CURRENT BIOCHEMISTRY

Original Article

In Silico Study of Active Compounds in Halosi (*Bidens pilosa*) as Androgen Inhibitors in Prostate Cancer

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Abstract: *Prostate cancer is a disease that affects men's prostate gland cells and can spread to other parts of the body, mostly bones and lymph nodes. It is the third most common type of cancer in men in Indonesia and the second most common type of cancer in men worldwide. This is an in silico study of the activity of chemical compounds in the leaves of the halosi plant (Bidens pilosa) to inhibit the androgen receptor (1E3G) in the treatment of prostate cancer. The androgen receptor (AR) is a receptor protein that plays an important role in the development of prostate cancer cells. A molecular docking method using Chemdraw 3D, AutoDockTools 1.5.6, BIOVIA Discovery Studio 2021, and LigandScout was used to conduct the tests. Results show that the Bidens pilosa compound andrographolide has a low ΔG free bond energy of -10.26 kcal/mol and interacts with several amino acid residues similar to oxymetholone, a natural ligand. Therefore, it can be concluded that the andrographolide compound from the halosi plant is the most able to inhibit androgen receptors.*

Keywords: *Bidens pilosa; halosi; in silico; prostate cancer*

Abstrak: *Kanker prostat dapat terjadi akibat terjadinya abnormalitas pertumbuhan yang memengaruhi sel-sel kelenjar prostat pria dan dapat menyebar ke bagian tubuh lainnya, terutama tulang dan kelenjar getah bening. Kanker prostat adalah jenis kanker yang paling umum ketiga pada pria di Indonesia dan jenis kanker yang paling umum kedua pada pria di seluruh dunia. Ini adalah studi in silico tentang aktivitas senyawa kimia dalam daun tanaman halosi (Bidens pilosa) untuk menghambat reseptor androgen (1E3G) dalam pengobatan kanker prostat. Reseptor androgen (AR) adalah protein reseptor yang memainkan peran penting dalam perkembangan sel kanker prostat. Metode docking molekuler menggunakan Chemdraw 3D, AutoDockTools 1.5.6, BIOVIA Discovery Studio 2021, dan LigandScout digunakan untuk melakukan pengujian. Hasil menunjukkan bahwa senyawa andrografolid dari tanaman Bidens pilosa memiliki energi ikatan bebas ΔG yang rendah sebesar -10,26 kkal/mol dan berinteraksi dengan beberapa residu asam amino yang mirip dengan oxymetholone, yang merupakan ligan alami. Oleh karena itu, dapat disimpulkan bahwa senyawa andrografolid dari tanaman halosi adalah yang paling mampu menghambat reseptor androgen.*

Kata kunci: *Bidens pilosa; halosi; in silico; kanker prostat*

Citation: Harahap AM, Priawan I, Siregar SR. 2025. In silico study of active compounds in halosi (*Bidens pilosa*) as androgen inhibitors in prostate cancer. *Current Biochemistry*. 12(1): 1-12.

Received: August 30th, 2024
Revised: September 4th, 2024
Accepted: June 2nd, 2025
Published: June 2nd, 2025

pISSN: 2355-7877
eISSN: 2355-7931

1. Introduction

Halosi contains secondary metabolites in the form of andrographolide, with a quantitative analysis using HPLC showing a content of 2.208%. Other literature also states that the halosi plant contains alkaloids, tannins, and saponins. Testing on the halosi plant's ethyl acetate extract using IR and UV-Vis spectrophotometry indicates the possible presence of triterpenoid functional groups at wavelengths of 278 nm and 202 nm. One of the compounds found in the halosi plant can be used as an anti-cancer agent (Garmus *et al.*, 2019). Cancer is an abnormal growth of body cells that often attacks other organs. Andrographolide, the active ingredient in the halosi plant, can fight cancer by blocking the TLR4 pathway through p50 against NF-kB synthesis. Finally, this halts the synthesis of target gene mediators necessary for cancer cells, which stops cancer cell development (Gilson *et al.*, 2016).

Prostate cancer is the most common type of malignancy and the leading cause of cancer-related deaths among men. According to data collected by the Ministry of Health of the Re-

public of Indonesia in 2018, prostate cancer is the third most common cancer in men in Indonesia, with a total of 1,102 cases reported in educational hospitals (Jakarta, Surabaya, and Bandung) over the past eight years. In men, the prostate is a small gland about the size of a walnut located at the bladder's base. This gland consists of two symmetrical lobes and surrounds the initial part of the urethra, which carries urine from the bladder to the penis. The urethra also transports semen, which is the fluid that contains sperm.

