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Lagerstroemia, Euphorbia Hirta, and *Kleinhovia Hospita* as Inhibitors of Heptad Repeat (HR) SARS-CoV-2 Spike Protein Based on an *In Silico* Study

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Abstract: Severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease-19 (COVID-19), has become a global health issue. Spike proteins from the virus have a vital role in infection. Herbal medicines such as *Lagerstroemia*, *Euphorbia hirta*, and *Kleinhovia hospita* have several pharmacological functions such as anticancer, antiviral, and antioxidant because of their bioactive compound content. Based on an in silico study, this research was conducted on the possibility of phytochemicals from herbal *Lagerstroemia*, *E. hirta*, and *K. hospita* to inhibit spike protein SARS-CoV-2. A three-dimensional (3D) compound structure of each herbal medicine was docked with HR protein using AutoDock Vina software. The docking result, which has the best binding energy value, is continued with the analysis of molecular dynamics simulation. Lagerine, rutin, and nicotiflorin compounds might bind to proteins with lower binding energy. Protein was unstable when complexed with compounds compared with control, as seen from the root-mean-square deviation (RMSD) value. Therefore, this research is pre-experimental to inhibit SARS-CoV-2 spike proteins by herbal medicines.

Keywords: COVID-19, *Euphorbia hirta*, *Kleinhovia hospita*, *Lagerstroemia*, severe acute respiratory syndrome-coronavirus 2.

基于计算机研究的紫薇、大戟属赫塔和克莱因霍维亚医院作为七肽重复(人力资源)非典-冠状病毒-2 刺突蛋白的抑制剂

摘要：严重急性呼吸综合征冠状病毒 2(非典-冠状病毒-2)是冠状病毒病 19(新冠肺炎)的病原体，已成为全球健康问题。来自病毒的刺突蛋白在感染中起着至关重要的作用。紫薇、大戟和小花等中草药因其生物活性化合物含量而具有抗癌、抗病毒和抗氧化等多种药理功能。基于一项计算机研究，这项研究是针对草药紫薇、E.希尔塔和 K.医院中的植物化学物质抑制刺突蛋白非典-冠状病毒-2 的可能性进行的。使用自动停靠维娜 软件将每种草药的三维(3D)复合结构与人力资源蛋白对接。结合能值最佳的对接结果继续进行分子动力学模拟分析。紫薇、芦丁和烟花苷化合物可能与结合能较低的蛋白质结合。从均方根偏差(RMSD)值可以看出，与对照相比，当与化合物复合时，蛋白质是不稳定的。因此，这项研究是通过草药抑制非典-冠状病毒-2 刺突蛋白的预实验。

关键词：新冠肺炎, 大戟, 克莱因霍维亚医院, 紫薇, 严重急性呼吸综合征冠状病毒 2。

1. Introduction

At the end of December 2019, in Wuhan, Hubei Province, China, a novel coronavirus disease (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was discovered. It is rapidly spreading from its origin to the rest of the world, causing a pandemic [1, 2]. As of November 2021, more than 260 million people have become infected, and over 5 million have died because of this pandemic [28]. Although several vaccines are already

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found, the war against COVID-19 continues, and complementary medicine and diet are needed to prevent the severity of the disease [2, 3].

Prevent replication and attachment respiratory of syndrome-coronavirus (SARS-CoV-2) become the main target in combating COVID-19 [4, 5]. Previous studies have shown that several proteins from SARS-CoV-2 have been modeled and used as a target to decrease the number of positive cases [6–8]. The primary key of SARS-CoV-2 to enter the host cells is the spike protein, consisting of parts, such as heptad repeat 1 (HR1) and HR2 inside the receptor-binding domain (RBD) to form a fusion of membrane after attachment [7, 9, 10]. Based on this critical role, HR1 and HR2 can be the main targets to evade viral entry and infection [7].

Several herbal medicines with active compounds used as antioxidants, anticancer, antibacterial, and antiviral drugs were reported [11]. Antioxidants are used to prevent damage to cells caused by free radicals. In China, herbal medicines are used as conventional therapies for patients infected with SARS-CoV-2 [12, 13]. In Indonesia, herbal medicines are expected to provide treatment for SARS-CoV-2 patients. The current study focused on three herbal medicines, *Lagerstroemia*, *E. hirta*, and *K. hospita*, as potential treatments for COVID-19. In addition, several compounds from herbal medicines, such as rutin and nicotiflorin, were reported to have antioxidant and antiviral effects [14-16].

2. Materials and Method

2.1. Ligand and Protein Selection

The compounds of herbal medicines were collected from the KNApSAcK database [30]. The structures of three-dimensional (3D) compounds were assessed through the PubChem database. The 3D structures of protein were obtained from the Protein Data Bank (PDB) [29]. The HR structures were retrieved from the PDB (PDB ID: 6LXT).

