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Original Article

IDENTIFICATION OF POTENTIAL ACTIVITY OF VOLATILE COMPOUNDS DERIVED FROM *POGOSTEMON CABLIN* BENTH AS ANTIVIRAL OF SARS-COV-2

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ABSTRACT

Objective: Coronavirus disease-19 (COVID-19) is global pandemic which caused by SARS-CoV-2 infection. Mechanism of infection is initiated by attachment between viral glycoprotein with ACE2 receptor in human cells. Furthermore, Indonesia had a massive diversity of plants with a high potency of drugs, such as *Pogostemon cablin* Benth. In brief, it contained of various volatile compounds with many therapeutic properties. Therefore, this research aimed to identify the ability of volatile compounds from *Pogostemon cablin* Benth as a potential inhibitor of SARS-CoV-2 spike glycoprotein.

Methods: SMILE notation of 22 volatile compounds of *Pogostemon cablin* Benth were collected from PubChem and the 3D structure of SARS-CoV-2 glycoprotein (PDB ID: 6VXX) was obtained from PDB database. Simulation of interaction between volatile compound and glycoprotein was conducted by using Pyrx molecular docking. Moreover, the complex of compounds-glycoprotein was depicted by using Chimera and the amino acid residue was analysed by using LigPlot. Selected potential compounds were identified for biological activity prediction, drug-likeness, and toxicity analysis.

Results: Analysis showed that among those volatile compounds, only caryophyllene oxide (-6.3 kcal/mol) naturally bind specific into RBD site as compared to the control. Furthermore, it had comparable hydrogen and hydrophobic interactions with glycoprotein. Further analysis showed it has strong potential biological function for antiviral with low toxicity.

Conclusion: Caryophyllene oxide is considered as promising candidate compounds that inhibited viral infection through SARS-CoV-2 glycoprotein.

Keywords: Inhibitor compound, Glycoprotein, Secondary metabolite, Viral infection

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INTRODUCTION

As mega diversity country, Indonesia is known as a tropical country with high flora and fauna variety. Various flora in Indonesia is considered for having a huge potency to be developed as a therapeutic agent [1]. There are about 7,500 medicinal plants which varied from wild plant, to cultivated, or introduced species in Indonesia [2, 3]. *Pogostemon cablin* Benth is known as aromatic plant with various therapeutic function. The volatile compound of its plant is widely used as aromatherapy for treatment, especially for patchouli oil. Among various variety of *Pogostemon cablin* Benth, the Sidikalang variant is considered as the most outstanding variant in Indonesia, which had international trade quality for production. It is commonly used for patchouli oil supply for various potential function in industry or the medicinal field [4]. However, another various volatile compounds of *P. cablin* Benth is also needed to be explored of its function.

The viral infection of SARS-CoV-2 was recently spread across the globe as a pandemic in 2019 up to the present. In brief, SARS-CoV-2 is enveloped virus with single-strand RNA that had several part, such as nucleocapsid protein, membrane, envelope, and glycoprotein [5]. Glycoprotein of SARS-CoV-2 is a transmembrane protein which located in the surface of virion with an essential function for infection. It mediated the attachment of interaction between SARS-CoV-2 with human cells. As structural, SARS-CoV-2 glycoprotein consisted of a receptor binding domain (RBD) with a specific receptor binding motif (RBM) as a site for infection [6]. Several treatments by using any FDA-approved drugs were utilized, such as chloroquine, hydroxychloroquine, and others [7]. Moreover, various strategy development, including chemical and compounds agent, were explored to identify the most potential antiviral which can be utilized as a drug for treatment. Therefore, this study aimed to identify the potency of volatile compounds derived from *Pogostemon cablin* Benth as a potential inhibitor to bind into RBD in SARS-CoV-2 for an inhibitory-based therapeutic agent.

