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IN SILICO ANALYSIS OF ETHYL ACETATE *Bruguiera gymnorhiza* LEAF EXTRACTS AS AN ANTI-INFLAMMATORY AGENT

Fifi Atma Nur Diana*, Putut Har Riyadi, Eko Susanto
Department of Fishery Product Technology, Faculty of Fisheries and Marine Science
Diponegoro University
Prof. Jacub Rais street, Tembalang, Semarang, Jawa Tengah, Indonesia 50275

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*Correspondence: fifidiana80@gmail.com

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Abstract

Bruguiera gymnorhiza is a mangrove plant that contains many bioactive compounds, which exhibit anti-inflammatory properties. This study aims to evaluate the anti-inflammatory potential of bioactive compounds extracted from *B. gymnorhiza* leaves using ethyl acetate, through in silico analysis. A literature review was conducted using internationally recognized electronic databases to identify the chemical profiles of these compounds. In silico analyses were performed using PASS Server to predict biological activity, SwissADME for drug discovery potential, and ProTox III for toxicity assessment. Molecular docking was performed using the IKKβ receptor. A literature review identified 15 compounds present in the leaves of *B. gymnorhiza*. PASS Server analysis revealed that all identified compounds exhibited anti-inflammatory properties. Further evaluation using SwissADME and ProTox III indicated favorable drug-likeness and absorption, distribution, metabolism, and excretion potential, with varying levels of toxicity; four compounds were classified as Class 3, five as Class 4, four as Class 5, and two as Class 6. Molecular docking results demonstrated that elemicin and lauric acid formed hydrogen bonds with IKKβ, with binding energies of -4.4 kcal/mol and -6.6 kcal/mol, respectively, suggesting significant anti-inflammatory activity. These findings provide a foundation for the development of anti-inflammatory drugs based on *B. gymnorhiza* leaf extracts.

Keywords: bioactive compound, IKKβ inhibitor, molecular docking, pharmacodynamic analysis, therapeutic potential

Analisis In Silico Ekstrak Etil Asetat Daun *Bruguiera gymnorhiza* sebagai Agen Antiinflamasi

Abstrak

Bruguiera gymnorhiza merupakan tanaman mangrove yang mengandung banyak senyawa bioaktif, yang menunjukkan sifat antiinflamasi. Penelitian ini bertujuan mengevaluasi potensi antiinflamasi senyawa bioaktif yang diekstrak dari daun *B. gymnorhiza* menggunakan etil asetat, melalui analisis *in silico*. Penelusuran komponen bioaktif dilakukan menggunakan basis data elektronik yang diakui secara internasional untuk mengidentifikasi profil kimia senyawa ini. Analisis *in silico* dilakukan menggunakan PASS Server untuk memprediksi aktivitas biologis, SwissADME untuk potensi penemuan obat, dan ProTox III untuk penilaian toksisitas. Selain itu, *docking molekuler* dilakukan dengan reseptor IKKβ. Lima belas komponen bioaktif mengandung gugus yang terdapat dalam daun *B. gymnorhiza*. Analisis PASS Server mengungkapkan bahwa semua senyawa yang diidentifikasi menunjukkan sifat antiinflamasi. Evaluasi lebih lanjut menggunakan SwissADME dan ProTox III menunjukkan kemiripan obat dan potensi penyerapan, distribusi, metabolisme, dan ekskresi yang baik, dengan berbagai tingkat toksisitas: 4 senyawa diklasifikasikan sebagai kelas 3; 5 sebagai kelas 4; 4 sebagai kelas 5 dan 2 sebagai kelas 6. Hasil *docking molekuler* menunjukkan bahwa elemisin dan asam laurat membentuk ikatan hidrogen dengan IKKβ, dengan energi pengikatan masing-

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masing -4,4 kkal/mol dan -6,6 kkal/mol, yang menunjukkan aktivitas antiinflamasi yang signifikan. Temuan ini memberikan dasar untuk mengembangkan obat antiinflamasi berdasarkan ekstrak daun *B. gymnorhiza*.
Kata kunci: analisis farmakodinamik, *docking molekuler*, inhibitor IKKβ, potensi terapeutik, senyawa bio aktif

INTRODUCTION

Bruguiera gymnorhiza is a prominent mangrove species widely distributed across the coastal regions of Asia and the Pacific (Zhu *et al.,* 2012). Commonly known as the lindur plant, *B. gymnorhiza* can grow up to 30 meters in height and is valued for its leaves, stem, and fruit (Jacoeb *et al.,* 2013). Despite its potential uses, the plant is often underutilized in coastal communities, leading to its perception as a nuisance, especially when fallen fruits accumulate and decay (Rochmadi & Rohmah, 2019).

Studies have shown that *B. gymnorhiza* leaves extracted with ethanol, ethyl acetate, and n-hexane yield higher amounts of polar compounds, particularly when using ethanol as a solvent. The bioactive compounds identified in ethyl acetate and ethanol extracts include steroids, phenols, triterpenoids, flavonoids, tannins, and saponins (Dia *et al.,* 2015). Ethyl acetate extracts of *Leucaena leucocephala* (Lamk.) de Wit leaves have demonstrated the ability to reduce inflammation in rats (Amirah *et al.,* 2014), suggesting that *B. gymnorhiza* leaf extracts may also possess significant anti-inflammatory potential.

The selection of ethyl acetate in leaf extraction is helpful for finding anti-inflammatory compounds. Ethyl acetate is soluble for compounds with low polarity found in many plant extracts such as flavonoids which are known to have strong anti-inflammatory activity (Mohammed *et al.*, 2021). The results showed that the ethyl acetate fraction of breadfruit leaves has a better ability to reduce oedema than the ethanol fraction. This makes it a better choice for isolating bioactive chemicals (Widhihastuti *et al*., 2021). A result of its high volatility, ethyl acetate makes it possible to separate components more effectively without damaging the intended active substances (Listina, 2024). The use of ethyl acetate increases the extraction yield and guarantees that the extracted anti-inflammatory chemicals will perform as well as possible in further studies.

Anti-inflammatory agents are crucial in managing inflammation and accelerating recovery, particularly during the COVID-19 pandemic when controlling inflammation has become a focus due to its role in disease progression. Ensuring drug safety, especially concerning toxicity and side effects, is vital in pharmaceutical development (Yang *et al.,* 2018). Clinical studies are necessary to establish acceptable limits for users, which will, in turn, support the practice of alternative medicine (Bouyahya *et al.,* 2017). Pharmacokinetic screening can determine whether a compound can interact with specific receptors in organs. Early pharmacokinetic profile analysis has been shown to prevent failures in later stages of drug development (Daina *et al.,* 2017). The estimated LD_{50} value is a key indicator of the potential toxicity of new compounds (Banerjee *et al.,* 2018).

In silico testing refers to experiments conducted using computer simulations, typically to explore the interaction between a substance and a target molecule, such as a receptor. This approach allows researchers to use computational techniques to analyze and predict the phytochemical content of substances (Setiawan & Istyastono, 2015). One key focus of such studies is the transcription factor NF-kB (Nuclear Factor kappa B), which plays a crucial role in regulating inflammation and immunity (Mulero *et al.,* 2019). Inhibitors of kappa B kinase (IKK) are master regulators of the nuclear factor kappa B (NF-kB) cascade, relaying pro-inflammatory and growth-stimulating signals that support vital cellular processes (Gamble *et al.,* 2012). IKK operates by disrupting the nucleocytoplasmic binding mechanism of NF-kB and inhibiting its DNA binding activity (Huxford *et al.,* 2011). Within the IKK complex, IkB kinase beta (IKKβ) is the primary subunit responsible for triggering the canonical activation of NF-kB

(Gan *et al.,* 2023). This study aims to evaluate the anti-inflammatory potential of bioactive compounds from *B. gymnorhiza* leaves extracted with ethyl acetate using in silico analysis. The goal is to assess drug likeness, absorption, distribution, metabolism, excretion, and toxicity as a preliminary step in drug development.

