Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia Vol. 11 No. 1 April 2024, 1-11
DOI: 10.20473/jfiki.v11i12024.1-11
Available online at https://e-journal.unair.ac.id/JFIKI/

In Silico Evaluation: Bioactive Compounds in Soursop Plant (*Annona muricata* L.) as Caspase-3 Inhibitor for Prostate Cancer

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Submitted: 14 June 2023
Revised: 18 February 2024
Accepted: 2 April 2024

Abstract

Background*: Prostate cancer has become one of the leading causes of death in men. Cancer patients often seek alternative treatments apart from chemotherapy, radiation therapy, and surgery. The use of medicinal plants in both preventive and curative actions in healthcare has been widely recognized. One of the plants known to have anticancer activity is the soursop leaf (Annona muricata L.).* ***Objective****: This study was conducted to explore the potential of active compounds contained in A. muricata as drug candidates for the inhibition of caspase-3 in silico.* ***Methods****: The research began with the prediction of Lipinski's Rule of Five and ADMET properties for the compounds found in A. muricata. The prediction process was followed by pharmacophore modelling and molecular docking simulations on caspase-3 (PDB: 1NME) as the target protein and 2-hydroxy-5-(2-mercapto-ethylsulfamoyl)-benzoic acid as the natural ligand using AutoDockTools 1.5.6.* ***Results****: Based on the molecular docking results, 22 test ligands were able to form bonds with the caspase-3 enzyme. The two best interactions were observed with the test ligands, Isolaureline and S-norcorydine, with binding energy values of -6.20 kcal /mol and -6.12 kcal /mol and inhibition constant values of 28.65 µM and 32.53 µM. In terms of receptor-target interactions, these two compounds also exhibited hydrogen bonding and van der Waals interactions similar to the natural ligand.* ***Conclusion****: The best bioactive compounds in A. muricata (Isolaureline and S-norcorydine) were predicted to have the ability to interact with caspase-3 and the potential to be used as prostate cancer drug candidates.*

Keywords*: Annona muricata, Caspase-3, Molecular docking, Prostate cancer*

How to cite this article:
Aristokrat, A., Rumawan, M. W. A., Hanifa, Z. A., Rahmani, S. L., Hanifah, Parameswari, N., Pribadi, A. P. A. & Aulifa, D. L. (2024). In Silico Evaluation: Bioactive Compounds in Soursop Plant (*Annona muricata* L.) as Caspase-3 Inhibitor for Prostate Cancer. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*, 11(1), 1-11. http://doi.org/10.20473/jfiki.v11i12024.1-11.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

INTRODUCTION

Cancer is a complex and life-threatening illness that has impacted the lives of countless individuals and families worldwide. It refers to a group of disorders characterized by abnormal cell growth and division within the body (Faubert *et al.*, 2020). Cancer can manifest in a variety of organs and tissues, disrupting the body's normal functions and posing significant physical and emotional challenges. The cancer journey is often difficult, requiring courage, resilience, and unwavering support. The causes of cancer are multifaced, including genetic, environmental, and lifestyle factors. Inherited or acquired genetic mutations can disrupt cell function and increase the risk of cancer. Tobacco smoke, radiation, certain chemicals, and infectious agents are all carcinogens that can contribute to the development of cancer. Furthermore, lifestyle factors such as diet, physical activity, tobacco, and alcohol use may all affect the risk of developing certain types of cancer (Anand *et al.*, 2008).

Early detection of cancer is critical for successful treatment and improved outcomes. Regular screenings and self-examinations are critical for early detection because they allow medical professionals to identify abnormalities and initiate appropriate diagnostic procedures. Imaging scans, biopsies, and laboratory tests provide valuable insights into the presence, extent, and characteristics of cancer, allowing for more personalized treatment plans. It is a multidisciplinary approach to treatment that is determined by the type, stage, and location of the disease, as well as the individual's overall health. Surgery to remove tumors, radiation therapy to target and destroy cancer cells, and chemotherapy to kill cancer cells throughout the body. Besides, the use of drugs or treatments that specifically target specific molecules or pathways involved in the development and progression of cancer is known as targeted therapy (Shuel, 2022).

Related to cancer treatment and drug development, targeted therapy and molecular docking are inextricably linked. Molecular docking is a useful tool for discovering, designing, and optimising targeted therapies. It is a computational prediction and modelling of molecular interactions between molecules, such as drug candidates and their target proteins. By stimulating the interactions between drug molecules and target proteins, molecular docking allows researchers to predict and evaluate the effectiveness of potential drug candidates (Suhandi *et al*., 2022).

