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The potency of blumeatin and luteolin as caspase-1 inhibitor by molecular docking

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Abstract: COVID-19 infection induces inflammation by increasing cytokines such as IL-1β, IL-6, IL-18, IFN-γ, and TNF-α. IL-1β is generated by the involvement of caspase-1. Therefore, caspase-1 inhibitor can be potential for inflammation therapy caused by COVID-19 infection. This study aims to determine the potential of blumeatin and luteolin as anti-inflammatory agents by inhibiting caspase-1 using a molecular docking approach. This study was carried out by caspase-1 (PDB ID: 1RWK) preparation, blumeatin and luteolin structure optimization, docking protocol validation, and docking of blumeatin and luteolin on caspase-1. Bluematin and luteolin had a binding affinity of -5,63 kcal/mol and -5,93 kcal/mol, lower than Q158 native ligand (-3.92 kcal/mol). Similar amino acid residues in hydrogen bonds interaction were observed between Q158 native ligand, blumeatin, and luteolin with caspase-1 (GLN 283 and ARG 179). Blumeatin and luteolin are potentially anti-inflammation agents through the inhibition of the caspase-1 *in silico*.

Keywords: blumeatin, caspase-1, cytokines storm, in silico, luteolin, molecular docking.

Introduction

COVID-19 infection causes inflammation of the lungs by increasing cytokines [1]. Cytokines have an important role in the immune response, including defense against viral infections. However, COVID-19 infection can result in an overproduction of cytokines (cytokine storm) that can develop into pneumonia. Cytokine storms play a role in causing acute respiratory distress syndrome (ARDS) [2]. ARDS is a critical condition that needs a prompt and appropriate therapeutic intervention to prevent acute lung damage, multi-organ failure, and death [3].

Cytokine storm is a critical, life-threatening condition that requires intensive care. Cytokine storms are characterized by the clinical presentation of excessive systemic inflammation, hyperferritinemia, hemodynamic instability, and multi-organ failure. The trigger for a cytokine storm is an uncontrolled immune response that results in the continuous activation and expansion of immune cells, lymphocytes, and macrophages. Clinical findings of a cytokine storm are caused by pro-inflammatory cytokines such as IL-1, IL-6, IL-18, IFN-γ, and TNF-α [4].

IL-1 is a potent inflammatory cytokine involved in immunological responses to both innate and adaptive immunity. There are two similar molecules of IL-1, IL-1α, and IL-1β [5]. IL-1β is expressed in many tissues, including lung tissues [6]. IL-1β is produced by interleukin-1β converting enzyme (ICE), also known as caspase-1, that can be activated due to OCVID-19 infection [7]. Caspase-1 is a cysteine protease that converts pro-inflammatory IL-1β into active and mature IL-1β [8].

The caspase-1/ICE inhibitor can potentially be used for lung inflammation therapy caused by COVID-19 infection. Caspase-1 may be inhibited by several compounds like natural flavonoids [9]. Blumeatin and luteolin (Figure 1) are natural flavonoid that can be found in sembung plant (*Blumea balsamifera* L.) [10]. Blumeatin has various biological activities such as hepatoprotective, superoxide radical scavenging, antioxidant, and antityrosinase [11,12]. Meanwhile, luteolin has superoxide radical scavenging, antioxidant and antityrosinase activity [12].

The inhibitory activity of blumeatin and luteolin against caspase-1 can be determined by using *in silico* molecular docking, a computational simulation used to know the binding between a ligand and protein [13]. This study aims to determine the potential effect of blumeatin and luteolin as anti-inflammation by