METHODS

Chemical Profile Screening

The first step involved a literature search to analyze the GC-MS results of *B. gymnorhiza* leaf extracts obtained using ethyl acetate as a solvent. Literature searches were conducted using internationally accredited electronic databases such as ScienceDirect, PubMed, SpringerLink, and Nature, focusing on publications from 2010 to 2023. The main search keywords included *B. gymnorhiza* leaves combined with anti-inflammatory properties, ethyl acetate, and GC-MS analysis. To refine the search, terms like *B. gymnorhiza* leaf, ethyl acetate, GC-MS, and anti-inflammatory were used. Inclusion criteria for the articles selected were as follows: studies on the extraction of *B. gymnorhiza* leaves, studies involving ethyl acetate as the solvent, GC-MS analysis of leaf extracts, publications from 2010 to 2023, and descriptive research designs (both quantitative and qualitative) in full-text journal articles. The selected articles were then analyzed, compared, and synthesized to conclude the literature review.

Ethyl acetate was chosen due to its semi-polar nature. As noted by Artini *et al.* (2013), ethyl acetate, with a solubility index of 4.4, effectively extracts both polar and nonpolar compounds. The GC-MS analysis of *B. gymnorhiza* allowed for the identification of various compounds present in the ethyl acetate extract. GC-MS has high sensitivity, enabling the detection of compounds even in small concentrations. Unlike LC-MS-MS, which is limited by sample volatility and stability, GC-MS does not require sample derivatization and is ideal for analyzing volatile components with high accuracy (Zhang *et al.,* 2018).

Computational Analysis (In Silico Testing)

The in-silico approach is a modern and necessary strategy to adhere to the 3Rs principle (Reduction, Refinement, and Replacement) in research, minimizing the use of laboratory animals (Huang *et al.,* 2021). The in-silico tests began with obtaining the canonical SMILES of the bioactive compounds from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) (Riyadi *et al.,* 2021). PubChem is a reliable tool for identifying molecular structures and ensuring accuracy (Hahnke *et al.,* 2018). These canonical SMILES were then used for further analysis in the PASS (Prediction of Activity Spectra for Substances) server (http://www.pharmaexpert.ru/passonline/index.php) to estimate the potential of biomaterial development (Riyadi *et al.,* 2020). SwissADME (http://www.swissadme.ch/) was used to predict the absorption, distribution, metabolism, and excretion (ADME) properties of the compounds (Riyadi *et al.,* 2021). ProTox III (https://tox.charite.de/protox3/index.php) was utilized as a reference in calculating the toxicity levels of the compounds (Riyadi *et al.,* 2023).

Molecular docking was performed to determine the anti-inflammatory activity of the compounds. The materials used for docking included the IKKβ protein, sulfasalazine (a standard drug), and compounds that met the drug-likeness criteria according to Lipinski, Ghose, Veber, Egan, and Muegge's rules. Inhibiting IKKβ activity is a new approach, as compounds from *B. gymnorhiza* leaf extracts could potentially suppress inflammation. Protein preparation was conducted using the protein data bank (https://www.rcsb.org/), with the 3D structures of compounds sourced from PubChem. Ligand preparation and processing were carried out using PyMOL (https://pymol.org/) and Discovery Studio V20. Finally, molecular docking was performed using the PyRx tool.

RESULTS AND DISCUSSION

The literature search revealed the chemical composition of *B. gymnorhiza*

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Table 1 Chemical structures of bioactive compounds in *B. gymnorhiza* leaves ethyl acetate extract based on GC-MS analysis

Tabel 1 Struktur kimia senyawa bioaktif dalam ekstrak etil asetat daun *B. gymnorhiza* berdasarkan analisis GC-MS

Chemical compounds	Area (%)	Pubchem CID	Molecular formula	Canonical SMILES
Palmitic acid	26.84	985	$C_{16}H_{32}O_2$	CCCCCCCCCCCCCCCC(=O)O
Tetracontane	13.39	20149	$C_{40}H_{82}$	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
Neophytadiene	11.83	10446	$C_{20}H_{38}$	CC(C)CCCC(C)CCCC(C)CCCC(=C)C=C
7-Tetradecenal	4.82	5283367	$C_{14}H_{26}O$	CCCCCCC=CCCCCCC=O
Tetracosane	4.77	12592	$C_{24}H_{50}$	CCCCCCCCCCCCCCCCCCCCCCCCCC
Squalene	3.95	638072	$C_{30}H_{50}$	CC(=CCCC(=CCCC(=CCCC=C(C)CCC=C(C)CCC=C(C)C)C)C)C
Stearic acid	2.89	5281	$C_{18}H_{36}O_2$	CCCCCCCCCCCCCCCCCC(=O)O
Tetratetracontane	2.44	23494	$C_{44}H_{90}$	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
Phytol	1.64	5280435	$C_{20}H_{40}O$	CC(C)CCCC(C)CCCC(C)CCCC(=CCO)C
Myristic acid	1.22	11005	$C_{14}H_{28}O_2$	CCCCCCCCCCCCCC(=O)O
Elemicin	1.19	10248	$C_{12}H_{16}O_3$	COC1=CC(=CC(=C1OC)OC)CC=C
4,8,12,16-Tetramethyl heptadecan-4-olide	1.02	567149	$C_{21}H_{40}O_2$	CC(C)CCCC(C)CCCC(C)CCCC1(CCC(=O)O1)C
Linoleic acid	0.86	5280450	$C_{18}H_{32}O_2$	CCCCCC=CCC=CCCCCCCCC(=O)O
Heneicosane	0.83	12403	$C_{21}H_{44}$	CCCCCCCCCCCCCCCCCCCCC
Lauric acid	0.76	3893	$C_{12}H_{24}O_2$	CCCCCCCCCCCC(=O)O

Data from Dahibhate *et al.* (2022)

extracted with ethyl acetate (3×150 mL) using the Soxhlet extraction method. Extraction method used Soxhlet. Table 1 shows the chemical compositions of palmitic acid (26.84%), tetracontane (13.39%), neophytadiene (11.83%), 7-tetradecenal (4.82%), tetracosane (4.77%), squalene (3.95%), stearic acid (2.89%), tetratetracontane (2.44%), phytol (1.64%), myristic acid (1.22%), elemicin (1.19%), 4,8,12,16-tetramethyl heptadecan-4-olide (1.02%), linoleic acid (0.86%), heneicosane (0.83%), and lauric acid (0.76%) (Dahibhate *et al.,* 2022). GC-MS is highly effective in separating volatile compounds (Mondello *et al.,* 2008) and offers more concise and readable mass spectra compared to LC-MS (Smith *et al.,* 2006). Additionally, GC-MS provides qualitative and quantitative data on the number and type of compounds in a sample, as well as a similarity index for compounds present (Makin *et al.,* 2023).

