Inhibition of Human Epidermal Growth Factor Receptor-2 (HER-2) from Pomelo (Citrus maxima) Flavonoid Compounds: an In Silico Approach

Roihatul Mutiah*, Tanaya Jati Dharma Dewi, Arief Suryadinata, Kesimira Qonita

Department of Pharmacy, Faculty of Medicine and Health Science, Universitas Islam Negeri Maulana Malik Ibrahim Malang, Malang, Indonesia

Abstract

Citrus maxima or pomelo is a plant that has potential as an anticancer because it contains flavonoids. One of the targets of breast anticancer receptors is the HER-2 protein. This research aims to determine the anticancer activity, the toxicity of the compound, and the prediction of physicochemical properties of flavonoids contained in Citrus maxima through in silico approach. Flavonoid compounds were screened using SwissADME with Lipinski’s rule of five, Torsion, TPSA, and P-Gp Non-Substrate. Compounds that passed the screening were carried out molecular docking to the HER-2 receptor (PDB ID: 3PP0) using the Molegro Virtual Docker (MVD). The HER-2 receptor (PDB ID: 3PP0) was declared valid because it had RMSD<2Å. The results showed that there were 11 flavonoid compounds that passed the screening and had a lower rerank score than the comparison compound Trastuzumab. Toxicity was predicted using the Protox II online tool and the results showed that the flavonoid compounds were in the safe limits, namely classes 5 and 3. Based on this research, it can be concluded that acacetin, diosmetin, honyucitrin, isosinensetin, nobiletin, sinensetin, and tangeretin can be candidates for breast cancer drugs based on natural ingredients.

Keywords: breast cancer, Citrus maxima, HER-2, in silico.

INTRODUCTION

Cancer is a disease caused by the growth of abnormal cells and can not be controlled. This causes tissue damage that is dangerous for cancer sufferers (WHO, 2018). Breast cancer is a malignant tumor found in breast tissue, such as connective tissue, fat tissue, mammary glands, and milk ducts (Mansjoer, 2002). Based on data from The Global Cancer Statistics 2020, the number of new cases of breast cancer is the highest in the world, with a percentage of 11.7% or 55.9 per 100,000 women (Sung, et al., 2021). The percentage of cases of death caused by breast cancer is 6.9%. More than 500,000 patients have died from breast cancer, with the highest incidence and mortality rates among other cancers in women (Kemenkes RI, 2015).

The Human Epidermal Growth Factor Receptor–2 (HER-2) protein is one of the receptors on breast cells (Sulasstri and Murti, 2017). Breast cancer cases with HER-2 positive can cause faster and aggressive tumor growth, triggering an increase

Submitted: December 29, 2021
Revised: February 15, 2022
Accepted: February 16, 2022

*Corresponding author: roiha@farmasi.uin-malang.ac.id
in the degree of malignancy of cancer cells, distant metastases to the brain and lungs. This causes the second-worst prognosis among other breast cancer subtypes, high recurrence rate, and lower life expectancy (Liao, 2016; Vu and Claret, 2012; Yersal and Barutca, 2014).

One of the breast cancer drugs that work as anti-HER-2 is Trastuzumab (HerceptinTM). Trastuzumab is a first-generation drug in the form of a monoclonal antibody that targets ovarian cells that have increased HER-2 protein or HER-2 overexpression (Jiang, et al., 2018). Trastuzumab or HerceptinTM has been recommended by The Food and Drug Administration (FDA) as a direct therapy for HER2. The results of the meta-analysis revealed that the effectiveness of using trastuzumab as an adjuvant therapy that targets HER-2 can reduce the risk of recurrence, reduce the incidence of local and distant metastases, and reduce overall mortality (Holla, et al., 2016). Long-term use of trastuzumab will cause harm to the body, which can cause cardiotoxicity such as heart failure followed by a decrease in the Left Ventricular Ejection Fraction (LVEF) (Huszno, et al., 2013). There are about 4-7% cases of decreased myocardial function due to the use of trastuzumab (Yeh, et al., 2004). Based on this, there is a need for treatment for cancer that is not only effective but also can reduce side effects due to the use of anticancer drugs. One source of medicine that needs to be developed is materials derived from nature.

