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Red Betel Leaf Bioactive Compounds as ERα Receptor Inhibitors *In Silico* and MCF-7 Cell Anticancer *In Vitro*

Ayu Tri Nursyarah[1], Mega Safithri[1,2*], Dimas Andrianto[1]

[1]*Departement of Biochemistry, Faculty of Mathematics and Natural Science, IPB University, Dramaga Campus, Bogor 16680, Indonesia*
[2]*Tropical Biopharmaca Research Center (Biopharmaca), Institute for Research and Community Service, IPB University, Bogor, 16128, Indonesia*

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ABSTRACT

Cancer is one of the leading causes of death in the world. Excess endogenous estrogen is a risk factor for breast cancer. Red betel leaf herbal plants have been used as an alternative cervical and colon cancer treatment. This study aimed to obtain the active compounds that play a role in ERα receptor inhibitors *in silico* and to determine the anti-breast cancer cytotoxic activity of the extract and fraction of red betel leaf against MCF-7 cells. We use *in silico* research method using the YASARA Structure software with anti-breast cancer receptors, namely 3ERT, and *in vitro* using the MTT test on the anti-breast cancer cytotoxic activity of MCF-7 cells. There are 38 compounds that were obtained from the research. The results of the in silico test showed that the bioactive compound that played a role in inhibiting the ERα was 2-(4-Hydro xyphenyl)-2-phenyl-N(3,3-diphenylpropyl)-acetamide (44578655), a compound from water fraction, with an inhibition constant of 2.82 × 10^{-8} µM and Gibbs free energy of –10.2880 Kcal/mol. *In vitro* results showed that the best cell growth inhibition value was obtained from the n-hexane fraction at a concentration of 500 ppm of 73.42%. The conclusions of this study indicate that the bioactive compound of red betel leaf is water fraction is the best fraction inhibition. However, the hexane fraction proved to have cytotoxic activity against breast cancer MCF-7 cells.

1. Introduction

Cancer is one of the leading causes of death in the world. Based on data from the Global Burden of Cancer (GLOBOCAN) released by the World Health Organization (WHO) in 2020, shows that breast cancer ranks first in new cases and fifth in deaths from breast cancer. The number of new cases of breast cancer was 2,261,419 (11.7%) from 19,292,789 new cases of all types of cancer, and the death rate from breast cancer was 684,996 (6.9%) of 9,958,133 deaths from all types of cancer. According to (Kemenkes 2019) the number of breast cancer deaths in Indonesia reached 9.6% in 2019. Breast cancer cases in postmenopausal women have higher estrogen levels than in premenopausal women. The role of endogenous estrogen as a carcinogen in premenopausal women is not as high as in postmenopausal women (Warjianto *et al.* 2020).

* Corresponding Author
E-mail Address: safithri@apps.ipb.ac.id

Estrogen levels in the body are caused by excessive leptin and are directly related to breast cancer-causing DNA damage that results in mutations in genes that control the process of cell division. Estrogen provides sexual characteristics in women, affects various specialized organs and tissues, and regulates cell proliferation and differentiation in women or men. It is suspected that excessive exposure to endogenous estrogens at the premenopause stage of a woman's life contributes to and is the cause of breast cancer (Yanger and Davidson 2006).

The conventional treatment commonly used by breast cancer patients is tamoxifen. Tamoxifen is a selective estrogen-receptor modulator (SERM) drug class and reduces breast cancer cell growth. The side effects of tamoxifen arise due to the selective nature of this drug's receptors. Tamoxifen can act as an estrogen antagonist in tissue while as an estrogen agonist in the there tissue. According to Schultink *et al.* 2015) research the most complained side effects

of tamoxifen are hot flashes and sweating, abnormal vaginal discharge, depression, and skin disorders such as itching and rash (Schultink *et al.* 2015). Therefore, herbal plant users have been used as an alternative treatment that does not cause side effects (Debbie *et al.* 2012). Red betel is one of the herbal plants shown to have anticancer activity (*Piper crocatum* Ruiz and Pav.).

