ORIGINAL ARTICLE

Bioactive phytoconstituents of ethanolic extract from *Chromolaena odorata* leaves interact with vascular endothelial growth factor and cyclooxygenase-2: A molecular docking study

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ABSTRACT

Chromolaena odorata is an invasive plant with a broad spectrum of medicinal properties, including wound healing. This study aimed to evaluate the interaction of the already identified bioactive phytoconstituents from ethanolic extracts of *C. odorata* leaves with two angiogenesis-related proteins – vascular endothelial growth factor (VEGF) and cyclooxygenase-2 (COX-2) *in silico*. A molecular docking protocol was performed on AutoDock Vina employing the molecular structure of VEGF (3HNG) and COX-2 (3LN1) downloaded from the Protein Data Bank. The results reveal that most of the phytoconstituents possess strong binding affinity, where β-tocopherol and squalene have the highest values. In conclusion, it is highly possible that the phytoconstituents of *C. odorata* from the ethanolic leaf extract perform an interaction with VEGF and COX-2 and affect their activities.

Key words: *In silico*, phytocompound, squalene, wound healing, β-tocopherol

INTRODUCTION

An invasive weed, *Chromolaena odorata*, has attracted the attention of phytomedicine experts due to its richness in bioactivities. A review article from 2021 has highlighted several common bioactivities of this plant including anti-inflammatory, antiparasitic, antimicrobial, anticancer, antidiabetic, antipyretic, antinociceptive, and wound healing.[1] Among its vast medicinal benefits, the wound-healing properties of this plant are considered promising. The first report of the wound-healing potential of *C. odorata* could be traced back to as early as 1998, where a study observed the increased growth of fibroblasts and endothelial cells upon exposure to aqueous extract of *C. odorata* leaves.[2] More recently, researchers have now focused on identifying its phytocompounds and wound-healing mechanisms.[3,4] Some studies even have used the *C. odorata* extracts as active agents in topical gel formulations.[5,6] Since the phytoconstituents of this plant have strong antioxidant activities, many reports suggest the involvement of oxidative stress attenuation as the main mechanism of its wound-healing activities.[4]

Wound healing involves four major steps including coagulation and homeostasis, inflammation, proliferation,

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and tissue remodeling. *C. odorata* is prominent in facilitating proliferation steps, especially by promoting angiogenesis, cell adhesion, and fibroblast proliferation.[2,5] In this step, vascular endothelial growth factor (VEGF) and cyclooxygenase (COX-2) work collectively or independently in inducing inflammation response, angiogenesis, and re-epithelization.[7] VEGF in particular has the ability to stimulate vasculogenesis, induce inflammation, and control vascular permeability. Meanwhile, COX-2 is upregulated in almost all tissue injuries and produces prostaglandins through arachidonic acid conversion.[8] Particular COX-2 inhibitors have been shown to promote cutaneous wound healing which was observed along with decreased expression of inducible nitric oxide synthase.[9] Increased COX-2 levels were documented to be affected by VEGF concentration in endothelial cells.[10] Therefore, in this study, we determined the interaction between VEGF or COX-2 and phytoconstituents from *C. odorata*. Molecular docking was used as a modality to investigate the interactions by providing data on binding affinity and the most probable binding locations. The rationale for using molecular docking was its accuracy in predicting the ligand–protein interaction without the necessity of consuming a lot of resources.[11,12]

MATERIALS AND METHODS

Plant specimen

The leaves of *C. odorata* were collected from Darussalam, Banda Aceh, Indonesia on May 10, 2021. The plant was identified for its taxonomy at the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia on October 20, 2021, by Dr. Rasnovi (voucher number: 559/UN11.1.8.4/TA.00.01/2021).