Citrus maxima or pomelo is a group of fruits that are commonly consumed by the public. A previous study reported that the ethanol fraction of leaves Citrus maxima caused 69.1% of HeLa cell death (Shivananda, 2013). Another study stated that pomelo fruit extract has potential as an anticancer breast with an IC\textsubscript{50} of 234 g/µL (Maritha, 2020). Previous research reports have never screened the affinity of flavonoid compounds in Citrus maxima was screened for HER-2 receptors through \textit{in silico} approach.

**MATERIALS AND METHODS**

**Materials**

The material used is the HER-2 (GDP ID: 3PP0) which was downloaded from https://www.rcsb.org/ and flavonoid compounds in Citrus maxima, including 4’-5-7-8-tetramethoxy-flavone, acacetin, apigenin trimethyl ether, cosmosin, diosmetin, diosmin, eriocitrin, hesperidin, honyucitrin, isosinensetin, luteolin, naringenin, naringin, naringin glucoside, narirutin, neodiosmin, neoricietin, nobiletin, neoponcirin, neohesperidin, poncirin, quercetin, rhoifolin, rutin, sinensetin, and tangeretin whose molecular structures were drawn using Chem Bio Draw Ultra version 12 (CambridgeSoft).

The tools used include hardware in the form of a set of ASUS laptops with specifications for Processor type Intel® Core\textsuperscript{TM} i7 and 8 GB RAM and operating system software Windows 10 Home Single Language, Chem Bio Draw Ultra version 12 (CambridgeSoft), Chem Bio 3D Ultra version 12 (CambridgeSoft), Molegro Virtual Docker version 6.0 (Molegro ApS), SwissADME, pkCSM, and Pro-tox Online Tool.

**Compound Screening**

The 2D molecular structures of 26 flavonoid compounds were drawn using the application Chem Bio Draw Ultra version 12. Then, copy the SMILES code in the online application SwissADME. After that, the compounds that complied with Lipinski’s Rule of Five were screened for Topological Polar Surface Area (TPSA), Torsion, and were P-Gp Non-substrate.

**Sample Preparation by \textit{In Silico}**

Sample preparation is done by making the compound 3-D structure of the compound with pro-
gram Bio Chem 3D Ultra version 12. Do energy minimization by pressing MMFF94 → Calculations → Perform → MMFF94 → Minimization. This is to determine the most stable stereochemical form of the compound. Then saved in mol2 {SYBYL2(*.mol2)} format. Receptor sample preparation was carried out by eliminating water molecules and the reference ligand and adding hydrogen atoms using the application Molegro Virtual Docker version 6.0.

Validation Method of Docking

The validation method of docking is done by docking a native ligand in the cavity receptor using the application Molegro Virtual Docker version 6.0. The results of the receptor validation were interpreted with the value of Root Mean Square Deviation (RMSD). Receptors can be said to be valid if they meet the criteria for the RMSD value 2Å (Jain and Nicholls, 2008).

Docking Ligand-Protein

Ligand-protein docking is done by detecting cavities where the drug will bind or interact with receptors. Place the 3-Dimensional structure of the compound into cavities selected. The docking of compounds on the receptor is done automatically by Molegro Virtual Docker. The parameter measured is the energy value in the form of rerank score.

Prediction of Compound Toxicity

Prediction of compound toxicity is done by copying the SMILES code in the application Pro-tox online tool (http://tox.charite.de/protox_II/) to predict the LD50. Then, predictions of Ames toxicity and Hepatotoxicity were carried out using the website pkCSM (http://biosig.unimelb.edu.au/pkcsm/prediction).

RESULTS

Compound Screening Results

Compound screening parameters in this study were P-Gp non-substrate, physicochemical properties, torsion, and Topological Polar Surface Area (TPSA). Screening results on P-Gp non-substrate parameters are shown in Figure 1. Another parameter that was carried out was a prediction of physicochemical properties. The results obtained on the screening of compounds based on the parameters of physicochemical properties and P-Gp non-substrate are shown in Table 1. There were 11 compounds that passed the screening based on the fulfillment of Lipinski’s rule of five and were P-Gp non-substrate. The eleven compounds were 4’-5-7-8-tetramethoxyflavone, acacetin, apigenin trimethyl ether, diosmetin, honycitrin, isosinensetin, luteolin, nobiletin, quercetin, sinensetin, and tangeretin.