Red betel (*Piper crocatum* Ruiz and Pav) is a popular traditional herbal plant in Asia commonly referred to as the Golden Heart of Nature. Based on previous studies *in vitro*, the best anticancer potential was found in red betel leaf simplicia which had the highest inhibitory activity of 85.72% in HeLa cells with a concentration of 800 ppm (Suci 2014). Ethanol extract and ethyl acetate fraction of red betel leaf showed the best inhibition results at a concentration of 500 ppm, which was 98.18±1.21% in colon cancer (Umar 2022). Red betel leaf can inhibit the proliferation of human breast cancer cells (T47D) by the MTT method, with the potential of inhibition of 50% (Wicaksono *et al.* 2009). This research is expected to reveal one of the potentials of red betel leaf by using the estrogen receptor alpha *in silico* with the molecular docking method and determine the cytotoxicity activity of the red betel compound by using the MTT assay indicator on MCF-7 cell culture *in vitro.*

2. Materials and Methods

2.1. Materials

The materials used in the *in silico* research were ligands from the chemical structure of the ERα with 38 ligands (bioactive compounds) from red betel leaf (*Piper crocatum* Ruiz and Pav.) from LCMS ethanol extract and 3 fractions (n-hexane, ethyl acetate and water) and 1 ligand comparison. We obtained based on the research (Afifah *et al.* 2020), (Amalia *et al.* 2020), (Anugrahwati *et al.* 2016), (Safithri *et al.* 2016), (Safithri *et al.* 2022) and (Septiani 2017). The materials used in the *in vitro* study were red betel leaf (*Piper crocatum* Ruiz and Pav.) obtained from the Special Region of Yogyakarta, 70% ethanol (Merck Germany), ethyl acetate (Merck Germany), n-hexane (EMSURE® EMD-Milipore Corporation), water, MCF-7 cancer cells, Dulbecco's Modified Eagle Medium (D-MEM) (Gibco, USA), Fetal Bovine Serum (FBS) (Hyclone, USA), Penisilin-streptomisin (Invitrogen, USA), Trypsin (Gibco, USA), DMSO 1% (Sigma, USA), MTT (Sigma, USA).

2.2. Methods

2.2.1. *In Silico*

2.2.1.1. Ligand and Receptor Preparation

Receptor and ligand preparation was carried out using YASARA Structures (Watching nature work) Test ligands from red betel leaf in SDF format with a three-dimensional shape were obtained from the PubChem Compound database on the page http://pubchem.ncbi.nlm.nih.gov. The test and comparison ligands were geometrically optimized using the energy minimization method with the em_runclean.mcr program in YASARA Structure software. Receptor preparation was carried out using YASARA Structure. The receptor used is an ERα with ID 3ERT on the RSCB PDB page https://www.rcsb.org/. The receptors used were target proteins from 3D modeling results of ER. Unneeded parts in the docking protocol were removed, such as water molecules, residues, and native ligands. After that, hydrogen atoms were added while aliphatic hydrogen was not displayed (Agistia *et al.* 2013).

2.2.1.2. Ligand Bioavailability and Toxicity Prediction

The prediction of pharmacokinetic properties, toxicity, bioactivity, and Lipinski of the test ligands and comparison ligands were carried out before the molecular docking simulation. The test ligands and comparison ligands were analyzed for physicochemical properties on the http://www.scfbioiitd.res.in/software/drugdesign/lipinski.jsp page based on Lipinski's five rules. Toxicity analysis of the test and comparison ligands was then carried out by uploading the SMILES structure of each ligand on the http://lmmd.ecust.edu.cn/admetsar1/predict/ page to determine the level of toxicity of a compound to the body. The results of the analysis were obtained to identify drug candidates with the best oral pharmacokinetics (Hamzah *et al.* 2015).

2.2.1.3. Validation and Molecular Docking Simulation

Validation is carried out by directed molecular docking using Yasara structure with a targeted docking technique. The ligand docking zone is limited by a gridbox around the active site on the

ligand and the estrogen receptor alpha (Agistia *et al.* 2013). Molecular docking of the test ligands (natural ligands/bioactive compounds) and the comparison ligands was carried out using the optimum gridbox obtained at the gridbox validation stage. The receptor preparation file is opened in the YASARA Structure software in the format '_receptor.sce'. All test ligands and comparison ligands are opened individually in PDB format. Then, the file is prepared by forming a ligand-receptor complex based on a predetermined gridbox, then saved in the format "_complex.sce". This step is carried out like the gridbox validation step. Docking results can be read in ".log" format on the notepad software. Other docking results are binding energy values stored in ".txt" format and non-covalent interactions of ligand-receptor complexes stored in ".yob" format (Yasin *et al.* 2020).

