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Research article

In Silico Analysis of Bioactive Compounds from Water Extract of *Gelidium spinosum* as COX-2 Inhibitor

Warsi Warsi[1*], Irwandi Jaswir[2,3]

[1]Faculty of Pharmacy, Ahmad Dahlan University, Yogyakarta. Indonesia
[2]International Institute for Halal Research and Training, International Islamic University Malaysia, Gombak 53100, Kuala Lumpur, Malaysia
[3]Department of Chemistry, Faculty of Sciences and Mathematics, Padang State University, Indonesia

*Corresponding Author: warsi@pharm.uad.ac.id

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Abstract

Water extract of *Gelidium spinosum* (WEGs) contains various bioactive compounds which potential as anti-inflammatory agent. This study was aimed to predict anti-inflammatory potential of bioactive compounds from WEGs as COX-2 inhibitor through in silico analysis. The in silico analysis was conducted using AutoDock Vina. The protein originated from the Protein Data Bank, and the metabolites were gathered from PubChem. 2-Acetyl-3-methylbenzo[b]thiophene (**1**) (-7.1 kcal/mol), and ethyl p-methoxycinnamate (**3**) (-7.1 kcal/mol) were demonstrated a higher binding affinity compared with the other ligands and phenidone (-6.7 kcal/mol). Potential of these ligands were weaker than tolfenamic acid (-9.1 kcal/mol). According to this in silico analysis, bioactive compounds from water extract of *G. spinosum* possesses an anti-inflammatory potential mediated COX-2 receptor inhibition.

Keywords: Anti-inflammatory, cyclooxygenase site, receptor, water extract, 5IKT

Introduction

A structure-based in silico method for modeling interactions between compounds and biological targets is known as molecular docking. Docking has shown to be a crucial technique for both learning how organic compounds interact with other molecular targets and for drug discovery and development. Furthermore, molecular docking has been widely used to identify structural elements necessary for efficient ligand-receptor binding and the advancement of accurate docking methods [1]. Primary goal of molecular docking is to determine the ideal configuration for the protein and ligand. Besides, this method aims to establish the basics of orientation between the two molecules in order to reduce the free energy of the whole process [2]. The search for a novel medication is a difficult undertaking. An in silico, chemical, and biological approach is a key element of contemporary drug discovery. Computer-aided techniques are becoming steadily more widely recognized, used, and popular in the drug research and development process. The in silico intermolecular interaction between two molecules is represented by molecular docking. The macromolecule functions as the protein (receptor) in molecular docking. The ligand molecule is a micromolecule that has the ability to act as an inhibitor [3].

Protein of Cyclooxygenase (COX) constitutes one of macromolecule that essential as key in inflammatory disease. COX protein is necessary for the transformation of arachidonic acid into prostaglandins, thereby mediating fever, inflammation and pain. COX originates in two isomeric forms: COX-1 and COX-2. In some tissues, Cyclooxygenase-1 (COX-1) is primarily expressed at a constant rate during the cell cycle [4]. Meanwhile, Cyclooxygenase-2 (COX-2) is thought to play relatively minimal physiological roles. It is principally responsible for the beginning and maintenance of the inflammatory process [5]. Nonsteroidal anti-inflammatory drugs (NSAIDs) have anti-inflammatory properties due to the inhibition of COX-2, nonetheless it also has adverse gastrointestinal side

effects since the inhibition of COX-1. COX-2 selective NSAIDs, such as rofecoxib was taken off the market in 2004, due to its relation to cardiovascular problems [6].

Thereby, investigating novel, relatively safe anti-inflammatory drug that minimize these adverse effects might provide more effective alternative for NSAIDs. Genus *Gelidium* such as *Gelidium spinosum* constitutes a marine macroalgae product containing various primary and secondary metabolite compounds which might be alternative as anti-inflammatory drug. The metabolites from this genus including alkaloids [7], flavonoids and polyphenols [8], and sulfated polysaccharides [9]**.** Based on previous research, *G. spinosum* has anti-inflammatory pharmacological activity [10]. Hence, the current study aimed to assess in silico analysis of bioactive compounds from *G. spinosum* to reveal particular binding sites with the enzyme that cause inflammation, especially COX-2.

