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RESEARCH ARTICLE

# The activity of bioactive compounds from bidara upas (*Merremia mammosa* (Lour) Hall. f.) as an inhibitor of SARS-CoV2 entry stage: In silico study

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## Abstract

**Background:** Covid 19 is a global pandemic caused by SARS-CoV2, a novel coronavirus. This virus enters target organ epithelial cells by utilising two host proteins; Transmembrane Serine Protease 2 (TMPRSS2) and Angiotensin Converting Enzyme 2 (ACE2). The inhibition of TMPRSS2 has shown to be a promising means to prevent viral infection. Molecular docking and Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) analysis will determine the activity of *Merremia mammosa* (Lour) Hall.f. secondary metabolites against the TMPRSS2 of SARS-CoV2. **Objective:** This study aimed to investigate the *in silico* activity of *Merremia mammosa* (Lour) Hall.f. active compounds against TMPRSS2 of SARS-CoV2. **Method:** Molecular docking was performed on 206 compounds obtained through metabolite profiling from a previous study on the SARS-CoV TMPRSS-2 protein (PDB id.7MEQ) using the Maestro Schrodinger software. **Result:** The results indicated there were 6 compounds (three of which were flavonoids: cynarine, phellodensin F, and gemixanthone A) with docking scores lower than standard drugs (nafamostat as a native ligand). ADMET analysis revealed that among 6 compounds, cynarine has the highest drug-likeness and the greatest inhibitory potential against TMPRSS2. **Conclusion:** Cynarine was found to be active and promising to be developed as an inhibitor of the SARS-CoV2 entry step.

## Introduction

COVID-19 is a global pandemic caused by the newly discovered coronavirus SARS-CoV-2 (WHO, 2020). Recent studies have revealed that the virus enters the target organ epithelial cells by utilising two host proteins, Transmembrane Serine Protease 2 (TMPRSS2) and Angiotensin Converting Enzyme 2 (ACE2), found on the cell surface (Parmar, 2021). Among the two proteins, the inhibition of TMPRSS2 has been shown to be the most promising and has been shown to prevent SARS–CoV2–driven lung cell access (Manjunathan *et al*., 2022). Therefore, developing therapeutic agents that inhibit the TMPRSS2 function is a promising strategy for combating current and future coronavirus epidemics (Manjunathan *et al*., 2022).

*Merremia mammosa* (Lour) Hall. for bidara upas is one of the medicinal plants traditionally used for the treatment of respiratory tract diseases, digestive disorders, and wounds (Purwitasari *et al*., 2023). Bidara upas has also been proven to be effective against the H1N1 flu virus and *Mycobacterium tuberculosis* (Purwitasari, 2020; Agil *et al*., 2021). Bidara upas also contains glycoside resin, such as merremoside, that been used to treat respiratory disease (Kitagawa, 1996).

## Methods

### *Design*

Using Agilent LC-MS/MS QTOF, a total of 206 compounds were predicted from 96% of its ethanol extract, butanol fraction, ethyl acetate fraction, and n-hexane fraction (Purwitasari, 2022). Through metabolite profiling were then subjected to a molecular docking study to predict the potential compounds that can inhibit the TMPRSS2 of SARS-CoV-2. The 206 compounds were successfully docked with the SARS-CoV-2 TMPRSS-2 protein binding site (PDB id. 7MEQ).

### *Hardware and software*

The computational study in this research was conducted using Maestro Schrödinger 2021-2 (Schrödinger, New York, NY, USA) software on Dell WorkStation, Linux Ubuntu 20.04.3 LTS OS, Intel Xeon(R) W-2223 CPU @ 3.60GHz octa-core; RAM 16 GB and GPU NVIDIA Quadro P2200.

### *Preparation of ligands and receptor*

The 2D structures from LC-MS/MS profiling were produced using Chemdraw, then optimised and transformed into 3D by LigPrep module in Schrodinger 2021-2 as well as protonated with Epik at pH of 7.4 and OPLS4 forcefield in attempt to re-establish improper or missing bonds, assign protonation, possible ionisation, and tautomeric states (Ikram *et al*., 2015; Zubair *et al*., 2021).

In the meantime, SARS-CoV-2 receptors were prepared with the Protein Preparation Wizard module in Maestro Schrodinger 2021-2 by removing the residual solvent, optimising hydrogen bond, protonating with ProtAssign and PROPKA, and adding partial charge with the OPLS4 forcefield (Schrodinger Release 2022-1, 2022c).