2.2.1.4. *In Silico* Data Analysis

The Parameter observed are molecular docking analyzed by looking at the value of Gibbs free energy (Binding affinity energy). The Gibbs free energy value or the binding affinity energy value is used to analyze the binding affinity between the ligand and the receptor. Further analysis of the molecular docking results in the form of complex two- or three-dimensional visualization formed between the ligand and receptor. The visualization includes analysis of hydrogen bonds, hydrophobic interactions, and bond distances performed with Discovery Studio (2D) and Pymol (3D) Software. (Susila *et al.* 2022 with modifications).

2.2.2. *In Vitro*

2.2.2.1. Cytotoxicity Activity of Breast Cancer MCF-7 Cells

The conserved cells that had been grown in T25 flask were sub-cultured, then the cells grown in 96 wells tissue culture plate with a number of 5,000 cells/well and incubated for 24 hours in growth medium at 37°C and 5% CO_2. Bioactive compounds at each concentration were added as much as 100 µL/well, untreated cells were included as control cells which were then re-incubated for 49 hours. Compound 3-(4,5-Dimethyliazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) was added and incubated for 4 hours at 37°C and 5% CO_2. Cell supernatant was removed, and the formazan crystals formed were dissolved in 70% ethanol. Reading optical density (OD) was carried out using a microplate reader with a that wavelength of 565 nm:

$$\%\text{Inhibition} = \frac{(\text{Control cell OD-Treatment cell OD})}{\text{Control cell OD}} = 100\%$$

2.2.2.2. *In Vitro* Data Analysis

Statistical analysis was performed using the One-Way ANOVA test with SPSS, with a significance level of 0.05 ($p = 0.05$) with a 95% confidence level ($\alpha = 0.05$). If $p>0.05$ means that there is no significant difference, on the other hand if $p<0.005$ means that there is a significant difference. Prior to the One-way ANOVA test, it is necessary to perform a normality test to see whether the sample and concentration data are normally distributed or not and to see their homogeneity. If normality is violated, the bootstrap can be used, but when homogeneity is violated, a post hoc test can be performed. Follow-up test using Duncan SPSS 20.

3. Results

3.1. *In Silico*

3.1.1. Ligand Bioavailability and Toxicity

There were 30 ligands out of 39 ligands that passed the bioavailability prediction stage. Analysis of bioavailability characteristics based on the reference of Lipinski's rule, namely relative atomic mass value < 500 Da, logP value < 5, hydrogen bond donor value < 5, hydrogen bond acceptor value < 10, and molar refractivity value between 40-130. The results of the analysis showed that 30 ligands complied with Lipinski's rule and only 9 ligands violated more than 2 Lipinski's rules (Table 1).

There were 27 ligands from 30 ligands that passed the toxicity prediction stage when making new drugs through the *in silico* approach. Toxicity testing aims to analyze the degree of damage or adverse effects that a compound or drug will cause when consumed. There are 3 control parameters of a drug candidate compound taken in this study, namely human ether a go-go related gene (herG), carcinogenic, and acute oral toxicity in rats. The results of the analysis showed 3 rule-breaking ligands for drug candidates from AdmetSAR (Table 2).

Table 1. Bioavailability in violation of 2 of Lipinski's rule

Code CID	Molecular Weight (Da)	LogP	Bond donor H	Bond acceptor H	Molar Refractivity
3414657	592	7.0649	0.0	0.0	166.964
44257372	698	-1.7971	8.0	17.0	162.439
10074228	488	6.8521	3.0	5.0	145.131
9985480	570	2.6818	2.0	10.0	143.329
71723026	599	6.91318	4.0	6.0	167.965
15940183	552	4.38089	0.0	8.0	144.790
4202426	510	4.99590	0.0	8.0	130.441
5486199	578	-1.9543	8.0	18.0	160.721
5280805	610	-1.8788	10.0	16.0	137.495

The bold: violates 2 Lipinski's rule

Table 2. Toxicity that violate AdmetSAR parameter

Code CID	Human Ether-A-Go-Go related gene (hERF) inhibition		Carcinogenicity		Acute oral toxicity	
	Category	Probability	Category	Probability	Category	Probability
2733199	Week Inhibitor	0.99	Carcinogen	0.51	III	0.65
188289	Week Inhibitor	0.97	Non-carcinogen	0.96	I	0.62
1032	Weak Inhibitor	0.96	Carcinogen	0.65	III	0.91

