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In silico Analysis of the Potential of Red Betel (Piper crocatum) Active Compounds as Anti-Inflammatory Drug Candidate

Analisis In silico Potensi Senyawa Aktif Sirih Merah (Piper crocatum) sebagai Kandidat Obat Anti-inflamasi

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Abstract. Inflammation is the body's response to tissue damage, but if uncontrolled, it can become pathological. Piper crocatum, commonly known as red betel, has long been recognized for its health benefits, yet scientific research on its active compounds remains limited. This study aims to predict the anti-inflammatory potential of red betel leaf's active compounds against pro-inflammatory proteins. Using an in silico molecular docking approach, this research targeted the TNF-α and TACE as a target receptor macromolecule. The processes of exploration, screening, docking, and visualization were conducted using RCSB PDB, PubChem, ADME Calculator, AutoDock, and Biovia Discovery Studio. The findings revealed that none of the active compounds from red betel leaves exhibited lower binding affinity values than the native ligand for the TNF-α protein. However, one compound showed a lower binding affinity compared to the native ligand for the TACE protein and demonstrated binding capability to the HIS 415 amino acid. Consequently, no red betel leaf compounds are predicted to be potential anti-inflammatory agents for inhibiting TNF-α activity. In contrast, one active compound from red betel leaves, *2(2-isopropyl-5-methylphenoxy)-N2-(4-carboxybenzylidene)*, displayed predicted potential for inhibiting TACE protein activity.
Keywords: *drug discovery; molecular docking; TNF-a; TACE*

Abstrak. *Inflamasi merupakan respon tubuh terhadap kerusakan jaringan, namun jika tidak dikendalikan dapat menjadi patologis. Piper crocatum, yang biasa dikenal dengan sirih merah, telah lama dikenal manfaatnya bagi kesehatan, namun penelitian ilmiah mengenai senyawa aktifnya masih terbatas. Penelitian ini bertujuan untuk memprediksi potensi anti inflamasi senyawa aktif daun sirih merah terhadap protein pro inflamasi. Dengan menggunakan pendekatan in silico molekuler docking, penelitian ini menargetkan TNF-a dan TACE sebagai makromolekul reseptor target. Proses eksplorasi, screening, docking, dan visualisasi dilakukan RCSB PDB, PubChem, ADME Calculator, AutoDock, dan Biovia Discovery Studio. Hasil penelitian menunjukkan bahwa tidak ada satu pun senyawa aktif dari daun sirih merah yang menunjukkan nilai afinitas pengikatan yang lebih rendah dibandingkan ligan asli untuk protein TNF-a. Namun, satu senyawa menunjukkan afinitas pengikatan yang lebih rendah dibandingkan dengan ligan asli untuk protein TACE dan menunjukkan kemampuan pengikatan terhadap asam amino HIS 415. Oleh karena itu, diperkirakan tidak ada senyawa daun sirih merah yang berpotensi menjadi agen anti-inflamasi dalam menghambat aktivitas TNF-a. Sebaliknya, satu senyawa aktif dari daun sirih merah, 2(2-isopropyl-5-methylphenoxy)-N2-(4-carboxybenzylidene), diperkirakan berpotensi menghambat aktivitas protein TACE.*
Kata kunci: *drug discovery*; *molecular docking*; TNF-α; TACE

INTRODUCTION

Indonesia possesses a wide array of plant species, which contributes to its abundant biodiversity. This richness has the potential to enhance health and serve as a primary ingredient for natural remedies. Nevertheless, the majority of information concerning the utilization of natural components in Indonesia relies solely on empirical data, which is widely trusted by the people but lacks scientific substantiation (Rachmawaty et al., 2016). According to Emrizal et al. (2014), only a small percentage, around 5-15%, of the estimated 500,000 documented plant species have undergone research and testing to determine their chemical and biological impacts. This suggests that the investigation of natural medicinal raw materials provides many resources for the advancement of natural therapies. The red betel plant (*Piper crocatum*) is a species that thrives in tropical climates,

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including Indonesia. Red betel has long been believed to be a remedy by the people for diabetes mellitus, hepatitis, kidney stones, and skin softening. Alkaloids, saponins, tannins, and flavonoids are phytochemical compounds commonly present in red betel leaves (Fadlilah, 2015). These four categories of compounds have been demonstrated to actively inhibit the inflammatory process. Nevertheless, there is a scarcity of studies that have examined the efficacy of the active compounds found in red betel leaves as anti-inflammatory agents. A study conducted by Audina et al. (2018) on sumambu leaves (*Hyptis capitata* Jacq.) shown that the application of *sumambu* leaf extract played a role in the anti-inflammatory process. Subsequent phytochemical analysis revealed the presence of alkaloids, saponins, tannins, and flavonoids in *semambu* leaves. These four groups of compounds also share similarities with the compounds found in red betel leaves. Hence, the active compounds present in red betel leaves may function as inhibitors of inflammatory pathways and hold promise in the field of inflammatory therapeutic research.