2.2. Molecular Docking

Protein-ligand docking was analyzed using AutoDock Vina integrated into PyRx 0.8 [17, 18]. Docking was performed on the active side of HR. Herbal compounds were screened using molecular docking. Protein-protein docking was performed using HEX 8.0, was run using default settings, and was operated under the Shape + DARS correlation type [19]. The results of docking and protein-ligand interactions were analyzed using BIOVIA Discovery Studio.

2.3. Molecular Dynamics Simulation

The best binding affinity from the docking results was continued to molecular dynamics simulation using the software Yet Another Scientific Artificial Reality Application (YASARA) [20]. The parameters used were in accordance with the cell physiological conditions (37°C, 1 atm, pH 7.4, and salt content of 0.9%) for 20 ns with autosave every 25 ps. The simulation was run using the md_runfast macro program, and the root-mean-square deviation (RMSD) was displayed using the macro md_analyze program.

2.4. Potential Compounds

Potential compounds from *Lagerstroemia*, *E. hirta*, and *K. hospita* were analyzed using Way2Drug Pass Server prediction to determine antiviral activity based on Pa value (Probability to Be Active).

3. Results and Discussion

Through the KNApSAcK retrieval, 22 compounds from *Lagerstroemia*, *E. hirta*, and *K. hospita* were collected. The results of the docking HR based on binding energy showed that a compound from each herbal medicine, such as lagerine, rutin, and nicotiflorin, has a lower binding energy value of −7.5, −6.9, and −7 kcal/mol for HR1 (Table 1) and −1175.3, −986.9, and −1027.8 kcal/mol for HR1-HR2 (complex) (Table 2). The binding energy of the HR complex after binding to this compound could decrease compared to that of the HR complex without the ligand (Table 2). This compound could also change the HR1–HR2 binding motif (Fig. 1a). Among the three compounds that have the best affinity to HR1, rutin was able to modify the HR1–HR2 interaction. This was described by the binding energy value of the HR complex with rutin, which had the highest value among the complexes, compared to the HR complex alone. Further analysis used molecular dynamics simulation to evaluate the interaction of compounds when interacting with HR1–HR2.

Table 1 Binding energy of *Lagerstroemia*, *Euphorbia hirta*, and *Kleinhovia hospital*

Herbal	Compound	CID	Binding Energy
			HR1
Lagerstroemia	Cryogenine	61002	-5.9
	Petunidin 3-arabinoside	6325802	-6.6
	Petunidin 3-O-beta-D-glucopyranoside	443651	-6.3
	Malvidin 3-arabinoside	12137511	-6.5
	Oenin	443652	-6.2
	Lagerstronolide	101445553	-6.1
	(+/-)-Epiepoxydon	331736	-5
	Methyl 3,4,5-trihydroxybenzoate	7428	-4.7
	Isoprene	6557	-4
	5-Epi-dihydrolyfoline	42640298	-7.1
	Dihydrolyfoline	42640297	-6.5
	Lagerine	101473393	**-7.5**
	Decamine	2993	-6.1
	Lagerstremine	78385190	-6
Euphorbia hirta	alpha-Amyrin	73170	-6.6
	beta-Amyrin	73145	-6.6
	Taraxerol	92097	-6.9
	Rutin	5280805	**-6.9**
	Ellagic acid	5281855	-6.2
	Euphorbianin	44259305	-6.4
Kleinhovia hospita	**Nicotiflorin**	5318767	**-7**
	Rutin	5280805	-6.9

Note: Compounds with high binding energy are marked in bold

Table 2 Binding energy of HR complex + ligand

Herbal	Compound	CID	Binding Energy HR Complex + ligand
Lagerstroemia	Lagerine	101473393	-1175.3
Euphorbia hirta	Rutin	5280805	-986.9
Kleinhovia hospita	Nicotiflorin	5318767	-1027.8
HR1 & HR2 (Control)		12137511	-3136.8

Molecular dynamics simulations were carried out to analyze the structural stability and conformational fluctuations of HR protein interaction with compounds. The important information regarding the stability and flexibility of protein-ligand complexes during the simulation is shown in RMSD. High deviation and variability indicate low stability [21]. Proteins that were stable during the simulation had RMSD values less than 3Å. An RMSD value greater than 3Å indicates that the protein has undergone some structural changes [22]. The molecular dynamics simulation results show that the protein has structural changes (Fig. 1b), and the HR complex with compound inside showed that the RMSD value was more than 3Å compared with the HR complex alone (Fig. 1c). The crucial step for SARS-CoV-2 fusion to membranes is the interaction of HR1–HR2 to form a helix bundle [23, 24]. Modification of the helix bundle formation has been studied to prevent viral entry [7]. A compound such as Lagerine, nicotiflorin, and rutin has an RMSD value of more than 3Å, which may have an excellent potency in inhibiting viral infection. The fusion process is essential for virus entry into the cell host. HR1 and HR2 are located in the S2 subunit regions, which, when exposed, form the fusion core. The formation of the fusion core induces virus membrane fusion with the host cell membrane. The herbal compound can bind to HR1 more strongly and prevent HR2 from binding to HR1 to form the fusion core (Fig. 2).