MATERIALS AND METHODS

Sample preparation

Twenty two data of volatile compounds derived from *Pogostemon cablin* Benth were collected from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The data of ID number and canonical simplified molecular-input line-entry system (SMILES) of each compounds were collected for analysis. Moreover, the 3D structure of each compound was collected in sdf. Format.

Moreover, the 3D structure of SARS-CoV-2 glycoprotein was obtained from Protein Data Bank (PDB) database (https://www.rcsb.org/) with PDB ID 6VXX. The non-essential molecules, such as water molecule and native ligand as NAG were removed by using Chimera. Furthermore, protein structure was added by hydrogen polar as preparation for further analysis. The MLN-4760 and sulabiroins A were selected as control for the analysis [8].

Simulation of molecular docking and visualization of complex compounds-glycoprotein SARS-CoV-2

The simulation of the interaction between 22 volatile compounds and glycoprotein of SARS-CoV-2 was conducted by using molecular docking analysis. The simulation process was performed by using Autodock Vina in Pyrx software. The molecular docking process was done by using blind docking in order to identify the natural ability of each compound to interact with the receptor-binding domain (RBD) of the glycoprotein of SARS-CoV-2. Furthermore, the results of the binding affinity score were compared to the control and other compounds to obtain a candidate of a potent inhibitor. The complexes of the glycoprotein of SARS-CoV-2 and potent compounds were visualized by using Chimera software. Moreover, the amino acid residue was analyzed by using LigPlot software.

Biological activity prediction of potential compounds derived from *Pogostemon cablin* Benth

The biological activity of potent compounds was identified by using PASS Online webserver (http://way2drug.com/passonline/). Several biological activities were selected for identification, such as antiviral, anti-inflammatory, and apoptosis agonists. Score of each probability of activity (Pa) and probability of inactivity (Pi) were collected. Each of score was varied from 0-1.0. In brief, score of $Pa<0.7$ suggested high potential, $0.5<Pa<0.7$ exhibited moderate potential, and $Pa<0.5$ showed poor potential [9-11].

Druglikeness analysis of potential compounds derived from *Pogostemon cablin* Benth

The druglikeness of potent compounds was identified by using SWISS ADME webserver (http://www.swissadme.ch/index.php). Several parameters were selected for analysis based on the Lipinski's rule of five, such as molecular weight (MW)<500 g/mol, H-bond donor<5, H-bond acceptor<10, calculated LogP (ClogP)<5, and molecular refractivity 40-130. Compound which fulfil each of parameter was considered with acceptable potency as a potential drug.

Toxicity prediction of potential compounds derived from *Pogostemon cablin* Benth

Selected potential compounds were addressed for analysis of toxicity prediction by using ProTox-II (https://tox-new.charite.de/protox_II/). The analysis was conducted by submitting the canonical SMILE of selected compounds [12]. Result of analysis was determined by six class of toxicity and predicted LD50.

RESULTS AND DISCUSSION

This study is focused to identify the potency of various volatile compounds of *Pogostemon cablin* Benth targeted the SARS-CoV-2 glycoprotein. SARS-CoV-2 is known as an essential protein which mediated the viral infection and pathogenesis. In brief, the glycoprotein is composed of 1260 amino acids with S1 subunit that contain of receptor-binding domain (RBD). This is an essential region which had ability to contact with ACE2 receptor in human cells. Recent research showed that 17 residues of RBD are in contact with 20 residues of ACE2 receptor. This interaction is suggested as virus-receptor engagement process [13].

Twenty two volatile compounds of *Pogostemon cablin* Benth were selected as a samples for analysis (table 1). Furthermore, MLN-4760 was selected as control and sulabiroins A was selected as reference control. Recent research demonstrated that sulabiroins A which derived from propolis had the most potential ability to inhibit SARS-CoV-2 protein related to the viral infection [8]. Therefore, the sulabiroins A is considered as a reference for comparation.