Prostate cancer is one of the prostate gland's conditions that continues to develop. The growth of prostate cells usually does not cause symptoms, but as it continues to develop, it can lead to pain and difficulty when urinating (Amadei *et al.*, 2023). One of the treatments for prostate cancer is to stop androgen activity at the androgen receptors. Androgen receptors play a role in controlling the proliferation of prostate cancer cells, which can be inhibited by blocking the receptors with compounds that can compete with estrogen hormones (Ahmed *et al.*, 2001). Surgery, radiation therapy, and chemotherapy are some methods of treating prostate cancer that have quite significant side effects.

Radiation therapy can cause DNA damage and cell death, which makes cancer cells worse than normal cells. Therefore, in silico methods are used on the halosi plant (*Bidens pilosa*), which is one of many plants used as medicinal herbs and serves as an alternative therapy that can minimize side effects. Andrographolide has been shown to inhibit cancer cell growth, with a 50% inhibition rate ranging from 10 to 28 μM, depending on the type of cancer cells tested (Bartolome *et al.*, 2013). The drug discovery process is a difficult and challenging path, so only a few candidates progress from compounds that successfully become commercially available products. This is caused by several factors, such as poor binding affinity, off-target effects, or physicochemical properties like solubility or stability.

This process is becoming increasingly complicated due to the high costs of research and development, as well as the time required. Therefore, it is important to optimize every step of the process to maximize the chances of success. As time goes by, there are now software and hardware advancements in computing that have revolutionized the use of in silico methods in drug design, with access to high-performance computers allowing for more complex calculations and larger datasets to be processed easily, such as modeling, simulations, or gene sequencing (Azadbakht *et al.*, 2003). In silico methods, which involve computational modeling or simulations, are considered quite effective in the discovery of new drugs. In silico studies can also be used to discover natural compound interactions with target proteins. Additionally, in silico studies require validated methods to identify potential interactions between ligand compounds and receptors (Benet *et al.*, 2016).

2. Methods

2.1. Lipinski Rules of Five Predictions

The physicochemical properties of the secondary metabolite chemicals present in sambiloto leaves were examined through application of the Lipinski Rule of Five (RO5). Using the SwissADME website, physicochemical parameters were predicted in order to estimate a compound's potential for oral administration and to evaluate its permeability.

2.2. Determination of ADME/Tox

By adding the test substance to the PreADMET site, the ADME/Tox profile, which comprises the evaluation of absorption, distribution, metabolism, excretion, and toxicity, can be determined. ADME prediction and toxicity prediction are two of PreADMET's predictive features. The metrics that were collected comprise the following: the BBB value (blood-brain barrier), Caco-2 cell permeability, PPB (plasma protein binding) value (which predicts the compound's ability to bind to plasma proteins), and HIA (human intestinal absorption) value (which predicts medication absorption in the intestines). The toxicity test determines the compound's mutagenic and carcinogenic characteristics.

2.3. Pharmacophore Modelling

The three steps of pharmacophore modeling are database preparation, pharmacophore modeling, and pharmacophore validation. Initially, the DUD-E website is accessed to download the database containing both active and dummy compounds, which is then saved in LDBF format. Clicking on the ligand-based view after resetting LigandScout on the all or

empty page completes the database preparation process for the test compounds. Next, using the Insert feature, each test compound is input one at a time, having its type changed to test, and saved in ldb format. LigandScout is then used for modeling, and the settings are reset by selecting 'Restore Default' under the 'File' section. Next, choose the previously established active database by clicking on the 'Ligand Based' section and opening the file. Making sure that every compound has been reduced is the next step. Cluster construction can then proceed, with one 'Training' chemical per cluster and the remaining compounds labeled as 'Ignored' until confirmation has been obtained. The 'Create Pharmacophore' option can then be selected to generate 1–10 pharmacophore models that are attached to a .pmz file. Once ten pharmacophore models have been obtained, each model can be evaluated independently by clicking "Copy to another perspective" and transferring the model. Next, move the model to the 'Screening' column by clicking the green icon in the center and selecting 'Screening Perspective'. In the screening column, click 'Load Screening Database' and choose the previously generated active and decoy databases. Subsequently, execute 'Screening' by marking the decoy with red and the active database with green. The optimal model will be identified by obtaining an AUC value and ROC curve at the conclusion of the process.