Palmitic acid (PA), a saturated fatty acid, is known to trigger inflammatory responses through various signaling pathways, some of which may interact or be specific to certain cell types (Korbecki & Bajdak, 2019; Hwangbo *et al.,* 2021). Neophytadiene is a nutraceutical that shows anti-inflammatory potential. Neophytadiene, a nutraceutical

derived from *Turbinaria ornata* has demonstrated anti-inflammatory potential, as shown in studies where it suppressed LPS-induced inflammatory reactions in RAW 264.7 macrophages and Sprague Dawley rats (Bhardwaj *et al.,* 2020). N-tetracosane, found in the methanol extract of *Leea indica* leaves, is among the best ligands for anti-inflammatory activity against prostaglandin D2 (PGD2) synthase, which is linked to hair loss (Sakib *et al.,* 2021). Squalene, extracted from virgin olive oil, has preventive effects against skin damage and possesses anti-inflammatory properties (Sánchez *et al.,* 2018). Stearic acid has shown anti-fibrotic effects by reducing cells producing alpha-smooth muscle actin and transforming growth factor beta-1, thereby inhibiting leucocyte collection and NF-kB activation in chronic liver damage models (Pan *et al.,* 2010). Tetratetracontane also exhibits anti-inflammatory activity in LPS-stimulated RAW 264.7 macrophages (Ano *et al.,* 2015). Phytol, found in the leaves of *Corchorus olitorius*, is a pharmacologically active compound with a range of activities, including anti-inflammatory, anticancer, and hepatoprotective effects (Shariare *et al.,* 2021). Lauric and myristic acids have demonstrated anti-inflammatory effects (Basson *et al.,* 2021), while elemicin, found in essential oils, also shows anti-inflammatory and cytotoxic activity (Wang *et al.,* 2016). Linoleic acid acts as a potent neuroprotective and anti-inflammatory agent, particularly in Parkinson's disease models (Gil *et al.,* 2022), and can be metabolized into 13-hydroxy-octadecadienoic acid (13-HODE), which modulates inflammation through various signaling pathways (Spector & Kim, 2015). Furthermore, linoleic acid can be converted into endocannabinoids, which regulate inflammation and immune responses through interaction with cannabinoid receptors CB1 and CB2 (Di Marzo, 2008). Linoleic acid contributes to the production of prostaglandin E2 (PGE2), which is known for its anti-inflammatory effects (Calder, 2013). Additionally, linoleic acid can inhibit the production of PGE2 by suppressing the expression of Cyclooxygenase-2 (COX-2), potentially reducing the inflammatory effects typically associated with PGE2. Despite its anti-inflammatory potential, linoleic acid can also be metabolized into arachidonic acid (ARA), a pro-inflammatory metabolite (Sergeant *et al.,* 2023). Heneicosane is the main component of *C. tinctorius* flower essential oil that has anti-inflammatory activity. Flower essential oil also has anti-inflammatory activity. Saturated fatty acids such as stearic, linoleic, oleic, and palmitic acids make up more than 80% of overall plasma-free fatty acids, and elevated levels of these acids are linked to inflammation, insulin resistance, and various obesity-related disorders (Almeida *et al.,* 2002; Boden, 2011).

Biological Potential as an Anti-inflammatory

The PASS server is a tool designed to predict the biological activity spectrum of organic compounds based on their structural formulas. With an accuracy rate above 95%, the PASS server provides valuable insights into the potential biological activities of new compounds, helping researchers prioritize candidates for further testing. The server estimates the probability of activity (Pa) and inactivity (Pi) for over 4,000 types of biological activities, allowing users to gauge the confidence and potential errors in the predictions (Filimonov *et al.,* 2014). The results of the PASS analysis on the anti-inflammatory potential of *B. gymnorhiza* leaf compounds are shown in Table 2.

Table 2 reveals that 7 out of 15 compounds have a Pa value greater than 0.7, indicating a high likelihood of anti-inflammatory activity, though they may share similarities with other compounds. Four compounds have Pa values between 0.5 and 0.7, suggesting probable activity with less similarity to known drugs. The remaining four compounds have Pa values below 0.5, indicating a lack of predicted activity (Lagunin *et al.,* 2000). The PASS platform allows for the prediction of possible biological activity profiles of organic compounds, such as drugs with molecular masses ranging from 50 to 1,250 Dalton, containing at least three carbon atoms, and being single-component and uncharged, based on their molecular formulas (Filimonov *et al.,* 2014).

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Table 2 Chemical profile and anti-inflammatory potential of compounds in *B. gymnorhiza* leaves
Tabel 2 Profil kimia dan potensi antiinflamasi senyawa dalam daun *B. gymnorhiza*

Chemical compounds	Probability value		Biological potential
	Active	Inactive	
Palmitic acid	0.727	0.002	Anti-inflammatory
Tetracontane	0.585	0.004	Anti-inflammatory
Neophytadiene	0.286	0.082	Anti-inflammatory
7-Tetradecenal	0.427	0.017	Anti-inflammatory
Tetracosane	0.585	0.004	Anti-inflammatory
Squalene	0.701	0.016	Anti-inflammatory
Stearic acid	0.727	0.002	Anti-inflammatory
Tetratetracontane	0.585	0.004	Anti-inflammatory
Phytol	0.299	0.080	Anti-inflammatory
Myristic acid	0.727	0.002	Anti-inflammatory
Elemicin	0.453	0.072	Anti-inflammatory
4,8,12,16-Tetramethyl heptadecan-4-olide	0.748	0.010	Anti-inflammatory
Linoleic acid	0.730	0.012	Anti-inflammatory
Heneicosane	0.585	0.004	Anti-inflammatory
Lauric acid	0.727	0.002	Anti-inflammatory

Physicochemical Properties, Drug-likeness, and Bioavailability

Physicochemical properties can be used for predicting the ecological impact, ecotoxicity, and human toxicity of chemicals. The physicochemical and bioavailability results for several bioactive compounds found in *B. gymnorhiza* leaves are presented in Table 3 and Figure 1.

Drug-likeness is a qualitative criterion used in drug development to assess how similar a compound is to an established drug, particularly in terms of bioavailability. This assessment is based on the molecular structure before the substance undergoes manufacturing and testing. Five key rules are applied to evaluate drug-likeness: Lipinski, Ghose, Veber, Egan, and Muegge rules. The drug-likeness results for *B. gymnorhiza* leaves are detailed in Table 4.

The physicochemical analysis shows that all compounds, except tetracontane and tetratetracontane, comply with Lipinski's rule. Lipinski's rules define five criteria that characterize small molecules based on their physicochemical profiles. A candidate drug must meet these criteria to be optimally absorbed by the body. According to Lipinski's rule, a drug-like compound should have a molecular mass below 500 Dalton, fewer than five hydrogen bond donors, molar refractivity between 40–130, fewer than ten hydrogen bond acceptors, and a log P value smaller than 5. Additionally, nitrogen and oxygen atoms serve as hydrogen bond acceptors, while nitrogen and oxygen with at least one hydrogen serve as hydrogen bond donors. Furthermore, aliphatic fluorine is an acceptor, while alanine nitrogen is neither a donor nor an acceptor.