As cancer treatment requires the development of new medication, herbal medicine is a global icon of alternative medication much needed as new drugs. Plants with medicinal properties have been used to treat a variety of ailments since the dawn of time. The key characteristics were medicinal plant chemical compounds that can have a physiological effect on the human system (Ilango *et al*., 2022). *Annona muricata*, also known as soursop, has been studied for its potential as a source of anticancer compounds. Several studies have been carried out in laboratories to investigate the effects of various extracts and isolated compounds from *Annona muricata* on cancer cells. There have been reports of widespread use of *A. muricata, of* which 80.9% of patients with prostate, breast, and colorectal cancer for their malignancies (Foster *et al*., 2017). *A. muricata* is also known as one of the liver cancer therapies that been used by 14% Peruvian patients to treat their cancer-related symptoms (Rojas *et al*., 2018).

This study aimed to predict the cytotoxic activity, inhibition of proliferation, and apoptosis induction activity of *A. muricata* leaf extract targeting the caspase-3 pathway of prostate cancer. An increase in caspase-3 can cause certain proteins in cells to become activated, which can accelerate the process of apoptosis. Via the intrinsic mechanism, an increase in caspase-3 enzyme expression will accelerate the PC-3 prostate cancer cell line's in vitro turnover (Ismy *et al*., 2020).

MATERIALS AND METHODS

Materials

Caspase-3 structure, with PDB ID number "1NME," was retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) at www.rcsb.org for use in this investigation. The bioactive substances used in the test ligands came from the soursop plant (*Annona muricata* L.). The three-dimensional structure of a natural ligand and the test compounds, which include the reference medication Sorafenib, were retrieved from PubChem (pubchem.ncbi.nlm.nih.gov) created using the program ChemOffice 2010.

Tools

The computer used in this investigation was an Acer Swift SF314-56G with an Intel ® CoreTM i5-8265U processor, 4.00 GB of RAM, and a Windows 11 Home Single Language 64-bit operating system. The docking positions and interactions were visualized using the Biovia Discovery Studio Visualizer. The

P-ISSN: 2406-9388
E-ISSN: 2580-8303

production of macromolecules and ligands, as well as the execution of docking studies, were done using AutoDock 1.5.6.

Method

Physicochemical and pharmacokinetic prediction

Physicochemical and pharmacokinetic predictions were conducted for both the test ligands. The ligand structures were obtained from the PubChem database (pubchem.ncbi.nlm.nih.gov) in (.sdf) format. The physicochemical predictions were performed using Lipinski's Rule of Five on the SwissADME website (http://www.swissadme.ch/). The pharmacokinetic predictions were carried out by submitting the structure of the test ligands to the PreADMET website (https://preadmet.webservice.bmdrc.org/).

Ligand preparation

For both test ligands, physicochemical and pharmacokinetic predictions were made. The ligand structures were downloaded in (.sdf) format from the PubChem database at pubchem.ncbi.nlm.nih.gov. On the SwissADME website (http://www.swissadme.ch), Lipinski's Rule of Five was used to make the physicochemical predictions. The test ligands' structures were entered into the PreADMET website (https://preadmet.webservice.bmdrc.org) to make pharmacokinetic predictions.

Receptor preparation

The Protein Data Bank's Caspase-3 3D structure (PDB ID: 1NME) was visualized using the BIOVIA Discovery Studio application. The protein chain was then isolated from the native ligand by removing those components as well as the water molecules surrounding the protein. The generated structure was stored as the receptor in the *pdb file format. In addition, the natural ligand's structure was recovered by eliminating the protein chain and saved in the *pdb file format. A software called AutoDockTools 1.5.6 was used to produce the native ligand as well as the protein. To proceed with this production, hydrogen atoms had to be added to the structure's polar side, Kollman charges had to be applied for the receptor, and Gasteiger charges for the native ligand.

Validation of the molecular docking method

The approach is validated to confirm that the docking parameters are appropriate for the docking process of the test ligand with the caspase-3 receptor. Re-docking, which includes inserting the dissociated native ligand back into the Caspase-3 receptor, is used to carry out this validation. A grid box with the dimensions 30 x 30 x 30 and the coordinates x = 42.197; y = 96.352; z = 24.611 is used in the re-docking procedure.

The Genetic strategy (GA) value is set to 10, and Lamarckian GA 4.2 is used as the docking strategy in the docking settings. The default settings are used for all other docking parameters.

The Root Mean Square Deviation (RMSD) is a parameter that can be taken into account in the re-docking outcomes. The RMSD value of 2.0 is considered acceptable (Istyastono, 2018).

Molecular docking

The reference drug and each of the twenty-two test compounds were docked against the Caspase-3 receptor after being optimized and produced. Using AutoDockTools 1.5.6, the receptor was created by separating it from its natural ligand and docking it in the same manner as in the technique validation.