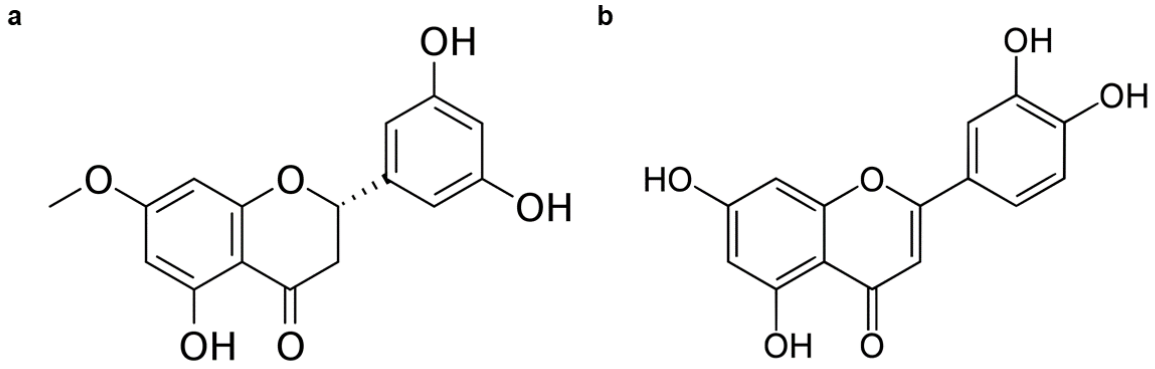

Figure 1. Natural flavonoids compounds (a) blumeatin, (b) luteolin

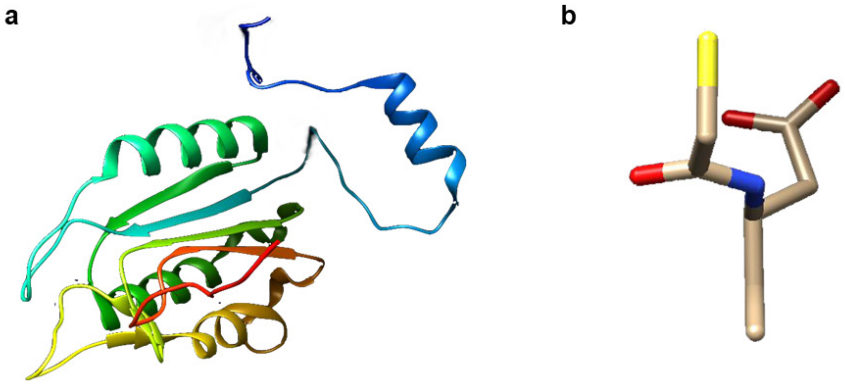

Figure 2. Structure of protein and native ligand. (a) caspase-1, (b) Q158

inhibition of caspase-1 using a molecular docking approach.

Methods

Preparation of the protein

Caspase-1 protein structure was downloaded from https://www.rcsb.org/. Caspase-1 (PDB ID: 1RWK) was prepared by using Chimera 1.10.1 and separated from 3-(2-mercapto-acetylamino)-4-oxo-pentanoic acid (Q158) native ligand.

Optimization of blumeatin and luteolin

The three-dimensional (3D) structures of blumeatin and luteolin were downloaded from https://pubchem.ncbi.nlm.nih.gov/. The structures were optimized using HyperChem 8 with Austin Model 1 (AM1) semi-empirical computational method and single-point calculations and geometry optimization.

Validation of docking method

The molecular docking procedure was validated using the Autodock Tools application (Autodock4 and Autogrid4) through redocking the native ligand Q158 to the prepared caspase-1 protein. The grid box size was set to x = 30 Å, y = 20 Å, z = 30 Å with the x, y, and z coordinate centers were 33.016 Å, 60.302 Å, and 4.934 Å, respectively. The validation parameter of the molecular docking method was the value of root mean square deviation (RMSD), which is valid if the value ≤ 2.0 Å [14].

Blumeatin and luteolin docking to caspase-1

The molecular docking of the prepared blumeatin and luteolin was performed using Autodock 4.2 program. The docking process was conducted with a similar grid box size as validation step. The docking results showed the conformations with the lowest binding energy in complex with caspase-1 protein.

