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The Study of Molecular Docking and Molecular Dynamics Simulation Chemical Compound of *Pycnarrhena cauliflora* Diels. as Proapoptosis in Cervical Cancer

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Abstract

Cervical cancer is one of the most common cancers among women worldwide and in Indonesia. B-cell lymphoma 2 (Bcl-2) can play a role in causing cancer by inhibiting apoptosis. The purpose of this study was to analyze the chemical compound of the sengkubak plant (*Pycnarrhena cauliflora* Diels.), which can act as antiapoptotic inhibitor by binding to the B-cell lymphoma 2 (Bcl-2) receptor. The research was conducted *in silico* with molecular docking methods and molecular dynamics simulations. Molecular docking used the AutoDock 4.2.6 software and visualization used Biovia Discovery Studio. Molecular dynamics simulation used Gromacs 5.1.2 software and result visualization used Grace. Longipinocarvone was the best test ligand with the smallest ΔGbind value of -6.99 kcal/mol compared to the positive control of Doxorubicin and other compounds which indicating Longipinocarvone's affinity for binding to the Bcl-2 receptor was better than Doxorubicin. The types of interactions involved in the molecular docking of the chemical compounds of the sengkubak plant and Doxorubicin including hydrogen bonds and hydrophobic interactions. The stability of the bond between the ligand-protein complex resulting from molecular docking was analyzed based on the parameters RMSD, RMSF, Radius of Gyration (Rg) values through molecular dynamics simulations. The results of the analysis showed that Longipinocarvone and Doxorubicin had a stable bond with Bcl-2 as indicated by the RMSD and RMSF values meeting the requirements, namely <3 Å (0.3 nm). The Rg graph showed both complexes are stable during simulation and have resemblant ligand-protein movements.

Keywords: *Cervical cancer, B-cell lymphoma 2 (Bcl-2),* Pycnarrhena cauliflora *Diels., molecular docking, molecular dynamics.*

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INTRODUCTION

Cervical cancer is a cancer that originates from the cervix which ranks second as the most common cancer in Indonesia. B-cell lymphoma protein (Bcl-2) acts as an antiapoptotic, which has an essential role in the regulation of apoptosis. An overexpression of antiapoptotic proteins is found in several types of cancer, including cervical cancer (Protrka, *et al.*, 2010). Doxorubicin is an example of a chemotherapeutic agent in the treatment of cervical cancer (Hasyim, *et al.*, 2023). This drug can induce apoptosis by downregulating antiapoptotic Bcl-2 protein expression (Pilco-Ferreto and Calaf, *et al*., 2016). In long-term use, resistance to chemotherapeutic agents can occur (Yusuf, *et al.*, 2022). The problems in cancer treatment provide impetus to develop alternative therapies for cancer using natural ingredients derived from medicinal plants or herbs (Mastura, *et al.*, 2021). Plants are the most dominant natural source utilized in medicine. Active compounds in plants that can be used in therapy are used as new entities and become an essential foundation in drug discovery and development. In drug development, the discovery of active compounds in plants and their derivatives with pharmacological activities and potential as medicinal ingredients can be proposed to be synthesized for further drugs production (Mathur, and Hoskins, 2017).

Discovering and developing a new drug requires a long process and high costs. In order to reduce the costs and timeframes, the technology breakthrough through drug design simulations with bioinformatics or in silico based on the Computer Aided Drug Design (CADD) method can be used. In this method, drug development is carried out based on information from previous known drugs and diseases as well as computational simulation studies of interactions between drug candidates and receptor targets. Molecular docking is a common method used to predict intermolecular complexes between drug molecules as ligands and target proteins or receptors in screening new drug compounds (Parikesit, *et al.*, 2019). The stability of the bond between the ligand and the receptor that occurs over time and space can be observed by validating the molecular binding process through Molecular Dynamics (MD) (Elfita, *et al.*, 2023).