Materials and Methods

Materials

This research using bioactive compounds from water extract of *Gelidium spinosum* which analysed using gas chromatography-mass spectrometry (GC-MS) [11].

These compounds including 2-acetyl-3-methylbenzo[b]thiophene (1), benzothiazole (2), ethyl p-methoxycinnamate (3), D-(-)-fructose (4), lauric acid (5), D-mannose (6), myristic acid (7), octadec-9Z-enol/ oleyl alcohol (8), palmitic acid (9), D-pinitol (10), propanoic acid (11), D-psicose (12), D-(+)-talose (13). Crytal structure of COX-2 protein was obtained from Protein Data Bank (PDB) code 5IKT [12] with 2-[(3-chloro-2-methylphenyl)amino]benzoic acid (tolfenamic acid/TLF) as a native ligand and phenidone as a standard drug.

Methods

Protein Preparation

The protein was prepared using AutoDock software. The six site chains of the COX-2 protein are A, B, C, D, E and F. The side chain A was employed as a protein in this investigation. Therefore, all of the B, C, D, E and F chains were eliminated from the crystal structure. The protein and an inhibitor (native ligand or co-crystallized) were in complex. The native ligands complex were removed from protein. The protein was then prepared which include removal of crystal water from protein. Hydrogen was then added, followed by merger of polar and non-polar hydrogens. The next steps were adding of missing residues, and Gasteiger charge [13]. The receptor was saved in *.pdbqt format [14].

Native Ligand Preparation

The native ligands of 5IKT protein were in complex which include four compounds: protoporphyrin ix containing Co, 2-[(3-chloro-2-methylphenyl)amino]benzoic acid (TLF), 2-acetamido-2-deoxy-beta-D-glucopyranose, phosphate ion. In this study, TLF was chosen as a native ligand for validation of the method of in silico analysis. Thus, the other ligands were removed using AutoDock tool. TLF was then prepared for torsion tree through menu of ligand in AutoDock tool. This required a number of actions, included detect root, choose root, and choose torsion [15]. The ligand was saved in *.pdbqt format [14].

Ligand Preparation

The ligands can be obtained as three dimensional (3D) in *.sdf format from the PubChem database. These ligands were then converted into *.pdb using Open Babel (2.4.1). The conformation of each ligand structure was then minimized using software of Avogadro. The setup of field was set at 5000 number of steps and followed by optimize geometry. The optimized geometries' final results were saved in *.pdf format. The next steps of this preparation which involved setting the torsion tree as preparation of the native ligand [16].

Setting up the Grid Box

Setting up the grid box was a constitute of validation in performance of in silico analysis. This validation was used AutoDock tool. The grid box center was needed to be set, in order to focus on the active site of the protein. For this purpose, amino acid residues were input into AutoDock. Essential amino acid residues for the COX-2 (5IKT) protein as the active site included Ala527, Gly526, Leu531, Leu352, Met522, Tyr355, Tyr385, Trp387, Ser353, Ser530, Val523, Val349. The residues were obtained from PDBsum. Creating the grid box of COX-2 protein which involved position of the x, y, and z centers. These parameters were set to 24 (x), 18 (y), and 18 (z), while an exhaustiveness was established at 12. The coordinates of the grid box were 165.756 (x), 183.886 (y), and 192.976 (z) at spacing of 1.000 Å. After that, these grid boxes were saved as config.txt in a notepad [15]. The Root Mean Square Deviation (RMSD) was then calculated using PyMOLTM 1.7.4.5. This value can be obtained by superimposing the re-docked with the co-crystallized of native ligands. Every RMSD value was verified as remaining under the 2.0 Å threshold [17].