![Figure 1. Results of P-Gp non-substrate flavonoid compounds and comparator compounds.](image)
Table 1. Results of screening for flavonoid compounds in *Citrus maxima* and comparison compounds.

| Name of Compounds                          | Parameters Lipinski’s Rule of Five | Application of Lipinski’s Rule of Five | TPSA (Å²) | Torsion | P-gp Substrate |
|-------------------------------------------|------------------------------------|----------------------------------------|------------|---------|----------------|
|                                           | MW (g/mol) | Log P | HBA | HBD | Yes | 67.13 | 5 | No |
| 4’-5-7,8- tetramethoxyflavonea             | 342.34     | 3.00  | 6   | 0   | Yes | 67.13 | 5 | No |
| Acacetina                                 | 284.26     | 2.52  | 5   | 2   | Yes | 79.90 | 2 | No |
| Apigenin trimethyl ethera                 | 312.32     | 3.10  | 5   | 0   | Yes | 57.90 | 4 | No |
| Cosmosinin                                | 432.38     | 0.52  | 10  | 6   | Yes | 170.05 | 4 | Yes |
| Diosmetina                                | 300.26     | 2.19  | 6   | 3   | Yes | 100.13 | 2 | No |
| Diosmina                                  | 608.54     | -0.52 | 15  | 8   | No  | 238.20 | 7 | Yes |
| Eriocitina                                 | 596.53     | -2.10 | 15  | 9   | No  | 245.29 | 6 | Yes |
| Hesperidina                                | 610.56     | -1.06 | 15  | 8   | No  | 234.29 | 7 | Yes |
| Honyucitinaa                               | 406.47     | 5.07  | 5   | 3   | Yes | 90.90  | 5 | No |
| Isosinensetinaa                            | 372.37     | 2.98  | 7   | 0   | Yes | 76.36  | 6 | No |
| Luteolinb                                 | 286.24     | 1.73  | 6   | 4   | Yes | 111.13 | 1 | No |
| Naringenin                                | 272.25     | 1.84  | 5   | 3   | Yes | 86.99  | 1 | Yes |
| Naringin                                  | 580.53     | -0.87 | 14  | 8   | No  | 225.06 | 6 | Yes |
| Naringin glucoside                        | 742.68     | -2.13 | 19  | 11  | No  | 304.21 | 9 | No |
| Narirutin                                 | 580.53     | -1.06 | 14  | 8   | No  | 225.06 | 6 | Yes |
| Neodiosimin                                | 608.54     | -0.35 | 15  | 8   | No  | 238.20 | 7 | Yes |
| Neoaeriocitrin                            | 596.53     | -1.15 | 15  | 9   | No  | 245.29 | 6 | Yes |
| Neohesperidin                             | 610.56     | -0.83 | 15  | 8   | No  | 234.29 | 7 | Yes |
| Neoponcirin                               | 594.56     | -0.48 | 14  | 7   | No  | 214.06 | 7 | Yes |
| Nobileinta                                | 402.39     | 3.02  | 8   | 0   | Yes | 85.59  | 7 | No |
| Poncirin                                  | 594.56     | -0.71 | 14  | 7   | No  | 214.06 | 7 | Yes |
| Quercetina                                | 302.24     | 1.23  | 7   | 5   | Yes | 131.36 | 1 | No |
| Rhofofin                                  | 578.52     | -0.81 | 14  | 8   | No  | 228.97 | 6 | Yes |
| Rutina                                    | 610.52     | -1.51 | 16  | 10  | No  | 269.43 | 6 | Yes |
| Sinensetinaa                               | 372.37     | 3.10  | 7   | 0   | Yes | 76.36  | 6 | No |
| Tangeretinaa                               | 372.37     | 3.02  | 7   | 0   | Yes | 76.36  | 6 | No |
| Trastuzumab                                | 298.26     | -2.48 | 7   | 6   | No  | 185.53 | 2 | No |

Description :
* : Compounds that comply with Lipinski’s rule of five and are P-Gp non-substrates.
The structure of 11 compounds that fulfill the Lipinski’s rule of five and a P-Gp non-substrate is presented in Figure 2.

