

Molecular Docking Study of Active Compounds in Java Turmeric (*Curcuma xanthorrhiza*) As Potential Inhibitors of The *B-Cell Lymphoma 2* (Bcl-2) Protein For Breast Cancer Therapy

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Temulawak (*Curcuma xanthorrhiza*) contains active compounds that have the potential to have anticancer activity. One potential target for the development of cancer therapy is the Bcl-2 protein which plays a role in maintaining the life of cancer cells so that the protein inhibits programmed cell death in the body. This study aims to analyze the potential of active compounds contained in the temulawak plant as potential inhibitors of Bcl-2 proteins in breast cancer using the molecular docking method and evaluate their toxicity properties. This study was conducted by identifying the active compounds contained in the temulawak plant then drawing them into 2D and 3D structures, downloading the bcl-2 protein macromolecules, positive controls (Venetoclax) and negative controls (Paracetamol) then prepared together with other test compounds, method validation, compound docking to the target protein, analysis of binding affinity values, analysis of amino acid residue interactions, and testing of toxicity parameters. The results showed that the Demethoxycyclocurcumin compound had the best binding affinity with a low energy value of -7.5 kcal/mol, had amino acid hydrogen bond interactions similar to the positive control with amino acid residues ARG A:127, ARG A:139, and TYR A:180, Pi-Cation interactions with ARG residue A:183, Pi-Alkyl with ALA residue A:131. Low toxicity parameters so that the compound can be said to be safe. From this study it can be concluded that the Demethoxycyclocurcumin compound has the potential to be a bcl-2 protein inhibitor, although further research is still needed, namely in vitro and in vivo in experimental animals to evaluate its effects on the growth of cancer-causing cells.

Keywords Bcl-2; Breast cancer; Curcuma; Docking.

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INTRODUCTION

Breast cancer is the most common cancer in women worldwide with high morbidity and mortality rates according to data from the Global Cancer Observatory in 2022 (1). Breast cancer is the fourth highest cause of cancer death worldwide with a case presentation of 6.8% of other cancer cases, while in Indonesia breast cancer is also the highest cancer case in 2022 with a percentage of 16.2% and is also the third cause of cancer death after lung cancer and liver cancer (2). Factors causing breast cancer are lifestyle, diet, exposure to environmental risks (3), in addition there are also other internal factors such as age, family history, excess weight, dense breasts, smoking, and drinking alcohol. Breast cancer begins with DNA damage, genetic mutations, and disruption of cell regulation (4).

Breast cancer is associated with the mechanism of carcinogenesis, namely the change of normal cells into cancer cells due to the accumulation of genetic mutations, these mutations destroy the cell's ability to stop dividing, attach to other cells and remain in place. Normal cells should commit cell suicide (apoptosis) when they are no longer needed, but in the case of cancer, this is protected by a group of certain proteins when the cell is ready to commit suicide (4). The B-Cell Lymphoma 2 (Bcl-2) protein is an anti-apoptotic agent by regulating the intrinsic apoptosis pathway or cell death. Anti-apoptotic proteins prevent cell death by inhibiting the release of cytochrome c, maintaining mitochondrial integrity, and often overexpression occurs so that it becomes dominant and forms cancer, this is because the cells are able to survive. The Bcl-2 protein is often found in various types of cancer, making cancer cells difficult to kill and is an important target in the development of anticancer drugs (5).

The use of natural materials as a source of bioactive compounds is increasingly being developed in anti-cancer drug research. One natural ingredient native to Indonesia that has potential is the Temulawak plant (*Curcuma xanthorrhiza*) which was found to contain several active compounds including curcumin, demethoxycurcumin, bisdemethoxycurcumin, xanthorrhizol, and demethoxycyclocurcumin. These compounds are reported to have pharmacological activities such as antioxidant, anti-inflammatory, antidiabetic, anticancer and antimicrobial (6). According to research by Noordam (2023), it is explained that the largest content of curcumin in ginger can be seen with its anti-breast cancer effect based on the docking value (7). According to Sativa et al. (2024) xanthorrhizol compound can trigger apoptosis (cell death) in cancer cells by increasing caspase-8, caspase-9, and caspase-3, xanthorrhizol inhibits the proliferation of cultured cancer cells and growth inhibition which is associated with cell cycle arrest and induction of apoptosis (8).