The Bold is toxic

3.1.2. Molecular Docking Simulation and Validation

Molecular docking validation (grid box validation) needs to be done to get the right grid box size or can cover the active site of the ERα. The grid box size used has a center value of x = 0.00; y = 0.00; and z = 50.00. After validating the grid box, a size of 1.0 A was obtained. A good grid box has a valid grid box molecular docking method parameter validation, namely the RMSD value < 2A. The grid box size gives an RMSD value of 0.1748 A. The ligand solubility and toxicity analysis results indicate that it meets the criteria, so that molecular docking is carried out to determine the binding affinity to the receptor. The results of the docking simulation show that the ligand with the highest affinity energy is 2-(4-Hydroxyphenyl)-2-phenyl-N-(3,3-diphenylpropyl)-acetamide of -10.288 kcal/mol by interacting hydrobic with the amino acid residues Leu346, Ala350, Asp351, Leu387, Arg394, Gly521, and His524 (Antagonist Binder) with an inhibition constant of 2.8×10^{-8}. The comparison ligand (drug) 4_hydroxytamoxifen has an affinity energy value of -9,224 kcal/mol with an inhibition constant (Ki) of 1.7×10^{-7}. The following are the results of the molecular docking simulation shown in Table 3 and Figure 1 the best result molecular docking visualization compounds piper betel leaf.

3.2. *In Vitro*

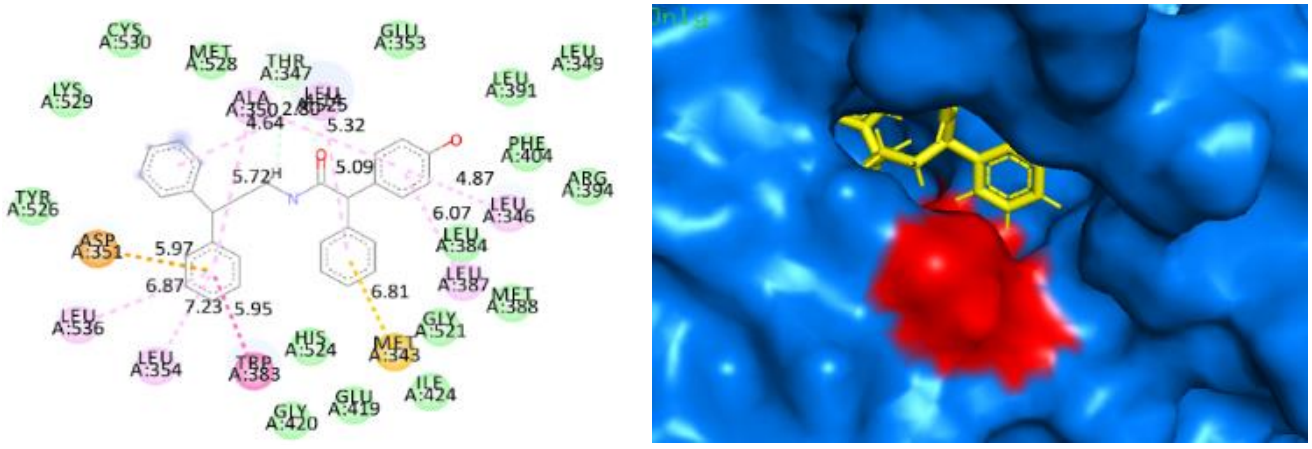
3.2.1. MCF-7 Cells Anti-breast Cancer

The data shown in Table 4 shows that the n-hexane fraction of red betel leaf has the highest average inhibition of 73.43±1.60a% at a concentration of 500 ppm. The average value of inhibition obtained by the positive control was 28.60±2.96% at a concentration of 3 ppm. The positive control used was tamoxifen 200 mg oral tablet. Testing the anti-breast cancer cytotoxic activity of MCF-7 cells in this study used ethanol extract, n-hexane fraction, ethyl acetate fraction, and water fraction. Based on Table 4, shows that the highest yield was obtained from he hexane fraction with a concentration of 73.43±1.60a%. Cancer cells need to be prepared first by making monolayer cells. In this study monolayer cells were used. The bioactive compound was added as much as 100 µL/well to the wells that had grown MCF-7 cells.