2.4. Molecular Docking

Preparing the receptor, ligand, molecular docking, analyzing the results, and visualizing the final product (composing, overlaid, two- and three-dimensional) are all included in molecular docking. The first step is to download the protein that matches the target receptor via the Protein Data Bank website. After that, receptors and ligands are prepared using Biovia Discovery Studio's features. Following completion of all necessary preparations, the AutoDock application may be used to perform molecular docking and provide findings that can subsequently be examined via the command prompt. Following that, four visualization stages were also completed. In the first visualization, an AutoDock complex is created and saved in a .pdb format, allowing Biovia to open it. The second visualization attempts to generate an overlay on the previously created complex file using Biovia in the .pdb format. The goal of the third visualization is to create a two-dimensional structure of the current compound; the finished product is stored as a picture. Using the same tool, Biovia, the final visualization's goal is to obtain a three-dimensional structure. The final file is then saved as an image.

3. Results

Following the discovery of eleven test chemicals in the halosi plant's leaves, the compounds' pharmacological or biological activity was evaluated using the Lipinski Rule of Five, and it was found to be sufficient for them to be safe and effective oral medication candidates. With this prediction, prospective compounds will be chosen early in the medication development process, saving time and money by concentrating research efforts on molecules with a higher chance of success in clinical trials.

According to Lipinski's guidelines, the number of hydrogen bond donors must be less than 5; the number of hydrogen bond acceptors must be less than 10; the molecular weight (MW) cannot exceed 500 Da; and the partition coefficient (logP) must be greater than 5. If there is only one infraction of the Lipinski guidelines, the tested compounds are deemed to satisfy the RO5 standards and are appropriate for development into oral formulations (Prasad *et al*., 2017; Shukla *et al*., 2020).

Bisandrographolide A and 5,2',3'-Trihydroxy-7,8-dimethoxyflavone-3'-glucoside fail the Lipinski Rule of Five because they fail multiple criteria. This means that these compounds are difficult to penetrate membranes via passive diffusion and cannot be formulated into oral preparations. Table 1 displays the results of the Lipinski Rule of Five. To reduce the possibility of drug development failure, ADMET analysis is the next step in the process of choosing a molecule as a possible medication candidate.

Table 1. Lipinski rules of five

No	Compound	Molecul weight (<500Da g/mol)	Log P (<5)	Ikatan hidrogen		Keterangan
				Donor (<5)	Aseptor (<10)	
1	Andrographolide	356.07	2.2	2	7	Qualified
2	Skullcapflavone	324.87	2.9	3	5	Qualified
3	Quinic acid	187.96	3.6	3	6	Qualified
4	Andrhographidin B	356.67	6.7	2	7	Not Qualified
5	3,19isopropylideneandrographolide	246.90	2.5	2	7	Qualified
6	Bisandrographolide A	643.97	3.8	6	8	Not Qualified
7	5,2',3'-Trihydroxy-7,8 dimethoxyflavone-3'-glucoside	344.09	0.9	1	5	Qualified
8	Anthocyanin	216.90	1.3	4	11	Not Qualified
9	Apigenine	346.98	1.2	3	13	Not Qualified
10	Swertiajaponine	449.99	3.2	1	5	Qualified

The PreADMET program was used to validate ADME and determine its toxicity features. A number of metrics are included in the ADMET analysis: PPB and BBB are used to evaluate distribution aspects, and HIA and Caco-2 scores are used to evaluate absorption aspects. The HIA score is divided into three levels. A score between 0 and 20% is regarded as low, a score between 20 and 70% as moderate, and a score between 70 and 100% as high. When an HIA is between 70 and 100 percent, it indicates that the intestines are properly absorbing the substance. According to the collected data, quinic acid has a low absorbance, whereas wogonin-5-glucoside and 5,2',3'-trihydroxy-7,8-dimethoxyflavone-3'-glucoside have a moderate absorbance. The other chemicals' absorbance is good. Next is human colon adenocarcinoma (Caco-2), which is used to estimate how well drugs will pass through colon adenocarcinoma epithelial cells. Only andrographolide was determined to have high permeability or absorption capacity in Caco-2 cells based on the data obtained, as it falls within the range of >70 nm/s.