Technological developments can be used to identify drug candidates more quickly and efficiently, one of which is through a computational approach with the molecular docking method carried out by analyzing the interaction of active compounds and the stability of the bonds between compounds and target proteins (9), in addition it is also used to evaluate the potential of active compounds as Bcl-2 protein inhibitors. Toxicity prediction is also needed in assessing the feasibility of compounds as drug candidates (10). The challenge in breast cancer treatment is the emergence of resistance to conventional therapy and side effects caused by long-term drug use, therefore by conducting research on the potential analysis of active compounds in the Temulawak plant (*Curcuma xanthorrhiza*) as Bcl-2 protein inhibitors using the molecular docking method and evaluating its toxicity parameters is expected to provide scientific information regarding potential candidates for anti-breast cancer agents and support the development of bioinformatics-based drugs before being carried out before in vitro or in vivo testing or to strengthen the research.

METHODS

A. Research Tools

Acer Aspire A514-53 laptop with an Intel(R) Core(TM) i3-1005G1 CPU @ 1.20GHz (1.19GHz), 4.00 GB of RAM, and 238 GB of storage. The software used included Windows 11, ChemDraw Professional 15.0, Chem 3D 15.0, Discovery Studio Visualizer, VegaZZ, PyMOL, PyRx-Python AutoDock Vina, and Toxtree.

B. Research Materials

The 3D structure of the Bcl-2 protein with the identity code PDBID: 6GL8 as the target macromolecule, the 2D and 3D structures of the active compounds or test ligands demethoxycurcumin, zedoarol, curcumin, dihydropyrocurenone, isovelleral, xanthorrhizol, salvigenin, bisacumol, demethoxycyclocurcumin, α -cubebene, the structure of the native ligand S-55746, the structure of the drug compound Venetoclax as a positive control, and the drug compound Paracetamol. as a negative control.

C. Research Steps

1. Preparation of Bcl-2 protein macromolecules and test ligands

Macromolecules were downloaded from the Protein Data Bank (RSCB PDB) which were stored in pdb format. The 2D structure of the test ligand was created using ChemDraw Professional 15.0 software, then converted to a 3D model using Chem3D 15.0, the structure was saved in pdb format. Macromolecules were prepared or separated by structure with ligands and other residues using the Discovery Studio Visualizer application, with the following steps: open the application, click file, then open, and search for the macromolecule file to be used, then a display of the 3D structure of the target protein will appear. Unnecessary molecular chains, ligands, and residues were then removed by clicking the script, clicking selection, select water molecules/ligands/protein chains, then selecting edit, and delete. The prepared macromolecule file was then saved in .pdb format (11).

Test ligands were prepared using the VegaZZ application, by opening the 3D structure of the compound, then adding hydrogen atoms in the edit menu, correcting the compound's charge by adding partial Gasteiger charges and then applying forcefield autodock. The compound was minimized by 3000 steps to obtain the most stable conformation. Then, each optimized compound was saved in .pdb format for docking tests and mol format for ADME and toxicity parameter analysis (11).

2. Validation of the Molecular Docking Method

Method validation is performed by re-docking the native ligand to a previously prepared and optimized target protein. Method validation uses the PyRx application with appropriate gridbox settings. The gridbox results are then saved for use when docking the test compound to the target protein. Afterward, overlap between the two is observed (12).