Data analysis

Analysis of in silico was carried out using software of AutoDock Vina 1.5.7 [18]. The grid box of protein as config.txt, the protein and ligands in *.pdbqt formats were inserted into a folder in desktop. The in silico analysis was executed through the command prompt. The in silico test's data were recorded in a notepad output format in a various of split mode. The energy of binding affinity was stated in kcal/mol. As a docking result, the ligand conformation with the highest binding affinity was determined [19]. Phenidone as a standard drug and TLF as the native ligand were also docked against COX-2. The Biovia Discovery Studio Visualizer 2021.1.0. was used as a visualization of result from in silico test [20].

Results and Discussion

The 13 bioactive compounds from WEGs by GC-MS analysis were assessed its potential as inhibitor of 5IKT. These metabolites which were selected for docking studies can obtained from PubChem database with a similarity at least 90%. This investigation was to find a potential binding mechanism that might exist in charge of inhibitory characteristics. This study's molecular docking approach required extensive validation to ascertain accuracy. The validation of this method was acquired RMSD value of 0.284Å. This result showed the effectiveness of the docking procedure accurately described the binding techniques found in experiment. Therefore, this method was proper to use in this investigation due to RMSD value less than 2Å [21]. Superimposed of re-docked and co-crystallized from TLF is displayed in Figure 1.

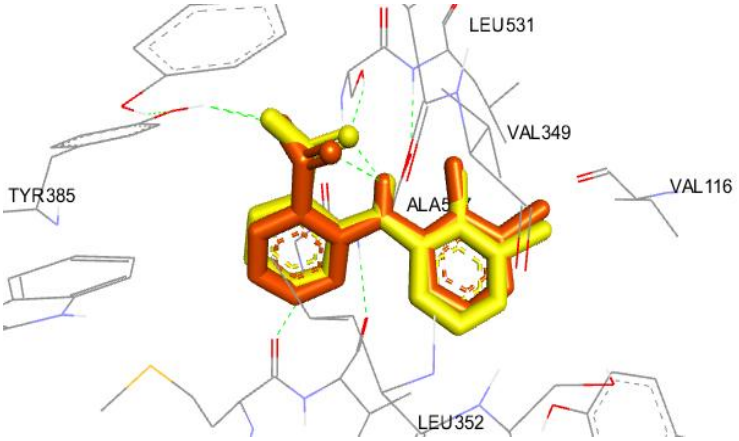

Figure 1. Superimposed 3D re-docked (red) and co-crystallized (yellow) of TLF on the COX-2 protein

The result of in silico analysis from the 13 bioactive compounds from WEGs is presented in Table 1.

Table 1. Results of in silico analysis of bioactive compounds from WEGs on the COX-2 protein (PDB ID: 5IKT)

No	Ligand CID	Ligand Name	Binding Affinity (kcal/mol)	Rotatable Bonds
1	519603	Ligand 1	-7.1	1
2	7222	Ligand 2	-5.7	0
3	5281783	Ligand 3	-7.0	5
4	5984	Ligand 4	-4.7	10
5	3893	Ligand 5	-6.1	11
6	18950	Ligand 6	-5.6	6
7	11005	Ligand 7	-6.2	13
8	5284499	Ligand 8	-6.4	16
9	985	Ligand 9	-6.5	15
10	164619	Ligand 10	-5.4	6
11	1032	Ligand 11	-3.7	2
12	441036	Ligand 12	-4.6	6
13	-	Ligand 13	-4.8	6
14	-	TLF*	-9.1	3
15	7090	Phenidone**	-6.7	1

Note: * TLF was acted as a native ligand, whereas **phenidone was used as a standard drug

Study of the 13 bioactive compounds from WEGs showed promise as COX-2 inhibitor using in silico i.e. molecular docking simulations. In this study, rotatable bonds were also determined for the 13 bioactive compounds. Result of this analysis was obtained rotatable bonds values ranged from 0 to 16. The greater the number of rotatable bonds, the more flexible of the ligand and resulted in a lower of binding energy [22]. Result of this study was not linear between number of rotatable bonds and the binding affinity value.