PPB and BBB are evaluated in the distribution analysis. Plasma Protein Binding, or PPB, is a distribution value that measures how well a medicine binds to plasma proteins. The PPB value is divided into two groups: strong and weak binding, respectively. A number above 90% indicates that the medication binds strongly to plasma proteins. A value below 90% indicates weak binding. If a medication can readily pass across membranes and reach its intended target instead of only attaching to plasma proteins, it is deemed effective (Bhattacharya *et al.*, 2023).

Based on the results obtained, the compounds Halosi, Apigenin, Neoandrographolide, Bisandrographolide A, and 3,19-isopropylideneandrographolide have strong bonds as they have values above 90%. BBB, or the Blood Brain Barrier, is a boundary used to assess whether a drug can cross this area. The blood-brain barrier is a crucial factor because compounds that are active in the central nervous system must be able to penetrate this area. However, if the drug target is not related to the activity of the central nervous system, then the drug should not cross the blood-brain barrier to avoid side effects on the central nervous system. A high ability to penetrate the brain can lead to problems. If the BBB value is less than 2, this indicates a moderate ability to cross the blood-brain barrier. Based on the data obtained, all compounds showed moderate penetration ability to the blood-brain barrier because their values were less than two (Van Der Spoel *et al.*, 2005; Liu *et al.*, 2010; Thien *et al.*, 2017; Deshpande *et al.*, 2023). Lastly, there is the aspect of toxicity. Toxicity testing was conducted using the Ames test to determine whether the test compound has mutagenic potential. In addition, the carcinogenic potential of the drug compounds was also tested on rats and mice to evaluate the possible cancer effects (Chang *et al.*, 2000; Chiasson *et al.*, 2002; Desai *et al.*, 2008; Zamiri-Akhlaghi *et al.*, 2011).

Table 2. Result of determination of ADME/Tox

No	Compound	Absorbtion		Distribution		Toxixicity		
		HIA (%)	Caco-2 (nm/sec)	PPB	BBB	Mutagene	Carcinogene	
							Rats	Mus
1	Andrographolide	89.8789	20.654	95.654	0.1734	Mutagene	+	-
2	Skullcapflavone	94.699	18.897	79.456	0.06245	Mutagene	-	+
3	Quinic acid	32.567	10.0984	8.6544	0.76454	Mutagene	-	+
4	Andrhographidin B	75.975	6.345	73.7455	0.06554	Mutagene	+	-
5	3,19isopropylideneandrographolide	84.5484	18.7655	69.7466	0.04757	Non Mutagene	-	-
6	Bisandrographolide A	92.864	24.83746	95.847	1.05746	Mutagene	-	+
7	5,2',3'-Trihydroxy-7,8 dimethoxyflavone-3'-glucoside	90.1232	10.232	99.8476	0.7635	Mutagene	+	+
8	Anthocyanin	83.4664	20.8464	95.8667	0.3474	Mutagene	+	-
9	Apigenine	39.8464	12.6534	56.884	0.0543	Non Mutagene	+	-
10	Swertiajaponine	98.8736	39.846	94.8476	0.21937	Mutagene	+	+

Mutagenicity and toxicity indicate whether the tested ligand has mutagenic and/or carcinogenic properties in mice and rats. First, let's look at its mutagenic properties. The data obtained indicate that the compounds andrographidin A and 5,2',3'-trihydroxy-7,8-dimethoxyflavone-3'-glucoside are not mutagenic. Six compounds have been proven to have mutagenic properties based on tests on rats using ADME/Tox predictions. In terms of carcinogenicity, four compounds exhibited carcinogenic properties, while four others did not. Test data on rats indicate that five compounds are carcinogenic, while five others are not. Thus, the carcinogenicity data show that several of the tested ligand compounds have carcinogenic properties in mice and/or rats. After the ADME/Tox analysis is complete, the process continues with pharmacophore modeling and screening, starting with the validation stage. The purpose of this validation is to measure the accuracy in distinguishing between active compounds and decoys (inactive ones). In the validation process, 500 compounds were used, consisting of 100 active compounds and 400 decoy compounds. The validation results, as shown in Figure 1, indicate the presence of 65 best hit compounds with an Area Under Curve (AUC) value of 78%. This AUC value demonstrates that the method used is valid and acceptable, as it meets the requirement of an AUC value that must be ≥ 70%. Testing was conducted on 10 compounds in the sambiloto plant, resulting in 9 hit compounds, namely wogonin-5-glucoside, 5,2',3'-trihydroxy-7,8-dimethoxyflavone-3'-glucoside, skullcapflavone, neoandrographolide, bisandrographolide A, apigenin, andrographolide, andrographidin A, and 3,19-isopropylideneandrographolide. The best compound from the pharmacophore screening results is andrographolide, with the highest pharmacophore fit score of 39.04.