Ghose's rule stipulates that a compound should have a molecular weight between 160-480 Dalton, an atomic number between 20-70, refractivity between 40-130, and a log P value between -0.4 to 5.6. Veber's rule requires a TPSA value under 140 and no more than 10 rotatable bonds, with fewer than 12 hydrogen bond donors and acceptors combined. Egan's rule specifies a WLogP value under 5.88 and a TPSA value below 131.6. Muegge's rule

Table 3 Physicochemical properties and bioavailability of compounds in *B. gymnorhiza* leaves
Tabel 3 Sifat fisikokimia dan bioavailabilitas senyawa dalam daun *B. gymnorhiza*

Chemical compounds	MW	HA	AHA	RB	HBA	HBD	MR	TPSA	BA
Palmitic acid	256.42	18	0	14	2	1	80.80	37.30	0.85
Tetracontane	563	40	0	37	0	0	194.39	0.00	0.17
Neophytadiene	278.52	20	0	13	0	0	97.31	0.00	0.55
7-Tetradecenal	210	15	0	11	1	0	69.14	17.07	0.55
Tetracosane	338.65	24	0	21	0	0	117.48	0.00	0.55
Squalene	410.72	30	0	15	0	0	143.48	0.00	0.55
Stearic acid	284.48	20	0	16	2	1	90.41	37.30	-
Tetratetracontane	619.19	44	0	41	0	0	231.62	0.00	0.17
Phytol	296.53	21	0	13	1	1	98.94	20.23	0.55
Myristic acid	228.37	16	0	12	2	1	71.18	37.30	0.85
Elemicin	208.25	15	6	5	3	0	60.02	27.69	0.55
4,8,12,16-Tetramethyl heptadecan-4-olide	324.54	23	0	12	2	0	102.27	26.30	0.55
Linoleic acid	280.45	20	0	14	2	1	89.46	37.30	0.85
Heneicosane	296.57	21	0	18	0	0	103.06	0.00	0.55
Lauric acid	200.32	14	0	10	2	1	61.57	37.30	0.85

MW = molecular weight; HA = the number of heavy atoms; AHA = the number of aromatic heavy atoms; RB = the number of rotatable bonds; HBA = the number of hydrogen bond acceptors; HBD = the number of hydrogen bond donors; MR = molar refractivity; TPSA = the topological polar surface area; BA = bioavailability.

calls for a molecular weight between 200-600 Dalton, no more than seven aromatic rings, more than one heteroatom, fewer than 10 hydrogen bond acceptors, fewer than five hydrogen bond donors, an XLogP between -2 to 5, fewer than 15 rotatable bonds, and a TPSA value under 150 (Lipinski *et al.,* 2001).

Palmitic acid, 7-tetradecenal, myristic acid, elemicin, and lauric acid satisfy both Ghose and Egan's rules, while elemicin and lauric acid also comply with Veber's and Muegge's rules. According to Benet *et al.* (2016), physicochemical properties are essential in determining whether a compound can be classified as orally active in humans, allowing it to be categorized as an oral drug. Chander *et al.* (2017) reported that 95% of approved drugs exhibit physicochemical properties within the following ranges: molecular weight of 130-725 Da, hydrogen bond acceptors between 2-20, hydrogen bond donors between 0-6, log P value between -2 to 6.5, and 0-15 rotatable bonds. Therefore, the chemical profile of *B. gymnorhiza* leaves falls within these ranges, classifying them as suitable for oral administration.

Bioavailability is a key factor in understanding a compound's absorption, tissue distribution, metabolism, and excretion in the human body. Bioavailability is influenced by molecular size, flexibility, polarity, saturation, lipophilicity, and solubility. The bioavailability scores for palmitic acid, myristic acid, linoleic acid, and lauric acid were 0.85, while neophytadiene, 7-tetradecenal, tetracosane, squalene, phytol, elemicin, 4,8,12,16-tetramethyl heptadecan-4-olide, and heneicosane had scores of 0.55. Tetracontane and tetratetracontane had scores of 0.17. According to Pires *et al.* (2015), a compound is considered to have good bioavailability if its value is 0.55 or higher. Riyadi *et al.* (2023) suggest that bioavailability is crucial for calculating the effective dosage of nutraceuticals to achieve the desired pharmacological effects while minimizing side effects and toxicity.

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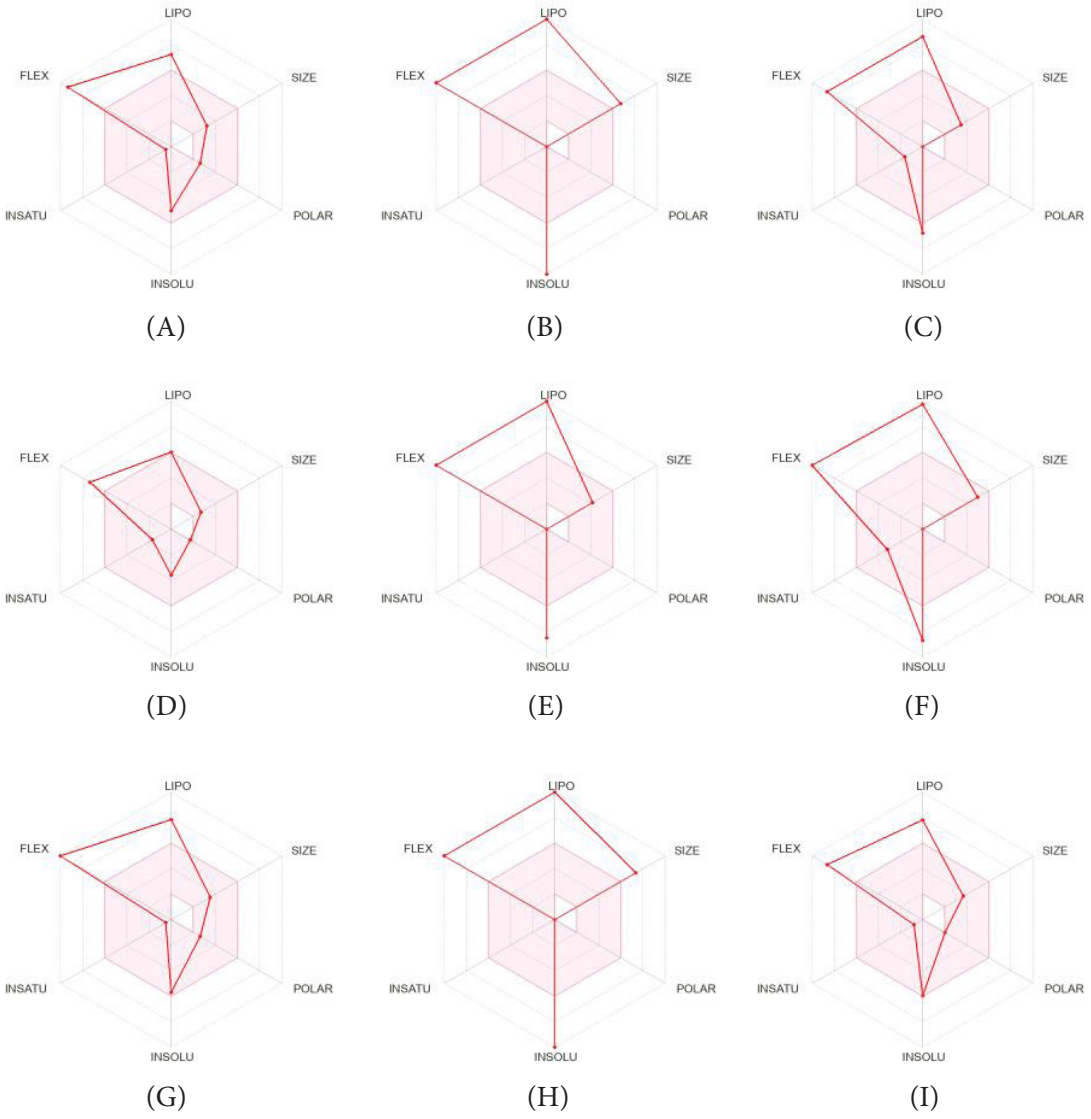