**Results of Compound Preparation and Receptors by In Silico**

In this study, the creation of a three-dimensional (3D) structure and the minimization of the energy of the compound ligand using the application of ChemBio 3D Ultra version 12. The results of the 3D structure and energy minimization of the sample compounds are shown in Table 2.

The downloaded protein is HER-2 with protein code 3PP0 and contains the ligand 2-{2-[4-((5-chloro-6-[3-(trifluoromethyl)phenoxy] pyridine-3-yl)amino)-5H-pyrrolo [3,2-d]pyrimidine -5-yl]ethoxy}ethanol. Receptor preparation was carried out by eliminating water molecules and reference ligands and adding hydrogen atoms using the application Molegro Virtual Docker 6.0. The following is a 3PP0 protein display on the application Molegro Virtual Docker 6.0.

**Method Validation**

The docking method can be said to be valid if the receptor meets the criteria for the RMSD value ≤2Å (Jain and Nicholls 2008). Based on the results obtained, cavity 3 has an average RMSD value of 0.6243Å which is lower than cavity 2 with an RMSD value of 0.8698Å. Therefore, cavity 3 with native ligand A was used for the process of docking next.

**Ligand Bonding with Receptors**

The docking of ligands with receptors can be seen through the results of the binding energy or Rerank score (CLCbio, 2013). In this study, it was found that the test compound that had the rerank...
Table 2. The minimum energy of flavonoid compounds in *Citrus maxima* that passed the screening.

| Name of Compound                        | Average Minimum Energy (Kcal/mol)±SD |
|-----------------------------------------|-------------------------------------|
| 4′-5-7-8-tetramethoxyflavone            | 5.8205±0.0002                       |
| Acacetin                                | 8.6829±0.0003                       |
| Apigenin trimethyl ether                | 8.3151±0.0002                       |
| Diosmetin                               | 44.582±0.02                         |
| Honyucitrin                             | 69.9004±0.4                         |
| Isoisinensetin                          | 112.936±0.04                        |
| Luteolin 3                              | 1.1005±0.0007                       |
| Nobiletin                               | 138.083±0.07                        |
| Kuersetin                               | 63.8500±0.10                        |
| Sinensetin                              | 117.243±0.02                        |
| Tangeretin                              | 120.449±0                           |
| Trastuzumab                             | 14.1956±0.001                       |

score lowest was sinensetin, while the one with the rerank score highest was quercetin. The results of the docking are shown in Table 4.

In this study, there was an interaction of the ligand with the active amino acid present at the 3PP0 receptor. Active amino acids with hydrogen bonds in native ligands are Asp 863(A), Thr 862(A), Met 801(A), Asn 850(A). Compounds that bind amino acids are the same as native ligands with hydrogen interactions, including acacetin, apigenin trimethyl ether, diosmetin, honyucitrin, isosinensetin, luteolin, nobiletin, quercetin, sinensetin, tangeretin, and trastuzumab. In addition, there are also amino acids with steric bonds in native ligands, including Met 774(A), Thr 862(A), Ala 751(A), Met 801(A), Val 734(A), Asn 850(A), and Asp 863(A). All test compounds that have passed the screening bind to the same amino acids as native ligands. Then, there are also amino acids in the electrostatic bond. However, in the results of this study, none of the amino acids in the electrostatic interaction were bound by native ligands, the test compound, or the comparison compound trastuzumab. Bonds with amino acids are shown in Figure 5.

![Figure 3. 3PP0 protein via MVD version 6.0.](image)

![Figure 4. Bonding of the test compound, comparison compound, and native ligand with the receptor.](image)
**Figure 5. Amino acid native ligand binding** of (A), 4’-5,7,8-tetramethoxyflavone (B), acacetin (C), apigenin trimethyl ether (D), diosmetin (E), honyucitrin (F), isosinensetin (G), quercetin (H), luteolin (I), nobiletin (J), sinensetin (K), tangeretin (L), trastuzumab (M) where blue lines represent hydrogen bonds, red line as steric bond, and green line as electrostatic bond.