The initial step in molecular docking is method validation by redocking the target protein against the natural ligand. In the docking process, the grid box setup is used to define the space where the ligand will bind. Considering the ligands that have been bound to the protein macromolecule during the download, the determination of the grid box is carried out. Validation results of the method show that the docking of the androgen receptor molecule (1E3G) with a natural ligand has an RMSD of 0.479 Å, indicating that the docking of the androgen receptor molecule with the native ligand is valid. Molecular docking methods are considered valid if the RMSD value is < 2 Å (Dwivedi *et al*., 2023). The smaller the RMSD value obtained, the smaller the error that occurs in the docking process. The grid box obtained results in a size of 40 x 40 x 40 with grid coordinates x = -0.086; y = 30.89; z = 4.301.

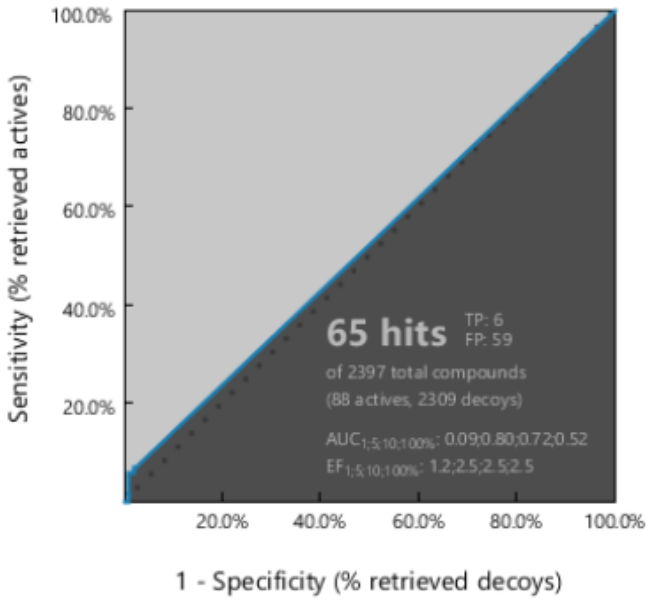

Figure 1. Validation curve (ROC curve, model 8).

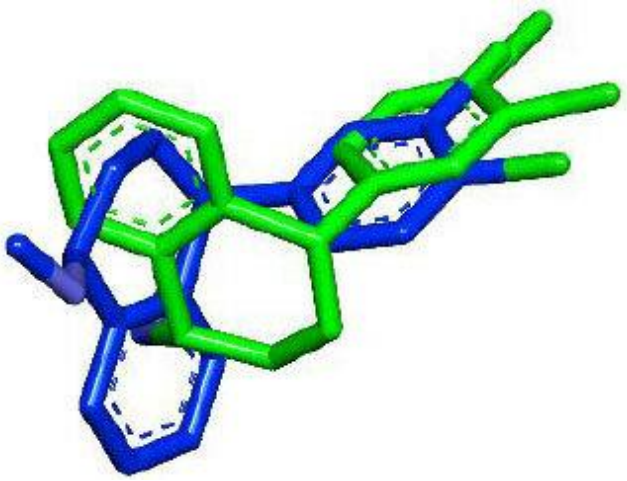

Figure 2. Overlay of the natural ligand before (green) and after (blue) the re-docking process.

Based on the method validation results, molecular docking was carried out on ten test compounds and oxymetholone as a natural ligand using the suitable grid box. Table 3 displays the outcomes of the molecular docking of the ten test chemicals. The characteristics employed in molecular docking include the conformation of the link between the test chemical and the receptor, the inhibition constant, and binding energy. The receptor utilised is 1E3G, the crystal structure of the androgen receptor's DNA-binding domain (Garmus *et al*., 2019). One of the causes of prostate cancer is overexpression of androgen receptors, which regulates the growth of prostate cancer cells in the prostate gland. Thus, ligands that are capable of substituting the original ligands at the androgen receptor are required (Genheden and Ryde, 2015; Gilson *et al*., 2016).