Inflammation is the body's innate defensive reaction to tissue damage, which can result from physical injury, harmful chemicals, or microbial activity. The purpose of this defensive reaction is to eradicate antigens from the body (Dewi et al., 2015). Cytokines play a crucial function in regulating one of the body's inflammatory processes. Cytokines are synthesized by many immune cell reactions, such as macrophages, dendritic cells, Natural Killer (NK) cells, and lymphocytes. The three cytokines that elicit the most significant pro-inflammatory response from the innate immune system are IL-1, TNF-α, and IL-6 (Pasaribu, 2021). Nevertheless, an atypical or exaggerated reaction to inflammation might potentially lead to severe illness if it is not well controlled.

Tumor Necrosis Factor alpha (TNF-α) plays a crucial role as a cytokine in the progression of inflammation. This cytokine is synthesized by not only activated macrophages, but also by monocytes, fibroblasts, mast cells, and NK cells. These cells are responsible for initiating signals that lead to the development of immunity. One possible strategy to reduce inflammation intensity is to decrease the activity of macrophages and T cells by suppressing TNF-α activity. Amarawati et al. (2019) elucidated in their study that TNF-α has the ability to engage with the Tumor Necrosis Factor Receptor (TNFR) during its active state. TACE regulates the conversion of pro-TNF-α into active TNF-α. Strategies for suppressing TACE activity can potentially decrease the production of active TNF-α. On the flip side, the TNF-α and TACE proteins are located in the transmembrane area of cells. This can be beneficial since transmembrane proteins play a crucial role as intermediates in a variety of biological processes, such as cellular signaling, chemical transport, and cellular connections. As such, they are frequently the primary targets of numerous medications. Additionally, transmembrane protein is more easily accessible to drug molecules compared to intracellular protein, making it an ideal candidate for drug research (Rudden and Degiacomi, 2021; You et al., 2021). Hence, the proteins TNF-α and TACE can serve as potential targets in endeavors to mitigate the dysregulation of inflammation.

In cases of inflammation, the often-employed therapy involves the administration of anti-inflammatory medicines, which can be either steroids (steroidal anti-inflammatory drugs; SAIDs) or non-steroids (nonsteroidal anti-inflammatory drugs; NSAIDs). Nevertheless, the administration of this medication may result in adverse reactions including gastrointestinal irritation, renal impairment, diarrhea, headaches, depression, pancreatitis, and other related symptoms (Dewi, et al., 2015). In addition to therapy including SAIDs or NSAIDs, disease-modifying antirheumatic medications (DMARDs) are also utilized. Nevertheless, these medications also induce adverse effects such as tuberculosis reactivation, cancer cell malignancy development, hematological diseases, and cardiovascular illness (Singh et al., 2020). In order to mitigate the negative consequences, it is imperative to investigate alternative pharmaceutical substances, namely bioactive compounds found in plants. These compounds possess the ability to regulate or diminish the inflammatory process, preventing it from becoming chronic and progressive.

One of the predictive stages in the strategy of drug discovery is to conduct computer experiments to assess the similarity of pharmacological characteristics (druglikeness) and perform molecular docking assays. The resemblance of drug characteristics can offer useful insights on the fundamental physicochemical features of a molecule within the organism, particularly in relation to its solubility, permeability, metabolic stability, and impact on transporters (Bickerton et al., 2012). The process of molecular docking in drug candidate development entails the prediction of molecular-level interactions between test compounds (ligand) and target receptor macromolecules. This approach enables the observation of the activity of test compounds towards specific protein binding sites, as well as the understanding of the fundamental biochemical pathways involved in these interactions (Agu et al., 2023). Hence, the objective of this work is to investigate the capability of active compounds

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found in red betel leaf to act as anti-inflammatory agents against pro-inflammatory proteins by in silico analysis.

MATERIALS AND METHODS

This research employed an analytical descriptive methodology, utilizing a computational approach. The focus of this study was on the proteins Tumor Necrosis Factor alpha (TNF-α) and TNF-α Converting Enzyme (TACE). These proteins were examined using a molecular docking test approach to evaluate the activity generated by the active compounds found in red betel leaves. The criteria examined encompass the binding affinity value and the congruity of the attachment locations of the amino acid produced. The 3D configurations of the TNF-α (**Figure 1a**) and TACE (**Figure 1b**) proteins were acquired from the RCSB PDB database website (https://www.rcsb.org/) using the PDB ID codes 2AZ5 and 3KME, respectively. The molecular structure of each red betel leaf compound was acquired from the PubChem database website (https://pubchem.ncbi.nlm.nih.gov/).

