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In silico study of Andrographolide from *Andrographis paniculata* (Burm F.) Ness as an anti-colorectal cancer agent

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Abstract

Cancer is one of the leading causes of death in the world, one of which is colorectal cancer, a malignant tumor in the epithelial tissue of the colon and rectum. Cancer treatment is long-term, so it has an impact on physical and psychological changes for patients. There was many exploration of anticancer drug candidates from natural ingredients such as andrographolide compounds. The in silico method can be done to predicts the absorption, distribution, metabolism, and excretion (ADME) of andrographolide compounds and meets Lipinski Rule of Five. The toxicity of andrographolide compounds was classified in class 4, which needs dose supervision. The prediction of activity potential is quite good based on the Structure-Activity Relationship (SAR). The results of molecular tethering of the three target receptors, namely Cyclooxigenase-2 (COX-2), caspase-3, and Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2), showed that caspase-3 has the best binding value or affinity of -5.36 kcal/mol, with quite a lot of hydrogen bonds of 7. The amino acids formed in andrographolide compounds are the same as the baseline ligands, so it can be concluded that andrographolide has colorectal anticancer activity and can increase caspase-3 activity in colorectal cancer cells.

Keywords: Andrographolide, Cancer, Caspase-3, Molecular docking

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1. Introduction

Up to now, cancer remains one of the leading causes of death worldwide. Cancer is a disease caused by abnormal growth of body tissue cells that develop rapidly and uncontrollably, pressing on body tissues and affecting organs. Worldwide, cancer-related deaths are estimated at 4.3 million cases annually, with 2.3 million of these cases occurring in developing countries. The annual global incidence of new cancer cases is 5.9 million, with three million found in developing countries (Dekker et al., 2019).

There are numerous types of cancer, one of which is colorectal cancer, ranking third globally with 935 thousand cases, while in Indonesia, it ranks fourth with 18 thousand cases. According to data from the Global Cancer Observatory in 2020, the annual global incidence of new colorectal cancer cases reaches 1.9 million, with 34 thousand cases in Indonesia, comprising 12 thousand cases in females and 24 thousand in males. The causes of colorectal cancer are not yet fully understood, but several risk factors contribute to its development, such as polyps, family history of colorectal cancer, genetic disorders, inflammatory bowel disease, unhealthy lifestyles including smoking, excessive alcohol consumption, high red and processed meat intake, obesity, diabetes mellitus, *Helicobacter pylori* and *Fusobacterium spp* infections (Dekker et al., 2019).

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Colorectal cancer is a malignant tumor found in the epithelial tissue of the colon or rectum (Kuipers et al., 2015). The colon and rectum are part of the large intestine in the digestive system known as the gastrointestinal tract, with the colon proximal and the rectum distal about 5-7 cm above the anus. Symptoms experienced by colorectal cancer patients are often nonspecific and develop gradually, including weakness, fatigue, weight loss, constipation, abdominal pain, and bloody, mucus-filled stools (Wu & Sun, 2015). In colorectal cancer cases, there is a significant increase in COX-2 expression compared to normal colorectal tissue, reaching 77.97 percent. Increased COX-2 expression in cancer is associated with tumor growth and spread, hindering apoptosis (programmed cell death) and disrupting cellular homeostasis, thereby inducing cancer (Farooqi et al., 2020; Wu & Sun, 2015).

Advancements in cancer treatment involve the search for active compounds that inhibit cancer cell growth. Sambiloto (*Andrographis paniculata* (Burm F.) Ness) is a plant widely used in traditional Indonesian medicine due to its pharmacological activities, including anticancer properties and potential as a colorectal cancer treatment. Andrographolide is one of the active compounds or secondary metabolites classified as diterpene lactones (Laksmiani et al., 2017; Mutiah et al., 2015; Olaosebikan et al., 2023).