Table 3. Results of the molecular docking of the test compounds.

Compound	Variable	Results
Oxymetholone (Native Ligand)	**Binding Energy (Kkal/mol)**	-12.76
	KI (nm)	1.87

Compound	Variable		Results
	Amino acid interaction	**H Afinity**	TYR A:857; SER 865; LYS 861; GLU 793; LEU 862
		VA Afinity	-
		Other afinity	Alkyl & Pi- Alkyl: LEU A: 701; MET A: 780; MET A: 742; LEU A: 704; MET A: 745; PHE A: 764; PHE A: 876
Andrographolide	**Binding Energy (Kkal/mol)**		-10.26
	KI (nm)		14.87
	Amino acid interaction	**H Afinity**	ASN A:705
		VA Afinity	LEU A: 701;PHE A: 891;MET A:895;THR A:877; META: 780;MET A: 787; VAL A:746; PHE A: 746; ARG A: 752; MET
		Other afinity	Alkyl & Pi- Alkyl: LEU A: 704; PHE A: 876; MET A: 742 LEU A: 873
Quinic acid	**Binding Energy (Kkal/mol)**		-6.45
	KI (nm)		254140
	Amino acid interaction	**H Afinity**	GLN A:711; ARG A: 752; GLY A: 708; LEU A: 704
		VA Afinity	MET A:787; MET A:780; MET A: 749: MET A: 745; MET A: 895; TRP A: 741
		Other afinity	Alkyl & Pi- Alkyl: LEU A: 707; PHE A: 764
Skullcapflavone	**Binding Energy (Kkal/mol)**		-10.26
	KI (nm)		698.44
	Amino acid interaction	**H Afinity**	ASN A:705; THR A 877
		VA Afinity	A: 745; MET A: 749; GLN A; 711; LEU A: 707; GLY A: 708
		Other afinity	Alkyl & Pi-Alkyl: MET A: 749 Pi-Pi T- shaped: PHE A: 764 Pi-Sigma&Pi- Sulfur: MET A: 745
Andrhographidin B	**Binding Energy (Kkal/mol)**		-10.26
	KI (nm)		698.44
	Amino acid interaction	**H Afinity**	ASN A:705; THR A 877
		VA Afinity	A: 745; MET A: 749; GLN A; 711; LEU A: 707; GLY A: 708

Compound	Variable		Results
		Other afinity	Alkyl & Pi- Alkyl: LEU A:707; MET A: 269; TAP A:741; MET A:895 ; EE A:899 Pi-Pi T-shaped: PHE A: 764 Pi- Sigma&Pi-Sulfur: MET A: 262; MET A:745
3,19-isopropyl-ideneandrographolide	**Binding Energy (Kkal/mol)**		-8.45
	KI (nm)		787.43
	Amino acid interaction	**H Afinity**	GLU A:872; MET A:745
		VA Afinity	VAL A:889; PHE A:878; LEUA:882; ILE A:899
		Other afinity	Unfavorable Bump & Unfavorable Donor Donor : LEU A:873; PHE A:876; MET
Bisandrographolide A	**Binding Energy (Kkal/mol)**		-7.36
	KI (nm)		985.44
	Amino acid interaction	**H Afinity**	ASN A:705; THR A 877
		VA Afinity	A: 745; MET A: 749; GLN A; 711; LEU A: 707; GLY A: 708
		Other afinity	Alkyl & Pi-Alkyl: MET A: 749 Pi-Pi T- shaped: PHE A: 764 Pi-Sigma&Pi- Sulfur: MET A: 745
5,2',3'-Trihydroxy-7,8 dimethoxyflavone-3'-glucoside	**Binding Energy (Kkal/mol)**		-12.26
	KI (nm)		132.44
	Amino acid interaction	**H Afinity**	ASN A:705; THR A 877
		VA Afinity	A: 745; MET A: 749; GLN A; 711; LEU A: 707; GLY A: 708
		Other afinity	Alkyl & Pi-Alkyl: MET A: 749 Pi-Pi T- shaped: PHE A: 764 Pi-Sigma&Pi- Sulfur: MET A: 745
Anthocyanine	**Binding Energy (Kkal/mol)**		-10.26
	KI (nm)		698.44
	Amino acid interaction	**H Afinity**	ASN A:705; THR A 877
		VA Afinity	A: 745; MET A: 749; GLN A; 711; LEU A: 707; GLY A: 708
		Other afinity	Alkyl & Pi-Alkyl: MET A: 749 Pi-Pi T- shaped: PHE A: 764 Pi-Sigma&Pi- Sulfur: MET A: 745
Apigenine	**Binding Energy (Kkal/mol)**		-9.26

