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In silico Study of Centella Asiatica Active Compounds as Anti-Inflammatory Agent by Decreasing Il-1 And Il-6 Activity, Promoting Il-4 Activity

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Abstract

Inflammatory is cascade process triggered by pro-inflammatory cytokines (IL-1 and IL-6) and anti-inflammatory cytokines (IL-4). *Centella asiatica* is known for has been studied fot its anti-inflammatory properties. The docking studies was used in the study to evaluate interaction between Centella asiatica active compound with IL-1α, IL-1β, IL-6, and IL-4.

Based on docking results, asiaticoside and batulinic acid had binding energy for IL-1α respectively -14.4199 kcal/mol and -12.3706 kcal/mol. In IL-1β docking, there were asiaticoside, madasiatic acid, and terminolic acid with binding energy -11.9288; -11.3074; and -11.2061 kcal/mol respectively. Asiaticoside bound into receptor active site Gln 141 of IL-1β. Asiatic acid, madecassic acid, and asiaticoside in IL-6 docking had binding energy -9.8244; -9.7071; and -9.0171 kcal/mol respectively. The three ligand bound into IL-6 receptor active site Arg179. IL-4 reacted to madecassic acid, termolinic acid, and asiaticoside with binding energy -12.6151; -12.5650; and -11.7490 kcal/mol respectively. The three ligands bound to receptor binding site IL-4 Lys102.

Centella asiatica active compounds (asiaticoside, termolinic acid, madecassid acid, asiatic acid, batulinic acid, and madasiatic acid) interacted significantly with inflammatory cytokines (IL-1α, IL-1β, IL-6, and IL-4). Madecassic acid, asiticoside, asiatic acid, madasiatic acid, and terminolic acid inhibit the pro-inflammatory cytokines by binding to receptor active site of IL-1β and IL-6. Madecassic acid, terminolic acid, and asiaticoside enhance anti-inflammatory cytokines by binding to IL4 receptor binding sites.

Keywords: Anti-inflammation, *Centella asiatica, In silico*, IL-1α, IL-1β, IL-6, IL-4.

1. INTRODUCTION

Cytokines is an intercellular protein produced by virtually all nucleated cell which used as signaling between cells. Cytokines act by binding to receptor membrane of targeted cell, thus trigger cascade pathway in targeted cell. This signaling pathway controls have immunomodulatory effect, controlling gene expression that leads to inflammatory cascade process.[1,2]

There had been 200 cytokines identified. These cytokines are classified into several groups, namely interleukin, growth factor, chemokines, interferon, hematopoietin, and colony stimulating factors. According to their role in inflammation process, cytokines separated into two groups, pro-inflammatory cytokines (i.e. IL-1, IL-6, TNF-α, TGF-β) and anti-inflammatory cytokines (i.e. IL-1Ra, IL- 4, IL-10, IL-13). Pro-inflammatory cytokines and anti-inflammatory cytokines works balancing each other in homeostatic environment. Disturbances in these cytokines cellular system have been studied significant in development of various diseases[1,3]

Interleukin-1 (IL-1) plays significant role as pro-inflammatory cytokines as it is secreted by macrophage and monocyte in response of inflammation mediator. IL-1 acts by affecting almost every cell in human body, usually works together with another pro-inflammatory cytokines, commonly tumor necrosis factor (TNF). Two types isoform of IL-1 have been studied for almost 30 years as pro-inflammatory cytokines, namely IL- 1α and IL-1β. Both of IL- 1α and IL-1β bind to IL-1 receptor type I (IL-1RI) and the IL-1 receptor accessory protein (IL-1RAcP) thus inducing stabilization of cytokine mRNA and the activation of transcription factors such as NFκB and AP-1 for inflammation process. Studies state that IL-1β has more potent role in inflammatory process however there isn't significant difference biological activity amongst them.[5-7]

IL-6 production by macrophage and monocytes is induced by IL-1β. IL-6 major action is responsible for further recruitment and differentiation of macrophage, anti-apoptosis of lymphocyte T, differentiation of lymphocyte T and B. IL-6 as pro-inflammatory cytokines is paradoxically involved in skin inflammation and regeneration. However, as a product from IL-1 signaling, IL-6 becomes an important negative feedback signaling for IL-1 production. Based on these capabilities, IL-6 is known as pleotropic cytokines, containing both pro-inflammatory and anti-inflammatory properties.[8,9]