Given the ongoing prevalence of colorectal cancer as a major global and Indonesian health issue, further studies on the activity of andrographolide against cyclooxygenase-2 (COX-2) receptors, caspase-3, and Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) as a colorectal cancer treatment are warranted (Laksmiani et al., 2018). Exploring potential anticancer drugs from natural sources can be conducted in silico by predicting absorption, distribution, metabolism, and excretion processes, toxicity, potential activity, and molecular docking interactions between bioactive compound components and selected receptor targets (Zhang & Cui, 2023). In silico molecular docking techniques can predict how active compounds interact with receptor proteins, thereby enhancing the effectiveness and efficiency of new drug development. This method reduces the risk of failure, saving time, costs, and effort in drug research by predicting molecular interactions of test compounds with target receptors beforehand (Grahl et al., 2021). In this study, an in silico test was conducted to predict the absorption, distribution, metabolism, and excretion (ADME) of andrographolide and to ensure that the compound complies with Lipinski's Rule of Five.

2. Methods

In this study, an Asus Pro 14 OLED laptop with the following specifications was used: 8 GB RAM, AMD Ryzen 5 5600H processor with Radeon Graphics 3.30 GHz. Computational tools included ADMETlab server (https://admet.scbdd.com/calcpre/indexsys/), ProTox-II server, Way2drugprediction server (Way2Drug - main), AutoDock Tools 1.5.6, and Biovia Discovery.

Protein structures for VEGFR (PDB ID: 1Y6A), COX-2 (PDB ID: 1CX2), and caspase-3 (PDB ID: 2XYG) were obtained from the RCSB PDB database, while the 2D structure of andrographolide (CID: 5318517) and its Canonical SMILES were retrieved from PubChem database. The study involved downloading the Canonical SMILES structure of andrographolide from PubChem and conducting ADME Structure Activity Relationship (SAR) screening to meet Lipinski Rule of Five (Ro5) criteria using ADMETlab server (Hussain et al., 2018; Lipinski et al., 2012). Toxicity prediction was carried out using ProTox-II server, followed by activity prediction (Probability to be Active) using WAYDRUG PASS Prediction server.

Molecular docking analysis was performed using AutoDock Tools (ADT) 1.5.7 and Biovia Discovery Studio 2021. Initially, the protein structures of the targets (VEGFR PDB ID: 1Y6A, caspase-3 PDB ID: 2XYG, COX-2 PDB ID: 1CX2) were prepared by separating solvents from ligands or non-standard residues in Biovia Discovery and saved in .pdb format. Subsequently, the protein structures were prepared using AutoDock Tools 1.5.7 by removing water molecules, adding hydrogen atoms, and separating native compounds and receptors present in protein complexes (Abdillah et al., 2017; Nur et al., 2023).

The ligands were coordinated with native ligands using Biovia Discovery Studio 2021 and saved as ligand documents. Receptors were prepared using AutoDock Tools and saved as .pdbqt documents (Nur et al., 2023). GridBox was then set up to determine receptor binding site coordinates and size. Grid parameters were saved as .gpf documents. Docking parameters were prepared by inputting ligand files into receptor.pdbqt and saved as docking.dpf documents.

Validation of the molecular docking method was conducted using previously downloaded receptors followed by redocking of native ligands bound to each target protein. Comparison of redocked native compound results with crystallographic results in the database was expressed in RMSD (Root Mean Square Deviation) values. Molecular docking was considered valid and usable if the RMSD value was below 2 Å (Veterini et al., 2021).

Further analysis of .dlg document format results was performed using Biovia Discovery Studio 2021. Visualization could be displayed in two-dimensional or three-dimensional structures. Parameters analyzed included interactions between andrographolide and VEGFR, COX-2, and caspase-3 receptors. Selection of the best docking pose involved choosing the ligand conformation with the lowest binding affinity energy.

3. Results and Discussion

3D structure and Canonical SMILES mining data of the receptors and test compounds were obtained from the RSCB PubChem and PDB servers (Table 1).

