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Molecular docking of ethanol extracts of katuk leaf (Sauropus androgynus) on functional proteins of severe acute respiratory syndrome coronavirus 2

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ABSTRACT

The COVID-19 pandemic has become a major health crisis globally. Alternative treatment approaches include using food sources rich in flavonoid compounds, such as the leaves of katuk plant (Sauropus androgynus). The purpose of this study was to analyze the characteristics of the flavonoid group present in active compounds of katuk leaves (Sauropus androgynus) and to study the mechanism underlying interactions (molecular docking) of these compounds with 3CLpro, Nsp1, Nsp3, RdRp, Nsp7_Nsp8 complex, and PLpro in SARS-CoV-2, and ACE2 in humans. In silico analysis was performed using Hex 8.0 software, which is primary tool of docking analysis. Interaction between the ligand and its receptors were analyzed using the software Discovery studio 4.1. The results of this study indicated that ABCD chains of 3CLpro had the highest bond energy with afzelin (-42.77 Kcal/mol), RdRp Nsp7_Nsp8 complex had the highest bond energy with trifolin (-310.87 Kcal/mol), PLpro had the highest bond energy with afzelin (-190.23 Kcal/mol), Nsp1 had the highest bond energy with trifolin (-334.97 Kcal/mol), and ACE2 had the highest bond energy with trifolin (-307.96 Kcal/mol). Thus, on comparison with conventionally used drugs, the active flavonoid compounds in katuk leaves (Sauropus androgynus) showed specific affinity for 3CLpro, Nsp1, Nsp3, RdRp Nsp7_Nsp8 complex, and PLpro in SARS-CoV-2 and ACE2 in humans. Thus, katuk leaves a potential herbal candidates to derive new drugs or complementary medicines for COVID-19.

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1. Introduction

The coronavirus disease 2019 (COVID-19) pandemic has become a major concern and problem in the world. In February 2021, there were 112,456,453 confirmed cases of COVID-19, including 2497,514 deaths (World Health Organization, 2021). As the number of cases increased, the symptoms found in patients increasingly varied (Carvalho et al., 2021; Polak et al., 2020).

In early 2021, the COVID-19 vaccination program began in Indonesia, however till date, its distribution is uneven and it will take a long time for the entire population to be vaccinated. Additionally, the development of COVID-19 treatment is time-consuming. Currently, only anti-viral drugs such as favipiravir and ribavirin, anti-HIV protease inhibitors such as ritonavir and lopinavir, and anti-inflammatory agent such as tocilizumab or dexamethasone have been used for treatment (Russo et al., 2020). Thus, there is a need to explore the biodiversity of Indonesia, which can potentially harbor natural resources with anti-COVID-19 properties (Park et al., 2017). This potential can be directed to virus engineering in the host and environment.

The Katuk plant (Sauropus androgynus) is a plant species that is widely found in Southeast Asia. Its leaves that can be consumed to increase breast milk production. The people of South Kalimantan recognize use katuk leaves as a special food called tapai green sticky rice. Katuk leaves have antibacterial and antiviral properties, and they have high flavonoid content (Andarwulan et al., 2010). Flavonoids have low systemic toxicity, and they can synergize with conventional drugs. Moreover, flavonoids are "pleiotropic" compounds, which means that their functional groups can interact with different cellular targets and inhibit several antiviral molecular pathways (Russo et al., 2020). However, the anti-COVID-19 effect of katuk (Sauropus androgynus) leaves has not been revealed. Therefore, we conducted this study to investigate the potential of the flavonoid group of active compounds from katuk leaves (Sauropus androgynus) as an antiviral agent for COVID-19 using target proteins for COVID-19 drugs.
2. Material and methods

2.1. Design

This is an in-silico study that aimed to study the mechanism (molecular docking) between the flavonoids group found in katuk leaves (Sauropus androgynus) against 3CLpro, Nsp1, Nsp3, RdRp Nsp7_Nsp8 complex, PLpro proteins in SARS-CoV-2 and ACE2 in humans. Further, a comparison of molecular docking between the active compounds and conventional drugs was also performed.

2.2. Preparation of extract

The katuk plant was obtained from the Kandangan area, South Kalimantan. Plant determination was conducted at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, Banjarbaru, South Kalimantan. After determination, 2500 g of leaf flour was soaked in 30 L of 96% ethanol, and it was then stirred for 30 min until well mixed. The mixture was then left for five nights to settle, and it was then filtered using a Buncher funnel to obtain the filtrate.