Figure 1 Bioavailability radar of compounds in B. gymnorhiza leaves: (A) palmitic acid, (B) tetracontane, (C) neophytadiene, (D) 7-tetradecenal, (E) tetracosane, (F) squalene, (G) stearic acid, (H) tetratetracontane, (I) phytol, (J) myristic acid, (K) elemicin, (L) 4,8,12,16-tetramethyl heptadecan-4-olide, (M) linoleic acid, (N) heneicosane, (O) lauric acid

Gambar 1 Radar bioavailabilitas senyawa dalam daun B. gymnorhiza: (A) asam palmitat, (B) tetrakontana, (C) neofitadiena, (D) 7-tetradekenal, (E) tetrakosana, (F) squalena, (G) asam stearat, (H) tetratetrakontana, (I) fitol, (J) asam miristat, (K) elemisin, (L) 4,8,12,16-tetrametil heptadekan-4-olida, (M) asam linoleat, (N) heneikosanea, (O) asam laurat

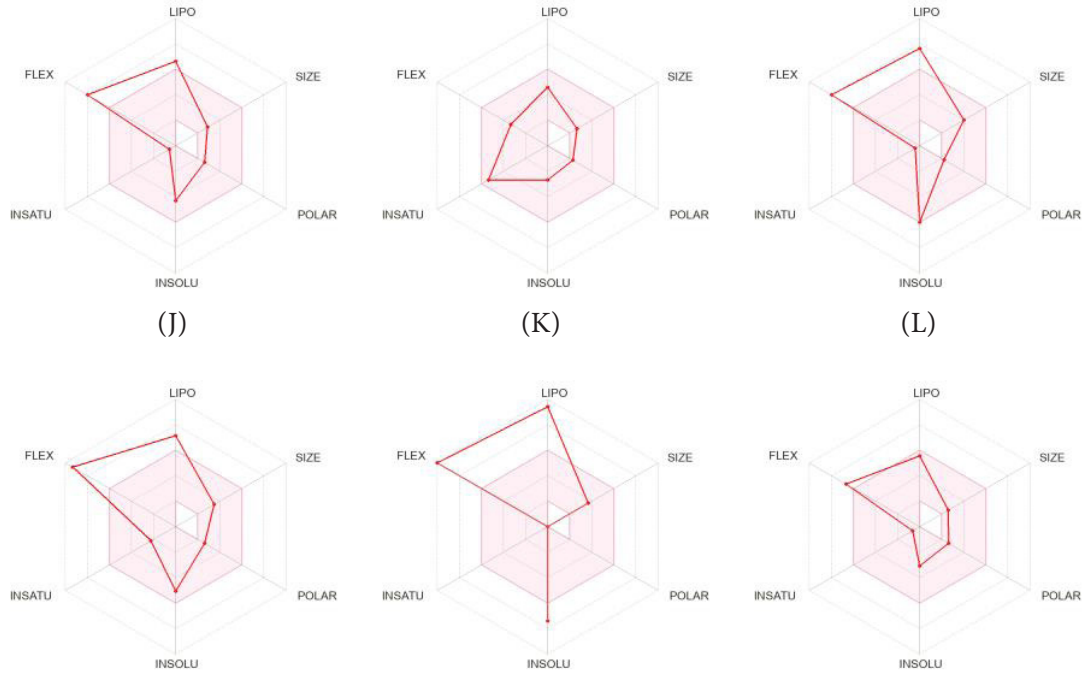

Figure 1 (J) myristic acid, (K) elemicin, (L) 4,8,12,16-tetramethyl heptadecan-4-olide, (M) linoleic acid, (N) heneicosane, (O) lauric acid

Gambar 1 (J) asam miristat, (K) elemisin, (L) 4,8,12,16-tetrametil heptadekan-4-olida, (M) asam linoleat, (N) heneikosanea, (O) asam laurat

Table 4 Druglikeness evaluation of compounds in *B. gymnorhiza* leaves
Tabel 4 Evaluasi kemiripan obat dari senyawa dalam daun *B. gymnorhiza*

Chemical compounds	Lipinski	Ghose	Veber	Egan	Muegge
Palmitic acid	√	√	-	√	-
Tetracontane	-	-	-	-	-
Neophytadiene	√	-	-	-	-
7-Tetradecenal	√	√	-	√	-
Tetracosane	√	-	-	-	-
Squalene	√	-	-	-	-
Stearic acid	√	-	-	-	-
Tetratetracontane	-	-	-	-	-
Phytol	√	-	-	-	-
Myristic acid	√	√	-	√	-
Elemicin	√	√	√	√	√
4,8,12,16-Tetramethyl heptadecan-4-olide	√	-	-	-	-
Linoleic acid	√	-	-	-	-
Heneicosane	√	-	-	-	-
Lauric acid	√	√	√	√	√

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Table 5 Pharmacokinetic parameters of compounds in *B. gymnorhiza* leaves
Tabel 5 Parameter farmakokinetik senyawa dalam daun *B. gymnorhiza*

Chemical compounds	GI absorption	BBB	Substrate P-gp	Inhibitor			Log Kp
				CYP1A2	CYP2C19	CYP2C9	
Palmitic acid	High	√	-	√	-	√	-2.77
Tetracontane	Low	-	√	-	-	-	5.37
Neophytadiene	Low	-	√	-	-	√	-1.17
7-Tetradecenal	High	-	-	√	-	-	-4.00
Tetracosane	Low	-	√	√	-	-	0.59
Squalene	Low	-	-	-	-	-	-0.58
Stearic acid	High	-	-	√	-	-	-2.19
Tetratetracontane	Low	-	√	-	-	-	6.57
Phytol	Low	-	√	-	-	√	-2.29
Myristic acid	High	√	-	√	-	-	-3.35
Elemicin	High	√	-	√	-	-	-5.77
4,8,12,16-Tetramethyl heptadecan-4-olide	Low	-	-	√	-	√	-2.70
Linoleic acid	High	-	-	√	-	√	-3.05
Heneicosane	Low	-	-	√	-	-	-0.31
Lauric acid	High	√	-	-	-	-	-4.54

GI is gastrointestinal absorption, BBB is blood-brain barrier permeability, Substrate P-gp refers to P-glycoprotein substrate permeability, and Log Kp is skin permeation coefficient.

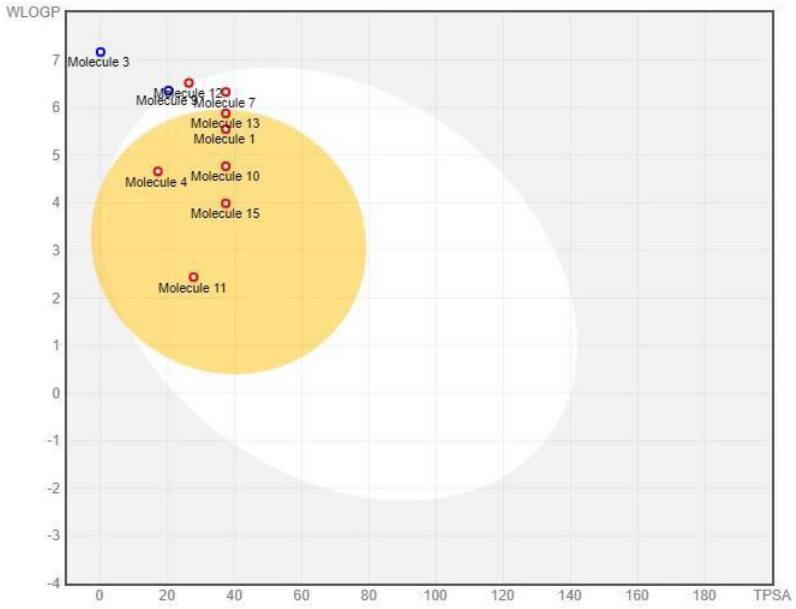

Figure 2 Boiled egg model for bioactive compounds in *B. gymnorhiza* leaves
Gambar 2 Model telur rebus untuk senyawa bioaktif dalam daun *B. gymnorhiza*

Pharmacokinetics

Pharmacokinetics is used to determine the course of a drug from the time it is taken to the time it leaves the excretory organs in the human body. This journey starts from adsorption, distribution, metabolism, and excretion. The pharmacokinetic parameters of bioactive compounds on *B. gymnorhiza* leaves are shown in Table 5.