Sengkubak plant which has the Latin name *Pycnarrhena cauliflora* Diels. is a wild plant found scattered in the interior of West Kalimantan (Iriani, *et al.*, 2022). This plant is part of the Menispermaceae family. The results of the phytochemical screening showed that the sengkubak plant contains terpenoid and alkaloid compounds in the roots, leaves, and stems (Masriani, *et al.*, 2019). Bioactive compounds from *P. cauliflora* extract detected through GC-MS analysis include α- Bergamotene, β-Sesquiphellandrene, α-Cubebene, Sabinene, α-santalene (Tricyclo[2.2.1.0(2,6)]heptane 1,7-dimethyl-7-(4-methyl -3-pentenyl)), Aristolene (1h-cyclopropa[a]naphthalene1a,2,4,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl), Trans(β)-caryophyllene, α-Humulene, Alloaromadendrene, Germacrene D, Longipinocarvone, Cis-Ocimene, β-Citronellol (Puspita, *et al.*, 2020).

Pycnarrhena cauliflora is also known has antibacterial, antioxidant, and cytotoxic activities (Masriani, *et al.*, 2015; Sarifati, *et al.*, 2020). The previous *in vitro* studies found that anticancer activities in *P. cauliflora* extract by inhibiting the growth of HeLa cancer cells through induction of apoptosis and cell cycle arrest in the G0/G1 phase (Masriani, *et al.*, 2013). The anticancer potency of *P. cauliflora* can be developed a new drugs design as *in silico* cervical anticancer agents. Therefore, this research was carried out to study the interaction of chemical compounds from sengkubak (*Pycnarrhena cauliflora* Diehls.) on B-cell lymphoma (Bcl-2) with PDB ID 4AQ3 as proapoptosis in cervical cancer computationally using molecular docking and molecular dynamics methods to find active compounds from sengkubak with a binding affinity that can inhibit antiapoptotic proteins.

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MATERIALS AND METHODS

Materials

The hardware used for molecular docking is in the form of a laptop set with Random Access Memory (RAM) specifications of 4 GB (Gigabyte), Processor Intel(R) Core (TM) i3-7020U CPU @2.30GHz 2.30 GHz, and Windows 10 (64 bit)). The hardware used for the molecular dynamics simulation is a Personal Computer (PC) with Ubuntu 18.04.1 LTS system specifications, AMD Ryzen 7 2700x Eight-Core Processor x 16, GNOME 3.28.2, 64-bit, 1 TB HDD connected to the internet. The software used is SCFBIO, pkCSM, Protein Data Bank, PubChem, PyMOL version 2.5.4, Autodock version 4.2.6, MarvinSketch, Biovia Discovery Studio, CHARMM-GUI, Gromacs version 5.1.2, and Grace. The materials used included Bcl-2 macromolecule, Doxorubicin as a positive control, and the test ligands which is active compounds from the sengkubak.

Methods

Molecular Docking

Bcl-2 Macromolecule Preparation

The Bcl-2 macromolecule was downloaded from the Protein Data Bank (PDB) with PDB ID 4AQ3 in (*.pdb) format. The 4AQ3 macromolecular complex was separated from the native ligand using the PyMOL software. The receptor was optimized in the AutoDock by adding hydrogen and charge and then saving it in (*pdbqt).

Ligand Preparation

Doxorubicin as a positive control, native ligand, and chemical compound test ligands of sengkubak were obtained from PubChem in (*.sdf) format. Ligands were converted into (*.pdb) format with MarvinSketch software. Ligands were optimized using Autodock by setting the torsion tree by choosing choose root, detect root, then choose torsion. Then, the ligands are saved in the format (*.pdbqt).

Analysis of The Rule Five of Lipinski (Ro5)

Ro5 analysis was performed via the SCFBIO website. Lipinski conditions consist of a molecular weight of ≤500 Da, hydrogen bond donors ≤5, hydrogen bond acceptors ≤10, Log *P*≤5.

Redocking and Molecular Docking

Redocking was carried out using a native ligand which has been separated and optimized (*.pdbqt). Binding sites were arranged with a gridbox orientation with a value of X=-22.779; Y=6.349; Z=-10.223 and the gridbox dimension size is 40 x 40 x 40. The grid box setting results are saved in the format (*.gpf) and the grid process is executed with autogrid.exe. The molecular docking stage was carried out by selecting the receptor and the test ligand, setting it with the genetic algorithm parameters, then setting the docking parameter 4.2, and the docking document was saved with Lamarckian GA 4.2 in (*.dpf) format. The docking process was run using autodock.exe by AutoDock version 4.2.6.