2.3. LC—HRMS analysis

The extract sample was diluted with a polar according to the solvent. Dilution was performed by looking at the sample density (not too concentrated and not too dilute) with a final volume of 1300 L. The sample was vortexed for 1 min and it was then spendown for 2 min. The supernatant was filtered using a 0.22 m syringe filter and injected into a vial. The sample in the vial was injected into an autosampler, and it was then subjected to the LC—HRMS. Analysis was conducted with LC—HRMS (Thermo Scientific Dionex Ultimate 3000 RSLcnano with microflow meter). Solvent A was 0.1% formic acid in water, and solvent B was 0.1% formic acid in acetonitrile. The analytical column uses Hypersil GOLD aQ 50 x 1 mm x 1.9 particle size. The analytical flow rate was 40 L/min, and run time was 30 min with a temperature of 30°C in the oven column.

2.4. Searching for amino acid sequence

Amino acid sequences that make up 3CLpro, Nsp1, Nsp3, RdRp Nsp7_Nsp8 complex, and PLpro proteins in SARS-CoV-2 and ACE2 in humans were obtained from The Research Collaboratory for Structural Bioinformatics Protein Data Bank database (https://www.rcsb.org). The 3D structure of protein was downloaded in *.pdb file format (Table 1).

2.5. Searching for the structure of active compound

The 3D structure of the active component of katuk leaf (Sauropus androgynus) was obtained from the PubChem Open Chemistry Database. The 3D structure of various compounds in the *.sdf file format was then converted into *.pdb files using OpenBabel software (O’Boyle et al., 2011).

2.6. Docking and visualization of protein-ligand interactions

Docking simulation between the active compound Sauropus androgynus and target protein was performed Hex 8.0 software. (Macindoe et al., 2010), and docking results were then visualized with the Discovery Studio 4.1 software.

2.7. Analysis of the binding interaction between protein and ligand

The results of the docking analysis were will then be visualized using the Discovery Studio 4.1 software (Laskowski and Swindells, 2011; Wolber & Langer, 2005). The interaction between proteins and ligands was studied to analyze the number and types of bonds, such as hydrogen, hydrophobic, and van der Waals bonds.

3. Results

Table 2 shows the interaction energy between the active compounds and protein target. Docking on 3CLpro revealed that the most negative interaction energy of the three active compounds was found on afzelin. The most negative energy was found in the AC (−346.70 Kcal/mol), BD (−347.65 Kcal/mol), and ABCD chains (−42.77 Kcal/mol) of 3CLpro structure. Against the protein complex RdRp Nsp7_Nsp8, trifolin had the most negative energy of the three compounds (−310.87 Kcal/mol). For PLpro and Nsp1 proteins, the most negative energy was found on interaction with afzelin (PLpro: −190.23 Kcal/mol) (Nsp1: −286.89 Kcal/mol). Nsp3 protein had the most negative on binding to trifolin (−334.97 Kcal/mol), and ACE2 protein had the most negative energy on binding to trifolin (−307.96 Kcal/mol).

As shown in Fig. 1, for AC and BD chains of 3CLpro, the most negative interaction energy was observed for afzelin as compared to conventional drugs (Table 4). Afzelin interacts with the AC 3CLpro chain composed of conventional hydrogens on LY55 and TRP207 and van der Waals bonds on ACE2 and SER284, GLU286, LEU282, TRP207, and GLU288; and the hydrogen-carbon bonds in GLU286 and GLY283. Additionally, an unfavorable bump

![Table 2](https://example.com/table2.png)

**Table 2** Identification of flavonoid compounds from Sauropus androgynus leaves.

| Compounds        | Chemical structure | PubChem CID | Group       |
|------------------|--------------------|-------------|-------------|
| Afzelin          | C_{21}H_{20}O_{10} | 5,316,673   | Flavonoid   |
| Kaempferol       | C_{15}H_{10}O_{6}  | 5,280,863   | Flavonoid   |
| Trifolin         | C_{15}H_{19}O_{11}| 5,282,149   | Flavonoid   |

![Table 1](https://example.com/table1.png)

**Table 1** Target proteins in SARS-CoV-2 and entry receptors in humans.

| Protein         | Function                        | Organism   | PDB ID | Reference                             |
|-----------------|---------------------------------|------------|--------|---------------------------------------|
| 3CLpro          | Virus replication               |            |        |                                       |
| Complex of RdRp Nsp7_Nsp8 | Virus replication               |            |        |                                       |
| PLpro           | Virus replication               |            |        |                                       |
| Nsp3            | Virus replication               |            |        |                                       |
| Nsp1            | Virulence factor and spreading agent |            |        |                                       |
| ACE2            | Receptor for SARS-CoV-2         | Human      | 1842   | (Wu et al., 2020)                     |

![Fig. 1](https://example.com/fig1.png)
was formed on PHE291 and Alkyl and Pi-Alkyl bonds in LEU283 and LYS55.