The binding affinity value was utilized as a parameter of in silico analysis. This parameter constitutes a scoring mode that was used to determine the binding free energy of receptor-inhibitor complex. The best-docked complex is considered to have a more negative binding affinity value. Ligand **1** (-7.1 kcal/mol), and ligand **3** (-7.0 kcal/mol) were shown the higher binding affinity compared with the other ligands and standard drug, phenidone (-6.7 kcal/mol). Nevertheless, potential of these ligands was weaker than native ligand (TLF, -9.1 kcal/mol). The potential bioactive compound as COX-2 inhibitor i.e. ligand **1** was produced two amide-pi stacked interactions through aromatic and thiophene moieties with Gly526 residue in the active site at distance of 4.18 and 3.81 Å. This compound was also interacted with residue of Ala527 via aromatic and thiophene moieties and produced three different types of interactions such as alkyl, Pi-alkyl and Pi-sigma at distance of 4.34, 5.27 and 3.90 Å, respectively. One pi-alkyl and pi-sigma interactions were generated by residue of Leu352 via aromatic and thiophene moieties. Two amino acid residues: Val349 and Val523 were interacted with thiophene moiety via alkyl and pi-alkyl bond interactions, respectively.

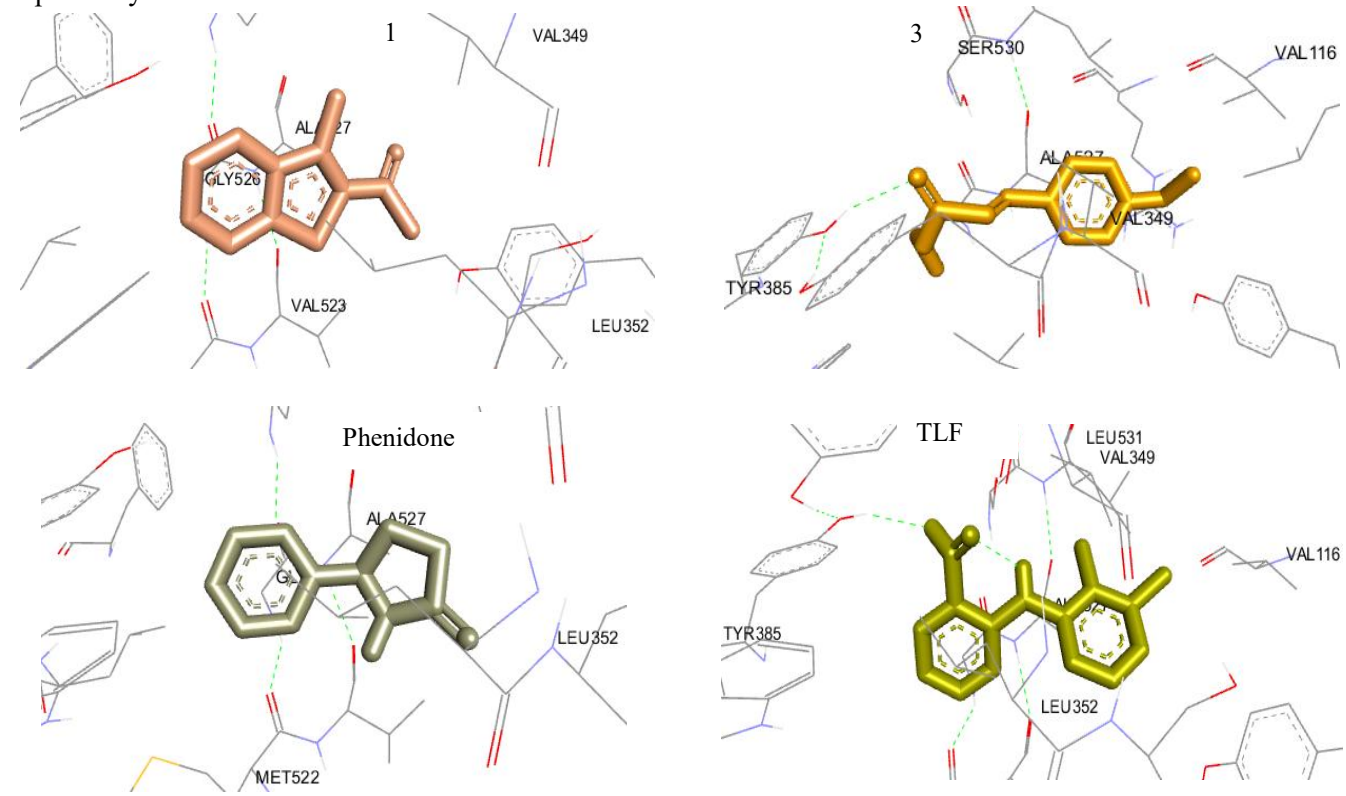

Figure 2. Simulated 2D binding modes of the promising compounds from WEGs's GC-MS analysis compared with the standard drug (phenidone) and the native ligand (TLF) on the COX-2 protein (PDB ID: 5IKT)