IL-4 is commonly known as anti-inflammatory cytokines based on IL-4 capability to inhibit production of major pro-inflammatory cytokines, IL-1 and TNF-α. IL-4 is produced by T-cell with functions merely controlling B-cell proliferation and differentiation. IL-4 role is important for limiting the inflammation process, that if uncontrolled capable to be destructive to host itself.[10]

Centella asiatica, commonly known as "Pegagan" in Indonesia, had been used among oriental traditional medicine practices for thousand years. *Centella asiatica* had been known to possess several benefits such as antipyretic, diuretics, antibacterial, antiviral, and cognitive enhancer. Its benefits have been studied extensively as its ever increasing usage in Eastern and Western countries. In dermatological fields, *Centella asiatica* is used in several cases including treating wound, burn wound, hypertrophic lesion, eczema, psoriasis, leprae, and lupus erythematosus.[11,12]

In vitro studies show asiatic acid and madecassid acid, active compound in *Centella asiatica*, played anti-inflammatory effect by inhibiting enzymes (iNOS, cyclooxygenase-2(COX-2)), interleukins (IL-6, IL-1β), and tumor necrosis factor (TNF-α).[13,14]

Molecular modelling through computational (in silico) method is widely applied and developed in pharmacology hypothesis testing. The docking method assess interaction between ligand and the biomolecular target by placing ligand in active site using model of protein-substrate interaction. In turn, docking method enable designing small molecule to inhibit target protein. Quality of information in architecture of active site is crucial for dictating the geometry of ligand that will bind within.[15] Orientation preference in the binding interaction between ligand and target which simulated in the method may predicts the strength affinity or binding affinity. Furthermore, docking method has been used for identifying physiochemical characterization of new drugs.[16,17]

This study performed in silico docking study to investigate interaction between *Centella asiatica* active compound with IL-1α, IL-1β, IL-6, and IL-4.

2. METHODS

The study was performed by software tool installed on PC Intel® core i7 3770 3,5 GHz, Quad Core-8 threaded, RAM 32 GB DDR3 10600. Software used in this study were MarvinBean® Suite, Open Babel®, PyMOL® verse 1.5.03 Open Source, PyRx-Autodock-Vina®, Chimera®, JChem® for Excel®.

2.1. Ligand and Protein Preparation

Three-dimensional structure of ligand (active compounds of *Centella asiatica)* structure used in this study was downloaded from Pubchem® database. The structure had been optimized in three-dimensional structure using Chemdraw® V-2000.

Crystallography protein was downloaded from protein bank data http://www.rcsb.org/pdb/results/ with IL-1β (6I1B), IL-1α (2KKI), IL-4 (4YDY) and IL-6 (1P9M). Chimera program was used for preparation of crystallography protein. Protein A and B was optimized using available docking preparation. Optimization was according to default, which using AMBER method hydrogen addition and charge adjustment. The residue was enhance through Drunbrack Rotamer Library® (Drunbrack, 2002). Based on interaction account from protein data bank, co-crystal ligand was selected as crystallography ligand then used in validation process. Co-crystal ligand was prepared with hydrogen addition and charge adjustment.

2.2. Docking Validation

Validation of crystallography ligand was conducted via four phases. Crystallography ligand protein was selected for docking. Ligand from docking process was saved and compared with crystallography ligand for measuring Root Mean Square Deviation (RMSD). The result from docking with RMSD $\leq$ 2 was selected. Docking protein and ligand derivatives inserted into PyRx program. Method used was similar with the docking process except there wasn't comparison with crystallography ligand. Result from docking was saved in .csv format and .sdf format. Analysis conducted based on interaction between residue and observed ligand along with binding affinity from molecular docking.

3. RESULTS AND DISCUSSION

Inflammatory response is a complicated process, which involves a complicated network of signalling molecules, mainly cytokines. Some of the cytokines exert their function as pro-inflammatory agents such as IL1, IL-6 and TNFα, whereas others have anti-inflammatory activities such as IL-4. However, these cytokines acts similar by binding to target cell receptor before alter the function of target cell. These binding requires specific site within the structure of cytokines known as receptor binding site and receptor active site. In this study, we tried to see *Centella asiatica* active compound interaction to these sites of cytokines.