Selection of ligands

Phytoconstituents were identified using gas chromatography–mass spectrometry (GC-MS) in the ethanolic extract from *C. odorata* leaves which were collected from Darussalam, Banda Aceh, Indonesia. The extraction procedure followed the suggestion from previously reported studies.[13] Briefly, crushed dried leaves were macerated using ethanol 70% for 24 h before the filtrate was collected and subsequently concentrated using a rotary evaporator. The extract was analyzed using GC-MS (QP2010 SE, Shimadzu, Kyoto, Japan) and each of the identified phytoconstituents was screened *in silico* for its bioactivities. Detailed characteristics of each phytoconstituent, including its chemical formula, are presented in Table 1. LogP, the number of hydrogen bond acceptors (nON), as well as the number of hydrogen bond donors (nOHNH) were estimated from the algorithm used by Molinspiration (https://www.molinspiration.com/). For the molecular docking study, the 3D structure of each phytoconstituent was downloaded from PubChem (https://pubchem. ncbi.nlm.nih.gov/). The ligand molecule was reformatted from.sdf to.pdb and added with hydrogens using Chimera 1.15.

Table 1: Molecular characteristics of the phytoconstituents identified in the ethanolic extract from *Chromolaena odorata* leaves

Name (formula)	MW (g/mol)	LogP	nON/ nOHNH
Longiverbenone ($C_{15}H_{22}O$)	218.34	4.06	1/0
Phytyl acetate ($C_{22}H_{42}O_2$)	338.58	7.46	2/0
Ledol ($C_{14}H_{23}O$)	207.34	3.49	1/1
Neomenthol ($C_{10}H_{20}O$)	156.27	3.33	1/1
Pentadecadien-1-ol ($C_{15}H_{28}O$)	224.39	6.09	1/1
Octadecanoic acid ($C_{18}H_{36}O_2$)	284.48	8.07	2/1
Squalene ($C_{30}H_{50}$)	410.73	9.62	0/0
β-tocopherol ($C_{28}H_{48}O_2$)	416.69	8.98	2/1
Octacosanol ($C_{28}H_{58}O$)	410.77	9.83	1/1

MW: Molecular weight, nON: Number of hydrogen bond acceptors, nOHNH: Number of hydrogen bond donors

Protein molecule acquisition and preparation

The molecular docking was performed on hardware with the following specifications: random access memory of 4 GB, 64-bit operating system, and Windows 10 operating system. Molecular structures of VEGF (3HNG) and COX-2 (3 LN1) were acquired from the Protein Data Bank (PDB, https://www.rcsb.org/). Threading of the foregoing structures was performed on Iterative Threading ASSEmbly Refinement. The protein structures were prepared using Chimera 1.15 to remove solvents and native ligands and to be fixed for missing amino atoms.

Molecular docking and visualization

Ligand docking onto the protein molecule was performed on AutoDock Vina 1.1.2 following the suggestions from a published report.[11] Priorly, all.pdb files were converted into.pdbqt using AutoDockTools. Gridbox size and position were adjusted according to the biggest tested ligand molecule and native ligand, respectively. The exhaustiveness was adjusted to 8. After the docking was performed, ligand–protein interaction was visualized on BIOVIA Discovery Studio Visualizer 21.1. To validate the docking procedure, the native ligand was redocked onto its protein molecule, where the root-mean-square deviation value was found below 2.

RESULTS AND DISCUSSION

Molecular characteristics of the phytoconstituents

Molecular characteristics of bioactive phytoconstituents of ethanolic extract from *C. odorata* leaves are presented in Table 1. Molecular weight, Log*P* value, nON, and nOHNH, according to Lipinski's rule of five, should be below or equal to 500 g/mol, 5, 10, and 5, respectively.[14] Herein, the violation was only found in terms of the Log*P* value (>5), as shown by phytyl acetate, pentadecadien-1-ol, octadecanoic

acid, squalene, β-tocopherol, and octacosanol. Violation of this rule suggests the poor bioavailability and absorption of the foregoing phytocompounds.