Compound	Variable		Results
	KI (nm)		398.44
	Amino acid interaction	**H Afinity**	ASN A:705; THR A 877
		VA Afinity	A: 745; MET A: 749; GLN A; 711; LEU A: 707; GLY A: 708
		Other afinity	Alkyl & Pi-Alkyl: MET A: 749 Pi-Pi T- shaped: PHE A: 764 Pi-Sigma&Pi- Sulfur: MET A: 745
Swertiajaponine	**Binding Energy (Kkal/mol)**		-14.26
	KI (nm)		986.44
	Amino acid interaction	**H Afinity**	ASN A:705; THR A 877
		VA Afinity	A: 745; MET A: 749; GLN A; 711; LEU A: 707; GLY A: 708
		Other afinity	Alkyl & Pi-Alkyl: MET A: 749 Pi-Pi T- shaped: PHE A: 764 Pi-Sigma&Pi- Sulfur: MET A: 745

4. Discussion

The parameters that will be obtained from molecular docking include binding free energy (ΔG), inhibition constant (KI), and ligand interactions with protein residues. A negative free energy value indicates that the reaction can occur spontaneously (Khanal *et al.*, 2023). In addition, ligand affinity towards the receptor can be indicated by free energy. When the value of ΔG is low, it results in a high affinity of the ligand for the receptor. The results of the molecular docking indicate that all test compounds do not have a lower free energy than the natural ligand. In bisandrographolide and neoandrographolide, the ΔG values are positive, indicating that the reactions occurring are non-spontaneous. The compound with the lowest ΔG value is andrographolide at -10.26 kcal/mol. However, the free energy of andrographolide is still greater than the free energy of oxymetholone as a natural ligand. This should be interpreted as andrographolide being produced with lower receptor affinity compared to that generated by natural ligands (Figure 3).

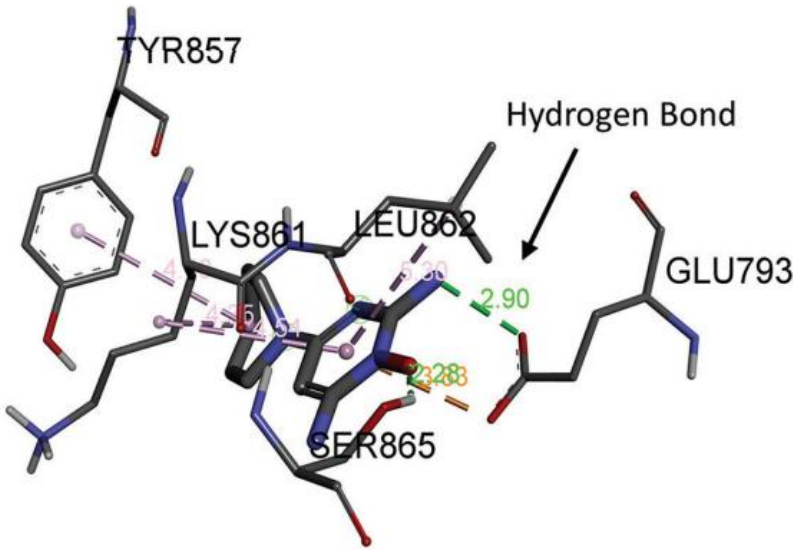

Figure 3. Visualisation of interactions between andrographolide and the androgen receptor (1E3G). Hydrogen bonds are represented by green bonds; andrographolide is represented by grey structures.

The inhibition constant (KI) is used to determine the concentration of inhibition required to reduce the rate of enzymatic reaction to half of its maximum rate (Kim *et al.*, 2019). The lower the KI value, the stronger the inhibitor's affinity for the target enzyme, indicating that a smaller concentration of the inhibitor is already effective in inhibiting enzyme activity. In the case of the test compound andrographolide, the inhibition constant (KI) value of 14.87 nM (Figure 4) shows that this compound has a very strong affinity for the target enzyme, suggesting that only a very small concentration is needed to effectively inhibit the enzyme's activity. In comparison, a natural ligand with a KI value of 1.87 nM has a much lower affinity for the target enzyme, requiring a higher concentration to achieve the same level of inhibition. The interaction between receptors and ligands will result in binding distances and the amino acid residues that are involved in the binding. Therefore, the visualization process is carried out to observe that interaction.

