In Silico Study : *Phyllanthus Niruri L* as Immunomodulator Against Covid-19

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Abstract

In December 2019, a mysterious case of pneumonia was first reported in Wuhan, Hubei Province. The source of transmission of this case is still unknown, but the first case was linked to the fish market in Wuhan. From 18 December to 29 December 2019, there were five patients treated with Acute Respiratory Distress Syndrome (ARDS). 2 From 31 December 2019 to 3 January 2020 this case increased rapidly, marked by 44 cases reported. In less than a month, the disease has spread to other provinces in China, Thailand, Japan and South Korea. This virus can be transmitted from human to human and has spread widely in China and more than 190 other countries and territories. On 12 March 2020, WHO announced COVID-19 as a pandemic. As of March 29, 2020, there were 634,835 cases and 33,106 deaths worldwide. While in Indonesia, 1,528 cases were confirmed with COVID-19 and 136 deaths. Phyllanthus niruri L (meniran) is one type of immunostimulator that can improve the immune system in animal experiments and humans. This study aims to determine Phyllanthus niruri L as an immunomodulator for Covid-19. From the results of research conducted that Phyllanthus niruri L can improve the activities and functions of several non-specific immune system components and specific immune systems, both soluble and cell-related forms. The conclusion from the results of this study is Phyllanthus niruri L as an immunomodulator for Covid-19.

Keywords: *Phyllanthus niruri* L, Immunomodulator, Covid-19

Introduction

In December 2019, a mysterious case of pneumonia was first reported in Wuhan, Hubei Province. The source of transmission of this case is still unknown, but the first case was linked to the fish market in Wuhan. From 18 December to 29 December 2019, there were five patients treated with Acute Respiratory Distress Syndrome (ARDS). 2 From 31 December 2019 to 3 January 2020 this case increased rapidly, marked by 44 cases reported. In less than a month, the disease has spread to other provinces in China, Thailand, Japan and South Korea [1,2].

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The sample studied shows the etiology of a new coronavirus. Initially, the disease was temporarily named as the 2019 novel coronavirus (2019-nCoV), then WHO announced a new name on February 11, 2020 namely Coronavirus Disease (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) virus.). This virus can be transmitted from human to human and has spread widely in China and more than 190 other countries and territories. On 12 March 2020, WHO announced COVID-19 as a pandemic. As of March 29, 2020, there were 634,835 cases and 33,106 deaths worldwide. While in Indonesia there have been 1,528 cases tested positive for COVID-19 and 136 cases of death[3,4,5].

Phyllanthus niruri L (meniran) is one type of immunostimulator that can improve the immune system in animal experiments and humans. The administration of Phyllanthus niruri L extract can increase the activity

and function of several components of the nonspecific immune system and specific immune system, both dissolved (humorous) and cell-related forms. Effects of nonspecific immune responses include increased phagocytosis and macrophage and neutrophil chemotaxis movements, NK cell cytotoxicity and complement hemolysis activities. Cellular immunity can increase the proliferation of T lymphocytes by increasing the secretion of TNFa, IFNg and IL-4, and decreasing the secretion of IL-2 and IL-10, whereas in humoral immunity, this drug can increase the synthesis of immunoglobulin M (IgM) and IgG, play a role in against infection[6,7,8,9,10,11] This study aims to determine Phyllanthus niruri L as an immunomodulator for Covid-19.

Materials and Methods

Phyllantus niruri L. Compound and Protein Target Preparation

The Bioactive component of Phyllantus niruri L. was obtained from a literature review and the component that could be identified was Cathechin, and Quercetin was then downloaded via pubchem in .sdf format, then changed the extension to .pdb using Discovery Studio Visualizer and the energy was minimized using Autodock.

The crystal structure of the target protein is TNF-alpha (PDB ID: 2AZ5), IL-1 (PDB ID: 2NVH), IL-6 (PDB ID: 1P9M) and iNOS (PDB ID: 1NS1). The protein is the main protein that plays a role in the signaling of inflammation that occurs in the case of Covid-19 and causes cytokine storm, causing symptoms of Acute Respiratory Distress Syndrome. The things that can interfere with docking results are water molecules because they can increase resolution and must be eliminated by using Discovery Studio Visualizer.