Ligand–Protein interactions

Molecular docking results of the phytoconstituents onto the molecular structure of VEGF and COX-2 are presented in Table 2. β-tocopherol appeared to have the highest affinity with VEGF or COX-2, followed by squalene. The binding affinity of β-tocopherol with VEGF and COX-2 were − 13.63 and −16.66 kcal/mol, respectively. Hydrogen bond contributes to that value was located at Asp1040 and Gln178, for VEGF and COX-2, respectively. In the case of squalene, the value was lower, where its binding affinity with VEGF and COX-2 was observed to be −11.93 and −14.50 kcal/mol, respectively, without involving a hydrogen bond. Both β-tocopherol and squalene had stronger affinities with VEGF or COX-2, as compared with the native ligand. In comparison, binding affinity >5 kcal/mol is usually used as an indication of the ligand's capability in forming an interaction with the protein.[11,12]

The locations of interaction formed between the ligand and protein could be observed in 3D or 2D illustration [Figure 1]. The native ligand of VEGF binds tightly at Cys912, Glu878, and Asp1040.[15] Meanwhile, in the case of COX-2, the

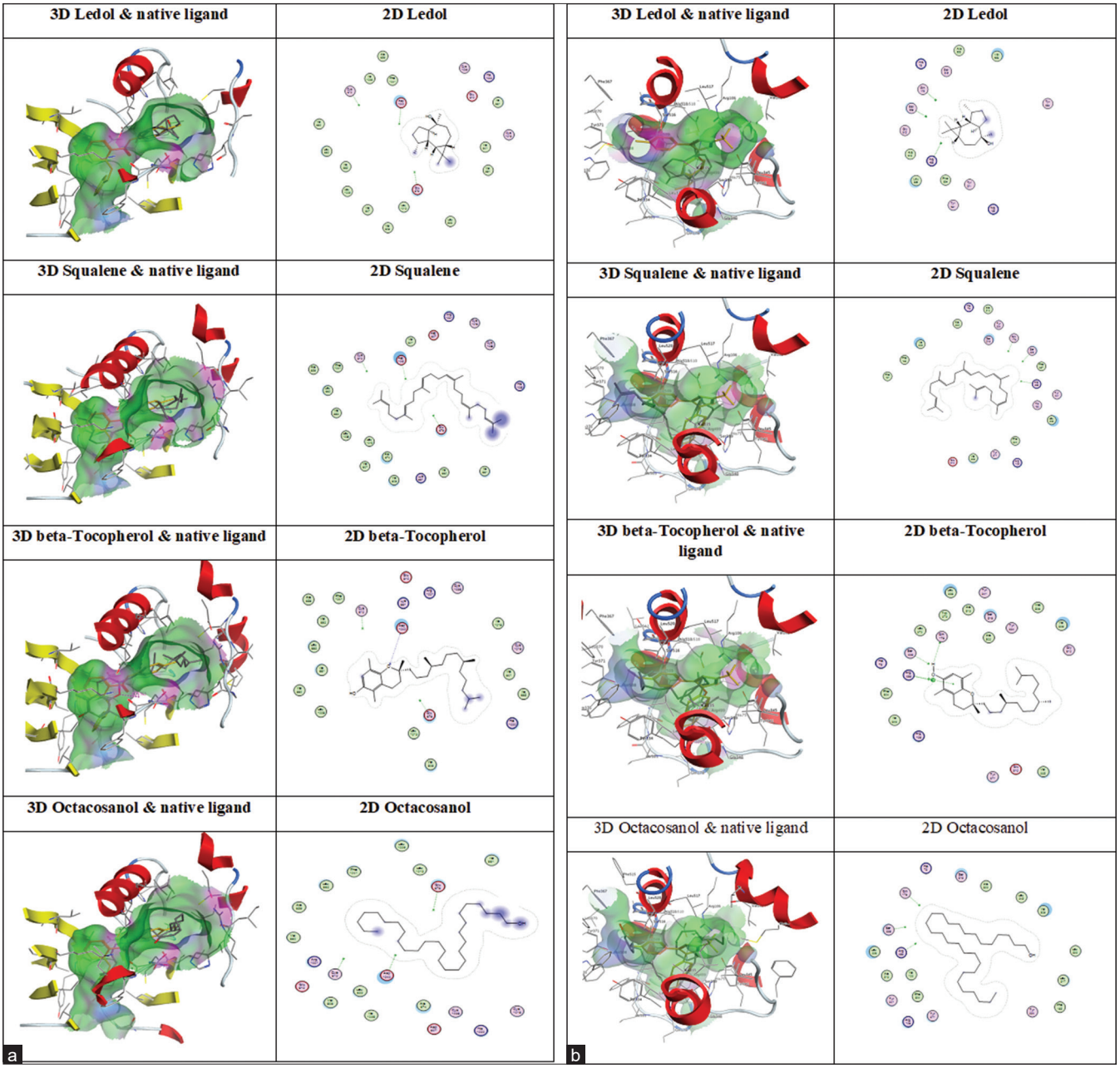

Figure 1: Visualization of molecular interactions of the *Chromolaena orodata* phytoconstituents with VEGF (a) and COX-2 (b). The phytoconstituents are ledol squalene, β-tocopherol, and octacosanol. VEGF: Vascular endothelial growth factor, COX-2: Cyclooxygenase-2

Table 2: Molecular docking parameters obtained from the interaction between the phytoconstituents (ligand) and vascular endothelial growth factor or cyclooxygenase-2 (receptor)

Ligand	Receptor (PDB ID)			
	VEGF (3HNG)		COX-2 (3LN1)	
	Affinity (kcal/mol)	Hydrogen bond	Affinity (kcal/mol)	Hydrogen bond
β-tocopherol	−13.63	Asp1040	−16.66	Gln178
Squalene	−11.93	-	−14.50	-
Longiverbenone	−10.51	Lys861	−9.99	Arg106
Phytyl acetate	−11.69	-	−12.05	-
Ledol	−9.38	-	−9.06	-
Neomenthol	−7.68	Cys1039	−9.02	Tyr301, Arg106
Pentadecadien-1-ol	−9.33	Lys861, Glu878	−10.26	Arg499, His75
Octadecanoic acid	−10.34	Asp1040	−11.51	-
Octacosanol	−10.61	-	−14.01	-
Native ligand (control)	−11.47	Cys912, Glu878, Asp1040	−14.30	Ser339, Gln178, Leu338

The absence of hydrogen bonds. VEGF: Vascular endothelial growth factor, COX-2: Cyclooxygenase-2, PDB: Protein Data Bank