Another potential bioactive compound as COX-2 inhibitor i.e. ligand **3** was generated two strong hydrogen bond interactions through carbonyl group in the active site by with Ser530 and Tyr385 at distance of 2.54 and 2.23 Å, respectively. This compound was also created two pi-sigma interactions with Ala527 and Val349. Four pi-alkyl interactions were produced by some amino acid residues: Leu351, Phe381, Trp387, and Tyr355. Two alkyl interactions were developed by residues of Leu359 and Val116. Interactions of these ligands were similar with the native ligand (TLF), which included interacted with residues of Ala527, Leu352, Leu531, Tyr385, Val116 and Val349 through pi-sigma, alkyl, pi-alkyl bond interactions. One strong hydrogen bond interaction was created in the docked-native ligand complex. Meanwhile, phenidone as the standard drug only interacted with four amino acid residues, such as Ala527, Leu352, Gly526 and Met522, through pi-alkyl, amide-pi stacked and pi-sulfur interactions. No hydrogen bond interaction was produced in this standard. Some ligands were also generated strong hydrogen bond interaction such as ligands **4**, **6**, **8**, **10**, **11**, at distance ranged from 2.09 to 2.97. It was said that an interaction is considered strong if the distance < 4 Å [23]. The bioactive compounds (ligands **1**-**13**) in *E. spinosum* interact with the COX-2 protein through

a variety of interactions, including alkyl, amide-pi stacking, carbon hydrogen bond, hydrogen bonds, pi-alkyl, pi-donor hydrogen bond, pi-sigma, and pi-sulfur interactions, is presented in Table 2.

Table 2. Interactions between bioactive compounds from WEGs and the COX-2 protein (PDB ID: 5IKT)