Permeability glycoprotein (P-gp) is an efflux transporter critical in drug delivery to various organs. P-gp controls the rate at which cells absorb foreign substances (Finch & Pillans, 2014). Identifying whether a compound is a P-gp substrate is essential to understanding its distribution and elimination. According to SwissADME results, all compounds except tetracontane, neophytadiene, tetracosane, tetratetracontane, and phytol are not substrates of P-gp, meaning P-gp does not significantly influence their distribution or elimination. P-gp substrates are usually expelled from cells by P-gp, primarily out of the body or into the lumen of organs like the intestine (Sharom, 2011).

Cytochrome P450 (CYP) isoenzymes play a vital role in drug metabolism and elimination (Testa & Krämer, 2007). Inhibition of these isoenzymes can prevent effective drug elimination, leading to toxicity (Ononamadu & Ibrahim, 2021). The analysis showed that none of the compounds inhibited CYP2C19. Tetracontane, neophytadiene, squalene, tetratetracontane, phytol, and lauric acid were also non-inhibitors of CYP1A2. Tetracontane, 7-tetradecenal, tetracosane, squalene, stearic acid, tetratetracontane, myristic acid, elemicin, heneicosane, and lauric acid did not inhibit CYP2C9, suggesting these compounds can be effectively metabolized by the liver and easily excreted.

Skin permeability analysis revealed that palmitic acid, 7-tetradecenal, myristic acid, elemicin, 4,8,12,16-tetramethyl heptadecan-4-olide, linoleic acid, and lauric acid exhibit good skin permeability, indicated by their Log Kp values of <-2.5 cm/s. The more negative the log Kp (with Kp in cm/s), the higher the molecule's skin permeability. This parameter is critical in drug discovery and development, particularly for transdermal drug delivery. According to Pires *et al.* (2015), a compound with log Kp > -2.5 cm/s is considered to have low skin permeability, whereas values below -2.5 cm/s indicate good skin permeability.

The BOILED-Egg model is a predictive tool used to estimate a compound's ability to be absorbed in the gastrointestinal (GI) tract and penetrate the blood-brain barrier (BBB). In the BOILED-Egg diagram (Figure 2), compounds that can cross the BBB without the mediation of P-gp are indicated in the red circle, while those in the yellow area can passively cross the BBB (Geldenhuys *et al.*, 2015). Gastrointestinal absorption refers to how well a drug is absorbed in the gut, and high absorption indicates effective distribution throughout the body, including to the brain. The BBB is a critical mechanism in drug delivery, helping to reduce side effects and toxicity or enhance the efficacy of drugs targeting the brain. Table 5 shows that compounds such as palmitic acid, 7-tetradecenal, myristic acid, elemicin, linoleic acid, and lauric acid are well absorbed in the GI tract and can cross the BBB without requiring P-gp mediation. In contrast, compounds like tetracontane, neophytadiene, tetracosane, tetratetracontane, and phytol are unlikely to be absorbed in the GI tract via P-gp.

Toxicity

Toxicity refers to the degree to which a substance is harmful to living organisms. Toxicity testing is crucial for assessing the potential hazards of compounds to both users and the environment. The results of toxicity tests on several bioactive compounds from *B. gymnorhiza* leaves are presented in Table 6.

LD_{50}, or the median lethal dose, is a standard measure of toxicity, representing the dose required to cause death in 50% of test subjects. According to Gadaleta *et al.* (2019), LD_{50} is the concentration of a toxic substance that results in 50% mortality in a specific type of animal and is often the first test conducted for any chemical to estimate its potential danger to humans.

Toxicity classes are categorized according to the Globally Harmonized System of Classification and Labeling of Chemicals (GHS), with LD_{50} values expressed in mg/

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Table 6 Toxicity assessment of compounds in *B. gymnorhiza* leaves
Tabel 6 Penilaian toksisitas senyawa dalam daun *B. gymnorhiza*

Chemical compounds	LD50	Class	Hepatotoxicity		Carcinogenicity		Immunotoxicity	
			+/-	Probability	+/-	Probability	+/-	Probability
Palmitic acid	900	4	-	0.52	-	0.63	-	0.99
Tetracontane	750	3	-	0.74	-	0.58	-	0.98
Neophytadiene	55,050	6	-	0.79	-	0.73	-	0.99
7-Tetradecenal	55,000	5	-	0.72	-	0.60	-	0.91
Tetracosane	750	3	-	0.74	-	0.58	-	0.98
Squalene	55,000	5	-	0.79	-	0.76	-	0.99
Stearic acid	900	4	-	0.52	-	0.63	-	0.99
Tetratetracontane	750	3	-	0.74	-	0.58	-	0.98
Phytol	55,000	5	-	0.79	-	0.76	-	0.99
Myristic acid	900	4	-	0.52	-	0.63	-	0.99
Elemicin	11,930	4	-	0.63	+	0.57	-	0.58
4,8,12,16-Tetramethyl heptadecan-4-olide	44,400	5	-	0.70	-	0.69	-	0.99
Linoleic acid	110,000	6	-	0.55	-	0.64	-	0.96
Heneicosane	750	3	-	0.74	-	0.58	-	0.98
Lauric acid	900	4	-	0.52	-	0.63	-	0.99

kg. Class I substances are fatal if swallowed ($LD_{50} \leq 5$ mg/kg), Class II substances are fatal if swallowed ($5 < LD_{50} \leq 50$ mg/kg), Class III substances are toxic if swallowed ($50 < LD_{50} \leq 300$ mg/kg), Class IV substances are harmful if swallowed ($300 < LD_{50} \leq 2{,}000$ mg/kg), Class V substances may be harmful if swallowed ($2{,}000 < LD_{50} \leq 5{,}000$ mg/kg), and Class VI substances are relatively harmless if swallowed ($LD_{50} > 5{,}000$ mg/kg).

ProTox III predicted that the bioactive compounds in *Bruguiera gymnorhiza* leaves fall into classes 3, 4, 5, and 6. Tetracontane, tetracosane, tetratetracontane, and heneicosane are classified as Class 3, indicating that they are toxic if swallowed. Compounds such as 7-tetradecenal, squalene, phytol, and 4,8,12,16-tetramethyl heptadecan-4-olide are classified as Class 5, meaning they may be harmful if swallowed. Neophytadiene and linoleic acid are in Class 6, which means they are non-toxic. Palmitic acid, stearic acid, myristic acid, elemicin, and lauric acid are classified as Class 4, indicating that they are harmful if swallowed. However, research by Burdock & Carabin (2007), showed that the LD_{50} value in oral studies for lauric acid can reach 10,000 mg/kg body weight, and for palmitic acid, it reaches 5,000 mg/kg. Additionally, the European Chemical Agency reported that the LD_{50} of stearic acid is above the tested dose (up to >6,000 mg/kg body weight).