Data analysis and visualization

The data presentation design in this study took into account various factors such as binding free energy, conventional hydrogen bonds, van der Waals bonds, and the number of hydrogen bonds. The BIOVIA Discovery Studio allows visualization of the receptor's complex conformations, ligand interactions, and contact amino acid residues in both two-dimensional (2D) and three-dimensional (3D) formats.

RESULTS AND DISCUSSION

Physicochemical and pharmacokinetic prediction

Physicochemical and pharmacokinetic prediction are two fields essential for drug discovery to explain how two chemical compounds interact in the human body. Physicochemical studies explain the physical and chemical properties of chemical compounds; therefore, pharmacokinetics predictions refer to scores of absorption, distribution, metabolism, excretion, and toxicity (ADMETox) predicted by how the chemical compounds work on the human body.

Lipinski's Rule of Five (Ro5) is one of the methods that can be used to predict the pharmacokinetics of drug-like chemical compounds designed for oral route administration. These rules are used to determine if chemical compounds have the potential to be drugs. The parameters include molecular weight (MW), partition coefficient (LogP), hydrogen bond donor (HBD), and hydrogen bond acceptor (HBA). The drug-like compound has a molecular weight under 500 Da. LogP value less than five represents hydrophobicity; no HBD is more than five, and no HBA is more than 10. (Chagas *et al.*, 2018).

P-ISSN: 2406-9388
E-ISSN: 2580-8303

On the other hand, the parameters of ADMETox are HIA (Human Intestinal Absorption) and $CaCO_2$, which define the capability of oral and transdermal administration of drug candidates to absorb. PPB (Plasma Protein Binding) is defined as the capacity of a chemical compound to bind with blood protein. BBB (Blood-Brain Barrier) is defined as the capability of a chemical compound to pass through the blood-brain barrier and reach the brain. Mutagenicity and carcinogenicity are parameters that determine the toxicity properties of potential drug candidates (Afinasari *et al.*, 2022).

Lipinski's Rules of Five and ADMETox evaluations are used as an early-stage evaluations for drug discovery. From the Lipinski prediction results (Table 1), one out of 22 chemical compounds from *A. muricata* L. did not pass Lipinski's Rule of Five and from the predicted result of the ADMETox (Table 2). Seven compounds did not fit the HIA criteria; only one compound did not fit the CaCO2 criteria; eight compounds did not fit the PPB criteria; and only three compounds passed the BBB criteria. Six compounds were found to have no toxicity, either carcinogenicity or mutagenicity.

Ligand preparation

ChemDraw Pro 12.0 software was used to construct 2D structures for the 22 test ligands during the preparation process. Afterwards, their 3D structures were created, and Chem3D Pro 12.0 software was used to optimize the test compounds' molecular mechanics (MM) geometry for stability.

To create the most stable structure that closely resembles the existing natural compound structures, the total energy of the molecules was minimized during the geometry optimization process (Roy *et al.*, 2015). These findings led to the creation of the test compound's optimal structures.

Receptor preparation

X-ray crystal structure of human Caspase-3 (PDB ID: 1NME) was chosen because it originated from humans (*Homo sapiens*), had no mutation, had a good resolution (<2 Å), and had a natural ligand. The Caspase-3 receptor was prepared by separating the protein from its original ligand using BIOVIA Discovery Studio software, resulting in a space or pocket that would be utilized during the docking simulation. The native ligand's structure without the protein was also discovered, in addition to the protein structure having a pocket for the test ligand.

Table 1. Lipinski prediction results of bioactive compounds in soursop plant (*Annona muricata* L.)

Compound	Parameter Lipinski rule of five				Application of Lipinski's rule of five
	Molecular weight (<500 Da)	LogP (<5)	Hydrogen donor (<5)	Hydrogen acceptor (<10)	
Annoionol A	230.34	1.73	3	3	Passed
Annoionol B	244.33	0.81	4	4	Passed
Anomuricine	329.39	2.65	2	5	Passed
Anomurine	343.42	3.08	1	5	Passed
Anonaine	265.31	2.88	1	3	Passed
Asimilobine	267.32	2.65	2	3	Passed
Chlorgenic acid	354.31	-0.39	6	9	Passed
Coclaurine	285.34	2.36	3	4	Passed
Coreximine	327.37	2.40	2	5	Passed
Epicathecine	290.27	0.83	5	6	Passed
Isoboldine	327.37	2.45	2	5	Passed
Isolaureline	265.31	3.12	0	4	Passed
Liriodenine	275.26	2.88	0	4	Passed
Myricyl alcohol	438.81	10.52	1	1	Passed
Myristic acid	228.37	4.45	1	2	Passed
N-methylcoclaurine	299.36	2.59	2	4	Passed
Palmitic acid	256.42	5.20	1	2	Passed
Polyphenol	458.37	1.01	8	11	Didn't pass
Remerine	279.33	3.13	0	3	Passed
Reticuline	329.39	2.64	2	5	Passed
S-norcorydine	327.37	2.61	2	5	Passed
Xylopine	295.33	2.88	1	4	Passed

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 2. The predicted results of the ADMETox profile of bioactive compounds in soursop plant (*Annona muricata* L.)