strong binding of the native ligand occurred at Ser339, Gln178, and Leu338.[16] Interestingly, β-tocopherol appears to form hydrogen bonds at Asp1040 and Gln178 which are the catalytic sites of VEGF and COX-2, respectively. It suggests the possibility of β-tocopherol acting as an inhibitor through substrate blockage. Even though squalene occupies noncatalytic parts of the protein, with such a strong binding affinity, it could inhibit or increase the activity of the proteins.[17] Taken altogether, β-tocopherol and squalene may affect the activities of VEGF or COX-2 since their binding affinities are high.

The interactions of β-tocopherol and squalene as the phytoconstituents of *C. odorata* could be responsible for the therapeutical properties of the ethanolic leaf extract. The ability of the *C. odorata* extract in assisting wound recovery could be derived from its anti-inflammation activities and ability to enhance mature tissue granulation through protein signaling.[18] COX-2 and VEGF have activities that could induce the inflammation occurred during tissue injuries. Meanwhile, the foregoing interaction formed by the presence of *C. orodata* phytoconstituents could assist the epithelization and collagen deposition during angiogenesis.[19]

CONCLUSION

Our molecular docking analysis reveals the strong interaction between the phytoconstituents of *C. odorata* leaf (particularly, β-tocopherol and squalene) and VEGF or COX-2. β-tocopherol may act as a blockage to VEGF and COX-2 since the hydrogen bonds are formed at the active sites. Squalene, another phytoconstituent with high binding affinities, may inhibit the protein activities by acting as a noncompetitive pathway or increase the activities by stabilizing the proteins' molecules. Since both β-tocopherol and squalene have a violation of Lipinski's rule of five, their development as drug candidates should involve an adequate drug delivery system. *In vitro* and *in vivo* investigations on *C. odorata* leaf extract as a wound-healing agent are warranted in future research.

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Conflicts of interest
There are no conflicts of interest.

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