Table 3. Molecular docking simulation

Code CID	Gibbs free energy (cal/mol)	Ki	Residu/visible amino acid	
			Hydrophobic interaction	Hydrogen bond
44578655	-10.2880	2.8×10^{-8}	LEU346 ALA350 ASP351 GLU353 LEU387 ARG394 GLY521[a] HIS524[b]	THR347 (2.80 A)
449459 (control positif)	-9.2240	1.7×10^{-7}	LEU346 ALA350 ASP351 GLU353 ARG394 MET421 GLY521[a] HIS524[b]	ARG394 (6.57 A) GLU353 (4.84 A)[a]
5282219	-8.5180	5.6×10^{-7}	LEU346 ALA350 ASP351 GLU353 ARG394 GLY521[a] HIS524[b]	HIS524 (3.87 A),GLY521 (3.25 A)
5281515	-8.5180	5.6×10^{-7}	LEU346 ALA350 ASP351 GLU353 LEU387 ARG394 MET421 GLY521[a] HIS524[b]	-
3754322	-7.5660	2.8×10^{-6}	LEU346 ALA350 ASP351 GLU353 LEU387 ARG394 MET421 GLY521[a] HIS524[b]	ALA350 (2.90)
959515	-7.4120	3.6×10^{-6}	LEU346 ALA350 ASP351 GLU353 LEU387ARG394 MET421 GLY521[a] HIS524[b]	THR346 (3.66) GLY521 (3.83)
90665169	-5.0880	3.4×10^{-5}	LEU346 ALA350 GLU353 LEU387ARG394 MET421 GLY521[a] HIS524[b]	LEU346 (3.69 and 4.61)[a] GLY420 (4.29) GLY521 (3.95)[a]

the symbol "a" active side, "b: antagonist binding side



A B

Figure 1. Molecular Docking Visualization of 2-(4-Hydroxyphenyl)-2-phenyl-N-(3,3-diphenylpropyl)-acetamide: (A) 2D diagram of hydrogen bonding and hydrophobic interaction between ligands and receptor, (B) 3D Binding Pocket

Table 4. MCF-7 cytotoxicactivity in 500 consentrasion

Sample	Inhibition average (%) ± SD
Ethanol extract	24.57±2.05[d]
N-Hexane fraction	73.43±1.60[a]
Ethyl acetate fraction	62.23±1.01[b]
Water fraction	48.13±1.30[c]
Tamofen (3 ppm) as positive control	28.60±2.96

4. Discussion

The process of making or designing drug designs carried out *in silico* requires information in the form of the physicochemical characteristics of a compound. These physicochemical characteristics determine the level of permeability of a drug. Physicochemical

characteristics can be referred to as Lipinski's Rule of Five. Analysis of the bioavailability of a ligand is used at the drug screening stage. If there are compounds that do not meet the rules (violating more than 2), they will be eliminated and do not pass to the toxicity prediction stage (Chen *et al.* 2020). The in silico toxicity analysis method was tested after the physicochemical analysis was carried out using the AdmetSAR web server. Based on the analysis of physicochemical tests showed that 33 ligands met the criteria to be used as drug candidates, so it is necessary to analyze the toxicity of the ligands. Identification of the safety of a compound at the beginning of drug development needs to be done to ensure the potential of the compound can reach target cells and work effectively without causing damage to body organs. The pharmacokinetic properties of drugs including absorption, distribution, metabolism, excretion, and toxicity (ADMET) play an important role in drug discovery (Moon *et al.* 2017).

Grid box validation used in 1.0 molecular docking. Based on the grid box size, the RMSD (Root Mean Squared Deviation) value of 0.1748 A from Yasara structure is obtained indicating the deviation of the ligand pose between the crystallographic structure and the structure resulting from the re-docking. RMSD compares positions between ligand atoms experimentally and based on algorithm predictions. The flexibility of the ligands can affect the accuracy of the position of the complex formed (Noviardi and Fachrurrazie 2015). The larger the RMSD value, the greater the deviation that occurs, namely the prediction error of the ligand pose on molecular docking and the crystallographic pose of the smaller RMSD value or close to zero (0), indicating the suitability of the ligand pose of the re-docking result is good or almost the same as the conformation of the crystallographic results. (Nauli 2014). Based on the results of the RMSD values obtained, the grid box size can be used in the molecular docking process because it has met the validity parameters of the molecular docking method by providing an RMSD value of < 2 A (Santoyoso *et al.* 2013). Molecular docking uses computational execution to find and determine the best compounds as drug candidates. The principle of virtual screening is to predict the bond pose and free energy (ΔG) of many ligands by using molecular docking at once in one run so that research can run effectively and quickly (Nugraha and Istyastono 2020).