**Compound Toxicity**

The toxicity parameters used were LD$_{50}$, Ames toxicity, and Hepatotoxicity. Based on the Globally Harmonized System (GHS) the level of toxicity is divided into classes I to VI. The toxicity class uses threshold values LD$_{50}$ of 5, 50, 300, 2000, and 5000 mg/kg body weight (El-Din, *et al.*, 2016). Compounds classified as toxicity class 5 (2000< LD$_{50}$≤5000) are compounds that have low toxicity with the category of possibly being harmful if swallowed, not mutagenic, and not toxic to the liver. Compounds at that level are Acacetin, Diosmetin, Honyucitrin, Isosinensetin, Nobiletin, Sinensetin, and Tangeretin. Prediction results of the toxicity of the test compounds are shown in Table 4.
DISCUSSION

The purpose of this study was to find candidate flavonoid compounds in agents *Citrus maxima* which have the potential as breast anticancer that act on the HER-2 target receptor. HER-2 is receptor tyrosine kinases which are located on the cell membrane and responds to a wide variety of ligands. Phosphorylation of the tyrosine kinase domain in the cytoplasm initiates downstream oncogenic signaling pathways such as PI3K/AKT pathway and Ras/MAPK pathway (Feng, et al., 2018). Flavonoid has showed beneficial results regarding the inhibition of HER-2 signaling. The mechanisms implicated are the inhibition of the AKT pathway (Saxena, et al., 2020). The parameters used in this study were to predict physicochemical properties, P-Gp non-substrate, an affinity for HER2. In addition, toxicity prediction was carried out to determine the safety of flavonoid compounds.

Screening is a preliminary test before docking the compound. The purpose of screening is to predict the physicochemical properties of a compound so that the best compound will be obtained that can bind to the receptor. Based on the results in Table 1, of the 26 flavonoid compounds in *Citrus maxima*, 12 compounds were P-Gp non-substrate. P-Gp is a member of the transporter superfamily ATP Binding Cassette (ABC), which is a determinant of various processes of penetration and absorption of drug compounds. P-Gp activity is highly dependent on ATP by forming a P-Gp-ATP complex. Inhibition of P-Gp activation and expression plays an important role in the success of cancer therapy (Finch and Pillans, 2014).

The next screening is based on Lipinski’s rule of five, where the rules can predict the biological activity of a compound designed for oral administration. According to the Lipinski rules, a drug intended for oral use must not violate more than one of the criteria contained in the Lipinski rules (Kartasasmita, et al., 2015). Based on these rules, drug compounds are required to have a molecular weight of <500g/mol, a log P value<5, a Hydrogen Bond Donors (HBD) value of ≤5, and a Hydrogen Bond Acceptors (HBA) value of ≤10. In a subsequent study, two additional rules were found, which aim to improve the bioavailability of...
Table 3. Results of docking and amino acid bonds of compounds passed screening and trastuzumab to 3PP0 receptors.