Docking Results Analysis and Visualization

Docking results on documents in (*.dlg) format were analyzed using AutoDock with parameters of binding energy (ΔGbind), inhibition constant (Ki), and RMSD. Ligand-receptor complex in (*.pdbqt) format was visualized in two dimensions and three dimensions in the Biovia Studio Visualizer.

Pharmacokinetic Properties Prediction

The pkCSM website (biosig.unimelb.edu.au/pkcsm/prediction) has been used to predict ADME properties of Longipinocarvone as the best tested ligands and Doxorubicin (Krihariyani, *et al*., 2019).

Molecular Dynamics

Receptor and Ligand Preparation

Molecular dynamics simulations were carried out using protein-ligan complex including

Bcl-2 receptor-Doxorubicin and Bcl-2 receptor-Longipinocarvone.

System Setup

Ligand-protein complex files were uploaded via the CHARMM-GUI website. System preparation was carried out by creating a topology, determining the shape of the box and solvation, adding ions (Na+ and Cl-), and selecting the force field.

System Minimization

System minimization was carried out to loosen the structural complex by reducing the potential energy so the system does not experience inappropriate collisions. The minimization process was carried out in 5,000 steps (Parchekani, *et al.*, 2022).

Equilibration

The temperature and pressure in the system are adjusted to a temperature that is set to 310 K while the pressure is arranged to 1 atm. The systems were equilibrated for 125,000 steps and 125 picoseconds (ps) (Meyer, *et al.*, 2020).

Production and Analysis of Molecular Dynamic Results

The protein-ligand complexes were simulated in molecular dynamics using Gromacs version 5.1.2 software. The production stage was run for 20 nanoseconds (ns) with 5000,000 steps. A time of 20 ns is good enough to obtain trajectories and illustrate protein-ligand stability during simulation (Lemkul, 2018). Production results were analyzed for several parameters including RMSD, RMSF, and radius of gyration (Rg), then visualized in trajectories form using Grace software.

RESULTS

The Drug- Likeness Analysis and Molecular Docking

The results of Ro5 analysis that listed in Table 1. The results of the Ro5 analysis show 12 test ligands meet the overall requirements of the Lipinski rule parameters. The α-Humulene test ligand has one violation and Doxorubicin has three violations of the criteria in the Lipinski rule.

Table I. Analysis results the rule of five.

Ligand	MW	HD	HA	Log*P*
	<500	<5	<10	<5
Doxorubicin	**543**	**7**	**12**	-0.464100
Alloaromadendrene	204	0	0	4.849299
α-Bergamotene	204	0	0	4.604679
α-Cubebene	204	0	0	4.270899
α-santalene	204	0	0	4.414999
α-Humulene	204	0	0	**5.035399**
β-Citronellol	156	1	1	2.751299
β-Sesquiphellandrene	204	0	0	4.891300
Aristolene	204	0	0	4.399459
Cis-Ocimene	136	0	0	3.474999
Germacrene D	204	0	0	4.891299
Longipinocarvone	218	0	1	3.593999
Sabinene	136	0	0	2,998699
trans β-Caryophyllene	204	0	0	4.725199

Note: Numbers in bold print mean that the grade does not meet the Lipinski requirements. MW: Molecular weight, HD: Hydrogen bond donor; HA: Hydrogen bond acceptor.

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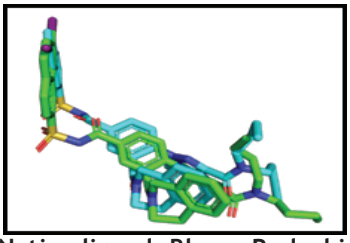
Molecular docking of the test compounds was carried out after the validation process through the redocking to determine the suitability for docking parameters, such as the coordinates of the receptor binding site by determining the gridbox value (Supandi, *et al.*, 2021). The results of the redocking stage were analyzed using PyMOL can be seen in Figure 1. This stage produces root-mean-square deviation (RMSD) values indicate the accuracy of the calculations, where the method used can be accepted or valid if the RMSD value obtained is <2 Å. The RMSD value resulting from the redocking stage is 1.887 Å. The values obtained from the result of this study meet the requirements and show that the atomic positions of the redocking ligands are not much different from those of the crystallographic ligands. The gridbox parameter can be used as a gridbox value and a binding site for molecular docking of the test compound (Aswad, M, *et al.*, 2020).