Visualization of the molecular interaction results from the docking results showed that 1 ligand had the best results based on Gibbs free energy, inhibition constants, and amino acid residues involved. Visualization of Discover Studio shows that the amino acid residues interact between the test ligands and comparison ligands. Amino acid residues that play an important role in receptor binding are Leu346, Ala350, Asp351, Glu353, Leu387, Arg394, Met421, Gly521, and His524 antagonist binding site (Table 3). Based on the results of the docking of the test ligands and natural ligands, attention should be paid to His524 residues in the search for more specific ERα receptor inhibitors. This is because His524 hydrogen bonding on the ligand is important in regulating receptor activity. According to (Muchtaridi *et al.* 2018), Helix-12 from ER (residue 536-544) plays a major role in determining the activity of agonist or antagonist ligands. When His524 is hydrogen bonded, it indicates that a ligand is an agonist. If His524 interacts with hydrophobic interactions, it shows that the compound is antagonistic, where the antagonist plays a major role in determining a good compound in inhibiting receptor activity.

Based on the results of the visualization analysis, there are 4 parameters used for the visualization analysis test, namely hydrogen bonding is an electrostatic bond that has been widely used in determining the strength of the bond between the ligand and the receptor. Ligands with more hydrogen bonds are hypothetically the easier and stronger a ligand will bind to the receptor's active site. The test ligands in (Table 4.) have a range of 2.80–6.57 Å. States that the hydrogen bond distance of more than 1.85 Å is weak, so it can be broken and converted into other bonds. This study's results can say that the hydrogen bonds are weak and can be converted into hydrophobic bonds. In addition to hydrogen bonds, hydrophobic bonds play an important role because they can stabilize the interaction between ligands and receptors. This is caused by protein folding so that most non-polar residues will be buried in the protein interior and protected (Arfi *et al.* 2020). Table 3 shows that there are 5 compounds and 1 positive control. Five compounds represent each fraction that gases the best results. However, the docking validation as the results of molecular docking obtained 1 best ligand, the water fraction with a Gibbs free energy value of - 10.2880 Kcal/mol.

The positive control used in this study was tamoxifen. It is a drug used for chemotherapy. Chemotherapy is the use of special drugs to kill cancer cells, chemotherapy prevents or kill cancer cells. Positive controls were

tested on MCF-7 cells, which are breast cancer cells. The control was used as a comparison of the selectivity of the extract and fraction of red betel leaf against MCF-7 cells. The results of the positive control test showed that tamoxifen inhibited the growth of more MCF-7 cells. Anticancer testing in this study was initiated by testing the cytotoxicity of the extract and fractions (n-hexane fraction, ethyl acetate fraction, and water fraction) of red betel leaf against MCF-7 cells using the MTT assay method. Cytotoxicity testing aims to determine the safety of the extract and the 3 fractions against MCF-7 cells. The ethanol extract and 3 fractions had different inhibition percentages. Ethanol extract had a inhibition percentage with cytotoxicity test results on cancer cells MCF-7 showed a very significant result between the sample concentration and the inhibition percentage. This can be caused by different concentrations contained in the compounds both in the extract and fractionation of red betel leaf. The highest percentage of inhibition was found in the fractionation of n-hexane, which inhibited the growth of the MCF-7 cancer cell population with a concentration of 500 ppm at 73.42±1.60 A. The lower the IC_{50} value, the higher the toxicity of the extract or fraction and it has higher antitumor properties (Ogugu *et al.* 2012).

Research conducted by (Wicaksono *et al.* 2009) showed that the methanol extract of red betel leaf has a cytotoxic effect with an IC_{50} value of 50% and has the potential to inhibit the proliferation of T47D breast cancer cells. Red betel leaf extract inhibited the growth of HeLa cells by 86% by the MTT method (Suci 2014). Based on the results obtained, both extracts and fractions of red betel leaf showed fluctuating results between concentration and inhibition percentage, this is presumably because the content of bioactive compounds contained in red betel leaf is still diverse and the levels of compounds contained at various concentrations are still different. Based on the positive control test results, the best anticancer potential is tamoxifen with a concentration of 3 ppm inhibition was 28.60 %. This study's conclusions indicate that the bioactive compound of red betel leaf is water fraction is the best fraction inhibition. However, the hexane fraction proven to have cytotoxic activity against breast cancer MCF-7 cells.