Table 1.
Data mining of receptors and test compounds

Roles	Compounds	Data	Details
Receptor	COX-2	PDB ID	1CX2
	VEGFR-2	PDB ID	1Y6A
	Caspase-3	PDB ID	2XYG
Test Compound	Andrographolide	Canonical Smiles	CC12CCC(C(C1CCC(=C)C2CC=C3C(COC3=O)O)(C)CO)O
		Pubchem CID	5318517

Prediction of absorption and permeability is based on the Lipinski Rule of Five with several physicochemical parameters. The required physicochemical parameters are Log P, molecular weight, Hydrogen Bond Acceptors (HBA), and Hydrogen Bond Donors (HBD). Ro5 is a set of ADME parameters globally linked to solubility, absorption, and permeability, thus assessing the potential of a molecule to become a drug based on its similarity to drug-like molecular properties (Lipinski et al., 2012).

Table 2.
ADME prediction results and fulfillment of Ro5 rules

Compounds	Parameters	Values	Details
Andrographolide	Molecular mass	350.21	✓ (Optimal 100-600)
	Lipophility	1.501	✓ (Optimal 0-3)
	Hydrogen donor	3	✓ (Optimal 0-7)
	Hydrogen acceptor	5	✓ (Optimal 0-12)
	Conclusion of compliance with the rules	Fulfilled	

The results of the toxicity prediction test for andrographolide compounds using the Protox-II server were carried out by entering Canonical SMILES of the test compounds on the server (Table 3).

Table 1.
Toxicity and LD_50 prediction results

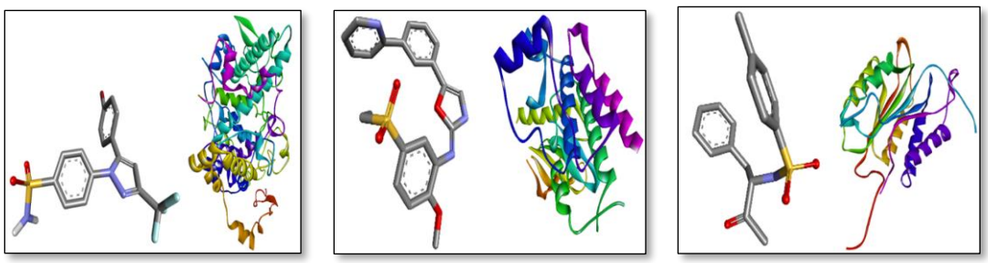
Compounds	Parameters	Values	Details
Andrographolide	Hepatotoxicity	0.93	negative
	Cytotoxicity	0.67	negative
	Immunotoxicity	0.82	negative
	Mutagenicity	0.71	negative
	Carcinogenicity	0.83	negative
	LD_50	1.890 mg/kg (Class 4)	
	Conclusion	Able to be researched further	

In the testing of predicting potential activity or Probability to be Active, data describing the potential activity of andrographolide was obtained using the Prediction of Activity Spectra for Substances (PASS) Online based on the relationship between compound structure and the likelihood of biological or pharmacological activity. This method is commonly referred to as Structure Activity Relationship (SAR) (Table 4).

Table 2.
Activity potential prediction results

Pa	Activity	Parameters	Descriptions
0.845	Anti-inflammatory	>7	The compound is predicted to have high activity potential, high similarity
0.909	Antineoplastic	>7	
0.909	Apoptosis agonist	>7	
0.708	Caspase-3 stimulant	>7	
0.587	Antimetastatic	0,3-0,7	Compounds are predicted to have potential activity, but similarity is low
0.538	Antineoplastic (Colorectal cancer)	0,3-0,7	
0.475	Anticarcinogenic	0,3-0,7	

Molecular docking of andrographolide to receptors begins with preparation, including adding polar hydrogen atoms because some hydrogen atoms may be lost during the isolation process of protein-ligand complexes. The ligand, once prepared, is further optimized by adding Gasteiger charges and torsion parameters, which will be used during molecular docking to adjust to the docking environment. Torsion is adjusted in its active site to determine active bonds that can rotate during the molecular docking process. The separation results of the receptor and natural ligand can be seen in Figure 1.