3. Molecular docking process

The docking process is carried out using the Pyrx application by anchoring the test ligand to the protein macromolecule. The application is opened then selected the folder then load molecule and selected the protein file and test ligand to be used, each file is right-clicked then autodock for the protein file selected make macromolecule and for the ligand selected make ligand. In the Vina Wizard section select star here then start, ensure the ligand and macromolecule are selected then forward to Run Vina. In the next section there is a gridbox that can be arranged in the view table, directed at the binding site as the midpoint, then recorded the x, y, and z coordinates. select Run Vina, then the docking process will start and wait until 100%, then the results will appear in the analyze result table. Select the ligand out with the smallest binding affinity then in the

navigator table select molecular, select the ligand out that has been selected in the analyze result table, then right-click save as PDB (11).

4. Toxicity Parameter Prediction

Toxicity parameter prediction is performed using Toxtree software. The following steps are performed: open the application, select file and open (search for the ligand file to be used), then select method to select the parameters to be calculated, select the parameters, and click estimate. The toxicity calculation results are displayed in the Classification Area. Use Method > View decision tree to view the details of the decision tree. The parameters examined are Cramer's rules, carcinogenicity (genotox and non-genotox), mutagenicity rulebase by ISS, and in vitro mutagenicity (Ames test) (12).

5. Analysis of Results

Validation of the method on native ligands was carried out by comparing the conformation of the docking results with the native ligand based on crystallographic data expressed in RMSD or Root Mean Square Deviation values. The RMSD value that is still acceptable is less than 5, so the closer to 0 the value, the better the alignment (13). Analysis of the molecular docking results was observed in the binding energy value with units of kcal/mol, the more minus the resulting value means there is a strong bond between the ligand and the target protein. The interaction of amino acid residues was displayed using Discovery Studio Visualizer, then observations were made and compared with the results of interactions with amino acids in native ligands or positive controls (14).

RESULTS

A. Validation of molecular docking method

Method validation or redocking was carried out between the bcl-2 protein and the previously separated native ligand by adjusting the gridbox in the Pyrx application, which can be seen in Table 1 and Figure 1. The overlap between the docking results and the previously optimized native ligand using the Pymol application can be seen in Figure 2. The results of the method validation expressed in RMSD and binding affinity values can be seen in Table 2.

Table 1 gridbox settings

	Gridbox					
	Center			Dimensi		
	X	Y	Z	X	Y	Z
S-55746	6,4199	2,0404	9,9047	38,4203	36,4203	42,3911

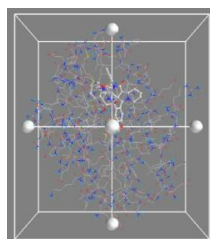


Figure 1 Gridbox view in the Pyrx application

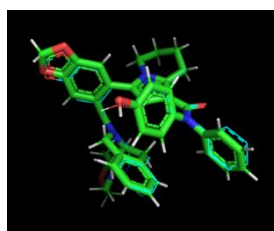


Figure 2 Overlapping view in PyMOL application

Table 2 RMSD values and binding affinity of native ligands from method validation results

Macromolecules	Application	Binding Affinity (kcal/mol)	RMSD
S-557746	PyRX	-9,4	1,543
	PyMOL	-	0,058

B. Molecular docking results

Molecular docking results are used to predict the binding ability between the test ligand and the target protein. The docking process uses the Pyrx application by docking the test ligand from the Javanese turmeric (*Curcuma xanthorrhiza*) plant to the target protein with gridbox sizes adjusted for method validation. The docking results to be analyzed include binding affinity, RMSD, and amino acid interactions, which can be seen in table 3.