Data analysis

The molecular docking results were binding energy and visualization of the interaction between blumeatin or luteolin and caspase-1 protein. The lower the binding energy, the stronger the interaction of the

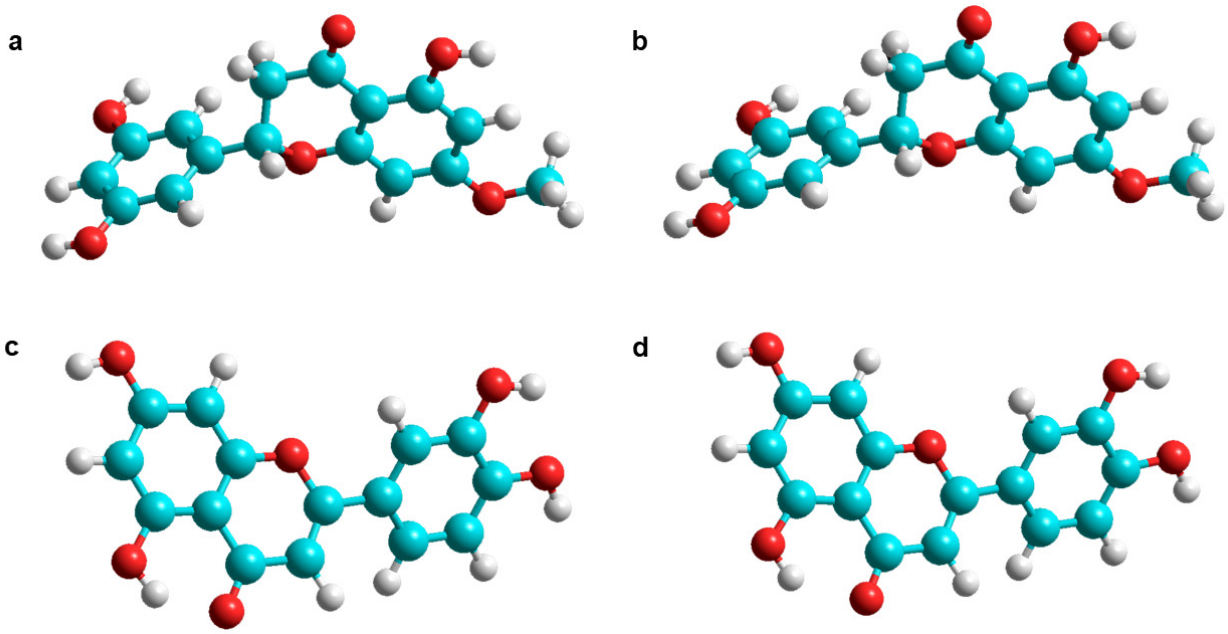

Figure 3. The results of single-point calculation and geometry optimization of the optimized structures. (a-b) blumeatin, (c-d) luteolin

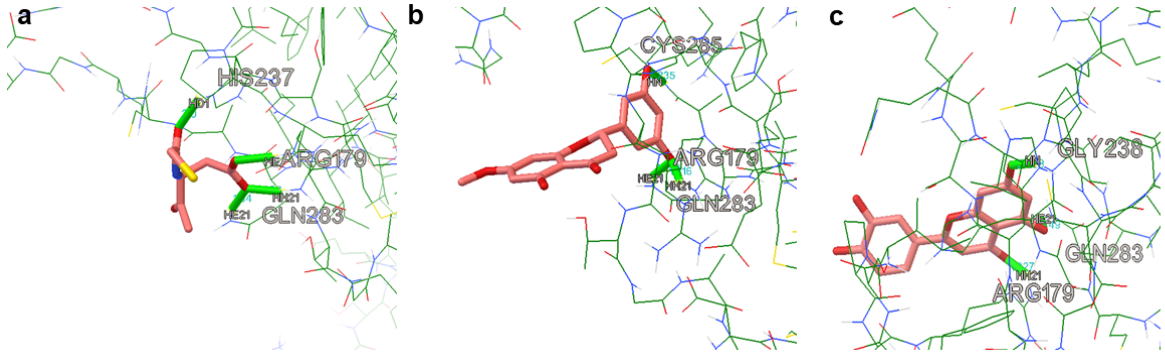

Figure 4. 3D visualization of docking results. (a) Q158, (b) blumeatin, (c) luteolin

compounds with protein, indicating the potential as anti-inflammatory agents.

Results

Caspase-1 preparation

The preparation of caspase-1 protein aims to separate the protein from Q158 native ligand as well as to obtain the Q158 for the docking validation. The prepared caspase-1 and Q158 native ligand are displayed in Figure 2.