(a) (b) (c)

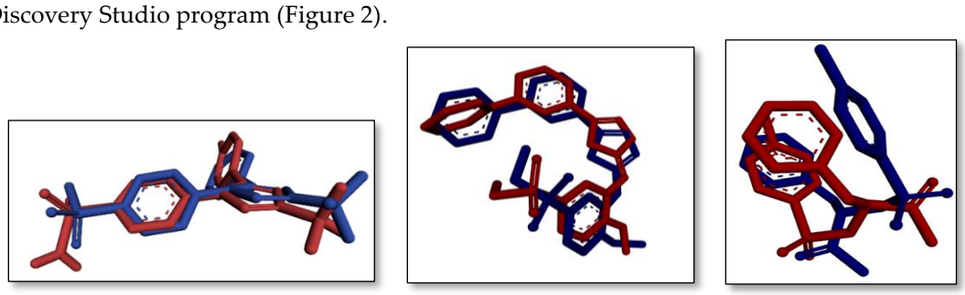
Figure 1. Ligand separation by receptor: (a) S58701 and 1CX2; (b) AAZ201 and 1Y6A; (c) TQ81176 and 2XYG

Validation of the molecular docking method is performed by redocking the active sites of each receptor using Autodock Tools server (Figure 2). The comparison of these redocking results is expressed in RMSD (Root Mean Square Deviation), which measures the deviation distance between the ligand binding positions with the protein after redocking compared to the position of the natural ligand binding.

Table 3.
Molecular docking validation results

Receptor	Grid box size			RMSD (Å)
	x	y	z	
1CX2 (COX-2)	26	36	50	1.250
1Y6A (VEGFR-2)	18	34	20	1.241
2XYG (*Caspase*-3)	14	34	18	2.087

Further analysis and visualization of the results was carried out using the Biovia Discovery Studio program (Figure 2).



(a) (b) (c)

Description
Red : natural ligand
Blue : ligands resulting from re-docking

Figure 2. Overlay visualization of: (a) 1CX2; (b) 1Y6A; and (c) 2XYG

The binding free energy obtained from the docking of molecules on the three receptors can be seen in Table 6. The next parameter, namely the inhibition constant or Ki, shows the concentration required for the ligand to inhibit the target protein.

Table 6.
Results of binding free energy and inhibition constants

Receptor	Results			
	ΔG Free Bond Energy		KI Inhibition Constant	
	Natural Ligand	Andrographolide	Natural Ligand	Andrographolide
COX-2 (1CX2)	-10.99 kkal/mol	-9.71 kkal/mol	8.81 nM	76.26 nM
VEGFR-2 (1Y6A)	-8.49 kkal/mol	-6.95 kkal/mol	601.68 nM	8050 nM
Caspase-3 (2XYG)	-4.85 kkal/mol	-5.36 kkl/mol	569.99 nM	118.05 nM

Molecular docking between andrographolide and the receptor shows the formation of hydrogen bonds and hydrophobic interactions (Table 7). Hydrophobic interactions can contribute to the formation of complexes between drug molecules and targets. These interactions occur because the compound and amino acid residues on the target have similar partial electric charges, resulting in attractive forces (Frimayanti et al., 2021). Nonpolar regions of the ligand strive to interact with nonpolar sides of the target, forming stable hydrophobic bonds. Enhancing the stability of drug-target complexes, the strength of hydrophobic bonds helps maintain the complex in the desired conformation and prevents premature dissociation.