Tabel 1 Hasil Docking menggunakan Pyrx

Ligand Name	Value Binding Affinity (kcal/mol) / RMSD	Category	Chemical Bonding	Amino Acid Residues / Bond Distance
Ligan natif (S-55746)	-9,4 / 1,543	Hydrogen Bond	Conventional Hydrogen Bond	GLN A:118
		Sulfur Interaction	Pi-Sulfur	MET A:115
		Aromatic Interaction	Pi-Pi T-Shaped	PHE A:104
		Hydrophobic	Pi-Sigma	LEU A:137
			Alkyl	ALA A:149
			Pi-Alkyl	LEU A:119
				VAL A:133
Positive control (Venetoclax)	-8,9 / 1,774	Hydrogen Bond	Conventional Hydrogen Bond	ARG A:107
			Carbon Hydrogen Bond	PHE A:153
			Pi-Donor Hydrogen Bond	ASP A:103
		Aromatic Interaction	Pi-Pi T-Shaped	TYR A:202
			Pi-Pi Stacked	PHE A:104
				PHE A:104
		Hydrophobic	Pi-Alkyl	VAL A:148
			Alkyl	PHE A:112
Negative control (Paracetamol)	-4,8 / 1,503	Unfavorable Interaction	Unfavorable Donor-Donor	ALA A:149
			Unfavorable Acceptor-Acceptor	ARG A:131
		Aromatic Interaction	Pi-Pi Stacked	ALA A:131
		Hydrophobic	Pi-Alkyl	TYR A:180
				VAL A:134
Demethoxycurcumin	-6,2 / 1,788	Hydrogen Bond	Conventional Hydrogen Bond	SER A:105
		Hydrophobic	Pi-Sigma	SER A:116
			Pi-Alkyl	GLU A:160
				VAL A:156
Zedoarol	-6,6 / 1,88	Hydrogen Bond	Conventional Hydrogen Bond	VAL A:159
		Unfavorable Interaction	Unfavorable Donor-Donor	LYS A:22
		Hydrophobic	Alkyl	ARG A:26
				ALA A:113
Curcumin	-5,9 / 1,886	Sulfur Interaction	Pi-Anion	ASP A:111
		Aromatic Interaction	Pi-Sulfur	MET A:115
				TYR A:108
			Pi-Pi Stacked	

Ligand Name	Value Binding Affinity (kcal/mol) / RMSD	Category	Chemical Bonding	Amino Acid Residues / Bond Distance
Dihidropyrocurrenon	-7,0 / 1,017	Aromatic Interaction	Pi-Pi T-shaped	PHE A:112
		Hydrophobic	Pi-Alkyl	LEU A:137 ALA A:149
		Hydrogen Bond	Carbon Hydrogen Bond	ASP A:111
		Aromatic Interaction	Pi-Pi Stacked	PHE A:112
			Pi-Pi T-shaped	PHE A:104
		Hydrophobic	Alkyl	PHE A:153
Isovelleral	-5,9 / 1,612		Pi-Alkyl	LEU A:137 ALA A:149
		Hydrogen Bond	Carbon Hydrogen Bond	TYR A:108
			Pi-Donor Hydrogen Bond	ASP A:111
		Hydrophobic	Alkyl	PHE A:104 PHE A:112
Xanthorrhizol	-5,7 / 1,415		Pi-Alkyl	ALA A:149
		Hydrophobic	Pi-Sigma	TYR A:108 PHE A:112
		Aromatic Interaction	Pi-Pi Stacked	PHE A:104
			Pi-Pi T-shaped	PHE A:112
Salvigenin	-6,4 / 2,109	Hydrophobic	Pi-Alkyl	ALA A:149
			Convention Hydrogen Bond	ARG A:146
		Hydrogen Bond	Pi-Donor Hydrogen Bond	TYR A:202
		Aromatic Interaction	Pi-Pi T-shaped	PHE A:104
		Hydrophobic	Alkyl	ALA A:100 ALA A:149
			Pi-Alkyl	VAL A:148
Bisacumol	-5,9 / 1,763		Pi-Sigma	TYR A:108
		Hydrophobic	Pi-Alkyl	LEU A:137 ALA A:149
			Conventional Hydrogen Bond	ARG A:127
Demethoxycyclocurcumin	-7,5 / 1,552	Hydrogen Bond	Carbon Hydrogen Bond	ARG A:139
			Pi-Donor Hydrogen Bond	TYR A:180
		Electrostatic Interaction	Pi-Cation	ARG A:183
		Hydrophobic	Pi-Alkyl	ALA A:131
α-cubebene	-7,1 / 1,415		Pi-Sigma	PHE A:112
		Hydrophobic	Alkyl	PHE A:104 TYR A:108
				MET A:115
			Pi-Alkyl	VAL A:133