Green = Native ligand; Blue = Redocking ligand
Figure 1. RMSD result.

Molecular docking produces a value of binding energy (ΔGbind) and inhibition constant (Ki), which are listed in Table 2. Several test ligands produce smaller ΔGbind values compared to the Doxorubicin as positive control including Longipinocarvone, Alloaromadendrene, Germacrene D, trans β-Caryophyllene, α-Cubebene, and α-Humulene. Research conducted by Matam, *et al.* (2020) and Al-Warhi, *et al.* (2020) have used Doxorubicin as a positive control for molecular docking with the Bcl-2 receptor target (PDB ID: 4AQ3).

Table 2. Results of molecular docking.

Ligand	ΔG_{bind} (kcal/mol)	Inhibition Constant (Ki)
Doxorubicin (positive control)	-6.4	20.33 µM
Longipinocarvone	**-6.99**	**7.55 µM**
Alloaromadendrene	**-6.91**	**8.66 µM**
Germacrene D	**-6.71**	**12.05 µM**
α-Cubebene	**-6.63**	**13.79 µM**
α-Humulene	**-6.59**	**14.72 µM**
trans β-Caryophyllene	**-6.56**	**15.44 µM**
β-Sesquiphellandrene	-6.1	33.97 µM
α-Bergamotene	-6.09	34.34 µM
α-santalene	-6.01	39.33 µM
Aristolene	-5.53	88.68 µM
Sabinene	-4.88	266.91 µM
Cis-Ocimene	-4.65	391.29 mM
β-Citronellol	-4.06	1.06 mM

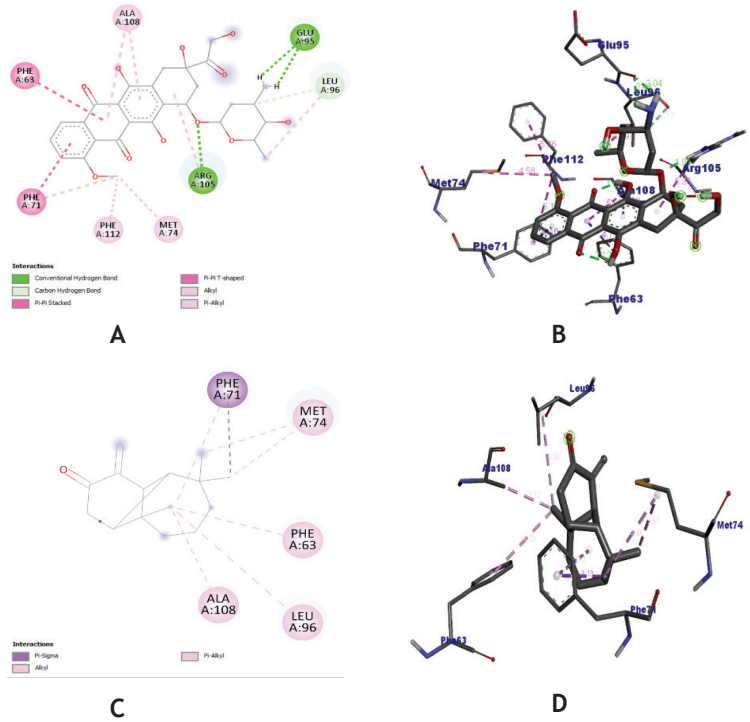
Note. Bold font: The value is smaller than the positive control.

The results of the docking visualization analysis using the Biovia Discovery Studio to determine the interaction between ligands and amino acids are attached in Table 3.

Table 3. Analysis results of interactions between macromolecules and ligands.