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References

Afifah, S., Lukiati, B., Maslikah, SI., 2020. The potentials of red betel (*Piper crocatum* Ruiz and Pav) terpenoid compounds as microsomal prostaglandin E synthase-1 (mPGES-1)enzyme inhibitor of rheumatoid arthritis through virtual screening. Proceedings of The 3rd International Seminar on Metallurgy and Materials (ISMM2019): Exploring New Innovation in Metallurgy and Materials;22April2020;AIPConference Proceedings2231.

Agistia, D.D., Purnomo, H., Tegar, M., Nugroho, A.E., 2013. Interaksi senyawa aktif dari *Aegle marmelos* Correa sebagai antiinflmasi dengan reseptor COX-1 san COX-2. *Traditional Medicine Jurnal.* 18, 80-87.

Amalia, A., Maslikah, S.I., Lestari, S.R. 2020. Virtual screening flavonoid compounds from red betel (*Piper crocatum* Ruiz and Pav.)as inhibitor of cyclooxygenase-2 (COX-2). Proceedings of The 3rd International Seminaron Metallurgy and Materials (ISMM2019): Exploring NewInnovation in Metallurgy and Materials; 22 April 2020;AIP Conference Proceedings2231;2020

Anugrahwati, M., Purwaningsih. T., Rustina, Manggalarini, J.A., Alnavis, N.B., Wulandari, D.N., Pranowo, H.D., 2016. Extraction of ethanolic extract of red betel leaves and its cytotoxicity test on HeLa cells. *Procedia Engineering.* 148, 1402-1407.

Arfi, A.S., Lestari, R.D., Damayanti, D.S., 2020. Studi *in silico* senyawa aktif rimpang kunyit (*Curcuma domestica*) terhadap penghambatan acetylcholinesterase, microtubulin (beta tubulin), dan aktivasi calcium channel sebagai terapi antelmintik. *JKK.* 8, 36-47. https://doi.org/10.20473/jmv.vol2.iss1.2019.55-59

Chen, X., Li, H., Tian, L., Li, Q., Luo, J., Zhang, Y., 2020. Analysis of the physicochemical properties of acaricides based on lipinski's rule of five. *Comput Biol.* 27, 1397-1406. https://doi.org/10.1089/cmb.2019.0323

Debbie, S., Graeme, L., Pierre, D., Elizabeth, W., Kelvin, C., 2012. Pharmacovigilance of herbal medicine. *J Etnopharmocal.* 140, 513-518. https://doi.org/10.1016/j.jep.2012.01.051

Hamzah, N., Rauf, A., Ariwati, 2015. Studi hubungan kuantitatif struktur aktivitas (HKSA) senyawa turunan 4-aminoquinolin piramidin, docking molekul, penelusuran farmakofor, virtual screening, uji toksisitas, dan profil farmakokinetik sebagai antimalaria secara *in silico*. *JK FIK UINAM*. 3, 176-186.

Moon, A., Khan, D., Gajbhiye, P., Jariya, M., 2017. *In silico* prediction of toxicity of ligands utilizing admetsar. *Pharma Bio Sci*. 8, 674-677. https://doi.org/10.22376/ijpbs.2017.8.3.b674-677

Muchtaridi, M., Dermawan, D., Yusuf, M., 2018. Molecular docking, 3D structure based pharmacophore modeling, and ADME prediction of alpha mangostin and its derivatives against estrogen receptor alpha. *Journal of Young Pharmacists.* 10, 252-259. https://doi.org/10.5530/jyp.2018.10.58

Nauli, T., 2014. Penentuan sisi aktif selulase *Aspergillus niger* dengan docking ligan. *JKTI.* 16, 94-100. https://doi.org/10.14203/jkti.v16i2.14

Noviardi, H., Fachrurrazie, 2015. Potensi senyawa bullatalisin sebagai inhibitor protein leukotrien a4 hidrolase pada kanker kolon secara *in silico. Fitofarmaka.* 5, 65-73. https://doi.org/10.33751/jf.v5i2.410

Nugraha, G., Istyastono, E.P., 2020. Pembuatan protokol penapisan virtual berbasis stuktur (pvbs) untuk identifikasi ligan inhibitor reseptor platelet-activating factor (PAF-r) sebagai target terapeutik asma menggunakan YASARA. *Riset Kimia.* 11, 35-42. https://doi.org/10.25077/jrk.v11i1.346

Ogugu, S.E., Kehinde, A.J., James, B.I., Paul, D.K., 2012. Assessment of cytotoxic effects of methanol extract of *Calliandra portoricensis* using brine shrimp (*Artemia salina*) lethality bioassay. *Global Journal of Bio-Science and Biotechnology*. 1, 257-260.