This discrepancy between the LD_{50} values from in silico methods and experimental literature on fatty acids could be attributed to variations in test methods, data quality, and other factors affecting toxicity. In silico methods, such as ProTox III, are predictive and have high validity in estimating compound toxicity. ProTox III relies on a collection of pharmacophores based on protein-ligand relationships to predict toxicity. These methods can also predict potential damage to specific organs and chronic health conditions that may arise from excessive fatty acid consumption. ProTox-II's hepatotoxicity prediction analysis showed an accuracy rate

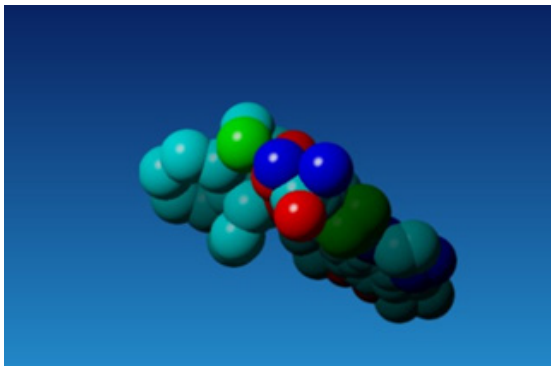
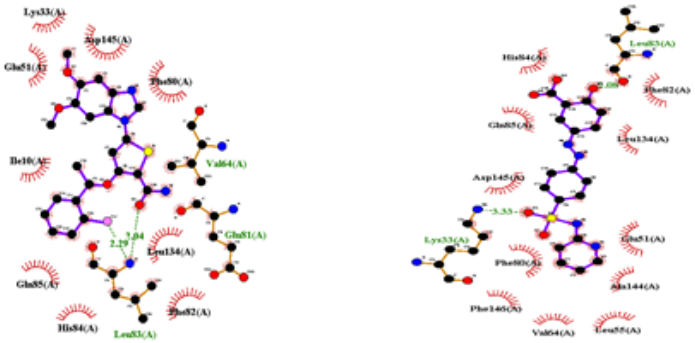


Figure 3 Visualization of IKKβ molecular docking with thiophene carboxamide inhibitor
Gambar 3 Visualisasi *molecular docking* IKKβ dengan inhibitor tiofen karboksamida



(A) (B)

Figure 4 Docking results visualization: (A) thiophene carboxamide inhibitor, (B) sulfasalazine
Gambar 4 Visualisasi hasil *docking*: (A) inhibitor tiofena karboksamida, (B) sulfasalazin

of 86% in external validation and a reliability of 82% in cross-validation (Chen *et al.,* 2016). However, it is essential to carefully evaluate these predictions and combine them with experimental data for more accurate results.

Some fatty acids and their salts have been approved by the United States Food and Drug Administration (FDA) as food additives for direct human consumption. The use of ProTox III is justified as the previous version of the ProTox web server has proven effective in real-world validation (Arulanandam *et al.,* 2022). ProTox has also been implemented as a lecture module in various universities (Giorgini *et al.,* 2023).

Estimates for hepatotoxicity and immunotoxicity targets of compounds like palmitic acid, tetracontane, neophytadiene, 7-tetradecenal, tetracosane, squalene, stearic acid, tetratetracontane, phytol, myristic acid, elemicin, 4,8,12,16-tetramethyl heptadecan-4-olide, linoleic acid, heneicosane, and lauric acid indicate inactivity. This suggests that these chemical compounds do not affect hepatotoxicity or immunotoxicity. The prediction tests showed inactivity with probability values between 0.70 and 1.00. The carcinogenic prediction was active for elemicin, with a probability value of 0.57, indicating a higher accuracy as it exceeds 0.5. This suggests that carcinogenic properties may be present in these compounds.

Molecular Docking

Molecular docking is a computational simulation used to calculate the bond between a ligand and a receptor (Pratama *et al.,* 2017). The purpose of molecular docking is to predict the structure of the ligand-receptor complex and assess binding affinity (Meng *et al.,* 2011). This method is widely used in drug discovery (Pinzi & Rastelli, 2019).

Molecular docking was performed using the IκB kinase β (IKKβ) receptor

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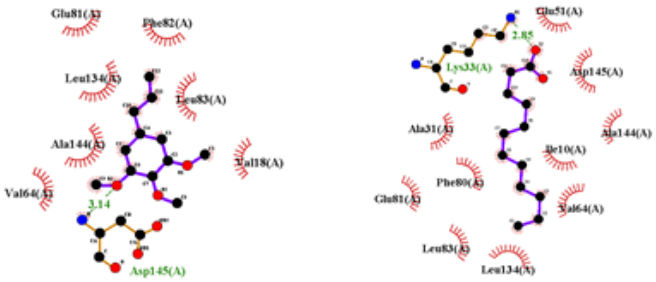


(A) (B)

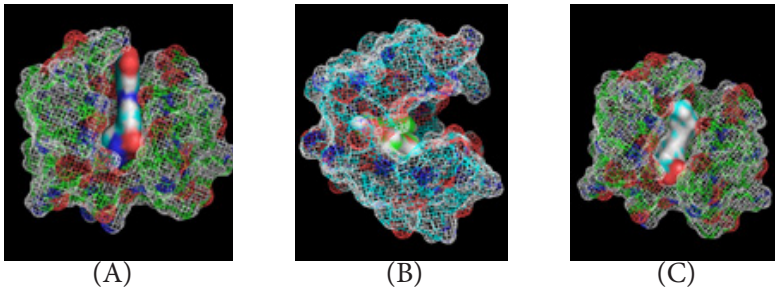
Figure 5 Docking results visualization: (A) elemicin, (B) lauric acid

Gambar 5 Visualisasi hasil *docking*: (A) elemisin, (B) asam laurat

(PDB ID: 2I40) downloaded from the RCSB PDB, which was bound with a thiophene carboxamide inhibitor. The test compounds were elemicin, lauric acid, and sulfasalazine as positive control. Elemicin and lauric acid were selected because they meet the drug-likeness criteria of Lipinski, Ghose, Veber, Egan, and Muegge, suggesting they can be easily developed and exhibit good activity. Sulfasalazine was chosen because it is a standard anti-inflammatory drug that reduces the production of inflammatory mediators. Sulfasalazine inhibits IKK activation, which is part of a larger inflammatory signaling pathway. IKK activates the IκB protein, allowing NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) to enter the cell nucleus and trigger the expression of pro-inflammatory genes. By inhibiting IKK, sulfasalazine reduces the expression of genes involved in the inflammatory process. Sulfasalazine has been shown to block NF-κB activation triggered by phorbol myristate acetate (PMA), tumor necrosis factor-α (TNF-α), or lipopolysaccharide (LPS), preventing the phosphorylation of IκBα and inhibiting IKKβ (Wahl *et al.,* 1998).

Target validation was carried out to ensure that the method meets the validity requirements and can be used for testing other compounds without causing errors or confusion. Validation was done by redocking the receptor and reference ligand. The method is considered valid if the resulting Root Mean Square Deviation (RMSD) value is ≤ 2 Å (Trott & Olson, 2010). The validation results of 2I40 receptor docking with the reference ligand thiophene carboxamide inhibitor showed valid results with an RMSD value of 2.0 Å. The shape and position of the validation can be observed in Figure 3.