As in the studies using molecular docking, binding energy used in this study as the primary parameter. Binding energy shows the strength and affinity of interaction between ligand and receptor where greater binding energy correlates with weaker interaction. So, ligand with the least amount of binding energy had the best affinity amongst the group.[18] Protein residue was used for determining specific site of hydrogen bonding interaction. Receptor active site and receptor binding site of the protein target from Protein Data Bank (PDB) was used in this study. Binding occurs in receptor active site showed ligand capability to act as inhibitor of target protein. Binding occurs in receptor binding site may leads to enhancement of the protein target. Location of receptor binding site and receptor active site of the target protein depicted in Table 1.

Based on the result of docking with IL-1α (Table 2), we can see that asiaticoside bound into IL1- α ligand with binding energy -14.4199 kcal/mol. This results showed asiaticoside had the highest binding affinity among other ligand. Batulinic acid also showed significant binding energy -12.3706 kcal/mol. Binding energies of other test ligand is depicted in the table.

This model binding also showed that asiaticoside and batulinic acid didn't bind to either receptor active site nor receptor binding site of IL-1α. This result made the mechanism of asiaticoside and batulinic acid in direct inhibition of IL-1α cannot be proved by this model. However, this result didn't hinder the ability of *Centella asiatica* as inhibitor of IL-1α, as inhibition may be achieved in different level. In study by Salim et al, *Centella asiatica* had been shown inhibited IL-1α by 23.7 ± 4.57 %.[23] This activity could be explained as several studies showed that IL-1α expression pathway, the nuclear factor NF-kB Pathway, was lowered by *Centella asiatica*. Madecassic acid and asiaticoside inhibited expression of IL-1α by annulment of inhibitory kappa B-α (IκB- α) degradation in the NF-kB pathway.[24]

Study analysis of docking with IL-1β is depicted in Table 3. The results showed that asiaticoside , madasiatic acid, and terminolic acid had top three binding energy respectively -11.9288, -11.3074, and -11.2061 kcal/mol. The common residue found in their interaction was Lys77. However this residue wasn't located in either binding site or active site of IL-1β. Asiaticoside was the only active compound that bind to IL-1β receptor binding site by binding to Gln141 position. Another ligand that bind to receptor binding site Gln141 was asiatic acid with lesser binding affinity -9.2626 kcal/mol.

Result of docking study in Table 4 showed there are top three active components of *Centella asiatica* potentially acted as inhibitor of IL-6. Ranking from the lowest binding energy were respectively asiatic acid, madecassic acid, asiaticoside with binding energy -9.8244, -9.7071 and -9.0171 kcal/mol.

Based on model, the common residues found from the ligand interactions were Arg 179 and Ser220. Arg 179 was located in the receptor active site of IL-6. Asiatic acid bound with the lowest binding energy to IL-6 receptor active site.

Based on the IL-4 docking result in Table 5, the three active compounds of *Centella asiatica* reacted with IL-4 with lowest binding energy were madecassic acid, terminolic acid, and asiaticoside. Madecassic acid had binding energy -12.6151 kcal/mol. Meanwhile, terminolic acid and asiaticoside reacted with binding energy respectively -12.5650 and -11.7490 kcal/mol.

Table 1. Active site and binding site of the protein target from literature [19-22]

Target Protein	Receptor Binding Site	Receptor Active Site
IL-1α	140,141,151,153,156,158,159,162,170,211,212,213,225,227,229,230,231,250	135,137,263
IL-1β	130,131,141,143,146,148,151,154,162,208,209,210,219,221,223,224,225,246	125,127,261
IL-6	30,31,33,52,78,81,105	179
IL-4	47, 50,51,54,58,59,60,61,62,63,64,99,100, 102,103,104,106	