Optimization of blumeatin and luteolin

The optimization of blumeatin resulted in total single point energy and geometry optimization of -3996 kcal/mol and -4006 kcal/mol, respectively. Meanwhile, the total single point energy and geometry optimization obtained of luteolin were -3606 kcal/mol and -3814 kcal/mol (Figure 3).

Validation of molecular docking

The docking method was validated by redocking Q158 native ligand to caspase-1. The validation produced 10 conformations with different RMSD values and binding energy. Conformation 6 had the lowest RMSD value of 1.97 Å (Table 2), suggesting the docking method is valid.

Docking of blumeatin and luteolin to caspase-1

The optimized blumeatin and luteolin were then docked to the caspase-1 protein. The docking process

produced ten conformations with binding affinity -5.63 kcal/mol and -5.93 kcal/mol for blumeatin and luteolin, respectively (Table 3). Visualization analysis indicated blumeatin and luteolin interacted with caspase-1 by hydrogen bonding through ARG 179 and GLN 283 residues (Figure 4).

Table 2. The results of molecular docking validation

Ligand	Conformations	Binding energy (kcal/mol)	RMSD (Å)	Amino acid residues	Groups in hydrogen bonds
Q158	1	-4.17	3.51	GLN 283 ARG 179 ARG 179	HE21-O11 HH21-O11 HE-O12
	2	-4.41	2.43	ARG 179 HIS 237 GLN 283 ARG 179	HE-N4 HD1-O8 HE21-O11 HH21-O11
	3	-4.31	2.71	ARG 179 HIS 237 GLN 283 ARG 179	HE-N4 HD1-O8 HE21-O11 HH21-O11
	4	-4.7	2.71	GLN 283 CYS 285 ARG 179	HE21-O8 HN-O12 HH21-O8
	5	-4.17	2.58	ARG 179 HIS 237 GLN 283 ARG 179	HE-N4 HD1-O8 HE21-O12 HH21-O12
	6*	-3.92	1.97	ARG 179 HIS 237 GLN 283 ARG 179	HE-O11 HD1-O13 HE21-O12 HH21-O12
	7	-4.2	2.3	CYS 285 GLN 283 GLY 238 ARG 179	HN-O12 HE21-O13 HN-O12 HH21-O13
	8	-4.63	2.4	GLN 283 CYS 285 HIS 237	HE21-O8 HN-O12 HD1-O11
	9	-4.34	2.35	CYS 285 GLN 283 GLY 238 ARG 179	HN-O11 HE21-O13 HN-O11 HH21-O13
	10	-4.71	2.74	CYS 285 ARG 179 GLN 283 HIS 237	HN-O12 HE-O13 HE21-O8 HD1-O11

Table 3. Results of blumeatin and luteolin molecular docking against caspase-1

Ligand	Conformations	Binding energy (kcal/mol)	RMSD (Å)	Amino acid residues	Groups in hydrogen bonds
Blumeatin	1	-5.38	0.18	GLY 238 CYS 285	HN-O HN-O
	2	-5.62	0.04	GLN-283 CYS-285 ARG 179	HE21-O HN-O HH21-O
	3*	-5.63	0.0	GLN-283 CYS-285 ARG 179	HE21-O HN-O HH21-O
	4	-5.43	0.17	CYS 285	HN-O
	5	-5.61	0.05	ARG 179 GLN 283	HH21-O HE21-O
	6	-5.6	0.03	ARG 179 HIS 237 GLN 283 ARG 179	HE-O11 HD1-O13 HE21-O12 HH21-O12
	7	-5.43	0.0	GLY 238 CYS 285	HN-O HN-O
	8	-5.62	0.01	ARG 179 GLN 283 CYS 285	HH21-O HE21-O HN-O
	9	-6.61	0.09	ARG 179 GLN 283 CYS 285	HH21-O HE21-O HN-O
	10	-5.62	0.21	ARG 179 GLN 283	HH21-O HE21-O
Luteolin	1*	-5.93	0.0	ARG 179 GLY 238 GLN 283	HH21-O HN-O HE21-O
	2	-5.93	0.05	ARG 179 GLY 238 GLN 283	HH21-O HN-O HE21-O
	3	-5.93	0.02	ARG 179 GLY 238 GLN 283	HH21-O HN-O HE21-O
	4	-5.93	0.05	ARG 179 GLY 238 GLN 283	HH21-O,O HN-O HE21-O
	5	-5.93	0.05	ARG 179 GLY 238 GLN 283	HH21-O,O HN-O HE21-O
	6	-5.92	0.1	ARG 179 GLY 238 GLN 283	HH21-O,O HN-O HE21-O
	7	-5.93	0.01	ARG 179 GLY 238 GLN 283	HH21-O,O HN-O HE21-O
	8	-5.93	0.02	ARG 179 GLY 238 GLN 283	HH21-O HN-O HE21-O
	9	-5.92	0.12	ARG 179 GLY 238 GLN 283	HH21-O,O HN-O HE21-O
	10	-5.92	0.09	ARG 179 GLY 238 GLN 283	HH21-O,O HN-O HE21-O