| Name of Compound       | Hydrogen Bonds | Amino Acid                           | Steric Bonds |
|------------------------|----------------|--------------------------------------|--------------|
| 4’-5-7-8-tetramethoxyflavone |                | Met 801(A)*, Leu 796(A)**            |              |
| Acacetin               |                | Asp 863(A)**, Thr 862(A)**, Ser 783(A)**, Lys 753(A)** |              |
| Apigenin trimethyl ether |                | Leu 785(A), Thr 862(A)**, Met 801(A)* |              |
| Diosmetin              |                | Asp 863(A)**, Thr 862(A)**, Lys 753(A)** |              |
| Honyucitrin            | Leu 726(A), Met 801(A)* | Val 734(A)*, Leu 726(A), Gly 804(A), Met 801(A)*, Ala 751(A)** |              |
| Diosmetin              | Leu 785(A), Lys 753(A)** | Asn 850(A)*, Asp 863(A)**, Thr 862(A)**, Val 797(A), Leu 796(A)**, Thr 798(A)** |              |
| Luteolin               | Arg 849(A), Asp 863(A)**, Thr 862(A)** | Arg 849(A), Asp 863(A)**, Asn 850(A)*, Thr 862(A), Leu 796(A)**, Val 797(A), Lys 753(A)** |              |
| Nobiletin              | Thr 862(A)**, Ser 783(A)** | Val 797(A), Leu 785(A), Ser 783(A)**, Thr 862(A)**, Leu 852(A), Leu 726(A), Cys 805(A) |              |
| Quercetin              | Lys 753(A)**, Leu 726(A), Met 801(A)*, Gin 799(A) | Met 801(A)*, Gin 799(A), Thr 798(A) | Leu 796(A)**, Val 734(A)*, Leu 726(A), Met 801(A)*, Gly 804(A), Gin 799(A), Thr 798(A) |
| Sinensetin             | Ser 783(A)**, Thr 862(A)** | Thr 862(A)**, Ser 783(A)**, Met 801(A)*, Asp 863(A)** |              |
| Tangeretin             | Cys 805(A), Met 801(A)* | Thr 862(A)**, Ala 751(A)**, Val 734(A)*, Cys 805(A), Gly 804(A), Met 801(A)* |              |
| Trastuzumab            | Glu 770(A), Asp 863(A), Leu 796(A), Ala 751(A), Lys 753(A), Thr 862(A), Thr 798(A), Ser 783(A) | Ala 771(A), Glu 770(A), Asp 863(A), Leu 796(A), Ala 751(A), Lys 753(A), Thr 798(A), Thr 862(A), Ser 783(A), Arg 784(A) |              |
| Native Ligand (SYR127063) | Asp 863(A), Thr 862(A), Met 801(A), Asn 850(A) | Met 774(A), Thr 862(A), Ala 751(A) | Met 801(A), Val 734(A), Asn 850(A), Asp 863(A) |              |

Description:

* : Amino acid same as the native ligand at the 3PP0 receptor.
** : Amino acid same as the comparison drug.
*** : Amino acids same as the native ligand and comparison drug.

drugs intended for oral use. The rules are Topological Polar Surface Area (TPSA) ≤140 and Torsion ≤10 (Chagas, et al., 2018). Based on the results in Table 1, there were 11 compounds that passed the screening marked by the fulfillment of all physicochemical parameters and were P-Gp non-substrate. These compounds include 4’-5-7-8-tetramethoxyflavone, acacetin, apigenin trimethyl ether, diosmetin, honyucitrin, isosinensetin, luteolin, nobiletin, querce tin, sinensetin, and tangeretin. These compounds can be predicted to have good absorption, distribution, and oral bioavailability (Silverman and Holladay, 2014).

Before docking the compound, first, determine the cavity to detect where the ligand interacts with the receptor. Next, validation of the method is carried out by docking native ligands in the cavity receptor. The method can be said to be valid if it meets the criteria for the RMSD value ≤2Å (Jain and Nicholls, 2008). There are two cavities that have native ligands, namely cavity 2 and 3. Based on the results obtained, cavity 3 with native ligand
Table 4. Predicted results of the toxicity of flavonoid compounds in *Citrus maxima*.

| Name of Compound                  | LD₅₀ (mg/kg)* | Toxicity Class* | Ames Toxicity** | Hepatotoxicity** |
|----------------------------------|--------------|-----------------|----------------|-----------------|
| 4’,5-7-8-tetramethoxyflavone     | 4000         | 5               | Yes            | No              |
| Acacetin                         | 4000         | 5               | No             | No              |
| Apigenin trimethyl ether         | 3919         | 5               | Yes            | No              |
| Diosmetin                        | 3919         | 5               | No             | No              |
| Honyucitin                       | 3919         | 5               | No             | No              |
| Isosinensetin                    | 5000         | 5               | No             | No              |
| Luteolin                         | 5000         | 5               | Yes            | No              |
| Nobiletin                        | 5000         | 5               | No             | No              |
| Quercetin                        | 159          | 3               | No             | No              |
| Sinensetin                       | 5000         | 5               | No             | No              |
| Tangeretin                       | 5000         | 5               | No             | No              |

A has an average RMSD value lower than cavity 2, therefore cavity 3 is used for the process *docking*. The lower RMSD value indicates that the ligand poses the predicted will be closer to the conformation native ligand (Saputra, 2018).