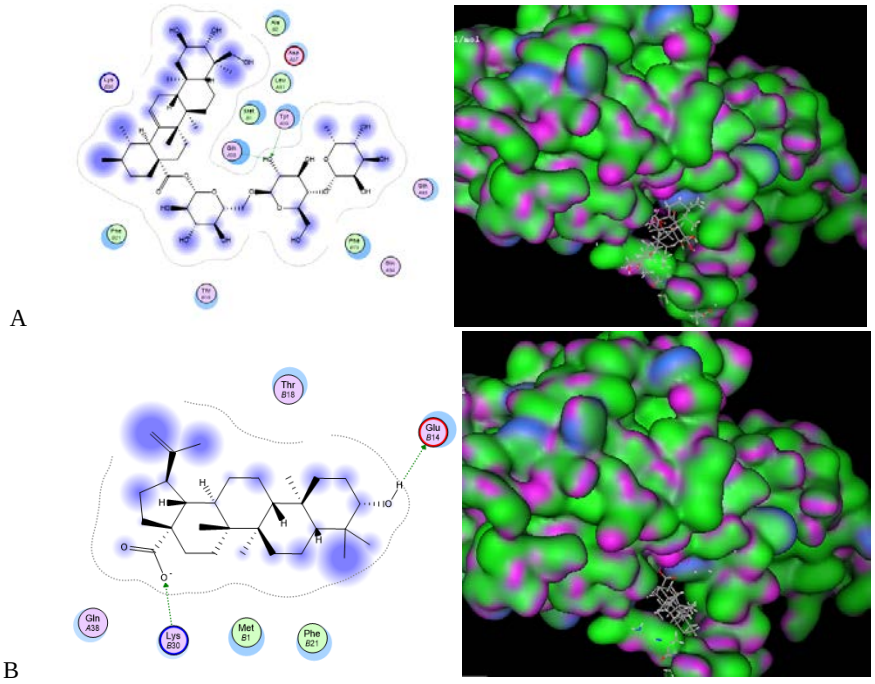

Figure 1. 2D and 3D model binding of ligand (A) ligand asiaticoside with IL-1α (B)batulinic acid with IL-1α

Table 2. Binding energy and residue components of each ligand with IL-1α

Molecules	ΔG (kcal/mol)	pKi	H don & H acc
Asiatic acid	-10.3779	7.651	Glu14, Glu14 , Lys30
Asiaticoside	-14.4199	7.982	Tyr39, Gln38, Lys30, Met1
Batulinic acid	-12.3706	7.894	Glu14, Lys30, Met1
Brahmol	-9.1118	7.562	Glu14 , Lys30
Centella sapogenol	-8.4881	5.692	Lys30
Isothankunik acid	-10.8180	6.721	Glu14 , Lys30
Madecassic acid	-11.0808	7.168	Glu14 , Lys30
Madasiatic acid	-9.6402	7.031	Lys30
Terminolic acid	-11.5765	8.059	Tyr39, Tyr39, Lys30

ΔG(mean binding energy);pKi(binding affinity);H don (Hydrogen donor); H acc (Hydrogen acceptor)

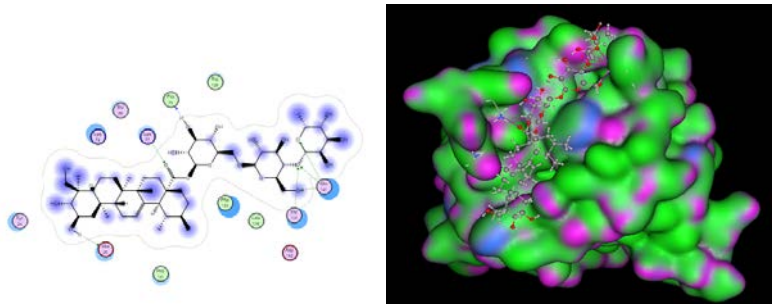

Figure 2. 2D and 3D model binding of ligand asiaticoside with IL-1β

Table 3. Binding energy and residue components of each ligand with IL-1β

Molecules	ΔG (kcal/mol)	pKi	H don & H acc
Asiatic acid	-9.2626	10.443	Thr137, Asp142, Lys72, Lys77, Gln141
Asiaticoside	-11.9288	8.212	Glu25, Pro78,Lys77, Gln141
Batulinic acid	-8.3510	5.266	Lys77
Brahmol	-9.5541	5.300	Tyr24, Glu25, Glu25
Centella sapogenol	-9.5742	5.668	Thr137, Thr137
Isothankunik acid	-10.4660	6.079	Lys77
Madecassic acid	-9.8571	6.642	Lys77
Madasiatic acid	-11.3074	7.439	Tyr24, Leu80, Lys77
Terminolic acid	-11.2061	6.368	Lys74, Lys77

ΔG(mean binding energy);pKi(binding affinity);H don (Hydrogen donor); H acc (Hydrogen acceptor)

Lys102 was the most prevalent residue from all of the active compound interactions, only absent in batulinic acid's. Lys102 was located in receptor binding site of IL-4. The top three binding energy compound madecassic acid, termolinic acid, and asiaticoside bind with lowest binding energy to Lys102. Madecassic acid also bind to Arg 47 which makes madecassic acid bind with two hydrogen bond in IL-4 receptor binding site.