Table 7.
Interactions of bonds and amino acids formed

Receptor	Control Variable				Test Variable (Andrographolide)			
	Number of Bonds		Amino Acid		Number of Bonds		Amino Acid	
	Hydrogen Bond	Hydrophobic Bond	Hydrogen Bond	Hydrophobic Bond	Hydrogen Bond	Hydrophobic Bond	Hydrogen Bond	Hydrophobic Bond
1CX2	5	10	His90 Arg120 Arg513* Arg120 Arg513*	Ser 353 Val523 Val523 Gly526 Val349 Leu359 Val349* Ala527* Val523 Val527	5	3	Tyr385 Arg513* Arg513* Leu352 Ser353	Val349* Ala527* Leu531
1Y6A	3	7	Asn921 Glu915* Lys18*	Val846 Val486 Leu838* Leu838 Phe916 Ala864 Val864	3	1	Cys917 Lys918* Glu915*	Leu838*
2XYG	2	3	His121* Gly122*	Met61* Met61	6	5	His121* Gly165 Gly165 Gly60 Thr59 Gly122*	Cys163 Cys163 Met61* His121 His121

The parameters analyzed from the two-dimensional visualization results are the interactions that occur between the andrographolide compound and the VEGFR, COX-2 and caspase-3 receptors. Determining the ligand conformation resulting from docking (best pose) is carried out by selecting the ligand conformation that has the lowest binding affinity (Figure 3).

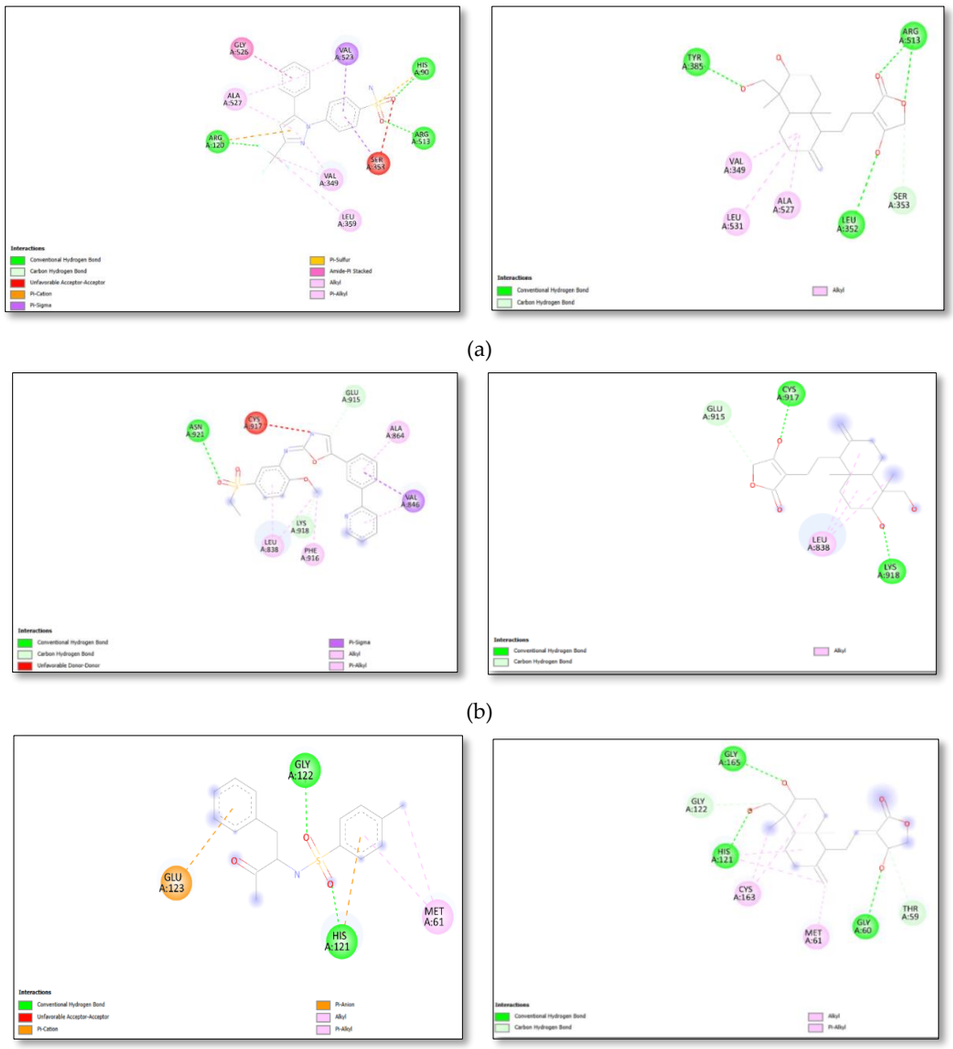


Figure 3. 2D visualization of the interaction between the ligands: (a) S58701; (b) AAZ201; and (c) TQ81176 with andrographolide