Compound	Parameter						
	Absorption		Distribution		Toxicity		
	HIA (%) [HIA > 70%]	Caco-2 (nm/sec) [CaCO2 > 5 nm/sec)	PPB (%) [PPB < 90%]	BBB [LogD > 2]	Mutagen	Carcinogen	
						Mouse	Rat
Annoionol A	80.621107	21.3756	78.685043	1.83591	mutagen	negative	negative
Annoionol B	68.686646	21.159	55.761220	0.426248	non-mutagen	negative	negative
Anomuricine	93.529812	29.5702	78.913882	0.558569	non-mutagen	negative	positive
Anomurine	95.858409	51.8528	76.072725	0.112353	non-mutagen	negative	negative
Anonaine	96.493990	47.6818	65.565065	0.984994	mutagen	negative	negative
Asimilobine	93.174009	26.308	63.678123	2.4684	mutagen	positive	negative
Chlorgenic acid	24.404298	19.2384	39.507803	0.0370233	non-mutagen	positive	negative
Coclaurine	89.643482	7.29997	97.170383	0.693997	mutagen	negative	negative
Coreximine	93.267525	14.641	84.091816	0.726882	non-mutagen	positive	negative
Epicathecine	66.707957	0.56962	100.000000	0.394913	mutagen	negative	negative
Isoboldine	93.265891	24.5565	61.235563	1.51842	non-mutagen	negative	negative
Isolaureline	99.658001	56.6616	71.572095	2.38425	mutagen	negative	negative
Liriodenine	97.767428	28.1451	49.719419	0.809938	mutagen	negative	negative
Myricyl alcohol	100.000	51.3386	100.00000	0.0875288	non-mutagen	positive	negative
Myristic acid	97.848313	24.0726	100.000000	5.03596	mutagen	negative	positive
N-Methylcoclaurine	93.072310	13.5813	91.190030	1.64191	non-mutagen	negative	negative
Palmitic acid	98.297110	26.0735	100.000000	8.21885	mutagen	negative	positive
Polyphenol	20.712498	12.0421	100.0000	0.0875288	non-mutagen	negative	positive
Remerine	100.000	56.7725	74.322985	1.80495	mutagen	negative	negative
Reticuline	93.26485	12.2566	83.959605	1.00893	non-mutagen	negative	negative
S-norcorydine	95.533986	29.2909	56.866176	1.00154	non-mutagen	negative	negative
Xylopine	95.782499	49.1829	60.230097	0.850866	mutagen	negative	negative

(a) (b)

Figure 1. The three-dimensional (3D) structure of Caspase-3 without a ligand (a) and native ligand 2-Hydroxy-5-(2-mercapto-ethylsulfamoyl)-benzoic acid (b)

Water molecules (H2O) must also be removed during this procedure. To ensure that only amino acid molecules are present in Caspase-3 and that only these amino acid molecules will interact with the test ligand. Water molecules must be removed. Additionally, eliminating water molecules can maximize the interaction between the protein and test ligand (Lemmon & Meiler, 2013). Figure 1 depicts the 3D architectures of Caspase-3 with its natural ligand separated and without it.

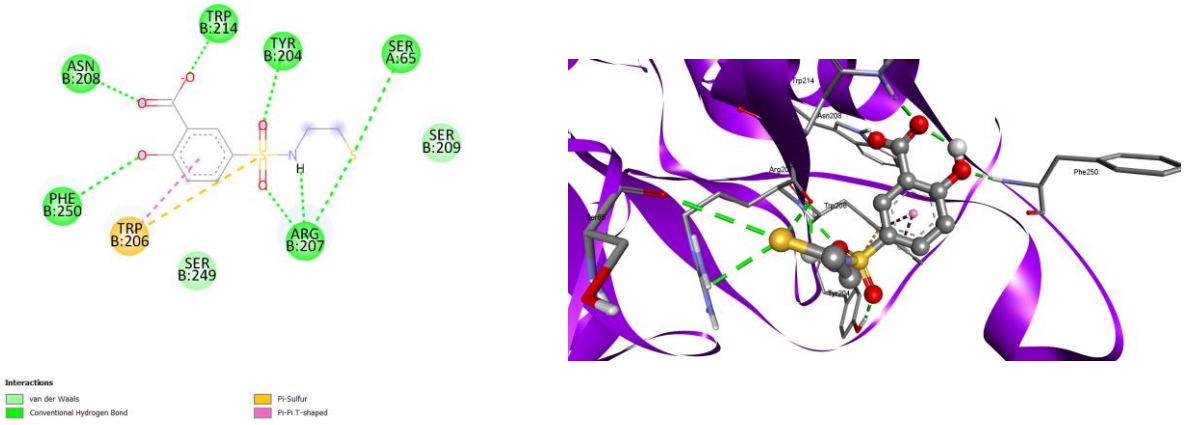
Validation of the molecular docking method