Table 4. The summary of docking results of Q158 native ligand, bluematin, and luteolin on caspase-1

Ligands	Binding energy (kcal/mol)	Amino acid residues	Groups in hydrogen bonds
Native ligand Q158	-3.92	ARG 179 HIS 237 GLN 283 ARG 179	HE-O11 HD1-O13 HE21-O12 HH21-O12
Blumeatin	-5.63	GLN-283 CYS-285 ARG 179	HE21-O HN-O HH21-O
Luteolin	-5.93	ARG 179 GLY 238 GLN 283	HH21-O HN-O HE21-O

Discussion

Our results showed that blumeatin and luteolin have a lower binding affinity (-5.63 kcal/mol and -5.93 kcal/mol) than Q158 native ligand (-3.92 kcal/mol) (Table 4). Blumeatin and luteolin have similar hydrogen-bonding interactions with Q158 native ligand through ARG 179 and GLN 283 residues. These results suggested blumeatin and luteolin docked in the same active site of Q158 native ligand in the caspase-1 target protein.

An *in silico* molecular docking study of rosmarinic acid (RA) to caspase-1 (PDB ID: 1RWK) showed that docking score was 25.0 ± 0.05 and showed interaction with GLY 238, HIS 237, SER 339, and ARG 341 residues [15]. Another docking study to caspase-1 (PDB ID: 1RWK) also showed compound 2-(4-{2-[(phenylthio) acetyl]-carbonohydrazonoyl}-phenoxy)acetamide has interaction through GLN 283, ARG 179, ARG 341, HIS 237, and ASP 288 [16]. An *in vitro* study of the anti-inflammatory activity of blumeatin and luteolin was carried out by the dual-luciferase assay method. The result showed that blumeatin (0.01 mmol/L and 0.1 mmol/L) and luteolin (0.01 mmol/L, 0.1 mmol/L and 1 mmol/L) have anti-inflammatory activity by inhibit NF-κB expression. Ingenuity pathway analysis (IPA) predicted that blumeatin has an anti-inflammatory effect related to Hif-1α [17]. Luteolin and luteolin-7-O-glycoside have been reported for anti-inflammatory effects due to TNF-α inhibition [18].

Based on this study, blumeatin and luteolin are predicted to have activity as the anti-inflammation agent. The interaction that occurs between blumeatin and luteolin on the active site of caspase-1 shows inhibitory activity so that it can inhibit the maturation of IL-1β, thereby decreasing the mature IL-1β expression.

Conclusion

The binding energy of blumeatin and luteolin with caspase-1 was lower than Q158 native ligand, indicating these compounds have an affinity for caspase-1. Blumeatin and luteolin are potentially anti-inflammation agents based on the *in silico* study against caspase-1.

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Declaration of interest

None.

Author contributions

IPAACP conceptualized the study design, IPAACP, IMHP, LWSP, KDMSD investigated the data, IPAACP, LWSP wrote original draft, IMPH, KDMSD, NPLL reviewed and edited final version, NPLL supervised all experiments. All authors have read the final manuscript.

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