Ligand	Interaction Type	
	Hydrogen Bonds	**Hydrophobic Interaction**
Doxorubicin (Positive Control)	ARG105; GLU95; LEU96	PHE71; PHE63; ARG105; LEU96; MET74; PHE112; ALA108
Longipinocarvone	-	**PHE71; ALA108**; **LEU96**; **MET74; PHE63**
Alloaromadendrene	-	**PHE71; ALA108; LEU96; MET74; PHE63**; TYR67
Germacrene D	-	**PHE71; LEU96**; **ALA108**; **MET74; PHE112**
α-Cubebene	-	**MET74**; **ALA108; PHE63**; **PHE71**; TYR67
α-Humulene	-	**ALA108**; **MET74**; VAL92; **PHE63**; **PHE71**; **PHE112**
Trans- β -Caryophyllene	-	**MET74**; **ALA108**; VAL92; **PHE63**; TYR67; **PHE71**; **PHE112**
β-Sesquiphellandrene	-	**LEU96**; **ALA108**; VAL92; **MET74;** TYR67; **PHE71**; **PHE112**
α-Bergamotene	-	**ALA108**; **MET74**; VAL92; **PHE63**; **PHE71**; **PHE112**
α-Santalene	-	TYR67; VAL92; **ALA108; LEU96**; **MET74; PHE63**; **PHE71**; **PHE112**
Aristolene	-	ALA59; ARG66; VAL107; **PHE63**; TYR67
Sabinene	-	**ALA108**; **MET74**; VAL92; **LEU96; PHE63**; **PHE71**
Cis-Ocimene	-	**ALA108**; **MET74**; VAL92; **LEU96; PHE63**; TYR67; **PHE71**; **PHE112**
β-Citronellol	VAL92	**PHE71; ALA108**; VAL92; **LEU96** ; **MET74;** TYR67; **PHE71**

Note: Bold font: The same amino acid residue interacts with positive control. ALA (Alanine), ARG (Arginine), GLU (Glutamic Acid), LEU (Leucine), MET (Methionine), PHE (Phenylalanine), TYR (Tyrosine), VAL (Valine).



A **B** **C** **D**

Figures 2. 2D (A) and 3D visualization of Doxorubicin (Positive Control) (B); 2D (C) and 3D visualization of Longipinocarvone (D).

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Two-dimensional and three-dimensional visualization of Doxorubicin and Longipinocarvone can be seen in Figure 2, there was interaction between ligands with amino acid at the receptor. Doxorubicin has hydrophobic interactions of the pi-sigma, pi-pi t-shaped, pi-pi stacked, pi-alkyl, and alkyl with amino acid residues including LEU96, PHE71, PHE63, MET74, PHE112, ALA108. The positive control Doxorubicin also interacts through conventional hydrogen bond and carbon hydrogen bonds with ARG105, GLU95, LEU96. Longipinocarvone has hydrophobic interactions of the pi-sigma, pi-alkyl, and alkyl with amino acid residues including PHE71, ALA108, LEU96, MET74, and PHE63.

Pharmacokinetic Properties Prediction

The result of pharmacokinetic properties prediction of Doxorubicin and Longipinocarvone are presented in Table 4.

Table 4. Results of pharmacokinetic properties prediction.

Parameter		Compound	
		Doxorubicin	Longipinocarvone
Absorption	Human intestinal absorption (HIA) (%)	62.372	95.972
Distribution	VDss (log L/kg)	1.647	0.563
	BBB permeability (Log BB)	-1.379	0.628
Metabolism	CYP2D6 substrate	No	No
	CYP2D6 inhibitor	No	No
Excretion	Total Clearence (log ml/min/kg)	0.987	0.891

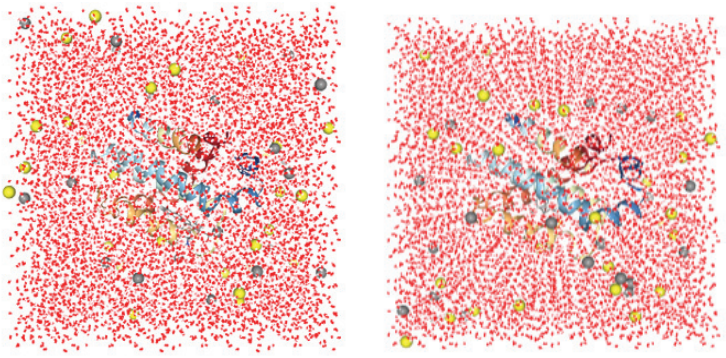
Longipinocarvone meets good HIA parameter values with values in the range 70-100%, while Doxorubicin does not. Both compounds are included in the high volume distribution with values >0.45. Longipinocarvone has a Log BB value in the range >0.3 so it is included in the category of fairly good penetration into the BBB. Doxorubicin has a Log BB value <-0.1 which indicates the compound has no target in the central nervous system (CNS).