Present study found active compound of *Centella asiatica* reacted with pro-inflammatory cytokines, IL-1α, IL-1β, and IL-6. *Centella asiatica* also had been showed reacted with anti-inflammatory cytokines IL-4. Based on 2D and 3D model, we had predicted the location of hydrogen binding formed by significant compound to cytokines. We found that *Centella asiatica* active compound bind into receptor active site of IL-1β, and IL-6. Therefore, these binding modeled their role as direct inhibitor to the inflammatory cytokines. In IL-4 docking study, the active compound bind into receptor binding site hence enhance the anti-inflammatory activity of IL-4. However, another study with another crystallography protein is required to ensure the method. Further investigation in laboratory studies is important for assessing *Centella asiatica* potency.

This study predicted *Centella asiatica* roles as anti-inflammatory agent by inhibiting pro-inflammatory cytokines and enhancing anti-inflammatory cytokines. Previous in vitro studies showed that the active compounds of *Centella asiatica* was able to reduce inflammation.[25-27] Study by Choi et al showed *Centella asiatica* extract treatment lowered IL-1β and IL-6 production in hepatocytes.[25] In addition, study by Won et Al showed madecassic acid and madecassoside (asiaticoside) isolated from *Centella asiatica* inhibited IL-1β and IL-6 production on macrophage cell assay.[26] Our study found that madecassic acid, asiticoside, asiatic acid, madasiatic acid, and terminolic acid bound to receptor active site of IL-1β and IL-6. This binding showed potential for direct inhibition of IL-1β and IL-6 which determines potential side of *Centella asiatica* anti-inflammation properties. In study by Masola et al, extract of *Centella asiatica* treatment boosted IL-4 level in diabetic rats brain tissue.[27] Our study had found *Centella asiatica's* madecassic acid, termolinic acid, and asiaticoside reacted with IL-4 receptor active site.

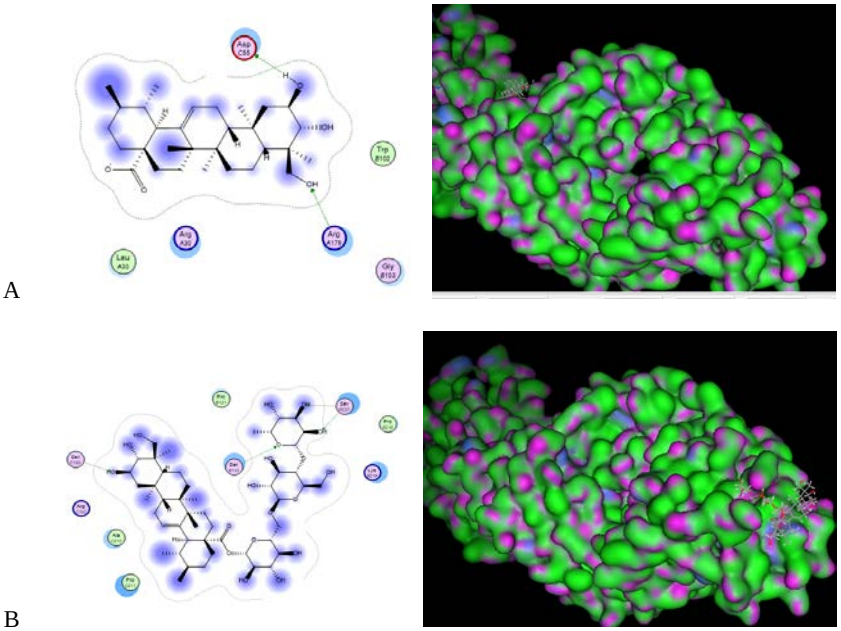

Figure 3. 2D and 3D model binding of ligand (A) asiatic acid with IL-6, (B) madecassic acid with IL-6

