). In silico pharmacology for drug discovery: methods for virtual ligand screening and profiling. *British Journal of Pharmacology*, *152*(1), 9–20. https://doi.org/10.1038/sj.bjp.0707305

Ervina, M., Pratama, M. R. F., Poerwono, H., Ekowati, J., Widyowati, R., Matsunami, K., & Sukardiman. (2021). In silico estrogen receptor alpha antagonist studies and toxicity prediction of Melia azedarach leaves bioactive ethyl acetate fraction. *Journal of Advanced Pharmaceutical Technology & Research*, *12*(3), 236–241. https://doi.org/10.4103/japtr.JAPTR_198_21

Elfita, L., Apriadi, A., Supandi, S., & Dianmurdedi, S. (2023). Studi penambatan molekuler dan simulasi dinamika molekuler senyawa turunan Furanokumarin terhadap reseptor estrogen alfa (ER-α) sebagai anti kanker payudara. *Jurnal Sains Farmasi & Klinis*, *9*(3), 255–264. https://doi.org/10.25077/jsfk.9.3.255-264.2022

Globocan. (2020). Cancer Incident in Indonesia. *International Agency for Research on Cancer*, *858*, 1–2.

Hamzah, N., Najib, A., Thahir, N., & Misqawati, I. (2014). Studi farmakofor reseptor COX-2 sebagai anti inflamasi. *Jurnal Farmasi UIN Alauddin Makassar*, *2*(3), 99–107.

Hartanti, I. R., Putri, A. A., AS, N. N. A., Triadenda, A. L., Laelasari, E., Suhandi, C., & Muchtaridi, M. (2022). Molecular docking senyawa xanton, benzofenon, dan triterpenoid sebagai antidiabetes dari ekstrak tumbuhan Garcinia Cowa. *Jurnal Kimia*, *16*(1), 72–83. https://doi.org/10.24843/JCHEM.2022.v16.i01.p10

Moo, T.-A., Sanford, R., Dang, C., & Morrow, M. (2018). Overview of breast cancer therapy. *PET Clinics*, *13*(3), 339–354. https://doi.org/10.1016/j.cpet.2018.02.006

Moussa, N., Hassan, A., & Gharaghani, S. (2021). Pharmacophore model, docking, QSAR, and molecular dynamics simulation studies of substituted cyclic imides and herbal medicines as COX-2 inhibitors. *Heliyon*, *7*(4). https://doi.org/10.1016/j.heliyon.2021.e06605

Nusantoro, Y. R., & Fadlan, A. (2020). Analisis sifat mirip obat, prediksi ADMET, dan penambatan molekular isatinil-2-aminobenzoilhidrazon dan kompleks logam transisi Co (II), Ni (II), Cu (II), Zn (II) Terhadap BCL2-XL. *Akta Kimia Indonesia*, *5*(2), 114–126. https://doi.org/10.12962/j25493736.v5i2.7881

Rabeta, M. S., Chan, S., Neda, G. D., Lam, K. L., & Ong, M. T. (2013). Anticancer effect of

underutilized fruits. *International Food Research Journal*, *20*(2), 551–556.

Sagitasa, S., Elizabeth, K., Sulaeman, L. I., Rafashafly, A., Syafra, D. W., Kristande, A., & Muchtaridi, M. (2021). Studi in silico senyawa aktif daun Singawalang (Petiveria alliacea) sebagai penurun kadar glukosa darah untuk pengobatan penyakit diabetes melitus tipe-2. *Chimica et Natura Acta*, *9*(2), 58–66. https://doi.org/10.24198/cna.v9.n2.34083

Suherman, M., Prasetiawati, R., Ramdani, D., Farmasi, P. S., Mipa, F., Garut, U., Jati, J., & Kaler, T. (2020). Skrining virtual senyawa aktif asam jawa (Tamarindus indica L.) terhadap selektif inhibitor siklooksigenase-2. *Jurnal Ilmiah Farmako Bahari*, 125–136. https://doi.org/10.52434/jfb.v11i2.879