Discussion

Data mining is the process of collecting necessary data during research. Canonical SMILES data is used for predicting ADME, toxicity, and activity potential based on structural similarity with databases as a specific treatment or Structure Activity Relationship (SAR) (Table 1). Meanwhile, 2D structures are used in molecular docking processes (Gadaleta et al., 2019).

Prediction of absorption and permeability is based on Lipinski's Rule of Five with several physicochemical parameters: Log P, molecular weight, Hydrogen Bond Acceptor (HBA), and Hydrogen Bond Donors (HBD). Log P (octanol/water partition coefficient) measures the lipophilicity of a compound. If the Log P value does not exceed 5, the compound tends to have suitable lipophilic properties. Lipophilicity indicates the compound's solubility in fats or water. A higher Log P value indicates higher hydrophobicity. Molecules that are excessively hydrophobic may have higher toxicity because they stay longer in the lipid bilayer and distribute more widely in the body, reducing binding selectivity to target enzymes. Conversely, excessively negative Log P values are undesirable because the molecule may not pass through the lipid bilayer (Syahputra et al., 2014).

The molecular weight should be ≤500 daltons because relatively small molecular weight can affect absorption through the digestive tract. Molecular weight relates to drug distribution in the body. Drug distribution occurs by penetrating biological membranes through diffusion processes. Drugs with a molecular weight over 500 daltons are relatively large, affecting the absorption time due to difficulty in penetrating biological membranes. Compounds with smaller molecular weights penetrate biological membranes more easily and have faster absorption times (Ruswanto, 2015). The number of hydrogen bond donors bound to nitrogen or oxygen atoms should be ≤5, and the number of acceptor atoms bound to nitrogen and oxygen should be ≤10. Hydrogen donors or acceptors make molecules more partitioned in strong hydrogen solutions like water compared to a lipophilic environment such as a lipid bilayer. Based on the test results, andrographolide compounds meet all Ro5 rule parameters. Thus, it can be said that andrographolide compounds are predicted to have good solubility, absorption power, and permeability so that they can be administered orally.

Toxicity testing is essential to obtain preliminary information before selecting drug candidates, as drugs are chemical compounds that may not be entirely safe and acceptable to the body. According to the Globally Harmonized System (GHS), LD50 is classified into 6 classes, where the higher the LD50 dose of the test compound, the lower its toxicity, and conversely, the lower the LD50 dose, the more toxic and hazardous the compound (Gadaleta et al., 2019).

The results of toxicity testing indicate that andrographolide is predicted to be inactive or non-toxic to the liver and cells, and it is not carcinogenic or mutagenic. However, caution is needed regarding the immune system, as the results suggest potential immunotoxic effects (Table 3). Further testing such as in vivo toxicity studies in animals or toxicity tests on human white blood cells is required to validate and confirm the potential immunotoxic effects. Additionally, in silico or in vitro prediction of immunotoxicity does not always reflect real-life conditions in the body, so toxicity testing in animals and clinical studies in humans are still necessary to confirm the potential immunotoxic effects and to evaluate health risks more comprehensively. The predicted LD50 value obtained is 1.890 mg/kg, falling into class 4, which ranges between 500-2000 mg/kg, indicating relatively low toxicity. A higher LD50 value corresponds to lower toxicity (Supandi et al., 2018).

In silico prediction of andrographolide compounds is based on their similarity to compounds in the PAS Online database, which are proven effective in treating certain diseases. The testing parameter is assessed based on the Probable Activity (Pa) value: if Pa > 0.7, the tested compound is predicted to have high potential due to its similarity to benchmark compounds proven effective in specific treatments listed in the database. If Pa falls between 0.3-0.7, the compound is predicted to have activity but with lower similarity to benchmark data. If Pa is less than 0.3, the compound has very low similarity to benchmark data, indicating low potential.