Table 1: Various volatile compounds derived from *Pogostemon cablin* benth

No	Compounds	PubChem ID	Canonical SMILES
1	Patchouli alcohol	10955174	CC1CCC2(C(C3CCC2(C1C3)C)(C)C)O
2	α-Patchoulene	521710	CC1CCC23C1CC(C2(C)C)CC=C3C
3	Beta-patchoulene	101731	CC1CCC2=C1CC3CCC2(C3(C)C)C
4	α-Guaiene	5317844	CC1CCC(CC2=C1CCC2C)C(=C)C
5	β-Guaiene	15560252	CC1CCC(=C(C)C)CC2=C1CCC2C
6	β-Caryophyllene	5281515	CC1=CCCC(=C)C2CC(C2CC1)(C)C
7	Trans-Caryophyllene	5354499	CC1=CCCC(=C)C2CC(C2CC1)(C)C
8	(E)-β-Caryophyllene [(+)-beta-Caryophyllene]	20831623	CC1=CCCC(=C)C2CC(C2CC1)(C)C
9	β-Elemene	6918391	CC(=C)C1CCC(C(C1)C(=C)C)(C)C=C
10	gamma-elemene	6432312	CC(=C1CCC(C(C1)C(=C)C)(C)C=C)C
11	Pogostone	54695756	CC1=CC(=C(C(=O)O1)C(=O)CCC(C)C)O
12	alpha-pinene	6654	CC1=CCC2CC1C2(C)C
13	beta-pinene	14896	CC1(C2CCC(=C)C1C2)C
14	Limonene	22311	CC1=CCC(CC1)C(=C)C
15	Seychellene	519743	CC1CCC2(C(=C)C3CCC2(C1C3)C)C
16	α-Humulene	6508206	CC1=CCC(C=CCC(=CCC1)C)(C)C
17	Germacrene D	6436582	CC1=CCCC(=C)C=CC(CC1)C(C)C
18	Aciphyllene	565709	CC1CCC(CC2=C(CCC12)C)C(=C)C
19	α-Bulnesene	6432384	CC1CCC2=C(CCC(CC12)C(=C)C)C
20	Norpatchoulenol	6451732	CC1(C2CCC3(C1(CC=CC3C2)O)C)C
21	Caryophyllene oxide	1742210	CC1(CC2C1CCC3(C(O3)CCC2=C)C)C
22	Pogostol	5320651	CC1CCC2C1CC(CCC2(C)O)C(=C)C

Table 2: Binding affinity between volatile compounds derived from *Pogostemon cablin* Benth. and SARS-CoV-2 glycoprotein

Compounds	Binding affinity (kcal/mol)
β-Guaiene	-7.4
α-Bulnesene	-7.2
(E)-β-Caryophyllene	-7.1
α-Guaiene	-7.0
Trans-Caryophyllene	-7.0
Sulabiroins A (reference control)	-6.8
α-Patchoulene	-6.8
gamma-elemene	-6.8
Pogostol	-6.8
Seychellene	-6.8
beta-Elemene	-6.7
Patchouli alcohol	-6.7
Germacrene D	-6.6
MLN-4760 (control)	-6.4
Aciphyllene	-6.4
Norpatchoulenol	-6.4
Caryophyllene oxide	-6.3
Beta-patchoulene	-6.2
αhumulene	-5.9
beta-Caryophyllene	-5.9
beta Pinen	-5.9
Alpha pinen	-5.8
Pogostone	-5.8
limonene	-5.5