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *71*(3), 209–249. https://doi.org/10.3322/caac.21660

Table 6. Results of lipinski's rule of five analysis, pharmacokinetics and toxicity analysis

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	Carsinogenity
Cyanidin-3,5-O-diglucosid	580	0	16	-3,69	2,16***	3,23***	47,73**	Non Mutagen	Negative
Quercitrin	428	0	11	0	24,94**	7,37**	64,95**	Non Mutagen	Negative
Peonidin 3,5-diglucosside	592	0	16	-1,77	4,27***	3,83***	29,76**	Non Mutagen	Negative
Pelargonidin-3-glucoside	412	0	10	-2,19	39,14**	6,59**	77,6**	Non Mutagen	Negative
Procyanidin B2	552	0	12	-0,71	19,51***	13,67**	100*	Non Mutagen	Negative
Peonidin-3-Glukosid	475	0	11	0	35,22**	6,84**	65,4**	Non Mutagen	Negative
Procyanidin B1	552	0	12	0	19,51***	13,67**	100*	Non Mutagen	Negative
Cyanidin-3-O-Glukosid	463	0	11	-1,28	19,72***	5,92**	79,61**	Non Mutagen	Positive
Cyanidin 3-glucoside	463	0	11	-1,28	19,72***	5,92**	79,61**	Non Mutagen	Negative
Procyanidin A2	552	0	12	-2,83	35,29**	9,23**	100*	Non Mutagen	Negative
2',4'-Dihydroxy-6'-Methoxy-3-Methyldihidrochalcone	268	0	4	0,57	92,76*	18,49**	96,08*	Mutagen	Negative
Stercurensin	268	0	4	0,57	93,03*	18,43**	92,06*	Mutagen	Negative
Kaempferol-3-glucoside	428	0	11	0	25,17**	11,14**	57,57**	Non Mutagen	Negative
Isorhamnetin-3-glucoside	456	0	12	0	21,6**	9,93**	47,83**	Non Mutagen	Negative
Isoquercitrin	444	0	12	0	11,77***	9,43**	59,15**	Non Mutagen	Negative
(-)-Epicatechin	276	0	6	-1,56	66,7**	0,65***	100*	Mutagen	Negative
Quercetin	292	0	7	0	63,48**	3,41***	93,23*	Mutagen	Negative
(+)-Catechin	276	0	6	-1,56	66,7**	0,65***	100*	Mutagen	Negative
Morin	292	0	7	0	63,49**	17,1**	91,62*	Mutagen	Negative
Myricitrin	444	0	12	0	11,64***	6,14**	65,37**	Non Mutagen	Negative
Chlorogenic Acid	336	0	9	0	20,42**	18,71**	41,96**	Mutagen	Positive
Myricetin-3-(3"galloylrhamnoside)	592	0	16	0	4,07***	7,47**	100*	Non Mutagen	Positive
Mearnsitrin	456	0	12	0	21,45**	6,21**	55,6**	Non Mutagen	Negative
(-)-Epicatechin gallate	424	0	10	-2,88	40,58**	13,21**	100*	Non Mutagen	Negative
Desmanthin1	592	0	16	0	4,07***	13,67**	100*	Non Mutagen	Positif
Rutin	**580**	**0**	**16**	**0**	**2,86*** **	**7,91****	**43, 89****	**Non Mutagen**	**Negative**

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	Carsinogenity
Ursolic Acid	408	0	3	0,42	95,99*	21,86**	100*	Non Mutagen	Positive

Information

HIA(%):	CaCo-2(nm)	PPB (%):
70-100 is well absorbed	>70 high permeability	>90 tightly bound
20-70 absorbed enough	4-70 medium permeability	<90 weakly bound
<20 poorly adsorbed	<4 low permeability	