To make sure the utilized approach satisfies the necessary standards and can be used for subsequent testing stages, the molecular docking method underwent validation. The Root Mean Square Deviation (RMSD) is the parameter used for validation. A metric called RMSD shows how much the protein-ligand interactions have changed between the crystal structure before and after docking. If the RMSD value is 2.0, the docking approach is regarded to be valid (Istyastono, 2018). The obtained RMSD value in this experimental technique validation is 1.85, demonstrating the validity of the docking approach used. The information is shown in Table 3. In Figure 2, which is based on the re-docking results, the interactions between the natural ligand and Caspase-3 are visualized in 2D and 3D. The ligand interacts with Caspase-3 through conventional hydrogen bonds, van der Waals bonds, Pi-Sulfur links, and Pi-Pi T-shaped bonds.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 3. The results of the method validation through re-docking with 2-Hydroxy-5-(2-mercapto-ethylsulfamoyl)-benzoic acid

PDB ID	Grid Box (x, y, z)	Validation		Binding Energy (kkal/mol)
		RMSD cluster (Å)	RMSD reference (Å)	
1NME	42.197 96.352 24.611	0.00	1.85	-5.28



(a) (b)

Figure 2. Visualization of the 2D interactions between Caspase-3 and the natural ligand (a) and visualization of the 3D interactions between Caspase-3 and the natural ligand (b)

Molecular docking

Using the same program, AutoDockTools 1.5.6, the test compounds continued to be docked to Caspase-3 after fulfilling the docking method's validation criteria. Since those coordinates corresponded to the region where the native ligand interacts with Caspase-3, they were modified to fit the grid box utilized during method validation when docking the test compounds on Caspase-3.

The procedure continued with docking the test compounds on 1NME using the same software, AutoDockTools 1.5.6, after witnessing the validated findings of the docking approach that satisfied the requirements. Since these coordinates indicated the site of the native ligand interaction with 1NME, they were modified to match the grid box utilized during method validation while docking the test compounds on 1NME. The test chemicals' docking with the 1NME receptor produced the following results: hydrogen bonding, inhibition constant, and binding energy.

The scoring function computation for the ligand conformation (I) inside the macromolecule/receptor under equilibrium conditions (conformational search) yields the binding energy. The Gibbs energy (G), also known as the binding energy, can be calculated from these variables using the equation [E+I] = [EI] (Limongelli, 2020). The binding energy reveals how well the test substances bind to the 1NME receptor. A lower value of the binding energy denotes a more solid interaction between the protein and the produced ligand (Manna *et al.*, 2017). The strength of a compound's ability to prevent a receptor's action is shown by its inhibition constant. On the other hand, the strength of inhibitory potency is indicated by a smaller value of the inhibition constant (Garcia-Molina *et al.*, 2022).

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 4. The docking results of bioactive compounds in the soursop plant (*Annona muricata* L.) against the Caspase-3 receptor