Molecular docking analysis is conducted by using a calculation approach to measure the interaction between protein to small molecule. The analysis could be used to identify the inhibition ability of small molecules [14]. The utilization of the volatile compound of *Pogostemon cablin* Benth as an inhibitor candidate of SARS-CoV-2 glycoprotein showed that only five volatile compounds had lower binding affinity as compared to the control. Those selected volatile compounds, such as β-Guaiene, α-Bulnesene, E)-β-Caryophyllene, α-Guaiene, and Trans-Caryophyllene (table 2). However, all of those compounds were not specifically bind into RBD site of glycoprotein. The result depicted that there is only caryophyllene oxide which naturally bind into RBD site of glycoprotein together with the control MLN-4760 (fig. 1). While, sulabiroins A was bind to another part of glycoprotein. Furthermore, the complex of compound caryophyllene oxide-SARS-CoV-2 glycoprotein was taken for visualization and further analysis. The visualization of this complex was served the information of specific location of interaction [15]. It is essential to define the ability of small molecules to induce the function of protein.

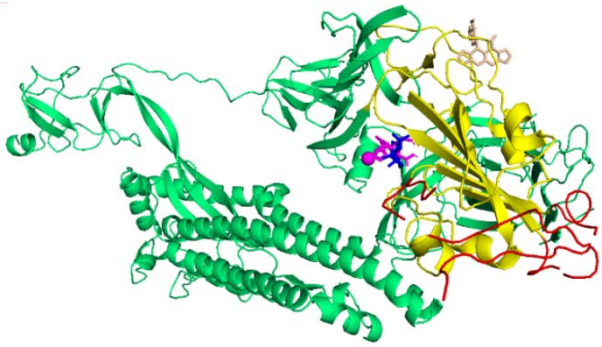

Fig. 1: Visualization complex of SARS-CoV-2 glycoprotein (PDB: 6VXX) with several ligand: Sularboins A (wheat), MLN 4760 (magenta), and caryophyllene oxide (dark blue). Location of the receptor-binding domain (RBD) is indicated by yellow color dan the receptor-binding motif (RBM) is indicated by red color (coil)

Complex of caryophyllene oxide and SARS-CoV-2 glycoprotein was addressed for further analysis to identify the amino residue within that interaction. Result showed that both hydrogen bonds and hydrophobic bonds were formed within the interaction (fig. 2). In brief, caryophyllene oxide shared several bonds, which similar as compared to MLN-4760. In a hydrogen bond, caryophyllene oxide shared interaction to Thr430 with stronger interaction in 3.20 Å as compared to MLN-4760. Furthermore, within hydrophobic interaction, caryophyllene oxide had a similar interaction with control, such as Glu516, Phe429, and Asp428 (table 3). This similar shared amino acid residue is revealed that both compounds was reside in the same site within structure of glycoprotein. Moreover, the hydrophobic bonds help for stable interaction [16].

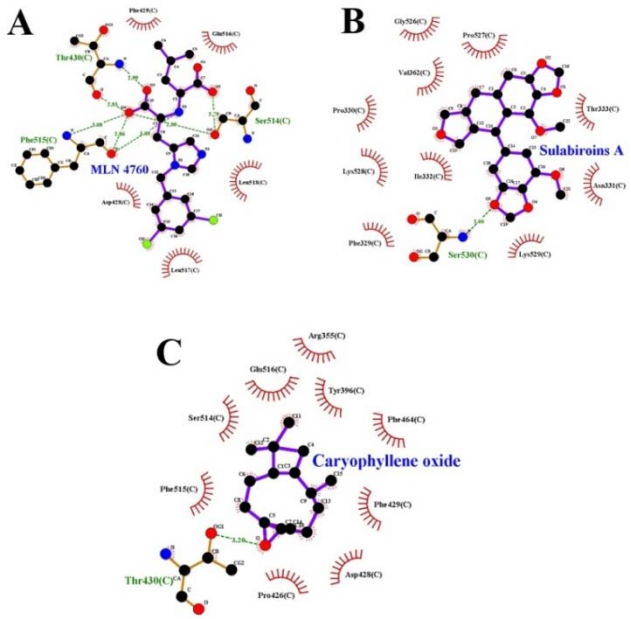

Fig. 2: Interaction of amino acid residue between (A) MLN-4760 (control), (B) Sulabiroins A (reference control), and (C) Caryophyllene oxide with the active site of SARS-CoV-2 glycoprotein