Molecular Dynamics

System setup and topology formation via the CHARMM-GUI website are carried out before molecular dynamics simulations simulation. A cubic shape is chosen at the stage of forming a box (Gajula, *et al.*, 2016). The water molecule used for solvation is the TIP3P water model, because it is a simple water model that has been used widely and compatible with CHARMM36 (Park, *et al.*, 2022). The solvation stage was carried out by adding 8,100 water molecules to the Doxorubicin box system and 8,109 to the Longipinocarvone box system. Then, 28 Na+ ions and 23 Cl- ions were added to neutralize the system (Muhammed, *et al.*, 2023). After that, the CHARMM36 force field is selected, because it is a force field supported by Gromacs and can generate the latest CHARMM force field (Apol, *et al.*, 2014).

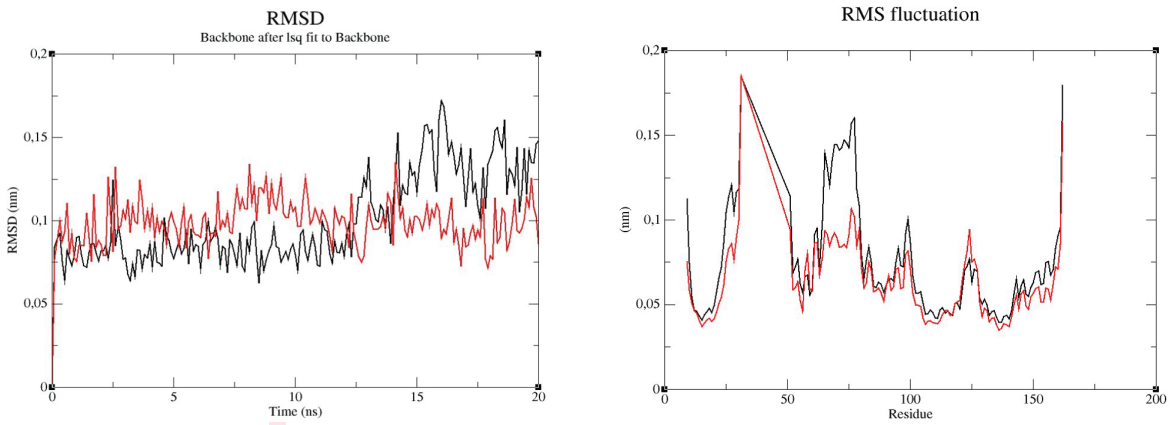
Based on the graph in Figure 4, The RMSD value of the protein-Longipinocarvone complex is in the range of 0.07-0.13 nm, while the protein-Doxorubicin complex is in the range of 0.06-0.17 nm. The RMSF values of the amino acid residues that interact in the protein-Longipinocarvone complex including PHE71, ALA108, LEU96, MET74, and PHE63 show a value range of 0.04-0.08 nm, while in the protein-Doxorubicin complex including ARG105, GLU95, LEU96, PHE71, PHE63, MET74, PHE112, ALA108 are in the value range of 0.05-0.14 nm. The Radius of Gyration (Rg) of the protein-Doxorubicin complex increased once to reach a value of 1.43 nm at 13600 ps, while the radius of gyration of the protein-Longipinocarvone complex increases once to 1.43 nm at 10400-10500 ps.

Aini, *et al.*, *2024*
Indones. J. Cancer Chemoprevent., **15**(1), 63-75

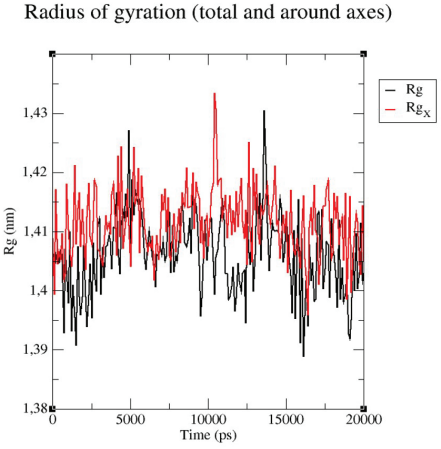


A B

Figures 3. A. Doxorubicin system; B. Longipinocarvone system.