No.	Compound	Binding Energy (kcal/mol)	KI (µM)	Bonding with amino acids		
				Hydrogen bonds	Van der waals bonds	Other bonds
1	Native ligand	-5.28	134.89	ASN 208, TRP 214, TYR 204, SER65, ARG 207, PHE 250	SER 209, SER 249	TRP 206
2	Sorafenib	-5.97	42.26	ARG 207, PHE 250, PHE 252	HIS 121, THR 62, SER 249, ASN 208, TRP 217, TRP 206, SER 205	TYR 204, SER 251
3	Annoionol A	-4.99	221.09	TYR 204, TRP 214, GLU 248, PHE 250	ASN 208, ARG 207, SER 249, SER 251	TRP 206
4	Annoionol B	-5.75	61.28	PHE 256, SER 209	HIS 257, SER 251, ARG 207, LYS 210, ASN 208, PHE 250, TRP 214, SER 249	TRP 206
5	Anomuricine	-5.40	109.72	-	ASP 253, SER 251, TYR 204, TRP 206, ASN 208, PHE 250, ASP 253, SER 251	PHE 252, PHE 256
6	Anomurine	-5.40	19.78	SER 209, PHE 250, TYR 204	ASN 208, SER 65, ARG 207, SER 205, SER 251, SER 249	GLU 248, PHE 256, TRP 206, TRP 214
7	Anonaine	-5.97	41.97	TYR 204, PHE 250	ASN 208, TRP 214, SER 249, SER 251	TRP 206, ARG 207
8	Asimilobine	-5.78	57.73	ASN 208	SER 209, SER 249, TYR 204	TRP 214, PHE 250, TRP 206, SER 205, ARG 207
9	Chlorgenic acid	-5.65	72.61	GLU 248, PHE 250, TYR 204, ARG 207, SER 209	SER 65, ASN 208, TRP 214, SER 249, TRP 206	ARG 207
10	Coclaurine	-5.14	171.23	ASN 208, ARG 207	SER 251, PHE 250, GLU 248, TRP 214, PHE 247, SER 63, SER 65, SER 209	SER 249, TRP 206
11	Coreximine	-5.63	75.01	ARG 207, SER 209, TRP 214, PHE 250	ARG 63, ARG 64, SER 65, ASN 208, PHE 247, TYR 204	SER 249, GLU 248, TRP 206
12	Epicathecine	-5.23	146.95	ARG 207, SER 209, PHE 250	SER 65, ASN 208, TRP 214, GLU 248, SER 249, TRP 204	ARG 207, TRP 206
13	Isoboldine	-5.61	76.84	TRP 214, PHE 250	ASN 208, ARG 207, SER 209, SER 251, GLU 248, PHE 247	PHE 256, TRP 206, SER 249
14	Isolaureline	-6.20	28.65	ARG 207, ASN 208	SER 249, PHE 247, PHE 250	TYR 204, TRP 206, TRP 214, SER 251
15	Liriodenine	-5.97	42.20	TYR 204, ARG 207, PHE 250	TRP 214, ASN 208, SER 249, SER 251	TYR 204, ARG 207, TRP 206
16	Myricyl alcohol	-0.10	842.90	-	SER 65, SER 209, TYR 204, PHE 256, ARG 207, SER 251, ASN 208, PHE 250, SER 249, GLU 248, TRP 214	PHE 252, TRP 206

P-ISSN: 2406-9388
E-ISSN: 2580-8303

17	Myristic acid	-3.38	3.31	ARG 207	GLU 248, ASN 208, SER 205, SER 249, PHE 250, TYR 204, SER 209	TRP 206, TRP 214
18	*N*-methylcoclaurine	-5.29	132.07	ARG 207, SER 206, PHE 250, GLU 248	ASN 208, SER 209, TRP 214, SER 249	TYR 204, ARG 207, SER 205, TRP 206
19	Palmitic acid	-3.37	3.36	ARG 207	TRP 214, SER 249, PHE 250, GLU 248, ASN 208, SER 209, TYR 204	TRP 206
20	Polyphenol	-5.7	63.80	PHE 250, SER 209, ARG 207, TYR 204	SER 249, SER 251, TRP 206, SER 65	ASN 208
21	Remerine	-5.38	113.09	PHE 250, ASN 208, SER 209, ARG 207, SER 65	SER 249, SER 251, PHE 256, TYR 204	TRP 206, TRP 214
22	Reticuline	-5.65	72.70	SER 209, SER 251	SER 65, TRP 206, SER 249, TRP 214	ARG 207, GLU 248, ASN 208, PHE 252, PHE 250
23	S-norcorydine	-6.12	32.53	PHE 250, GLU 248	ARG 207, ASN 208, SER 249, SER 251	TYR 204, TRP 206, TRP 214
24	Xylopine	-5.58	80.58	GLU 248, PHE 250	ARG 207, ASN 208, PHE 247	SER 249, TRP 214, TRP 206