C. Toxicity parameters

Toxicity testing of test ligands includes Cramer's rules (level of toxicity), the compound's ability to trigger cancer (carcinogenicity), the compound's ability to damage the cell's genetic material (mutagenicity), and the compound's ability to cause genetic mutations (amest test). The predicted results of the toxicity test can be seen in table 5.

Table 5 Toxicity Parameter Test Results

Compound	Cramer Rules	Parameters Carcinogenicity dan Mutagenicity	In Vitro Mutagenicity (Amest Test)
Demethoxycurcumin	High (Class III)	Structural Alert for genotoxic carcinogenicity Negative for nongenotoxic carcinogenicity	Structural Alert for <i>S.</i> typhimurium mutagenicity
Zedoarol	High (Class III)	Structural Alert for genotoxic carcinogenicity Negative for nongenotoxic carcinogenicity	Structural Alert for <i>S.</i> typhimurium mutagenicity
Curcumin	Low (Class I)	Negative for genotoxic carcinogenicity Negative for nongenotoxic carcinogenicity	No alerts for <i>S.</i> typhimurium mutagenicity
Dihydropyrocurzerenon	High (Class III)	Negative for genotoxic carcinogenicity Negative for nongenotoxic carcinogenicity	No alerts for <i>S.</i> typhimurium mutagenicity
Isovelleral	High (Class III)	Structural Alert for genotoxic carcinogenicity Negative for nongenotoxic carcinogenicity	Structural Alert for <i>S.</i> typhimurium mutagenicity
Xanthorrhizol	Low (Class I)	Negative for genotoxic carcinogenicity Negative for nongenotoxic carcinogenicity	No alerts for <i>S.</i> typhimurium mutagenicity
Salvigenin	High (Class III)	Structural Alert for genotoxic carcinogenicity Negative for nongenotoxic carcinogenicity	Structural Alert for <i>S.</i> typhimurium mutagenicity
Bisacumol	Low (Class I)	Negative for genotoxic carcinogenicity Negative for nongenotoxic carcinogenicity	No alerts for <i>S.</i> typhimurium mutagenicity
Demethoxycyclocurcumin	Low (Class I)	Negative for genotoxic carcinogenicity Negative for nongenotoxic carcinogenicity	No alerts for <i>S.</i> typhimurium mutagenicity
α -cubebene	Low (Class I)	Negative for genotoxic carcinogenicity Negative for nongenotoxic carcinogenicity	No alerts for <i>S.</i> typhimurium mutagenicity

DISCUSSION

Molecular docking research requires method validation or re-docking to prove and ensure that the method used has met the validity requirements and can be used in testing other compounds so that errors can be minimized. Method validation is carried out between the Bcl-2 protein and a previously separated native ligand (15). Gridbox settings in the Pyrx application to determine the ligand docking space in the molecular docking process, the space is determined based on the ligand that has bound to the molecule when downloaded. Gridbox settings include center x, center y, and center z to determine the position of the box parameters on the macromolecule, in addition to size_x, size_y, and size_z to determine the size of the gridbox that will be used to bind the test ligand (16). The gridbox size setting must be precise, namely determined based on the coordinates of the native ligand in the protein structure so that the search is focused on the known active area, the size must be sufficient to cover all native ligands and amino acid residues that play a role in binding, the gridbox setting affects the

success of the native ligand pose as shown by the RMSD (Root Mean Square Deviation) value ≤ 2.0 Å (17).