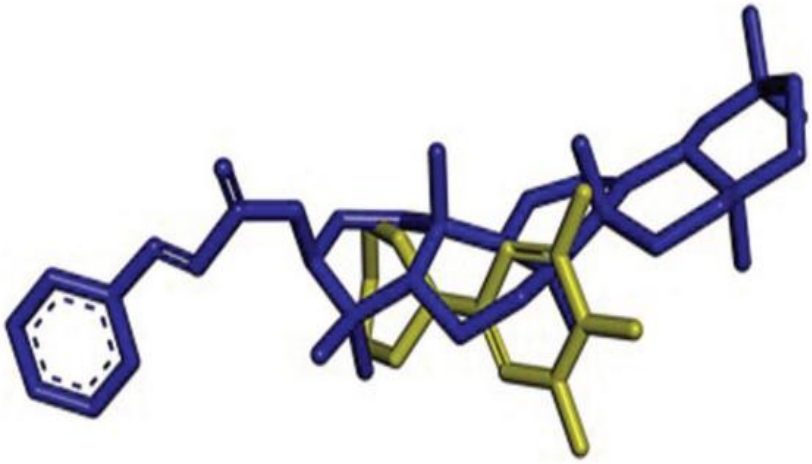

Figure 4. Overlay of the docked poses of the test compounds on that of the natural ligand.

Table 3 shows the interaction between ligands and amino acids. Among the bonds that can occur in this interaction are hydrogen bonds, Van der Waals bonds, and other bonds. Hydrogen bonds are stronger than Van der Waals bonds because they can form even when the distance between the ligand and the receptor is relatively far (Krivák and Hoksza, 2018). To understand the interactions that occur between ligands and receptors, it is necessary to observe the amino acid residues (Kumari and Kumar, 2014). The natural ligand oxymetholone has amino acid residues in the form of hydrogen bonds with amino acids THR A:877 and ASN A:705, and has other amino acid bonds as listed in Table 3. Among the test compounds, at least one amino acid is the same as the natural ligand residue in the compounds andrographolide, skullcapflavone, andrographidin A, apigenin, 5,2',3'-trihydroxy-7,8-dimethoxyflavone-3'-glucoside, and 3,19-isopropylideneandrographolide. The results indicate that the active compounds in sambiloto leaves have the potential to bind with androgen receptors (1E3G) because they have the same type of binding as that formed between natural ligands and receptors.

Based on the parameters of the molecular docking results, the test compound with values closest to the natural ligand is andrographolide. The binding energy of andrographolide is -10.26 kcal/mol, while the natural ligand oxymetholone has a lower (more negative) binding energy of -12.76 kcal/mol. The more negative binding energy value of oxymetholone indicates that it has a more stable interaction with the receptor compared to andrographolide. However, the inhibition constant (KI) of andrographolide is 13.72 nM, which is significantly lower compared to oxymetholone, which has a KI of 1.87 nM. This indicates that although the binding energy of oxymetholone is greater, andrographolide has a higher affinity for the target enzyme, as it requires a much lower concentration to achieve effective inhibition.

In conclusion, based on the results of the in silico study of sambiloto leaves — including the Lipinski Rule of Five, ADME/Tox predictions, and molecular docking on ten active compounds in sambiloto leaves — andrographolide emerged as the most promising active compound as a prostate cancer agent due to its best interaction with receptor 1E3G. Andrographolide demonstrated androgen receptor inhibition activity with the lowest ΔG of -10.26

kcal/mol, approaching the free energy of the natural ligand, and an inhibition constant (KI) value of 14.87 nM, indicating a high affinity that surpasses that of natural ligand compounds. Andrographolide also interacts with the same amino acid residues as the natural ligand, forming hydrogen bonds with ASN A:705 and other amino acids such as PHE A:876 and MET A:742.

5. Acknowledgements

The authors are grateful for the financial support provided by the Faculty of Health, Universitas Haji Sumatera Utara.

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