(a) (b)

Figure 1. 3D structures of the proteins (a) Tumor Necrosis Factor alpha (TNF-α; PDB ID: 2AZ5) and (b) TNF-α Converting Enzyme (TACE; PDB ID: 3KME). Each subunit or chain of each protein is distinguished by color. Subunits A, B, C, and D for each protein are colored blue, green, orange, and red, respectively.

The research procedure was conducted utilizing the methodology derived from Pratama et al. (2021) with certain modifications. Initially, the collection of data on the compounds present in red betel leaves involved doing a thorough review of existing research on the identification and analysis of these compounds. This was done by examining the results obtained from Gas Chromatography-Mass Spectrometry (GC-MS) analysis of ethanol extracts of red betel leaves. After red betel leaf compounds' component names were determined, the PubChem database website (https://pubchem.ncbi.nlm.nih.gov/) was used to search for the Simplified Molecular Input Line Entry System (SMILES) structure. The compound screening method is thereafter conducted according to druglikeness attributes. The druglikeness parameter employed is Lipinski's rule of five, which encompasses a molecular weight below 500 g/mol, a partition coefficient (logP) value below 5, a maximum number of hydrogen donors of 5, and a maximum number of hydrogen acceptors of 10 (Riyaldi et al., 2022). The process of filtering Lipinski's rule of five is performed using the ADME Calculator database website (https://armakovic.com/online-tools/adme-calculator/). After successfully completing the screening process, compounds have their three-dimensional structures obtained from the PubChem database website. This compound structure serves as a test ligand in the molecular docking experiments conducted.

The protein preparation starts with retrieving the three-dimensional structure of Tumor Necrosis Factor alpha (TNF-α; PDB ID: 2AZ5) and TNF-α Converting Enzyme (TACE; PDB ID: 3KME) proteins from the RCSD PDB database website (https://www.rcsb.org/). Using the AutoDock software, the protein structure is isolated from its native ligands, followed by the removal of water molecules and the addition of charges. The TNF-α protein structure utilizes a native ligand encoded with ID 307, which is *6,7-dimethyl-3-[(methyl{2-[methyl({1-[3-(trifluoromethyl)phenyl]-1h-indol-3-yl}methyl)amino]ethyl}amino)methyl]-4h-chromen-4-one*. On the other hand, the TACE protein structure's native ligand, encoded with ID Z59, is *(2R,3R)-2,3-dihydroxy-4-oxo-4-[(2R)-2-phenylpyrrolidin-1-yl]-N-(thiophen-2-ylmethyl)butanamide*. The two inherent ligands of each protein structure are empirical synthetic inhibitors of TNF-α and TACE (He et al., 2005; Rosner et al., 2010). The target receptor macromolecule used in the molecular docking test is the protein structure that has been isolated from the native ligand and undergone charge addition and water molecule removal.

AutoDock software was used throughout the entire molecular docking procedure. The molecular docking approach employed is specific docking. For the docking operation to be executed, a grid box or dimensions are required. The grid box can be detected and altered using the AutoDock

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program by inputting the preparation results of the target receptor macromolecules together with the native ligands in a simultaneous manner. Table 1 displays the measurements of the validation grid box employed in the molecular docking experiment. After confirming its docking procedure validity, the molecular docking test process commences by inputting the preparation outcomes of both the target receptor macromolecules and the test ligands simultaneously. The docking process script is modified based on data including the target receptor macromolecule's identification, the ligand being tested, the number of modes generated, and the known dimensions of the docking process (grid box). Subsequently, the molecular docking outcomes were visualized using the Biovia Discovery Studio software. The visualization findings attempt to accurately show the interactions between the tested compounds and the target receptor macromolecules.

Table 1. Grid box used for the molecular docking test process between the test ligand and the target receptor macromolecule.

Target Receptor Macromolecules	Ligand Code	Grid Box Coordinates					
		Size			Center		
		x	y	z	x	y	z
TNF-α	*Native ligand* 307 (NL 307)	15	15	15	-9.18	67.36	20.05
TACE	*Native ligand* Z59 (NL Z59)	15	15	15	49.49	33.45	41.46

The data obtained from this study was subjected to descriptive analysis, focusing on the druglikeness properties of the compounds according to Lipinski's rule of five. The analysis also involved comparing the binding affinity values and the similarities in the binding positions of the amino acids between the test ligand (red betel leaf compound) and the target receptor macromolecules (TNF-α and TACE). Compounds in red betel leaves that show characteristics in accordance with Lipinski's rule of five, low binding affinity values, and binding positions that are similar to the native ligand of the target receptor macromolecule indicate that these compounds are predicted to have the potential to become anti-inflammatory drug candidates by inhibiting TNF-α and TACE protein activity.