Safithri, M., Faridah, D.N., Ramadani, F., Pratama, R., 2022. Antioxidant activity of ethanol extract and fraction of *Piper crocatum* with Rancimat and Cuprac methods. *Turki Journal Biochemistry*. 47, 795-801. https://doi.org/10.1515/tjb-2021-0300

Safithri, M, Kurniawati, Syaefudin,. 2016. Formulaof *Piper crocatum, Cinnamomum burmanii*, and *Zingiber officinale* extracts as a 37 functional beverage for diabetics. *Intenational Food Research Journal.* 23, 1123-1130.

Santoyoso, A.H., Barajas, A.Y.T., Altuzar, V., 2013. *Protein-Protein and Protein-Ligand Docking*. In Tech.

Septiani, R., 2017. Ekstrakdan Fraksi Daun Sirih Merah (Piper crocatum Ruiz and Pav) sebagai Antioksidan dengan Metode2,2-difenil-1-pikrilhidrazil [Skripsi]. Bogor, Indonesia: Institut Pertanian Bogor.

Schultink, A., De Vries, H.M., Zwart, W., Linn, S.C., Beijnen, J.H., Huitema, A.D.R., 2015. Effects of pharmacogenetics on the pharmacokinetics and pharmacodynamics of tamoxifen. *Clin Pharmacokinet.* 54,797-810. https://doi.org/10.1007/s40262-015-0273-3

Suci, D.A.P., 2014. Potensi antikanker ekstrak daun sirih merah (*Piper crocatum*) terhadap sel HeLa [Skripsi]. Bogor, Indonesia: Institut Pertanian Bogor.

Susila, R.P., Safithri, M., Andrianto, D., 2022. Active compound of red betel (*Piper crocatum* Ruiz and Pav) as activator of cu/zn superoxide dismutase *in silico. Indonesian Journalof Applied Research*. 3, 85-94. https://doi.org/10.30997/ijar.v3i2.212

Umar, M.A., 2022. Kajian *in vitro* dan *in silico* daun sirih merah sebagai antikanker kolon pada sel widr serta proliferator sel limfosit [Thesis]. Bogor, Indonesia: Institut Pertanian Bogor.

Warjianto, Soewoto ,W., Alifanto, U., Wujoso, H., 2020. Hubungan reseptor estrogen, reseptor progesteron, dan ekspresi Her-2/Neu dengan grading histopatologi pada pasien kanker payudara di RSUD dr. Moewardi Surakarta. *SMedJour*. 3, 96-104. https://doi.org/10.13057/smj.v3i2.35228

[WHO] World Health Organization, 2020. Cancer. Available at: https://www.who.int/news-room/fact-sheets/detail/cancer. [Date accessed: 20 Agustus 2021]

Wicaksono, B.D., Handoko, Y.A., Arung, E.T., Kusuma, I.W., Yulia, D., Pancaputra, A.N., Sandra, F., 2009. Antiproliferative effect of the methanol extract of *Piper crocatum* Ruiz and Pav leaves on human breast (T47D) cells i*n-vitro. Tropical Journal of Pharmaceutical Research*, 8, 345-352. https://doi.org/10.4314/tjpr.v8i4.45229

Yanger, J.D., Davidson, N.E., 2006. Mechanism of disease estrogen carsinogenesis in breast cancer. *New England Journal of Medicine*. 354, 270-278. https://doi.org/10.1056/NEJMra050776

Yasin, S.A., Azzahra, A., Ramadhan, N.E., Mylanda, V., 2020. Studi penambatan molekul dan prediksi ADMET senyawa bioaktif beberapa jamu Indonesia terhadap SARSCoV-2 main protease. *BIMFI.* 7, 24-41. https://doi.org/10.48177/bimfi.v7i2.45