The results of ligand docking in the Pyrx application were compared with the position of the ligand in the crystal structure using the Pymol application, if there was an overlap between the two, it would affect the RMSD value (14). In this study, there was an overlap between the ligand docking results and the position of the ligand in the crystal structure, then the RMSD value in the Pymol application was 0.058 Å and the RMSD value of the docking in the Pyrx application was 1.543 Å. The RMSD results in this study have met the criteria of ≤ 2.0 Å, so the molecular docking method used in this application is valid so that the method can be accepted for further research using the molecular docking method (25).

When the method is said to be valid, the next step is the docking process, namely anchoring the test ligand to the Bcl-2 protein with a gridbox size adjusted to the validation method to analyze the binding affinity value, RMSD, and amino acid interactions of the test compound from the Javanese turmeric (*Curcuma xanthorrhiza*) plant. Binding affinity or the free energy value of the bond is the main parameter in this method because it describes how strongly the compound in the Javanese turmeric (*Curcuma xanthorrhiza*) plant can bind to the active site of the Bcl-2 target protein so that it can produce a biological effect as an inhibitor of cancer cell apoptosis. The more negative the binding affinity value, the better the stability between the compound and the target protein, so that the bond that occurs is stronger (18).

The molecular docking method requires a comparison, namely a positive control, namely the drug compound venetoclax and a negative control, namely the drug compound paracetamol. The positive control uses the drug compound venetoclax to validate the method and compare the strength of the interaction between the test ligand and the target protein, which is already known to have biological activity against the target protein (19). The negative control uses the drug compound paracetamol because it is known not to have specific activity against the Bcl-2 protein so it is expected to have a lower binding energy value than the test compound, therefore the potential of the test compound can be assessed without any interference from other activities. Paracetamol is widely available in terms of structure and molecular data, making it easy to use as a comparison in molecular docking (20).

In this study, the binding affinity value was obtained with a range of 5.1-7.5 kcal/mol and RMSD value with a range of 1.017-2.109 Å. The RMSD result value is in accordance with the literature, namely RMSD less than 3 for structural conformation alignment is still acceptable but the most optimal value is ≤ 2 Å, in line with the theory of Listyani et al., 2024 which states that the smaller the binding affinity value, the better the interaction with the reference compound. The binding affinity results were compared with the native ligand with a binding affinity value of -9.4 kcal/mol and an RMSD value of 1.543, the positive control, namely Venetoclax with a binding affinity value of -8.9 kcal/mol and an RMSD value of 1.774, and the negative control, namely Paracetamol with a binding affinity value of -4.8 kcal/mol and an RMSD value of 1.503, it was found that none of the test ligands were higher than the reference control or the positive control. From the docking process that has been carried out, the most negative binding affinity value and closest to the comparison control and positive control is the Demetoxycyclocurcumin compound, namely -7.5 kcal/mol, then the RMSD value is 1.552 Å where this value has met the requirements, namely a value of ≤ 2 Å.

Visualization of amino acid residues aims to see or identify amino acid interactions when the docked test ligand is docked with the Bcl-2 protein receptor using the BIOVIA Discovery Studio Visualizer application. Identification of amino acid residues involved in the interaction is carried out to describe the binding mode of the test ligand and its compatibility with key residues in the active site through comparison with native ligands or positive controls (21). One of the amino acid interactions is the hydrogen bond which is the strongest non-covalent bond

and is most widely used in creating pharmacological actions, hydrogen bonds help maintain the position of the ligand in the active pocket of the target protein (12).

The interaction that occurs in the test compounds against the Bcl-2 protein shows that none of the compounds have amino acid residue interactions in the same hydrogen bonds as the native ligand but some have similarities with the positive control, the similarity is in the amino acid residue ARG or Argyrin. These compounds are Zedoarol, Salvigenin, Zedoaraldehyde, and Demethoxycyclocurcumin. Of these compounds, the Demethoxycyclocurcumin compound has the most hydrogen bonds, this is in line with the theory of the research of Listyani et al (2024) which states that the more favorable interactions, the lower the free energy of the bond (the more negative) indicates a better binding affinity, this is in accordance with the results of the lowest binding affinity value of the compound, which is -7.5 kcal / mol, in addition, the similarity of residues with the positive control is an important indicator that the Demethoxycyclocurcumin compound is able to bind to the active pocket of the target protein similar to the positive control (Venetoclax), so it has the potential to show better inhibitory activity and can be considered as a potential Bcl-2 target protein inhibitor candidate (22).