RESULTS

Based on the results of the exploratory literature review that was carried out, 30 betel leaf compounds were obtained which were used as test ligands for the molecular docking tests that were carried out. The Simplified Molecular Input Line Entry System (SMILES) structure was then searched for each test ligand used on the PubChem database webserver. The results of all exploration data for the test ligands used are presented in Table 2.

Table 2. All test ligands from red betel leaves used for the molecular docking test process

Compound Name	Ligand Code	Molecular Formula	PubChem CID	SMILES
1H-Phenalen-1-one, hydrazone	L1	$C_{13}H_{10}N_2$	5375369	C1=CC2=C3C(=C1)C=CC(=NN)C3=CC=C2
4,4'-diaminostilbene	L2	$C_{14}H_{14}N_2$	5284646	C1=CC(=CC=C1C=CC2=CC=C(C=C2)N)N
Acethydrazide,2(2-isopropyl-5-methylphenoxy)-N2-(4-carboxybenzylideno)	L3	$C_{19}H_{22}N_2O_2$	9600027	CC1=CC(=C(C=C1)C(C)C)OCC(=O)NN=CC2=CC=CC=C2
C-(1-(4-methoxyphenyl-2-phenylethene))	L4	$C_{15}H_{14}O$	139728	COC1=CC=C(C=C1)CCC2=CC=CC=C2
Euclein	L5	$C_{22}H_{14}O_6$	308140	CC1=CC2=C(C(=C1)O)C(=O)C=C(C2=O)C3=C(C4=C(C=C3C)C(=O)C=CC4=O)O
Naphthalene	L6	$C_{10}H_8$	931	C1=CC=C2C=CC=CC2=C1
Phenol, p-1-indanyl	L7	$C_{15}H_{14}O$	221137	C1CC2=CC=CC=C2C1C3=CC=C(C=C3)O
Phytol	L8	$C_{20}H_{40}O$	5280435	CC(C)CCCC(C)CCCC(C)CCCC(=CCO)C
Vitamin E	L9	$C_{29}H_{50}O_2$	86472	CC1=C(C(=C(C2=C1OC(CC2)(C)CCCC(C)CCCC(C)CCCC(C)C)C)OC(=O)C)C
6-Octadecenoic acid, methyl ester, (Z)	L10	$C_{20}H_{38}O_2$	5362717	CCCCCCCCCCCC=CCCCCC(=O)OC

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Compound Name	Ligand Code	Molecular Formula	PubChem CID	SMILES
Alpha- Humulene	L11	$C_{15}H_{24}O$	5281520	CC1=CCC(C=CCC(=CCC1)C)(C)C
Beta- Bisabolene	L12	$C_{15}H_{24}$	10104370	CC1=CCC(CC1)C(=C)CCC=C(C)C
Gamma, terpinene	L13	$C_{10}H_{16}$	7461	CC1=CCC(=CC1)C(C)C
Methyl palmitate	L14	$C_{17}H_{34}O_2$	8181	CCCCCCCCCCCCCCCC(=O)OC
Octadecenoic acid	L15	$C_{18}H_{34}O_2$	5282750	CCCCCCCCCCCCCCCC=CC(=O)O
Oleic acid	L16	$C_{18}H_{34}O_2$	445639	CCCCCCCCC=CCCCCCCCC(=O)O
Palmitic acid	L17	$C_{16}H_{32}O_2$	985	CCCCCCCCCCCCCCCC(=O)O
Trans-beta-farnesene	L18	$C_{15}H_{24}$	5281517	CC(=CCCC(=CCCC(=C)C=C)C)C
Elemicin	L19	$C_{12}H_{16}O_3$	10248	COC1=CC(=CC(=C1OC)OC)CC=C
Neophytadiene	L20	$C_{20}H_{38}$	10446	CC(C)CCCC(C)CCCC(C)CCCC(=C)C=C
Propionic acid	L21	$C_3H_6O_2$	1032	CCC(=O)O
1,2,3-Propanetriol	L22	$C_3H_8O_3$	753	C(C(CO)O)O
2,4(1H,3H)-Pyrimidinedione	L23	$C_4H_4N_2O_2$	1174	C1=CNC(=O)NC1=O
4H-Pyran-4-one	L24	$C_5H_4O_2$	7968	C1=COC=CC1=O
2-Furancarboxaldehyde	L25	$C_5H_4O_2$	7362	C1=COC(=C1)C=O
2-Methoxy-4-vinylPhenol	L26	$C_9H_{10}O_2$	332	COC1=C(C=CC(=C1)C=C)O
2,6-Dimethyl-3-(methoxymethyl)-p-benzoquinone	L27	$C_{10}H_{12}O_3$	6430513	CC1=CC(=O)C(=C(C1=O)C)COC
1,2-Benzenedicarboxylic acid	L28	$C_8H_6O_4$	1017	C1=CC=C(C(=C1)C(=O)O)C(=O)O
1- Methylphenazine 5-oxide	L29	$C_{13}H_{10}N_2O$	610909	CC1=CC=CC2=[N+](C3=CC=CC=C3N=C12)[O-]
9-Octadecenoic acid	L30	$C_{18}H_{34}O_2$	637517	CCCCCCCCC=CCCCCCCCC(=O)O