Table 3: Amino acid residues between selected compounds with SARS-CoV-2 glycoprotein

Compounds	Hydrogen bond	Hydrophobic bond
MLN-4760 (Control)	Ser514: 2.80; 2.79Å Phe515: 3.08; 2.86; 3.08 Å Thr430: 2.99; 2.83 Å	Glu516, Phe429, Leu518, Asp428, Leu517
Sulabiroins A (Reference control)	Ser530: 3.08 Å	Lys529, Asn331, Phe329, Ile332, Lys528, Thr333, Pro330, Val362, Pro527, Gly526
Caryophyllene Oxide	Thr430: 3.20 Å	Phe515, Ser514, Glu516, Tyr396, Phe464, Phe429, Asp428, Pro426

Further analysis showed that Sulabiroins A as reference control did not bind to specific site in RBD as, similar to MLN-4760 and caryophyllene oxide. Therefore, only MLN-4760 and caryphyllene oxide which addressed for further analysis, including the drug-likeness analysis and toxicity analysis. The drug-likeness analysis exhibited that based on Lipinski's rule of five, the caryophyllene oxide had fulfilled all five parameters, including hydrogen donor, hydrogen acceptor, molecular weight, molecular refractivity, and CLogP as compared to control (table 4). Based on the Lipinski's rule of five, the appropriate compound for the drug should fulfil all those parameters or four parameters [17], Result showed that caryophyllene oxide had small molecular weight about 220.35 g/mol which suggested the possibility for appropriate absorption. Recent research showed that as the weight increased so that the absorption would be decrease [18]. Therefore, lower molecular weight of the compound is preferable to be developed as potential drugs. While the optimum hydrogen donor and hydrogen acceptor also essential for passive diffusion through cell membranes, including absorption and distribution [19].

Table 4: Druglikeness of *Pogostemon cablin* Benth compound based on the lipinski rule of five

No	Compounds	PubChem ID	Lipinski's rule of five				
			Ha (≤10)	Hd (≤5)	MR	MW (g/mol)	CLogP
1	MLN-4760	448281	6	3	107.76	428.31	2.62
2	Caryophyllene oxide	1742210	1	0	68.27	220.35	3.68

Further identification was addressed for toxicity analysis in order to identify the safety level of compounds for clinical study. Result showed that the caryophyllene oxide had low toxicity which is indicated by the tolerance of high doses of compound in 5000 mg/kg (table 5). In brief, it belongs to the toxicity class 5 with means that the compound might be harmful if swallowed between 2000 mg/kg<LD50 ≤ 5000 mg/kg. Results also demonstrated that caryophyllene oxide is less toxic as compared to control. This is an interesting result for further analysis *in vivo*.

Table 5: Toxicity class of *Pogostemon cablin* benth compound

No	Compounds	PubChem ID	Toxicity analysis		
			LD50 (mg/kg)	Toxicity class	Accuracy (%)
1	MLN-4760	448281	3000	5	67.38
2	Caryophyllene oxide	1742210	5000	5	100

CONCLUSION

Simulation on silico analysis showed that most of the volatile compounds of *Pogostemon cablin* Benth had the potential ability to interact with SARS-CoV-2 glycoprotein. Among those compounds, caryophyllene oxide demonstrated the most potent inhibitor by specifically bind to the RBD site of glycoprotein as compared to the control. Furthermore, caryophyllene oxide also showed high druglikeness ability based on the Lipinski rule of five and low toxicity with 100% accuracy. Therefore, caryophyllene oxide derived from *Pogostemon cablin* Benth is considered as a potential candidate of an inhibitor agent for SARS-CoV-2 glycoprotein.

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AUTHORS CONTRIBUTIONS

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CONFLICTS OF INTERESTS

Declared none

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