From the results of testing using the PASS Online server, andrographolide shows potential in several biological activities related to colorectal cancer, such as anti-inflammatory, antineoplastic, apoptosis agonist, and caspase 3 stimulant (Table 4). However, for colorectal cancer antineoplastic, antimetastatic, and anticarcinogenic activities, andrographolide shows potential but its similarity is relatively low because the Pa value is below 0.7. Therefore, for more accurate predictions, further testing using molecular docking methods is necessary.

Before conducting molecular docking between compounds and their receptor proteins, preparation is necessary. This stage is crucial for ensuring successful molecular docking and requires meticulous attention as it significantly affects the docking outcomes. Once 3D structure data of the target protein or receptor is obtained from data mining, preparation is carried out using the Biovia Discovery Studio server. During this process, separation between the protein and its natural ligands is conducted to prevent interference with the binding of the test compound. This step also involves cleaning the target protein from unnecessary molecules considered as residues, which typically include water molecules and metal ions. The presence of water molecules can slow down the docking process due to the increased variables that need to be addressed. The separated protein and natural ligand are saved in separate pdb files for further preparation.

After separating the protein and natural ligand, the preparation continues with both the protein and ligand. Validation of the molecular docking method is performed by redocking the active sites of each receptor using the Autodock Tools server. Comparison of these redocking results is expressed in terms of RMSD (Root Mean Square Deviation). According to several literatures, an RMSD value is considered valid if it is ≤2 Å, indicating that the docking parameters used are valid for the docking of test compounds. Another view suggests that an RMSD value for acceptable structural alignment is less than 3 Å, but the optimal value is less than 2 Å, indicating better alignment (Veterini et al., 2021). The RMSD value is also influenced by the resolution of the receptor protein and the receptor modeling method used. The validation process is conducted on the ligand binding site pocket with 100 replications. The more replications performed, the better the application is for docking (Muchtaridi et al., 2018).

On the COX-2 receptor, the RMSD value obtained was 1.583 Å, on VEGFR-2 it was 0.861 Å, and on caspase-3 it was 2.087 Å (Pasha et al., 2021). Additionally, during this stage, gridbox settings were adjusted using Autodock Tools software, which serves as the docking site for ligands and proteins. The size of the gridbox is adjusted to cover the ligand, meaning the gridbox size varies for each receptor. The gridbox sizes for receptors were as follows: (1) 1CX2 receptor was 26, 36, 50; (2) 1Y6A receptor was 18, 34, 20; and (3) 2XYG receptor was 14, 34, 18 (Table 5).

Andrographolide was docked to three target receptors known to influence cancer cell growth, particularly colorectal cancer. Docking of the compound to the target receptors was performed using Autodock Tools server, beginning with preparation and followed by docking

stages using validated receptors and ligands. The first parameter observed from the docking results was the binding free energy (ΔG). The binding free energy indicates the strength of the interaction between the protein and ligand. A lower (more negative) ΔG value indicates a stronger binding interaction, suggesting that the compound requires less energy for binding, thus indicating greater potential for strong interactions with the target protein.

A good inhibition constant is represented by a lower Ki value. The smaller the Ki value, the better the compound. The stability of ligand-receptor interactions correlates with the binding potential of the compound, indicating that ΔG can predict the inhibitory ability of a compound against a protein. The binding free energies obtained for COX-2 and VEGFR-2 receptors with natural ligands were more negative compared to ΔG values for andrographolide. However, for caspase-3 receptor, andrographolide showed a more negative ΔG value compared to its natural ligand. This difference can also be influenced by the number of hydrogen bonds and hydrophobic interactions present in the ligands of these receptors. The research findings indicate that andrographolide has the smallest Ki value on the caspase-3 receptor, suggesting that andrographolide has the best affinity for inhibiting caspase-3 due to its numerous interactions, specifically 6 hydrogen bonds and 5 hydrophobic bonds.