The acquired test ligands are subsequently proceeded to a selection process. The process of selecting the test ligand utilizes the druglikeness properties outlined in Lipinski's rule of five. Molecular weight, partition coefficient (LogP), hydrogen acceptors, and hydrogen donors are some of these druglikeness traits. Table 3 displays the druglikeness predicted results of the used test ligands. Any ligands that successfully pass the selection results can proceed to the molecular docking test step.

Table 3. Prediction of druglikeness of test ligands from red betel leaves used.

Ligand Code	Druglikeness Lipinski's rule of five's			
	Molecular weight (g/mol) x < 500	**Hidrogen acceptors** x < 10	**Hidrogen donors** x < 5	**LogP** x < 5
L1	194.237	2	1	2.529
L2	210.28	2	2	3.021
L3	310.397	3	1	3.648
L4	212.292	1	0	3.48
L5	374.348	6	2	3.112
L6	128.174	0	0	2.84
L7	210.276	1	1	3.47
L8	296.539	1	1	6.364
L9	472.754	3	0	9.06
L10	296.495	2	0	6.197
L11	204.357	0	0	5.035
L12	204.357	0	0	5.035
L13	136.238	0	0	3.309
L14	270.457	2	0	5.641
L15	282.468	1	1	6.109
L16	282.468	1	1	6.109
L17	256.43	1	1	5.552
L18	204.357	0	0	5.202
L19	208.257	3	0	2.441
L20	278.524	0	0	7.168
L21	74.079	1	1	0.481
L22	92.094	3	3	-1.67
L23	112.088	2	2	-0.94
L24	96.085	2	0	0.64

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Ligand Code	Druglikeness Lipinski's rule of five's			
	Molecular weight (g/mol) x < 500	Hidrogen acceptors x < 10	Hidrogen donors x < 5	LogP x < 5
L25	96.085	2	0	1.092
L26	150.177	2	1	2.044
L27	180.203	3	0	1.047
L28	166.132	2	2	1.083
L29	210.236	2	0	2.33
L30	282.468	1	1	6.109

Note: The yellow highlight indicates a discrepancy in the prediction results with the standard value of Lipinski's rule of five druglikeness character

All test ligands that passed the druglikeness screening process against the target receptor macromolecules are subjected to a molecular docking test, which yields binding affinity values. However, it is necessary to establish and determine the coordinate dimension of the docking process (grid box) prior to conducting the docking process between the test ligand and the target receptor macromolecule. The coordinate dimensions for the docking process can be determined, established, and verified by a repeated docking (re-docking) validation procedure. The positional configurations for the dimensions of the gridbox for each target receptor macromolecule utilized are documented in Table 1. Figure 2 displays the visualization and Root Mean Square Deviation (RMSD) values obtained from the re-docking validation of each native ligand (NL 307 for TNF-α protein and NL Z59 for TACE). These values are useful for conducting molecular docking tests between the test ligand and the target receptor macromolecules.

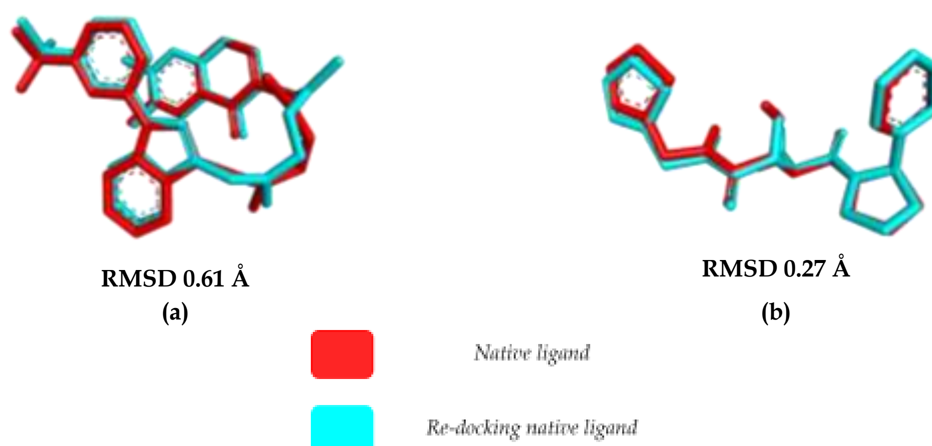

Figure 2. Visualization of validation results of native ligand re-docking **(a)** NL 307 and **(b)** NL Z59