As shown in Fig. 2, for the RdRp Nsp7_Nsp8 complex, the most negative interaction energy was found in chloroquine Table 4. This interaction was organized by hydrogen carbon bonds at ASP161, ASP164, and LYS798 and van der Waals bonds at TYR163, GLU167, ARG553, VAL792, PHE793, MET794, and SER795. For PLpro the most negative bond energy was found in chloroquine. This bond comprised covalent bonds at HOH546 and van der Waals bonds at LYS92, ALA107, HOH526, HOH529, HOH 538, HOH546, and HOH608.

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**Table 3**
Free energy from docking between flavonoids and target proteins.

|                  | 3CLpro AC | Chain BD | Chain ABCD | RdRp NSP7-NSP8 complexes | PLpro | NSP3 | NSP1 | ACE2 |
|------------------|-----------|----------|------------|--------------------------|-------|------|------|------|
| Afzelin          | –346.70*  | –347.65* | –42.77*    | –307.16                  | –190.23* | –299.19* | –286.89* | –303.50 |
| Kaempferol       | –258.32   | –254.16  | –23.28     | –238.45                  | –149.74* | –276.66* | –219.84* | –259.27 |
| Trifolin         | –329.22   | –337.34  | –40.02     | –310.87*                 | –182.42* | –334.97* | –270.22  | –307.96* |

* the most negative interaction energy compared to other compounds for one type of target protein.

**Table 4**
Free energy from docking between ligands or conventional drugs to target protein.

|                  | 3CLpro AC | Chain BD | Chain ABCD | RdRp NSP7-NSP8 complex | PLpro | NSP3 | NSP1 | ACE2 |
|------------------|-----------|----------|------------|------------------------|-------|------|------|------|
| Afzelin          | –346.70*  | –347.65* | –42.77*    | –307.16                | –190.23* | –299.19* | –286.89* | –303.50 |
| Kaempferol       | –258.32   | –254.16  | –23.28     | –238.45                | –149.74* | –276.66* | –219.84* | –259.27 |
| Trifolin         | –329.22   | –337.34  | –40.02     | –310.87*               | –182.42* | –334.97* | –270.22  | –307.96* |
| Chloroquine      | –284.80   | –283.47  | –51.33     | –338.56*               | –231.72* | –320.33* | –286.97  | –230.93 |
| Favipiravir      | –191.77   | –177.20  | –52.08     | –254.56                | –136.52* | –204.09* | –235.71  | –130.32 |
| Ribavirin        | –222.16   | –242.95  | –99.10*    | –335.94                | –174.10* | –317.39* | –302.35* | –66.80 |

* the most negative interaction energy compared to active compounds and conventional drugs for one type of target protein.

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**Fig. 1.** Molecular docking between afzelin with AC chain 3CLpro (A), afzelin with chain BD 3CLpro (B), and ribavirin with chain ABCD 3CLpro (C).

**Fig. 2.** Molecular docking between chloroquine with RdRp NSP7-NSP8 complex (A) and chloroquine with PLPro (B).
For the Nsp3 protein, the most negative interaction energy was found in trifolin Table 4. As shown in Fig. 3, these interactions were organized by hydrogen-carbon bonds at LEU126 and van der Waals bonds at LEU127, SER128, GLY130, ALA154, PHE156, ASP157, and LEU160. This interaction occurred outside the substrate-drug binding pocket at R166, L185, L199, V202, E203, M206-M208, and K232 (Yan and Gao, 2021).

In the ACE2 protein, the most negative interaction energy was found in trifolin. This interaction was organized by conventional hydrogen bonds at LYS187 and ASP509 and van der Waals bonds at GLY104, SER106, LEU120, TYR202, TRP203, ASP206, GLU398, ASN508, TYR510, and SER511.

As shown in Fig. 4 and Table 4, for Nsp1, the most negative interaction energy was obtained for ribavirin. These interactions were organized by conventional hydrogen bonds at LEU37, carbon hydrogen bonds at LYS2 and van der Waals bonds at THR3, VAL5, LEU7, ARG34, GLY40, LYS116, and HOH234.