Table 4. Binding energy and residue components of each ligand with IL-6

Molecules	ΔG (kcal/mol)	pKi	H don & H acc
Asiatic acid	-9.8244	8.478	Asp55,Arg30,Arg179, Arg179
Asiaticoside	-9.0171	9.045	Ser220, Ser133, Ser133, Ser220, Ser220
Batulinic acid	-8.7648	6.579	Arg179, 1rg179
Brahmol	-5.0335	4.201	Tyr170
Centella sapogenol	-6.6113	8.838	Asp62, Ser63, Glu99, Gly101, Ser63
Isothankunik acid	-7.6477	5.840	Asp55, Gln175
Madecassic acid	-9.7071	6.559	Lys206, lys206
Madasiatic Acid	-8.6699	5.872	Glu99, Gly101
Terminolic acid	-8.0815	6.024	Arg179, Arg179

ΔG(mean binding energy);pKi(binding affinity);H don (Hydrogen donor); H acc (Hydrogen acceptor)

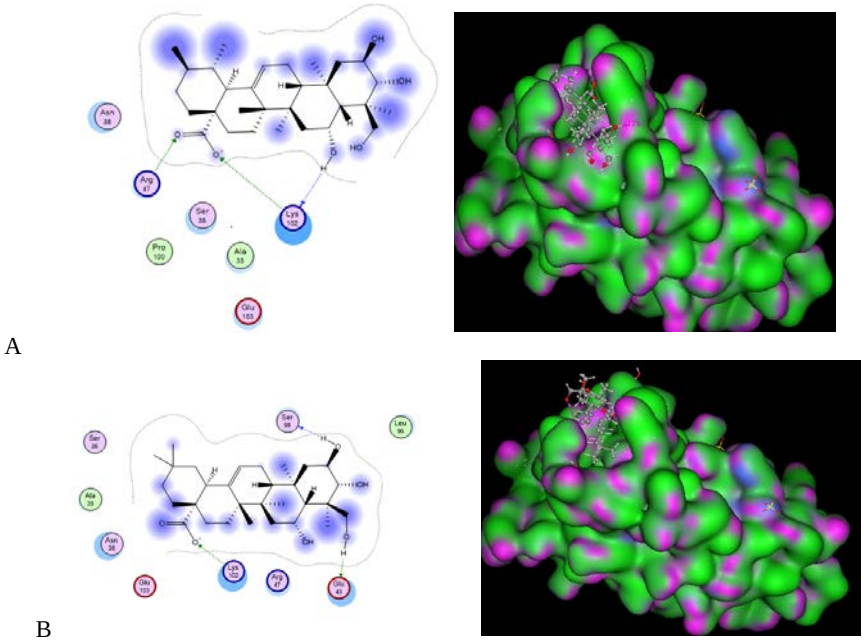

Figure 4. 2D and 3D model binding of ligand (A) madecassic acid with IL-4 (B) termolinic acid with IL-4

Table 5. Binding energy and residue components of each ligand with IL-4

Molecules	ΔG (kcal/mol)	pKi	H don & H acc
Asiatic acid	-11.7158	6.245	Lys102
Asiaticoside	-11.7490	9.612	Glu43, Glu122,Glu122, Lys102
Batulinic acid	-10.2716	6.389	Glu43, Ser36, Ser36
Brahmol	-11.2074	6.978	Asn38, Asn38, Thr39, Glu43,Glu43, Lys102
Centella sapogenol	-10.9736	6.482	Glu103, Glu103
Isothankunik acid	-10.8386	7.092	Lys102
Madecassic acid	-12.6151	7.322	Lys102, Arg47, Lys102Ala35
Madasiatic Acid	-10.2344	6.043	Asn38, Lys102
Terminolic acid	-12.5650	7.508	Glu43,Ser98, Lys102

ΔG(mean binding energy);pKi(binding affinity);H don (Hydrogen donor); H acc (Hydrogen acceptor)

4. CONCLUSION

In summary, *Centella asiatica's* interaction between its active compound with inflammatory cytokines IL-1α, IL-1β, IL-6, and IL-4 had been assessed by *in silico* docking method. Asiaticoside and batulinic acid had bind significantly with IL-1α. Asiaticoside, madasiatic acid, and terminolic acid bound to IL-1β, with asiaticoside bound into receptor active site of IL-1β. Asiatic acid, madecassic acid, and asiaticoside bound with receptor active site of IL-6. In the other hand, IL-4 reacted with madecassic acid, terminolic acid, and asiaticoside in receptor binding site. Result in this study predicted *Centella asiatica's* role as anti-inflammatory agent. However, further investigation is required for testing this study probable pathway of *Centella asiatica's* role as anti-inflammatory agent through *in vitro* studies.

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