No	Ligand Name	Amino Acid Residue	Bond Distance (Å)	Bond Type
1	Ligand 1	Ala527	4.34; 5.27; 3.90	Alkyl; Pi-Alkyl; Pi-Sigma
		Gly526	4.18; 3.81	Amide-Pi Stacked
		Leu352	5.22; 3.59	Pi-Alkyl; Pi-Sigma
		Val349	3.66	Alkyl
		Val523	5.06	Pi-Alkyl
2	Ligand 2	Ala527	3.93; 5.28	Pi-Alkyl
		Leu352	3.66; 5.19	Pi-Sigma; Pi-Alkyl
		Gly526	4.12; 3.86	Amide-Pi Stacked
		Met522	5.99	Pi-Sulfur
		Val349	5.42	Pi-Alkyl
		Val523	5.38	Pi-Alkyl
3	Ligand 3	Ala527	3.48	Pi-Sigma
		Leu351	5.39	Pi-Alkyl
		Leu359	4.22	Alkyl
		Phe381	5.45	Pi-Alkyl
		Ser530	2.54	Hydrogen bond
		Trp387	5.18	Pi-Alkyl
		Tyr385	2.23	Hydrogen bond
		Tyr355	5.03	Pi-Alkyl
		Val116	4.53	Alkyl
		Val349	3.44	Pi-Sigma
4	Ligand 4	Ala527	2.95	Hydrogen bond
		Tyr385	2.27	Hydrogen bond
5	Ligand 5	Ala527	4.42	Alkyl
		Leu352	4.82; 5.49; 4.83	Alkyl
		Leu384	5.38	Alkyl
		Met522	5.24	Alkyl
		Trp387	4.97	Pi-Alkyl
		Val523	3.78; 4.82	Alkyl
6	Ligand 6	Val523	2.79	Hydrogen bond
		Ser530	2.42; 2.75; 2.74	Hydrogen bond
7	Ligand 7	Ala527	4.17; 3.61	Alkyl
		Leu351	4.48	Alkyl
		Leu352	4.47; 5.08; 5.28	Alkyl
		Trp387	5.23	Pi-Alkyl
		Tyr385	5.05	Pi-Alkyl
		Val349	3.39; 4.50	Alkyl
		Val523	4.03	Alkyl
8	Ligand 8	Ala527	4.21; 4.66; 4.10	Alkyl
		Leu352	4.87; 4.63; 5.44	Alkyl
		Leu359	5.28	Alkyl
		Leu531	3.75	Alkyl
		Phe518	5.06	Pi-Alkyl
		Trp387	4.90	Pi-Alkyl
		Tyr355	4.92	Pi-Alkyl
		Tyr385	4.75; 2.09	Pi-Alkyl; Hydrogen bond
		Val349	4.88; 3.85	Alkyl
		Val523	4.13; 4.28	Alkyl
9	Ligand 9	Ala527	3.64; 3.48	Alkyl
		Leu352	4.87; 4.84	Alkyl
		Leu359	4.47	Alkyl
		Leu531	4.75; 4.84	Alkyl
		Met522	5.05	Alkyl
		Phe518	4.77	Pi-Alkyl
		Val116	4.83	Alkyl
		Val349	4.07	Alkyl
		Val523	4.86; 4.43	Alkyl

Table 2. (continued)

No	Ligand Name	Amino Acid Residue	Bond Distance (Å)	Bond Type
10	Ligand 10	Tyr385	3.19	Pi-Donor Hydrogen bond
		Val523	2.28	Hydrogen bond
11	Ligand 11	Ile277	5.17	Alkyl
		Phe381	5.26	Pi-Alkyl
		Phe209	3.62	Pi-Sigma
		Ser530	2.97	Hydrogen bond
12	Ligand 12	Ser530	3.63	Carbon Hydrogen bond
13	Ligand 13	Met522	3.68	Carbon Hydrogen bond

The COX-2 enzyme has two catalytic active sites: cyclooxygenase site and peroxidase site. The cyclooxygenase site transforms arachidonic acid (AA) into prostaglandin G2 (PGG2), whereas the peroxidase site catalyzes the activity that decreases PGG2 to prostaglandin H2 (PGH2) [24]. In this study, COX-2 protein code 5IKT was used as a receptor. This receptor was in complex with 2-[(3-chloro-2-methylphenyl)amino]benzoic acid as a native ligand at a cyclooxygenase site. This compound is known as tolfenamic acid (TLF). This compound functions as a substrate-selective inhibitor of COX-2 (5IKT), a nonsteroidal anti-inflammatory drug (NSAID). Besides, the 5IKT was also in a complex with protoporphyrin IX at a peroxidases site. The peroxidase site is a catalytic site of COX-2 protein which not active if absent an activator [24]. TLF was generated one strong hydrogen bond with Tyr385 at distance of 2.22 Å and two alkyl interactions by Leu531 (4.30 Å and 3.82 Å). The interactions with these amino acid residues constitute a hydrophobic pocket location of COX-2 protein within hydrophobicity of 2 [25]. TLF was also produced pi-sigma interaction with aromatic moiety by Leu352 residue. This location constitutes a specific receptor binding pocket of COX-2 protein [26]. The superimposing 3D of all bioactive compounds from WEGs's GC-MS analysis on the COX-2 protein is shown in Figure 2.