4. Discussion

Afzelin had the most negative interaction energy of the three active compounds when docked on 3CLpro. The AC (−346.70 Kcal/mol), BD (−347.65 Kcal/mol), and ABCD chains (−42.77 Kcal/mol) of 3CLpro structure had the maximum negative energy. Trifolin had the largest negative energy (−310.87 Kcal/mol) against the protein complex RdRp Nsp7_Nsp8. When PLpro and Nsp1 proteins interacted with afzelin, the highest negative energy was obtained (PLpro: −190.23 Kcal/mol; Nsp1: −286.89 Kcal/mol). When Nsp3 protein bound to trifolin, it had the largest negative energy (−334.97 Kcal/mol).
mol), and when the ACE2 protein bound to trifolin, it produced the maximum negative energy (−307.96 Kcal/mol). These findings indicated that afzelin was multi-targeted against 3CLpro, PLpro and Nsp1 proteins. Additionally, trifolin was also multi-targeted against the protein complex RdRp, Nsp7-Nsp8 and Nsp3. For the first time, this simulation extended the findings of previous studies, thus proving the interaction between afzelin and 3CLpro (Ouassaf et al., 2021; Joshi et al., 2021).

To compare the efficacy of the active compound with conventional drugs, we simulated docking of three active compounds and compared it to that of conventional drugs with target proteins, 3CLpro, RdRp complex Nsp7-Nsp8, PLpro, Nsp3 and ACE2. The conventional drugs we chose included chloroquine, favipiravir, and ribavirin. Previous studies have shown that chloroquine can interact with 3CLpro/MPpro protein, PLpro, RdRp Nsp7-Nsp8 complex, and ACE2 (Hosseini et al., 2021; Deshpande et al., 2020; Joshi et al., 2021). For favipiravir targeting 3CLpro, RdRp Nsp7-Nsp8 complex, and PLpro (Furuta et al., 2017; Yadav et al., 2021; Balkrishna et al., 2021), and ribavirin targets the 3CLpro, ACE2, and Nsp3 proteins (Deshpande et al., 2020).

Ribavirin had the highest negative interaction energy for the 3CLpro ABCD chain, which was consistent with previous findings (Deshpande et al., 2020). Ribavirin interacted with the ABCD chain via strong bonds, namely covalent links THR 226, ASP229, PHE230, VAL233, LYS269, conventional hydrogen bond PHE230, via strong bonds, namely covalent links THR 226, ASP229, PHE230, VAL233, LYS269, conventional hydrogen bond PHE230, via strong bonds, namely covalent links THR 226, ASP229, PHE230, VAL233, LYS269, conventional hydrogen bond PHE230, via strong bonds, namely covalent links THR 226, ASP229, PHE230, VAL233, LYS269, conventional hydrogen bond PHE230, via strong bonds, namely covalent links THR 226, ASP229, PHE230, VAL233, LYS269, conventional hydrogen bond PHE230, via strong bonds, namely covalent links THR 226, ASP229, PHE230, VAL233, LYS269, conventional hydrogen bond PHE230, via strong bonds, namely covalent links THR 226, ASP229, PHE230, VAL233, LYS269, conventional hydrogen bond PHE230, via strong bonds, namely covalent links THR 226, ASP229, PHE230, VAL233, LYS269

Chloroquine had the highest negative interaction energy for RdRp Nsp7-Nsp8 complex. The hydrogen carbon bonds ASP161, ASP164, and LYS196 and the van der Waals bonds TYR163, GLU167, ARG553, VAL792, PHE793, MET794, and SER795 contribute to this interaction. Chloroquine also had the highest negative bond energy for PLpro. This interaction comprised covalent bonds HOH546 as well as van der Waals bonds LYS92, ALA107, HOH526, HOH529, HOH 538, HOH546, and HOH608. This observation supports prior studies, indicating that chloroquine interacted with RdRp, Nsp7 Nsp8, and PLpro complexes (Hosseini et al., 2021; Deshpande et al., 2020; Joshi et al., 2021).

Overall, the simulation comparison of the active chemical with conventional medications revealed that afzelin had a greater unfavorable effect on AC and BD chain 3CLpro proteins as compared to the three standard pharmaceuticals. Trifolin had a higher unfavorable interaction with Nsp3 and ACE2 proteins than the other three standard medicines. In other words, while further research is needed, the interaction of active molecules may be complementary to the work of the three standard medications. As flavonoid molecules have low systemic toxicity and can synergize with conventional medications, this study has a lot of potential (Russo et al., 2020).

5. Conclusions

It was concluded that the active flavonoid compounds in katuk leaves (Saururus androgynum) had affinity for 3CLpro, Nsp1, Nsp3, RdRp Nsp7-Nsp8 complex, and PLpro in SARS-CoV-2, and ACE2 in humans when compared to conventional medications. Thus, katuk leaves can be used employed as herbal candidates for new COVID-19 medications or as complementary medicines, as well as for virus engineering in the environment.

Declaration of Competing Interest

None.

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