Molecular Docking Analysis

The docking was carried out by Pryx and the results of docking is in Kcal/mol. Docking is performed on all protein targets with different ligands. Then the results of Molecular Docking are analyzed and visualized the results through PyMOL and LigPlot+ to determine binding pocket and residual amino acids that bind into ligand.

Results

Molecular docking was carried out using PyRx software on TNF-alpha, IL-1, IL-6 and iNOS proteins that had been prepared before and using Phyllantus niruri L. bioactive components, namely Cathecin and Quercetin which had also been prepared before. Molecular docking is carried out in certain grid boxes with different sizes for each protein. For TNF-α use a gridbox (x: y: z) with centers -14,286: 65,875: 26,349, ntps 126: 126: 126, and spacing 0.644. For IL-1 center 46,251: 14,257: 69,836, ntps 100: 114: 124, and spacing 0.481. In IL-6 protein center -57,152 175,358 45,212, ntps 126 126 126 and spacing 1,000 and finally in iNOS Protein using center 52,683 47,596 52,073, ntps 126 126 126 126, and spacing 1,000.

The strength of molecular docking can be seen from the value of binding affinity expressed by Gibbs free energy (ΔG). The binding energy is strongly influenced by the amino acid residues of the target protein which bind and react hydrophobically to the ligand and follow the energy equation as follows:

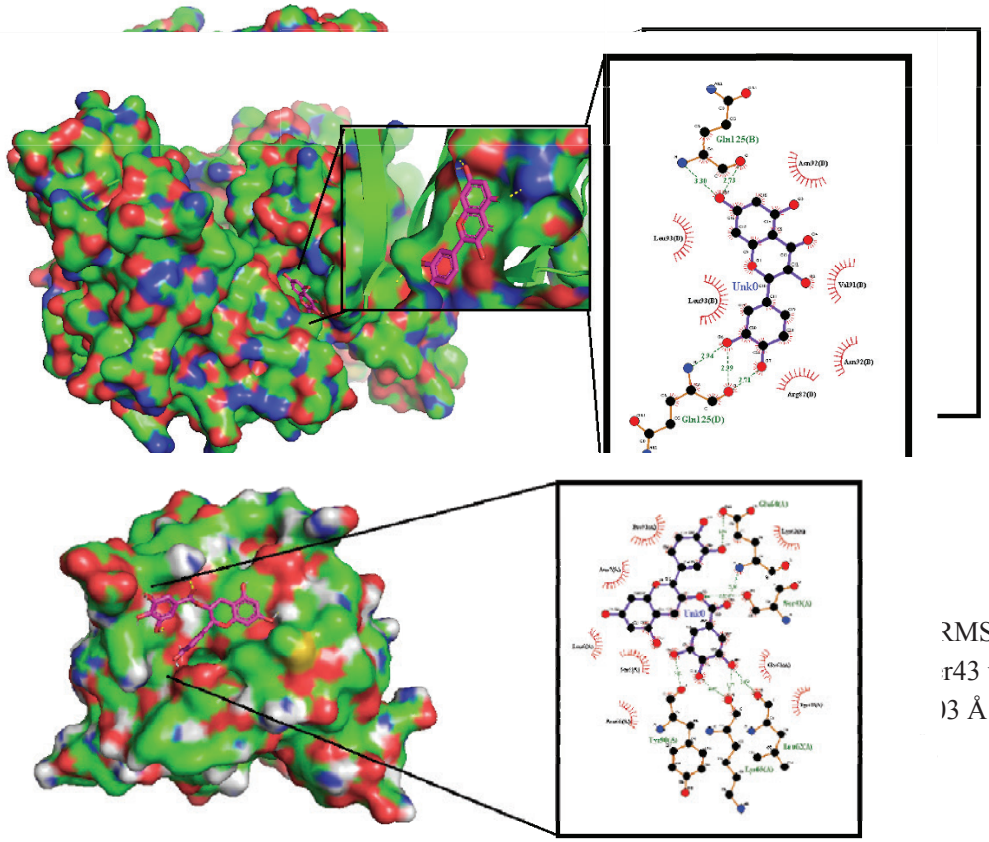
$$\Delta G_{binding} = \Delta G_{Gauss} + \Delta G_{Repulsi} + \Delta G_{HBond} + \Delta G_{Hydrophobic} + \Delta G_{Torsion}$$ [12].