Sulfasalazine has -8.4 kcal/mol and was used as a positive control because it is a disease-modifying antirheumatic drug (DMARD) that blocks IKKα and IKKβ by preventing adenosine triphosphate (ATP) binding (Weber *et al.,* 2000). The molecular formula of sulfasalazine is C18H14N4O5S with PubChem CID 5339. Sulfasalazine forms hydrogen bonds with the amino acid residues LEU83 and LYS33. The reference ligand, thiophene carboxamide inhibitor,



(A) (B) (C)

Figure 6 Binding site visualization: (A) sulfasalazine, (B) elemicin, (C) lauric acid

Gambar 6 Visualisasi situs pengikatan: (A) sulfasalazin, (B) elemisin, (C) asam laurat

also forms a hydrogen bond with LEU83. The similarity in hydrogen bonds suggests that both compounds may have similar or nearly identical mechanisms of action. Hydrogen bonds can stabilize the ligand-target complex, potentially leading to pharmacological effects akin to those observed with the reference ligand (Safa *et al.,* 2023). Visualization of the docking results for the reference ligand and sulfasalazine is presented in Figure 4.

Elemicin's binding energy with IKKβ was -4.4 kcal/mol, forming a hydrogen bond at amino acid residue ASP145 and hydrophobically interacting with LEU83. Lauric acid's binding energy with IKKβ was -6.6 kcal/mol, forming hydrogen bonds with amino acid residue LYS33 and hydrophobically interacting with LEU83. Neither test compound shares the same hydrogen interaction with the reference ligand. However, lauric acid maintains the same interaction with sulfasalazine at LYS33. Sulfasalazine has a more negative binding energy than elemicin and lauric acid due to the greater number of hydrogen interactions formed. Compounds with the lowest binding energy demonstrate a greater number of hydrogen interactions with target amino acid residues (Aswad *et al.,* 2020). Visualization of the docking results for elemicin and lauric acid is shown in Figure 5.

Furthermore, elemicin and lauric acid do not sufficiently fill the binding site compared to sulfasalazine. Binding sites are areas on a protein where specific molecules or ions, such as ligands, bind with high chemical specificity and affinity (Pu *et al.,* 2019). Sulfasalazine, being more voluminous and larger, is more suited for its target (IKKβ) compared to elemicin and lauric acid. Based on these data, it can be concluded that the IKKβ receptor is more stable when binding with sulfasalazine compared to elemicin and lauric acid. Sulfasalazine remains a superior anti-inflammatory agent. However, it is possible to optimize elemicin and lauric acid by modifying their structure to increase their volume or improve their physicochemical properties, enhancing their activity and interaction with the 2I40 target. Structural modification can enhance the biological activity of a compound (Shofa *et al.,* 2022). The binding sites of sulfasalazine, elemicin, and lauric acid are shown in Figure 6.

Inflammation is controlled by numerous molecules that recognize danger, transmit intracellular signals, and trigger the body's immune response to threats (Leiba *et al.,* 2023). Inbuilt sensors are important in the immune response to pathogens, known as pathogen-associated molecular patterns (PAMPs). These sensors, referred to as pattern recognition receptors (PRRs), include Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs), C-type lectin-like receptors (CLRs), and DNA sensors (Mahla *et al.,* 2013). For example, complement activation produces proinflammatory supercomplement anaphylatoxins that promote histamine release, vasodilation, and adaptive immunity through G protein-coupled receptor (GPCR) signaling (Klos *et al.,* 2009). Additionally, signal transduction by PRRs triggers signaling cascades and activates transcription factors such as nuclear factor-κB (NF-κB), activator protein-1 (AP-1), and interferon regulatory factor (IRF), leading to the regulation of various genes encoding inflammatory cytokines, chemokines, or interferons (Takeuchi & Akira, 2010).

The NF-κB signal transduction pathway controls various cellular functions, influencing the balance between cell survival and death. This cascade is activated by multiple stimuli, both innate and external, and is relayed by adaptor proteins that phosphorylate the IκB kinase complex (IKK). This process subsequently phosphorylates the inhibitory protein IκBα, leading to its proteasomal degradation and triggering nuclear reactions in response to the initial stimuli (Amaya *et al.,* 2014). NF-κB activation occurs via two main signaling pathways: the canonical and non-canonical pathways (Hayden & Ghosh, 2008). The canonical pathway is triggered by diverse stimuli detected by cell surface receptors such as tumor necrosis factor-alpha receptor (TNFR), Toll-like receptor (TLR), and antigen receptors. The signal is then passed on to the TNF receptor-associated factor (TRAF) adaptor protein, leading to TANK-binding kinase 1 (TBK-1) phosphorylating IKKα and

IKKβ. IKK-mediated phosphorylation of IκBα results in proteasomal degradation, triggering the nuclear translocation of the NF-κB p65/p50 heterodimer, which then either activates or inhibits target gene expression (Amaya *et al.,* 2014).

The non-canonical NF-κB pathway is activated by stimuli such as lipopolysaccharide. In this pathway, NF-κB-inducing kinase (NIK) phosphorylates IKKα homodimeric bonds, mediating between phosphorylation and the widespread activation of p100/RelB. This results in the conversion of p100 into a simpler p52 isoform, with the p52/RelB heterodimer translocating to the nucleus to regulate target gene expression (Amaya *et al.,* 2014). Functionally, the canonical NF-κB pathway is involved in almost all aspects of the immune response. In contrast, the non-canonical NF-κB pathway is believed to have evolved as an additional signaling axis that interacts with the canonical pathway to regulate specific functions of the adaptive immune system (Sun, 2012).

Activation of NF-κB via the canonical pathway depends on IKKβ. The activation of this cascade leads to the phosphorylation of IκBα, resulting in its degradation by proteasomes. This degradation allows the NF-κB complex to translocate to the nucleus (Khongthong, 2019). Inhibition of IKKβ can decrease NF-κB activity. One approach to achieving this is through small-molecule inhibition. Bay 11-7082, a synthetic small molecule, has potential as an inhibitor of the NF-κB pathway. These compounds inhibit IκB kinase (IKK), preventing NF-κB from translocating into the nucleus and triggering the release of target genes involved in inflammation and immune response (Cook *et al.,* 2022). Molecular docking studies indicate that elemicin and lauric acid have an affinity for IKKβ due to their ability to form hydrogen bonds with the kinase. As IKKβ inhibitors, elemicin and lauric acid could potentially prevent the phosphorylation of the IκB protein, thereby keeping NF-κB in the cytoplasm and preventing it from entering the nucleus. These findings suggest that elemicin and lauric acid possess anti-inflammatory activity.

CONCLUSION

Predictions using the PASS server indicated that all these compounds have potential anti-inflammatory properties. Thirteen of the compounds adhere to Lipinski's rule of five, making them viable candidates for oral drugs. Most of the compounds exhibit favorable absorption, distribution, metabolism, and excretion profiles. Toxicity predictions classified four compounds as belonging to class 3, five to class 4, four to class 5, and two to class 6. All compounds were found to be inactive against hepatotoxicity, carcinogenicity, and immunotoxicity. Molecular docking results revealed that elemicin and lauric acid could form hydrogen bonds with IKKβ. The binding energy of elemicin with IKKβ was calculated to be -4.4 kcal/mol, while the binding energy of lauric acid with IKKβ was -6.6 kcal/mol. These findings suggest that these compounds possess anti-inflammatory activity, providing a basis for developing anti-inflammatory drugs.

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