Data analysis and visualization

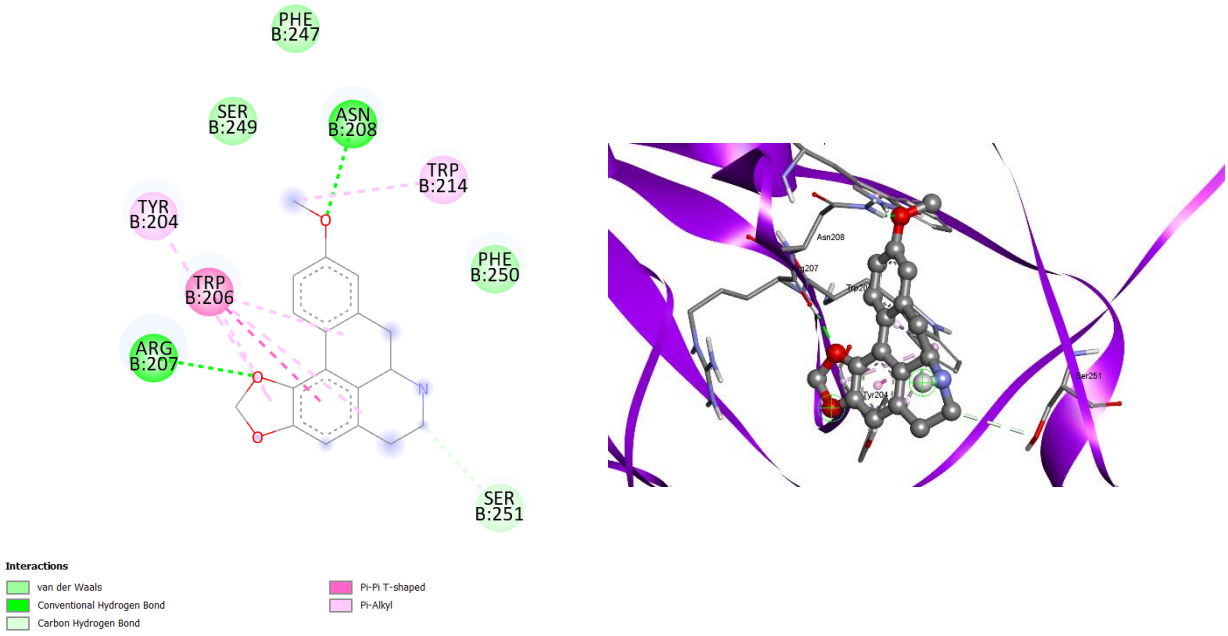
Molecular docking is used to predict the effectiveness of a ligand in interacting with the target cell. In this study, twenty-four ligands were likely present in the *A.muricata* plant to see the effectiveness of its anticancer activity against the caspase-3 enzyme (PDB ID: 1NME). Caspase-3 plays a role as the major mediator of apoptosis activated.

The first parameter observed from the docking results is the binding energy. Analysis and interpretation of the energy were done to data provided by AutoDockTools 1.5.6. According to AutoDock, the binding energy is the sum of the intermolecular forces acting upon the receptor-ligand complex (Lin *et al.*, 2011). A good binding energy is represented by a more negative (lower) value. While a low binding energy suggests that the compound requires less energy during binding, indicating that a low binding energy signifies a greater potential for interaction and the formation of a strong bond with the target protein (Rena *et al.,* 2022). The second parameter is inhibition constant (KI). Inhibition constant indicates the concentration required by the ligand to inhibit the target protein. A good inhibition constant is represented by a smaller value of KI. (Rena *et al.,* 2022).

In addition to the energy binding value and inhibition constant value, the interaction between residues and ligands, such as hydrogen bonding, is also taken into consideration. Hydrogen bonds play a crucial role in protein structure because the stability of a protein's structure depends on hydrogen bonds (Suryani *et al.,* 2018). The binding location of the ligand on the protein is determined by the extent of residue-ligand interactions. A greater number of interacting residues leads to a stronger bond between the ligand and the protein. (Rena *et al.,* 2022).

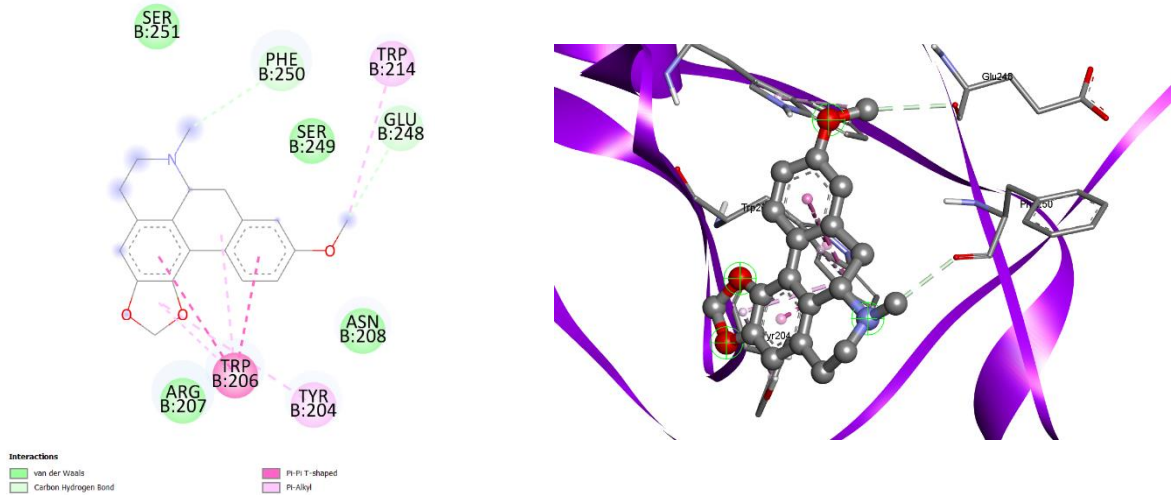
A total of twenty-two test ligands from *A. muricata* under research were employed and docked onto the caspase-3 receptor. Each ligand produced conformation based on binding energy values, inhibition constant, and hydrogen bonding. Based on Table 4, the test ligands, Isolaureline and S-norcorydine, had a binding energy value of -6.20 kcal /mol and -6.12 kcal /mol and inhibition constant value of 28.65 µM and 32.53 µM. Those compounds have lower binding energy values (-5.28 kcal/mol) and inhibition constant (134.89 µM) from the native ligand. The visualization can be seen in Figure 3 and 4.