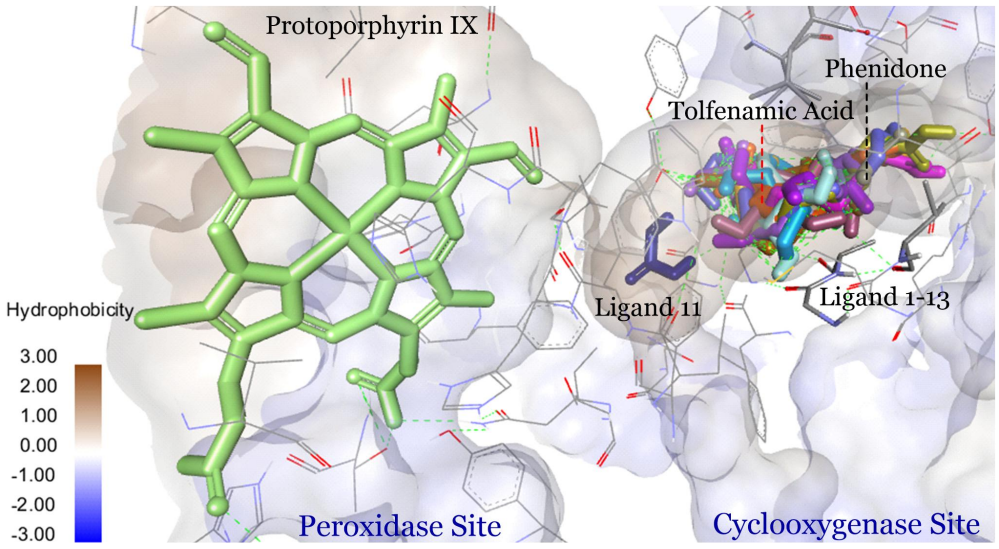

Figure 2. Superimposed 3D of all bioactive compounds from WEGs's GC-MS analysis compared with the standard drug (phenidone) and the native ligand (TLF) on the COX-2 protein (PDB ID: 5IKT) at the catalytic site

This superimposing was used to determine a possible binding mechanism that might be responsible for its inhibitory actions. Based on the Figure 2, all the bioactive compounds were bind at the cyclooxygenase site, except ligand 11. The illustration indicates that all the bioactive compounds have the same inhibitory mechanism as a native ligand and a standard drug.

Molecular docking has emerged as a essential component of in silico drug development. This analysis can predict interaction between inhibitor and protein, as well estimate binding affinity at the atomic level. This in silico analysis allows researchers to investigate the way small compounds, like nutraceuticals in the water extract of *G. spinosum*, interact inside a target protein's binding site i.e. COX-2 and comprehend the basic biochemical mechanism that underlies this interaction. However, these approximations can cause molecular docking to miss active compounds

especially for compounds with short structures or predict strong affinity for inactive ones for chemicals with long structures.

Conclusion

The study performs in silico analysis as evidence that the bioactive compounds from water extract of *G. spinosum* might possess anti-inflammatory properties as COX-2 inhibitor. Compounds of 2-acetyl-3-methylbenzo[b]thiophene (1), and ethyl p-methoxycinnamate (3) were exhibited the potential bioactive compounds by inhibiting COX-2 receptor protein as anti-inflammatory mechanism. All the bioactive compounds from water extract of *G. spinosum* have the similar inhibitory mechanism as native ligand and the standard drug, except ligand 11. *G. spinosum* is a prospective candidate for drug discovery, nutritional usage, and nutraceutical development due to its natural source.

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Declarations

Author contribution	:	Warsi Warsi setting topics of in silico analysis, performing docking, writing manuscript, presenting visualization and analysis of docking result; Irwandi Jaswir review and editing the grammar.
Funding statement	:	There is no funding available for this study.
Conflict of interest	:	The authors declare that no conflict of interest.
Ethics Declaration	:	We confirm that this work has been written based on ethical research principles in compliance with our university's regulations and that the necessary permission was obtained from the relevant institution during data collection. We fully support Clips commitment to upholding high standards of professional conduct and practicing honesty in all academic and professional activities.
Additional information	:	There is not any more information available for this paper.

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