The binding energy of the compound with the receptor can be known through the *rerank score* (CLCbio, 2013). The *rerank score* was obtained from the calculation of the total energy of all bonds. Based on the results in Table 4, the *rerank score* of 11 test compounds is lower than the comparison compound trastuzumab, it can be concluded that all test compounds that passed the screening have the same amino acid binding as native ligands and comparison compounds. The similarity of amino acids bound by the test compound with the comparison compound and native ligand indicates that the compound is predicted to have the same activity as the comparison compound and native ligand, this is because amino acids are the active site on the receptor (Puspita, 2020).

Toxicity is a condition that indicates that there is a poison or toxic effect on the ingredients contained in drug preparation. The LD₅₀ is the concentration of a compound that can cause 50% death in experimental animals (Kesuma, *et al*., 2018). The Ames Toxicity test is a test used to determine the presence of mutagenic potential in compounds. If a positive test result is obtained, it indicates that the compound is mutagenic and can act as a carcinogen (Kesuma, *et al*., 2018). Test Hepatotoxicity aims to determine the ability of a compound to damage the liver. Based on the results in Table 5, the compounds Acacetin, Diosmetin, Honyucitin, Isosinensetin, Nobiletin, Sinensetin, and Tangeretin are classified as toxicity class 5, are not mutagenic, and do not cause liver toxicity. Compounds 4’,5-7-8-tetramethoxyflavone, Apigenin trimethyl ether, and Luteolin are classified as toxicity class 5, they do not cause liver toxicity.
but are mutagenic. Meanwhile, quercetin is classified as a toxicity class 3 with a toxic category when ingested, is not mutagenic, and does not cause liver toxicity.

**CONCLUSION**

Acacetin, Diosmetin, Honyucitrin, Isosinensetin, Nobiletin, Sinensetin, and Tangere-tin have met all physicochemical parameters, have a lower *rerank score* than the comparison compound trastuzumab, and are classified in class 5 toxicity, are not mutagenic, and do not cause liver toxicity. Therefore, these compounds can be candidates for breast cancer drugs based on natural ingredients.

**REFERENCES**

Chagas, C.M., Moss, S., and Alisaraie, L., 2018, Drug Metabolites and Their Effects on the Development of Adverse Reactions: Revisiting Lipinski’s Rule of Five, *International Journal of Pharmaceutics*, 549(1-2), 133-149.

El-Din, H.M.A., Loutfy, S.A., Fathy, N., Elberry, M.H., Mayla, A.M., Kassem, S., and Naqvi, A., 2016, Molecular Docking Based Screening of Compounds against VP40 from Ebola Virus, *Bioinformation*, 12(3), 192–196.

Feng, Y., Spezia, M., Huang, S., Yuan, C., Zeng, Z., Zhang, L., *et al.*, 2018, Breast Cancer Development And Progression: Risk Factors, Cancer Stem Cells, Signaling Pathways, Genomics, and Molecular Pathogenesis, *Genes & diseases*, 5(2), 77-106.

Finch, A., and Pillans, P., 2014, P-Glycoprotein and Its Role in Drug-Drug Interactions, *Australian Prescriber*, 37(4), 137-39.

Ha, C.H.H., Fatima, A., and Gaurav, A., 2015, *In Silico* Investigation of Flavonoids as Potential Trypanosomal Nucleoside Hydrolase Inhibitors, *Advances in Bioinformatics*, 2015, 826047.

Holla, S.N., Nayak, V., Bairy, K.L., Tripathy, A., and...
Holla, S., 2016, Her-2 Gene, Receptors and Drug Target: A Systematic Review, *International Journal of Pharmacy and Pharmaceutical Sciences, 8*(4), 4-9.

Husznio, J., Les, D., Sarzyczny-Slota, D., and Nowara, E., 2013, Cardiac Side Effects of Trastuzumab in Breast Cancer Patients-Single Centere Experiences, *Contemp Oncol (Pozn)*, 17(2), 190-195.