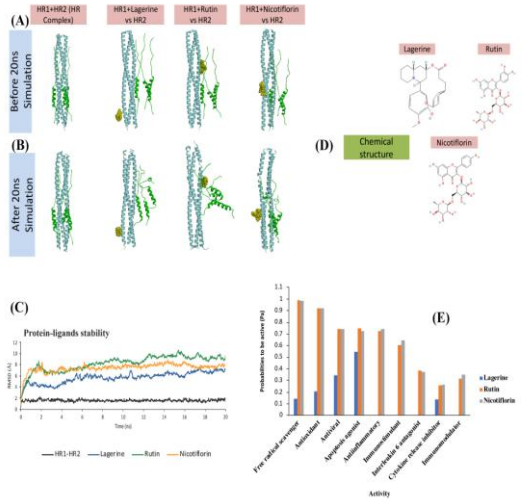

Fig. 1 Structural orientation and binding energy of the heptad repeat (HR) complex after being bound with phytochemical ligands (A) and structural alignment of HR before simulation (B) and after being bound with phytochemical ligands after simulation. The cyan ribbons in Fig. A represent the HR protein complex, and the yellow spheres describe the phytochemical ligand. (C) The stability of protein-ligand complex interactions. (D) Chemical structure of the ligand. (E) Probability to be active and activity of the ligands

Furthermore, the herbal compound was analyzed with Way2Drug Pass Server. Probability to be active (Pa) > 0.7 indicated that the compound is predicted to have a high potential as an immunomodulator. Pa > 0.3 and less than 0.7 indicates that the compound has the lowest potential as an immunomodulator. The results of the Way2Drug analysis have shown that compounds such as rutin (Pa 0.743) and nicotiflorin (Pa 0.742) have potential as antivirals (Fig. 2d), and lagerine (Pa 0.549) has potential as an apoptosis agonist. The highest activity is with free radical scavengers with a Pa value of 0.988 for rutin and 0.984 for nicotiflorin and antioxidants (0.923, 0.924). Stress conditions caused by lipid oxidation will be responded to in the body with an inflammatory mechanism. If stress occurs continuously, it would be chronic inflammation. The role of herbal medicines as anti-oxidative stress is supported by Pa values as those of antioxidants and free radical scavengers. Cytokine storms are inflammation that occurs through the deregulation of the immune response, leading to the disturbance of tissue homeostasis and severe organ damage [25, 26]. Herbal medicines such as *E. hirta* and *K. hospita* are potential anti-inflammatory drugs with Pa values of 0.728 and 0.743. From another perspective, immunostimulants enhance the immune system to suppress inflammation [27]. Rutin and nicotiflorin have immunostimulant activity (with Pa values of 0.607 and 0.642).

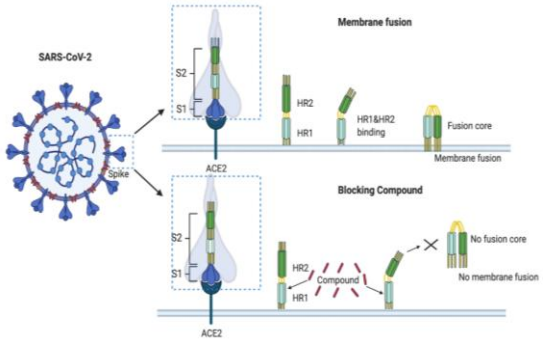

Fig. 2 Fusion core formation mechanism and blocking effect of an antivirus. The S1 subunit contains one receptor-binding domain (RBD). The entry of the virus is mediated by the S2 subunit for the virus/cell membrane fusion. The RBD of the S1 subunit binds to the host angiotensin-converting enzyme 2 receptor when the SARS-COV-2 is in contact with the cell membrane. The fusion of S2 is exposed and inserted into the target cell membrane. HR1s and HR2s combine to form the fusion core, pulling the viral membrane to fuse with the host cell membrane. The designed and computationally optimized antivirus can bind to HR1 more tightly, preventing the HR1s and HR2s from forming the fusion core

4. Conclusion

Three compounds from *Lagerstroemia*, *E. hirta*, and *K. hospita* have good treatment potential based on binding energy with spike protein HR. Lagerine, rutin, and nicotiflorin compounds showed potential to prevent viral–host fusion by modifying the HR complex structure. In addition, Rutin and nicotiflorin are good immunomodulators. The results of this study

can be used as pre-experimental evidence to support the use of herbal medicines against COVID-19.

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