The more amino acid residues that interact with the ligands, the smaller the value of ΔG will be (negative) and the reaction will take place spontaneously and bind very strongly so that the inhibitory effect it creates will also be stronger [13].

To see the validity of the docking results, the value of the root mean square deviation (RMSD) can be used to assess the error rate or linearity of a compound to be tested with a compound that is made as a standard. The smaller the RMSD value, the less error is considered. So the RMSD value <2.00 Å is considered the validation measurement standard value [14].

Table 1. The binding affinity of molecular docking between the bioactive compound Phyllantus niruri against the proinflammatory cytokine target protein.

Bioactive Compound	Target protein	ΔG (Kcal/mol)	RMSD (Å)
Cathecin	TNF-α	-8,3	2,383 Å
	IL-1	-7,3	1,682 Å
	IL-6	-8,6	2,014 Å
	iNOS	-10	1,037 Å
	TNF-α	-8,3	1,067 Å
			1,869 Å
			1,729 Å
			1,861 Å



ıl with RMSD 2,383
ıd forms a hydrogen
125 (B) with a bond
SD 1.067 Å with the

RMSD of 1.682 Å and for
r43 with bond distances of
)3 Å and in quercetin binds

Figure 2. The docking result of Cathecin (Top) and Quercetin (Bottom) with IL-1

From the IL-6 obtained the binding affinity for Cathecin was -8.6 Kcal/mol with RMSD 2.014 Å and -8.1 Kcal/mol for Quercetin with RMSD 1.729 Å. The cathecin have hydrogen bonds with Phe168 with a distance of 3.09 Å, Phe134 with 2.81 Å, 3.16 Å, 3.12 Å, and 2.85 Å. Gln135 with 2.76 Å, Leu64 with 3.13 Å and Leu62 with 2.76 Å. Where the quercetin binds to Phe136 with a bond distance of 2.97 Å, Ala152 with 2.71 Å and 2.93 Å, Gly127 with 3.14 Å, Arg128 with 2.84 Å and Thr130 with 2.98 Å..