P-ISSN: 2406-9388
E-ISSN: 2580-8303



(a) (b)

Figure 3. Visualization of the 2D interactions between Caspase-3 and Isolaureline (a) and visualization of the 3D interactions between Caspase-3 and Isolaureline (b)



(a) (b)

Figure 4. Visualization of the 2D interactions between Caspase-3 and S-Norcorydine (a) and visualization of the 3D interactions between Caspase-3 and S-Norcorydine (b)

Table 4 shows that Isolaureline forms hydrogen bonds with the amino acid ARG 207 and ASN 208, and S-Norcorydine forms hydrogen bonds with the amino acid PHE 250 and GLU 248. Hydrogen bonding plays a major role in the stability of molecular interactions, resulting in an abundance of hydrogen bonds that increase the bond energy between the enzyme and the substrate (Arwansyah *et al.*, 2014). In addition, these two compounds formed amino acid residue interactions in the form of hydrophobic interactions; specifically, Van Der Waals bonds with the residue. Isolaureline form Van Der Waals bonds with the amino acid SER 249, PHE 247, and PHE 250. On the other hand, S-Norcorydine form Van Der Waals bonds with the amino acid ARG 207, ASN 208, SER 249, and SER 251. The results indicated that the bonds

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E-ISSN: 2580-8303

of the three compounds were nearly as strong as those of natural ligand–as observed from the numerous similarities in amino acid residues formed. Based on all the parameters, these two compounds (Isolaureline and S-norcorydine) have the potential as candidates for anticancer agents.

CONCLUSION

Twenty-two compounds from *A. muricata* were successfully docked and then analyzed based on their free energy binding and intermolecular interactions with the caspase-3 binding site. The results of molecular docking showed that two compounds, namely Isolaureline and S-norcorydine, showed the best results, giving the lowest binding energy value and lowest inhibition constant with the most preferred interaction. A further study can be conducted to investigate the anticancer activity of these compounds via in vivo and in vitro research.

ACKNOWLEDGMENT

The author would like to thank the Faculty of Pharmacy, Universitas Padjadjaran for the support and good facilities.

AUTHOR CONTRIBUTIONS

Conceptualization, A.A., M.W.A.R., Z.A.H., S.L.R., H., N.P., A.P.A.P., D.L.A.; Methodology, A.A., M.W.A.R., Z.A.H., S.L.R., H., N.P., A.P.A.P., D.L.A.; Software, A.A., M.W.A.R., Z.A.H., S.L.R., H.; Validation, A.A.; Formal Analysis, A.A., M.W.A.R., Z.A.H., S.L.R., H.; Investigation, A.A., M.W.A.R., Z.A.H., S.L.R., H.; Resources, A.A., M.W.A.R., Z.A.H., S.L.R., H.; Writing - Original Draft, A.A., M.W.A.R., Z.A.H., S.L.R., H.; Writing - Review & Editing, A.A., M.W.A.R., Z.A.H., S.L.R., H.; Visualization, A.A., M.W.A.R., Z.A.H., S.L.R., H.; Supervision, A.A., M.W.A.R., Z.A.H., S.L.R., H., N.P., A.P.A.P., D.L.A.; Project Administration, A.A., M.W.A.R., Z.A.H., S.L.R., H., N.P., A.P.A.P., D.L.A.; Funding Acquisition, A.A., M.W.A.R., Z.A.H., S.L.R., H., N.P., A.P.A.P., D.L.A.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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P-ISSN: 2406-9388
E-ISSN: 2580-8303