Toxicity testing of test compounds was carried out using the toxtree application with the observed parameters being Cramer Rules which are used to eliminate non-carcinogenic chemicals and determine the toxicity level based on TTC (Threshold of Toxicological Concern) or the safe exposure threshold for humans (23). The toxicity level is divided into 3 classes, namely Class I-Low, Class II-Intermediate, Class III-High. Class I (low) means the substance has a safe structure, class II (intermediate) means the substance has a structure that is still safe compared to class I, while class III (high) means the substance contains a structure whose safety aspects have a weak initial effect or do not guarantee safety in their use (23). From the research that has been carried out, it was found that the test compounds that have a low or good level of toxicity are Curcumin, Xanthorrhizol, Bisacumol, Demethoxycyclocurcumin, α -cubebene.

The next parameter is Carcinogenicity (genotox and nongenotox) and Mutagenicity Rulebase by ISS used to assess the potential of compounds in triggering genetic (genotoxic) or cancer induction through non-genotoxic mechanisms. From the tests that have been carried out, several compounds were found to show negative results in the Carcinogenicity (genotox and nongenotox) and Mutagenicity parameters, namely Curcumin, Dihydropyrocuzerenon, Xanthorrhizol, Bisacumol, Demethoxycyclocurcumin, α -cubebene compounds. Negative results for genotoxic carcinogenicity indicate that the compound predictively does not trigger DNA damage or changes in genetic material that can initiate the carcinogenesis process, in addition, negative results for nongenotoxic carcinogenicity indicate that the compound is not expected to cause cancer through other mechanisms such as chronic oxidative stress, hormonal disorders, or prolonged cell proliferation without direct involvement of DNA damage. The results obtained are in accordance with the theory of the research of Listyani et al. (2024) where the compound profile was negative for genotoxic carcinogenicity and negative for nongenotoxic carcinogenicity supported candidate compounds with low carcinogenic risk based on the toxicological predictions used.

The next parameter is In Vitro Mutagenicity (Amest Test) Alert by ISS used as an initial stage to detect the potential genotoxicity of compounds, namely the ability to cause genetic mutations in DNA, which generally uses the Salmonella typhimurium strain test system (24). Compounds that show no alert results for S. typhimurium mutagenicity are Curcumin, Dihydropyrocuzerenone, Xanthorrhizol, Bisacumol, Demethoxycyclocurcumin, α -cubebene. These compounds indicate that based on the prediction model there is no activity that leads to the potential for causing mutations in Salmonella typhimurium bacteria, so the risk of in vitro mutagenicity is estimated to be low for these test compounds.

In this study, the Demethoxycyclocurcumin compound showed the best molecular docking and toxicity parameters compared to other active compounds in Temulawak (Curcuma

xanthorrhiza). Overall, the Demethoxycyclocurcumin compound was considered the most potential candidate for an anti-breast cancer agent based on Bcl-2 protein inhibitors for further research through in vitro and in vivo tests.

CONCLUSION

It was found that the most potential active compound of the Temulawak plant (*Curcuma xanthorrhiza*) as a Bcl-2 protein inhibitor is the Demethoxycyclocurcumin compound, because all parameters showed good results and were in accordance with the literature, namely having a binding affinity value of -7.5 kcal/mol, a Root Mean Square Deviation (RMSD) value of 1.552 Å, and the interaction of the amino acid Hydrogen Bond with the residues ARG A:127, ARG A:139, and TYR A:180, Pi-Cation interaction with the residue ARG A:183, and Pi-Alkyl interaction with the residue ALA A:131. In addition, toxicity parameter testing showed a low level of toxicity.

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