A B



C

Notes: Black = Doxorubicin (positive control); Red = Longipinocarvone (test ligand).
Figures 4. The RMSD (A); RMSF (B); Rg Graph of molecular dynamics results.

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DISCUSSION

The Drug- Likeness Analysis and Molecular Docking

The Rule of Five Lipinski (Ro5) is a rule to analyze the physicochemical properties of the test ligand and positive control. All test ligands meet the molecular weight requirements Ro5 of ≤500 Da, H-donor ≤5, and H-acceptor ≤10. The molecular weight show distribution process of the compound, while HBA and HBD describe the energy required in the absorption process. Therefore, all test ligands are easier to pass through biological membranes and do not require a lot of energy in the absorption process. The log*P* parameter describes the ability of the compound to penetrate the lipid bilayer. Based on the results in the Table 1, 12 test ligands meet the log*P* requirements of Ro5 with value <5 so that the ligands were able to cross the lipid bilayer (Syahputra, *et al*., 2014).

The best docking result was seen based on the smallest ΔGbind value and predicted inhibition Constant (Ki). The obtained binding energy value is a conformational stability parameter between the ligand and receptor, while the inhibition constant value is a prediction of the inhibitory ability of compound (Puspita, *et al*., 2022). All the ligands in the Table 2 give negative ΔGbind values, therefore it can be seen that all compounds interacted with the active site of the receptor (Safithri, 2022). The Longipinocarvone compound became the best ligand with a ΔGbind value of -6.99 kcal/mol and a predicted Ki value of 7.55 μM.

The value of the binding energy which is increasingly negative and the value of the inhibition constant which is getting smaller, indicates the ligand's affinity or the ligand's ability to bind higher (Elfita, *et al.*, 2023). Based on the analysis, Longipinocarvone has a higher affinity than Doxorubicin. Longipinocarvone is a compound in the sesquiterpene group which is known to show therapeutic potential in cancer treatment through reducing the progression of cancer (Abu-Izneid, *et al.*, 2020). However, there have been no experimental studies testing Longipinocarvone's activity against cancer cells.

The previous study has discovered amino acid residues in the Bcl-2 protein binding site with potent binding activity against native ligands include ARG105, LEU96, PHE157, TYR161, VAL92, TYR67, ASP70, MET74, ARG66, PHE71, PHE63, PHE112, ALA108, ASN102 (Mala, *et al.*, 2015; Al-Warhi, *et al.*, 2020). Doxorubicin has interactions with 8 amino acid residues in the Bcl-2 binding site including ARG105, LEU96, PHE71, PHE63, ALA108, MET74, GLU95, and PHE112. Longipinocarvone interacts with the same 5 amino acid residues as Doxorubicin and native ligand in the Bcl-2 active site including PHE71, ALA108, LEU96, MET74, and PHE63. Interactions on the same amino acid residues show similarity of test ligand activity with the native ligand and Doxorubicin towards the receptor (Elsiana, *et al.*, 2023).

Hydrogen bonds in doxorubicin which are not found in Longipinocarvone do not yet reflect conformational stability because the ΔGbind value is influenced by other factors, for example ΔGhydrophobic. Doxorubicin and Longipinocarvone have hydrophobic interactions with 5 amino acids. Hydrophobic interactions can help bind the ligand to the receptor by stabilizing the interaction between the ligand and the protein by lowering ΔGbind (Aswad, *et al*., 2020). Pi-type hydrophobic interactions are associated with stability at the binding site, such as pi-sigma and pi-alkyl interactions, which aid in drug insertion at the receptor binding sites (Arthur, D and Uzairu, 2019).

Parameters in predicting pharmacokinetic properties include absorption, distribution, metabolism, excretion (ADME). The ability of a compound to be absorbed by human gastrointestinal is seen through the HIA parameter. Longipinocarvone can be absorbed well, while

Doxorubicin has moderate absorption ability. A compound is said to have good absorption capacity if the percentage is in the range of 70 - 100% (Fitri, *et al.*, 2023). The steady state volume of distribution (VDss) parameter shows the steady state volume of distribution or the amount of drug distributed to tissue rather than plasma. Doxorubicin and Longipinocarvone are distributed evenly in the tissues to achieve the same concentration as blood plasma because they have VDss value >0.45. (Krihariyani, *et al*., 2021).