In molecular docking, hydrophobic interactions play a crucial role in forming complexes between target molecules (such as enzymes or receptors) and drug molecules or ligands. Drug molecules with nonpolar regions can interact with nonpolar sides of target molecules through hydrophobic bonds. This interaction helps to form stable complexes between the drug and its target. Strong hydrophobic interactions can enhance binding affinity and complex stability, whereas weak interactions may contribute less to binding. However, some compounds may not form hydrogen bonds during molecular docking, depending on the chemical nature and target ligand interactions (Frimayanti et al., 2021).

From the analysis of andrographolide with the three receptors, it is evident that andrographolide has higher potential and can interact effectively with the caspase-3 receptor. This is supported by the ΔG value of andrographolide, which is lower compared to other receptors at -5.36 kcal/mol, and the Ki value of andrographolide at 118.05 μM (Wanandi et al., 2020). These results align with literature indicating that a lower (more negative) binding free energy (ΔG) indicates less energy required for binding, and a smaller Ki value signifies better affinity. Andrographolide forms the most hydrogen bonds, specifically 6 hydrogen bonds with amino acids HIS121 and GLY122, similar to those formed by the natural ligand. This confirms that the active site between the natural ligand and andrographolide is appropriately bound, capable of inhibiting caspase-3 (Figure 3).

Several preclinical studies indicate that andrographolide possesses the ability to inhibit cancer cell growth, induce apoptosis, and inhibit the formation of new blood vessels that support tumor growth (angiogenesis) (Li et al., 2022). Additionally, andrographolide can inhibit COX-2 expression and prostaglandin production. Excessive COX-2 expression is associated with chronic inflammation and various types of cancer, including colorectal cancer. COX-2 has been identified as a potential target for cancer treatment due to its role in triggering inflammation and affecting the growth, invasion, and metastasis of cancer cells (Peng et al., 2018). Furthermore, andrographolide can affect the apoptosis pathway and increase caspase-3 activity in colorectal cancer cells, which in turn can induce programmed cell death in cancer cells (Wanandi et al., 2020). Apoptosis is a critical mechanism for regulating cell growth and preventing uncontrolled cancer cell growth. By inducing apoptosis, andrographolide can help inhibit tumor growth and reduce tumor size in colorectal cancer.

Several studies also suggest that andrographolide can inhibit the formation of new blood vessels that support tumor growth (angiogenesis). This ability can help limit the blood supply and nutrients to colorectal tumors, thereby affecting tumor growth. Notably, in addition to caspase-3, high expression of VEGFR2 in colorectal tumors is associated with increased angiogenesis and the tumor's ability to grow and spread. Andrographolide has the ability to inhibit angiogenesis by inhibiting VEGFR-2 expression and reducing VEGF signaling (Dai et al., 2017). By inhibiting VEGFR-2, andrographolide can reduce the growth of new blood vessels that supply colorectal tumors, thereby limiting the blood supply and nutrients to the tumor and inhibiting its growth.

4. Conclusion

From the results of the conducted tests, andrographolide is classified under LD_50 toxicity class 4, indicating its potential for oral use with consideration for its impact on the immune system. Molecular docking of andrographolide on three receptors showed that the compound has a strong affinity for the caspase-3 receptor. Andrographolide is predicted to have biological potential as an anti-inflammatory, antineoplastic, apoptosis agonist, and caspase-3 stimulant, and it is potentially effective as an agent for colorectal cancer as an antineoplastic, antimetastatic, cytoprotective, and anticarcinogenic, although its structural

similarity remains relatively low. To strengthen these predictions, further in vitro and in vivo studies are needed to complete the data on andrographolide's potential as a candidate for colorectal cancer treatment. These studies will provide a deeper understanding of its mechanisms of action, potential side effects, and efficacy in inhibiting the growth and spread of colorectal cancer cells.

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