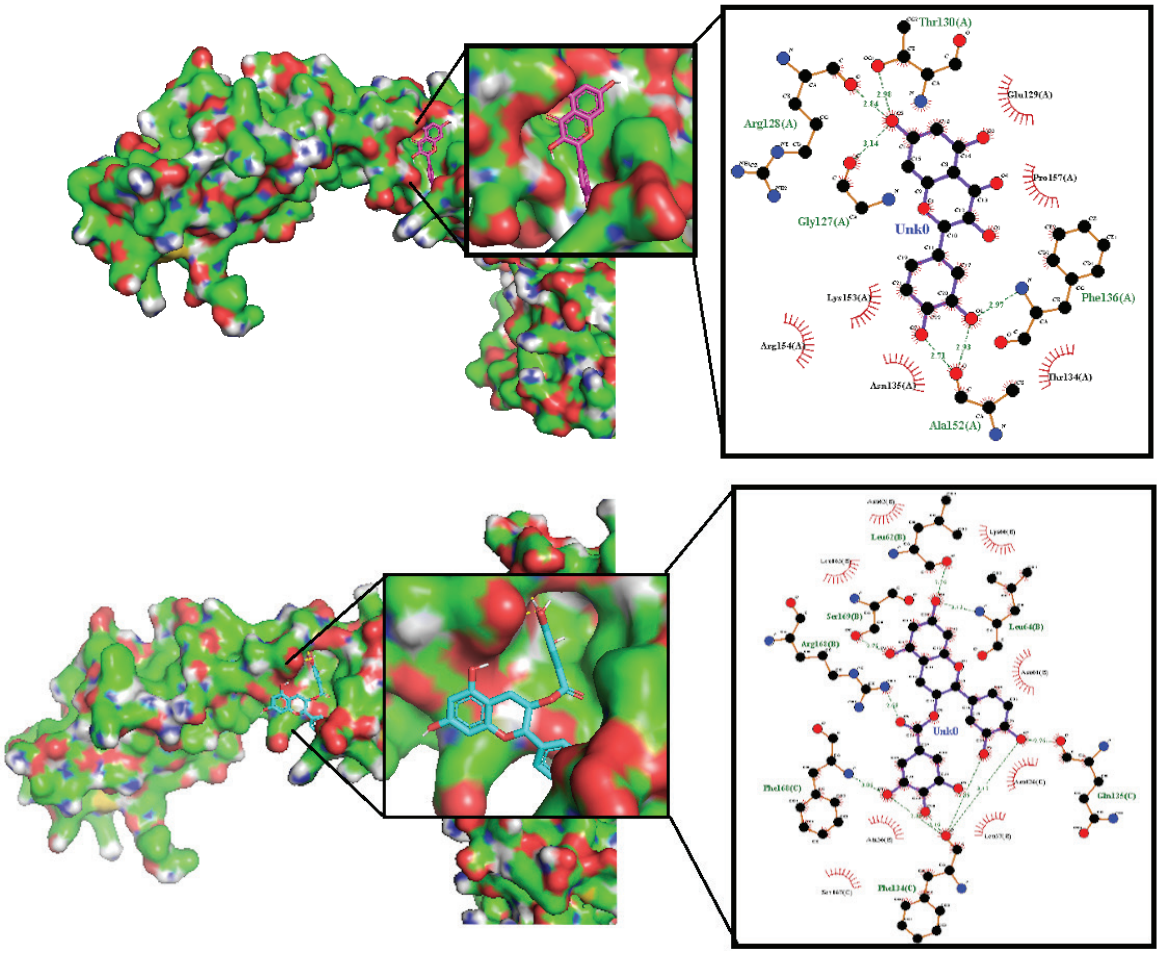
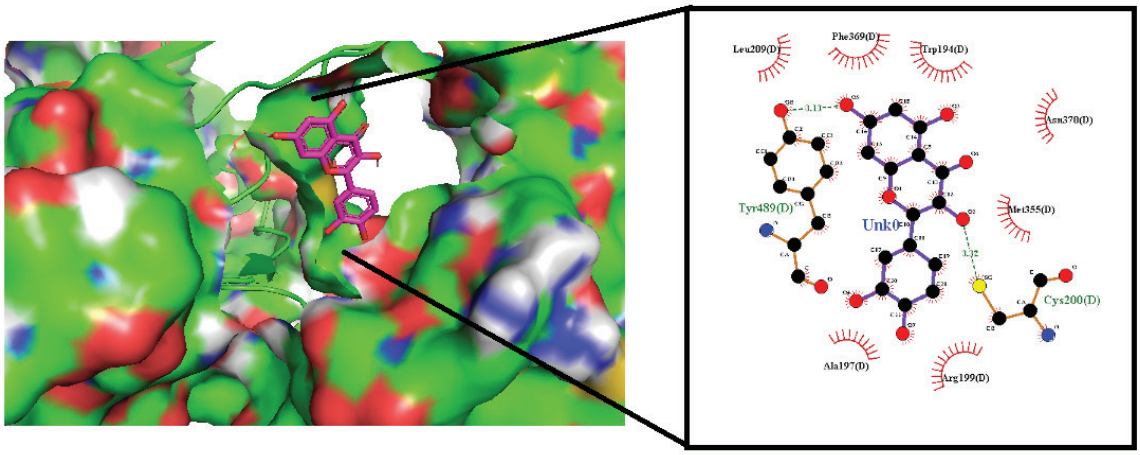


Figure 3. The docking result of Cathecin (Top) and Quercetin (Bottom) with IL-6

In iNOS, the binding affinity of -10 Kcal /mol was obtained for Cathecin with RMSD 1,037 Å and -8.3 Kcal / mol with RMSD 1,861 Å. For cathecin it have hydrogen bonds with Trp372 with a bond distance of 3.02 Å, Asn370 with 2.75 Å and Tyr489 with 2.70 Å. In quercetin it binds with Cys200 with 3.32 Å and Tyr489 with distance 3.13 Å.