Jain, A.N., and Nicholls, A., 2008, Recommendations for Evaluation of Computational Methods, *Journal of Computer-Aided Molecular Design, 22*(3-4), 133-139.

Jiang, N., Lin, J-J., Wang, J., Zhang, B-N., Li, A., Chen, Z-Y., et al., 2018, Novel Treatment Strategies for Patients with HER2 Positive Breast Cancer Who Do Not Benefit from Current Targeted Therapy Drugs, *Experimental and Therapeutic Medicine, 16*(3), 2183-2192.

Kartasasmita, R.E., Anugrah, R., and Tjahjono, D.H., 2015, Kajian Docking Dan Prediksi Beberapa Aspek Farmakokinetika Desain Molekul Turunan Kuinin Sebagai Upaya Menemukan Kandidat Senyawa Antimalaria Yang Baru, *Kartika Jurnal Ilmiah Farmasi, 3*(1), 6–13.

Kementerian Kesehatan Republik Indonesia, 2015, *Buletin Kanker.* Jakarta: Pusat Data dan Informasi Kementerian Kesehatan RI.

Kesuma, D., Siswandono, S., Purwanto, B.T., and Hardjono, S., 2018, Uji in Silico Aktivitas Sito-toksik dan Toksisitas Senyawa Turunan N-(Benzoil)-N' Feniltiourea Sebagai Calon Obat Antikanker, *Journal of Pharmaceutical Science and Clinical Research, 3*(1), 1-11.

Liao, N., 2016, HER2-Positive Breast Cancer, How Far Away from the Cure?-On the Current Situation of Anti-HER2 Therapy in Breast Cancer Treatment and Survival of Patients, *Chinese Clinical Oncology, 5*(1), 41.

Puspita, D., 2020, Studi In Silico Senyawa dalam Ekstrak Etanol 96% Daun Krisan (Chrysanthemum cinerariifolium (Trev.)) terhadap Reseptor Estrogen Alfa (SW9C) [Skripsi], Malang: Program Studi Farmasi UIN Maulana Malik Ibrahim Malang.

Saxena, A.R., Ilic, Z., Sripada, V., and Crawford, D.R., 2020, Lower Concentrations of Curcumin Inhibit Her2-Akt Pathway Components In Human Breast Cancer Cells, and Other Dietary Botanicals Potentiate This and Lapatinib Inhibition, *Nutrition Research, 79*, 93-104.

Shivananda, A., Muralidhara R.A.O.D., and Jayaveera K.N., 2013, Anticancer Activity of Extracts of Various Fractions of *Citrus maxima* (J. Burm.) Merr. on HeLa Cell Line, *Journal of Pharmacy and Chemistry, 7*(1), 3-7.

Silverman, R.B., and Holladay, M.W., 2014, The Organic Chemistry of Drug Design and Drug Action, Third Edition, 123-163, Academic Press.

Sulastri, H., and Murti, K., 2017, Hubungan Antar Overekspresi Vascular Endothelial Growth Factor Dengan ER, PR, HER-2, Ki67 Pada Subtipe Molekular Karsinoma Payudara Invasif Tidak Spesifik, *Jurnal Kedokteran dan Kesehatan, 4*(1), 25-34.

Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jamal, A., and 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.

Thavana pong, N., 2006, The Essential Oil From Peel and Flower of Citrus maxima. [Tesis]. Master of Pharmacy, Silpakorn University.

Vu, T., and Claret, F.X., 2012, Trastuzumab: Updated Mechanisms of Action and Resistance in Breast Cancer, *Frontiers in Oncology, 2*, 62.

World Health Organization. 2018. Cancer. https://www.who.int/health-topics/cancer#tab=tab_1. (accessed: 28 Februari 2021).

Yeh, E.T.H., Tong, A.T., Lenihan, D.J., Yusuf, S.W., Swafford, J., Champion, C., et al., 2004,
Cardiovascular Complications of Cancer Therapy: Diagnosis, Pathogenesis, and Management, *Circulation*, 109(25), 3122-3131.

Yersal, O., and Barutca, S., 2014, Biological Subtypes of Breast Cancer: Prognostic and Therapeutic Implications, *World Journal of Clinical Oncology*, 5(3), 412-424.