The BBB permeability value describes the ability of a compound to penetrate the blood brain barrier (BBB). Longipinocarvone has a log BB value >0.3 so it can penetrate the BBB and is well distributed, while Doxorubicin has a log BB value <-1 so does not distribute well into the BBB. Metabolism is predicted through the activity of compounds against CYP450 cytochrome enzymes, *i.e*, CYP2D6. Doxorubicin and Longipinocarvone, are neither CYP2D6 substrates nor inhibitors so they do not inhibit the metabolic process by CYP450. The total clearance parameter describes the rate of excretion of compounds. The greater the total clearance value, the faster the rate of drug elimination from the body. The total clearance value of Doxorubicin is greater than Longipinocarvone, so the elimination rate of Doxorubicin is faster than Longipinocarvone (Krihariyani, *et al.*, 2021).

Molecular Dynamics

Molecular dynamics simulations were carried out to determine the stability of the interaction between ligand and receptor from molecular docking result in a certain time and space. Minimization stage aims to avoid clashes between and avoid geometric mismatches (Yu, *et al.*, 2020). Potential energy results must be negative to meet the system simulation requirements. Each of the potential energies generate from the minimization stage of protein-Doxorubicin complex is -4.1063975e+05 kJ/mol and protein-Longipinocarvone complex is -4.18321283+05 kJ/mol. The equilibration stage is carried out using the NVT and the NPT ensemble. Equilibration aims to adjust the system in order that it is thermodynamically stable for any required conditions and in accordance with the conditions in the body (Elfita, *et al.*, 2023).

The stability of the interaction is known through analysis of the RMSD, RMSF, and Rg parameters. Root Mean Square Deviation (RMSD) is a standard calculation of the protein structure deviation (Elfita, *et al.*, 2023). The stability of the protein-ligand complex can be seen through the RMSD value, where stable molecular movement is indicated by RMSD value that <3 Å (0.3 nm) (Manna, *et al.*, 2017). Based on the results, the RMSD value of protein-Longipinocarvone and protein-Doxorubicin complexes have good stability because they meet the RMSD value requirements of < 3 Å (0.3 nm).

Root Mean Square Fluctuation (RMSF) is a value that describes the conformational shifts or fluctuations of each amino acid residue so that the flexibility of the receptor or protein can be determined (Elfita, *et al.*, 2023). The ligand-receptor interaction can be said to be stable during the simulation if the resulting RMSF value is <3 Å (0.3 nm) (Yeni, *et al.*, 2020). Based on the results, Longipinocarvone complex and Doxorubicin complex has a RMSF value range 0.04-0.08 nm and 0.05-0.14 nm respectively, which fulfill the criteria for a stable interaction between ligand and amino acid residue, namely <0.3 nm.

The Radius of Gyration (Rg) describes compactness of the protein structure. During the simulation, both complexes have a low level of fluctuation so that both have good compactness (Zohora, 2022). Based on the analysis of the results, both complexes are stable in terms of the compactness of the ligand-protein complex because the movements of the respective ligand-protein complexes resemble one to another (Elfita, *et al.*, 2023). In this study, molecular dynamics simulations were only carried out on the smallest test ligand result. Further molecular dyanmics

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studies can be carried out to determine the stability of the docking results with other test ligands that have ΔGbind values smaller than Doxorubicin.

CONCLUSION

The results of molecular docking showed that Longipinocarvone had the smallest ΔGbind value of -6.99 kcal/mol indicating that Longipinocarvone had a higher affinity than Doxorubicin and other test compounds. Longipinocarvone as the best test ligand with the positive control Doxorubicin has the same amino acid residues involved in interactions including PHE71, ALA108, LEU96, MET74, and PHE63 through hydrophobic interactions. Regarding the analysis of the parameters RMSD, RMSF, and Radius of Gyration (Rg) molecular dynamics simulation results, the protein-Longipinocarvone complex and the protein-Doxorubicin complex have good stability in binding to Bcl-2 because they produce RMSD values and RMSF that complied with the requirements, namely <3 Å (0.3 nm). The Rg which showed both complexes are stable and the movement of each ligand-protein complex resembled one another.

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