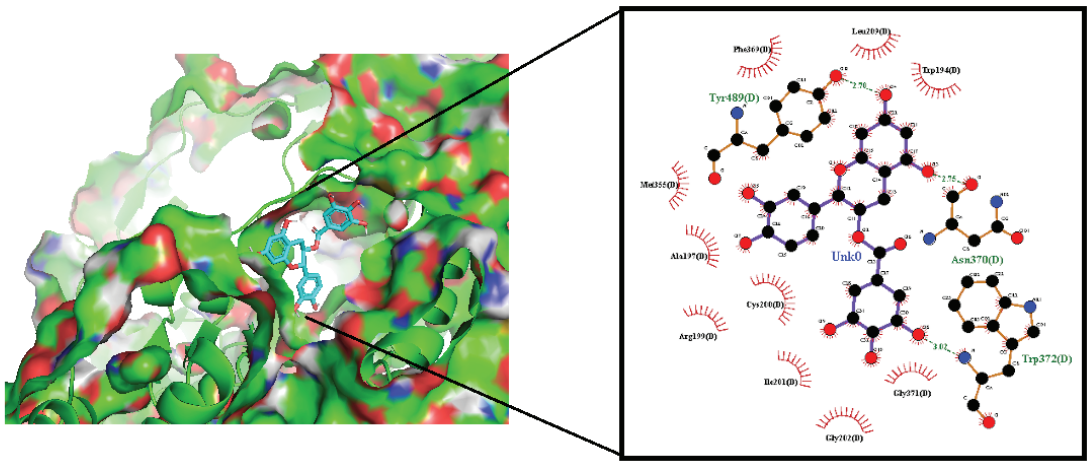


Figure 4. Hasil docking Cathecin (Atas) dan Quercetin (Bawah) dengan iNOS

Discussion

In the activation of the immune system, macrophages and monocytes produce Tumor Necrosis Factor-Alpha (TNF-α) and will bind to TNFR1 or TNFR2 and will stimulate the formation of degenerative protein caspase 3 and cause apoptosis of cells because they have death domains in the carboxyl terminal[15]. Overexpression of TNF-α will cause excessive cell apoptosis and stimulate the process of excessive inflammation [15]. Cathechin and quercetin bind to TNF-α with binding energy -8.3 Kcal / mol and inhibit the expression of TNF-α and inhibit excessive apoptosis of cells.

Cytokines produced by Presenting Cell Antigens such as macrophages are very important in the stimulation of T cells and B lymphocytes. Helper T cells (Th) will produce cytokines IL-2, IL-6 and IL-1 which are important in celullar immunity and humoral immunity [16]. IL-1 as a proinflammatory cytokine that plays a role in acute and chronic inflammation. Responsible for the febrile process and also sepsis and activation of neutrophils in the non-specific immune system [16]. Cathecin and quercetin in Phyllantus niruri bind to IL-1 with binding energy -7.3 Kcal / mol and -7.1 Kcal / mol. Both of these bioactive compounds inhibit the overexpression of IL-1 and have anti-inflammatory effects.

Activation of IL-6 as a proinflammatory cytokine will activate the inflammatory process and autoimmune response and generally occurs in the pathogenesis of immune mediated arthritis [17]. Cathechin and quercetin bind to IL-6 with binding energy -8.6 Kcal / mol and -8.1 Kcal / mol and have anti-inflammatory effects.

Inducible nitric oxide synthase (iNOS) functions to synthesize Nictric Oxide (NO) from L-Arginine. iNOS plays a role in NO biosynthesis preventing bacterial growth and inhibiting inflammation by inhibiting T cells [18]. Excessive NO production will induce inflammatory diseases [18]. Cathecin and quercetin bind to iNOS with bond energies of -10 Kcal / mol and -8.3 Kcal / mol. These compounds will inhibit the production of NO thereby preventing the effects of excessive inflammation.

Conclusion

Catechin and Quercetin in Phyllanthus niruri can inhibit the expression of TNF-α, IL-1, IL-6, and iNOS thus inhibiting the process of excessive inflammation and functioning as an immunomodulator

Conflict of Interests: The authors declare that they have no conflict of interest.

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Ethical Approval : This study was approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia (No. 2.KE.142. 08. 2018).

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