Once the grid box is determined and the re-docking validation has been confirmed, the molecular docking test is conducted between the test ligand and the target receptor macromolecule. Table 4 displays the outcomes of the molecular docking test conducted on the test ligand and the target receptor macromolecule.

All the test ligands exhibit greater binding affinity values than the native ligand for the TNF-α protein (NL 307) (Table 4), which has a binding affinity value of -9 Kcal/mol. A test ligand, L3-coded test ligand or *acethydrazide, 2(2-isopropyl-5-methylphenoxy)-N2-(4-carboxybenzylideno)*, on the other hand, had a lower binding affinity value for the TACE protein than the native ligand. This ligand has a binding affinity value of -8.6 Kcal/mol, whereas the native ligand TACE (NL Z59) has a value of -8.3 Kcal/mol. In addition to evaluating the binding affinity value, it is also important to assess the similarity of binding locations as additional indicator. Molecular docking visualization provides data on the precise location where the amino acids of the test ligand and the target receptor macromolecule link together. Figure 3 depicts a 2D visual representation of the specific location where the *acethydrazide, 2(2-isopropyl-5-methylphenoxy)-N2-(4-carboxybenzylideno)* or L3-coded test ligand binds to the TACE target protein.

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Table 4. Binding affinity values from the results of the molecular docking test between the test ligand and the target receptor macromolecule used.

Target Receptor Macromolecules			
TNF-α		TACE	
Ligand Code	***Binding affinity* (Kkal/mol)**	**Ligand Code**	***Binding affinity* (Kkal/mol)**
NL 307	-9	NL Z59	-8.3
L1	-7.3	L1	-6.3
L2	-6.7	L2	-7
L3	-7.8	L3	-8.6
L4	-6.8	L4	-7.6
L5	-8.7	L5	-7.9
L6	-5.9	L6	-7.1
L7	-7.8	L7	-7.7
L8	-5.9	L8	-6.4
L9	-7.5	L9	-7.5
L10	-5.8	L10	-5.4
L11	-7.1	L11	-4.6
L12	-6.9	L12	-7.4
L13	-5.7	L13	-6.7
L14	-5.2	L14	-5.4
L15	-5.4	L15	-5.5
L16	-5.5	L16	-5.7
L17	-5.3	L17	-5.8
L18	-5.9	L18	-6.7
L19	-5.4	L19	-5.9
L20	-5.9	L20	-6.2
L21	-3.4	L21	-4.2
L22	-3.7	L22	-4.2
L23	-4.6	L23	-5.5
L24	-4.1	L24	-5
L25	-3.9	L25	-4.9
L26	-5.5	L26	-6.6
L27	-5.5	L27	-5.5
L28	-6	L28	-6.8
L29	-7.5	L29	-6.7
L30	-5.3	L30	-5.9

Note: The binding affinity value of the test ligand that is lower than the binding affinity value formed by the native ligand for each target receptor macromolecule (NL 307 and NL Z59) is marked with a green color.

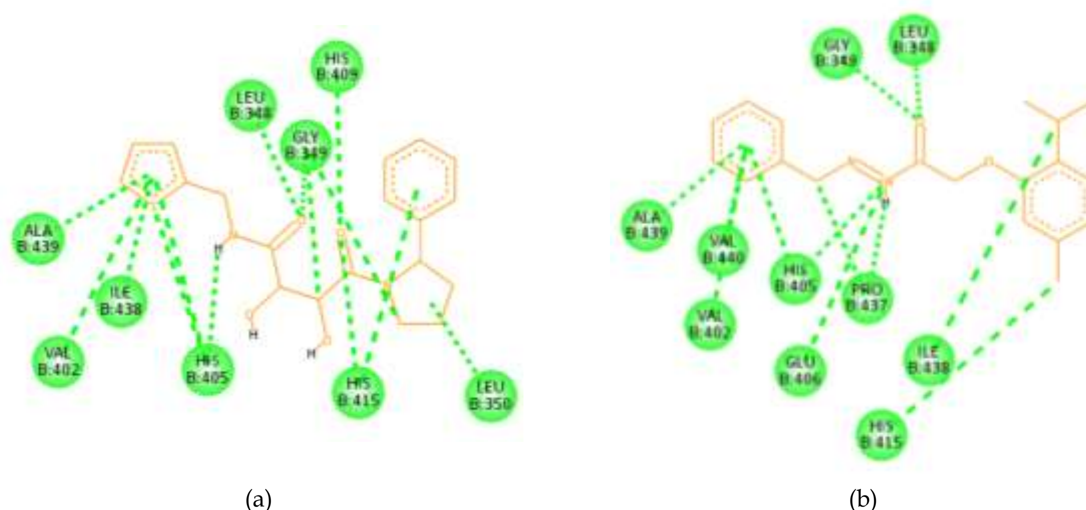

Figure 3. 2D visualization results of the molecular docking test carried out between (a) the native ligand NL Z59 and (b) the test ligand *acethydrazide, 2(2-isopropyl-5-methylphenoxy)-N2-(4-carboxybenzylideno)* with the TACE target receptor macromolecule.

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DISCUSSION

Lipinski's rule of five is a fundamental criterion used in predictive research to assess the similarity of a molecule to standard drugs. These characteristics encompass molecular weight parameters, the count of hydrogen acceptors and donors, and the lipophilicity value. Compounds with a molecular weight of 500 g/mol or above will have challenges in achieving appropriate distribution throughout the body. This is due to the fact that molecules with an excessive molecular weight would find it difficult to diffuse across the intestinal mucosal cell membrane due to their enormous size (El-Kattan, 2017; Rashid et al., 2021). Furthermore, if a compound exhibits fewer than 10 hydrogen acceptors and fewer than 5 hydrogen donors, it suggests that the compound can be readily absorbed by the body and takes minimal energy to traverse the membrane (Syahputra et al., 2014; Wang et al., 2016). Last but not least, the lipophilicity criterion assesses a molecule's capability to dissolve in fat and reveals its polarity. The lipophilicity of a chemical can be assessed by calculating its partition coefficient or logP value. The number represents the ratio of the concentration of the molecule's neutral form in two mixed phases, typically water and octanol (Pratiwi et al., 2023). If the logP value surpasses the threshold of 5, it indicates a greater propensity for the molecule to readily dissolve in fat while being less soluble in water, thereby classifying the compound as hydrophobic or non-polar (El-Kattan, 2017; Curie et al., 2022). According to the druglikeness prediction tests conducted and displayed in Table 3, there are 12 active compounds found in red betel leaves that fail to match one of the standard criteria specified in Lipinski's rule of five. The incompability criteria for these 12 chemicals are based on the standard lipophilicity criterion, where the partition coefficient value exceeds the usual threshold. Hence, it is imperative to exert further endeavors to enhance the solubility characteristics of these 12 compounds, thereby rendering them more soluble in aqueous environments. Nevertheless, it should be noted that the twelve compounds did not fail the druglikeness prediction test alone because they violate only one criterion of Lipinski's rule of five. Typically, a drug molecule or drug candidate should have less than two breaches of all the Lipinski's rule of five criteria (Sreelakshmi et al., 2017). Hence, out of the 30 red betel leaf compounds identified in the previous literature research, all of them were deemed eligible as potential medication candidates due to their adherence to Lipinski's rule of five, or having less than two violations.

The validation method for the docking approach involves multiple steps, one of which is the determination of the dimensions of the grid box, which serves as the docking place for the ligand. This determination can be ascertained by examining the attachment location of the native ligand to the receptor of the target macromolecule, as well as the information regarding the binding site of the target receptor macromolecule. The region on a protein that possesses the ability to attach to a molecule and subsequently impact the protein's function is referred to as the binding site of a protein (Frimayanti et al., 2021). The TNF-α protein's binding site (PDB ID: 2AZ5) is located in protein subunits C and D at the 119th tyrosine amino acid position (TYR 119). This role has the capability to impede the physiological activity of TNF-α. On the flip side, the TACE protein binding site (PDB ID: 3KME) is located at the 415th histidine amino acid position (His 415) on the B protein subunit. The TACE protein binding site can also affect the physiological function of TACE by suppressing its performance, similar to the influence of the TNF-α protein binding site (He et al., 2005; Rosner et al., 2010). The coordinate information for both TNF-α and TACE target receptor macromolecules is provided in Table 1.

The Root Mean Square Deviation (RMSD) is a parameter utilized in the validation phase of the docking procedure. This indicator quantifies the degree of similarity in the positions of the native ligand from the protein crystal structure (target receptor macromolecule) and the redocked native ligand. A docking method is deemed legitimate if its RMSD value is less than or equal to 2 Å. This guarantees that the docking strategy employed is applicable to the docking procedure involving the specific test ligand utilized (Sari et al., 2020). The purpose of conducting RMSD analysis is to assess the degree of change in the protein-ligand complex and to verify the stability of its structure (Muttaqin et al., 2019). The docking procedure's validation results indicate that the TNF-α and TACE proteins have validation values of 0.61 and 0.27 Å, respectively (Figure 2). This suggests that the current position and docking approach employed are acceptable and can be further utilized for the docking procedure of the experimental ligand.

The molecular docking approach is utilized to forecast the anti-inflammatory capabilities of the active compounds found in red betel leaf against TNF-α and TACE proteins. The assessment of potential activity was conducted by considering the binding affinity values and the similarity of amino acid binding sites. Binding affinity is a measure of the strength of the interaction between a

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ligand and a target receptor macromolecule. A smaller number suggests a greater interaction between the two (Naufa et al., 2022). The primary emphasis is placed on the specific spot where amino acids bind to the target protein. The stability of the ligand's position and its impact on protein activity are directly proportional to the similarity of the amino acid binding position (Frimayanti et al., 2021).

The results of the molecular docking test for the test ligand were compared to those of the native ligand for each target receptor macromolecule. The native ligand protein TNF-α (NL 307) binds to amino acids LEU 57, TYR 119, and GLY 121 in subunit C and TYR 59, TYR 119, and TYR 151 in subunit D. Its binding affinity value is -9 kcal/mol. The native ligand protein TACE (NL Z59), on the other hand, binds to amino acids LEU 348, GLY 349, LEU 350, VAL 402, HIS 405, HIS 409, HIS 415, ILE 438, and ALA 439 on subunit B with a binding affinity value of -8.3 kcal/mol. NL 307 and NL Z59 are novel synthetic compounds that have been developed to suppress the activity of TNF-α and TACE proteins in experimental settings. According to He et al. (2005) and Rosner et al. (2010), NL Z59 has to bind to amino acid HIS 415 in subunit B of the TACE protein in order to exert its inhibitory action, whereas NL 307 is effective when binding to amino acid TYR 119 in subunits C and D of the TNF-α protein. The binding affinity values and amino acid binding locations of these two natural ligands serve as crucial standards for assessing the effectiveness of docking test ligands.

According to the results of the molecular docking test, the *acethydrazide, 2(2-isopropyl-5-methylphenoxy)-N2-(4-carboxybenzylideno)* or L3 ligand has the lowest binding affinity value (-8.6 kcal/mol) when interacting with the TACE protein, compared to the binding affinity value of the native ligand protein (NL Z59), which is -8.3 kcal/mol. Figure 3 displays the various binding locations of the L3 test ligand with TACE. There are seven amino acid binding locations that share the same binding position as the NL Z59 amino acid, including the amino acid HIS 415. Meanwhile, the binding affinity value L3 is -7.8 kcal/mol when interacting with the TNF-α protein. The value exceeds the binding affinity of the native ligand, NL 307, for the TNF-α protein. Furthermore, all of the test ligands exhibit a binding affinity for the TNF-α protein that is greater than the binding affinity of the its native ligand (-9 kcal/mol). L3 is predicted to have the potential to be an anti-inflammatory substance candidate that can inhibit TACE activity, according to the low binding affinity value and the compatibility of the main amino acid's binding location of the target receptor major macromolecule TACE. Nevertheless, there has not been much prior investigation into the utilization of this molecule. Therefore, this presents a new prospect to delve into and enhance research into the possible application of *acethydrazide, 2(2-isopropyl-5-methylphenoxy)-N2-(4-carboxybenzylideno)*, particularly as a TACE protein-mediated anti-inflammatory medication. The remaining test ligands had less favorable outcomes, particularly with regards to the stability of the established binding. The instability of these compounds is attributed to their high binding affinity compared to the native ligand of each target protein macromolecule. This suggests that these compounds may be predicted to have limited potential as anti-inflammatory agents, particularly for those mediated by the TNF-α and TACE proteins.

CONCLUSION

The active compounds in red betel leaves examined in this study have no potential as therapeutic candidates for reducing inflammation, especially involving TNF-α protein, as compounds examined had a binding affinity value higher than that of the native ligand protein TNF-α. On the one hand, red betel leaves contain a single active compound that is predicted to have potential as a potential anti-inflammatory therapeutic mediated via the TACE protein; *acethydrazide, 2(2-isopropyl-5-methylphenoxy)-N2-(4-carboxybenzylideno)*. Additional research is required to examine and validate the precise effects of each active chemical found in red betel leaves as an anti-inflammatory medicine in laboratory experiments.

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