### *Molecular docking*

The molecular docking procedure was executed with glide under conditions of rigid receptors and flexible ligands in extra-precise (XP) mode. In addition, to determine which compounds have the highest binding affinity and actively inhibit SARS-CoV-2 receptors. For scoring the docked position, the molecular mechanics-generalised Born surface area (MM-GBSA) was used (Schrödinger Release 2022-1, 2022a; 2022b).

## Results

Out of the 206 compounds, six compounds revealed MMGBSA values ranging from -58,8942 to -52,5815, significantly lower than standard drugs, the lowest of which was nafamostat at -33,40. (Table I). It indicated that the compound was more active than the ligand controls. The greater the negative value of this energy, the lower the free energy and the stronger the binding. Figure 1 shows molecular interactions of cynarine against the TMPRSS SARS-CoV-2 receptor. Preliminary prediction analysis of physicochemical and absorption, distribution, metabolism, excretion and toxicity properties of the lowest MMGBSA value compound from bidara upas using PKCSM can be seen in Table II.

**Table I: Top compounds with the lowest MMGBSA value of bidara upas and standard drug against TMPRSS of SARS-CoV2**

| Compounds | MMGBSA |
|---|---|
| Nafamostat (1.0019 Å) (7MEQ) | -33.40 |
| Cynarine (1) | -58.8943 |
| N-[(3-Cyano-5,6-dihydro-4H-cyclopenta[b]thiophen-2-yl)carbamothioyl]-2-(3-isopropoxyphenyl)-4-quinoline-carboxamide (2) | -55.9942 |
| N,N'-(Oxydi-4,1-phenylene)bis(4-nitrobenzamide) (3) | -55.9332 |
| 6-[(4-Amino-2-methyl-6-quinolinyl)amino]-2-{[(2S)-5-(diethylammonio)-2-pentanyl]amino}-4-methylpyrimidin-1-ium (4) | -55.1054 |
| Phellodensin F (5) | -54.0013 |
| Gemixanthone A (6) | -52.5815 |

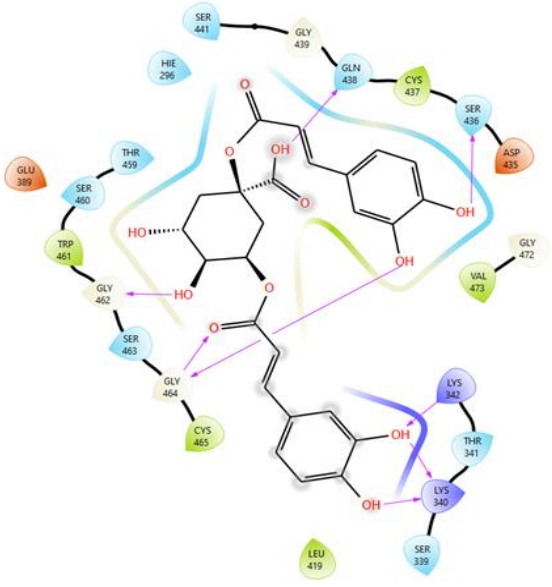


**Figure 1: Molecular interactions of cynarine against TMPRSS SARS-CoV-2 receptor**

**Table II: Preliminary prediction analysis of physicochemical and absorption, distribution, metabolism, excretion and toxicity properties of the lowest MMGBSA value compound from bidara upas using PKCSM**

| Compounds | | Cynarine |
|---|---|---|
| **Chemical structure properties** | MW | 512.66 |
| | LogP | 6.23768 |
| | HBA | 6 |
| | HBD | 2 |
| **Predicted $LD_{50}$ (mg/kg)** | | 1500 |
| **Predicted toxicity class** | | 4 |
| **Cytotoxicity** | | Inactive |
| **Absorption** | $CaCO_2$ permeability | 0.555 |
| | Intestinal absorption (human) | 92.509 |
| **Distribution** | VDss (human) | -0.848 |
| | Fraction unbound (human) | 0.028 |
| | BBB permeability | -0.258 |
| | CNS permeability | -1.547 |
| **Metabolism** | CYP2D6 substrate | No |
| | CYP3A4 substrate | Yes |
| | CYP2D6 inhibitor | No |
| | CYP3A4 inhibitor | Yes |
| **Excretion** | Total clearance | -0.18 |
| | Renal OCT2 substrate | No |

MW: Molecular weight (<500 Da); LogP: Coefficient partition (<5)
HBD: Hydrogen bond donor(<5); HBA: Hydrogen bond acceptor (<10)
Vdss: Volume of Distribution at Steady State; BBB : Blood Brain Barier
CNS: Central Nervous System; CYP2D6 : Cytochrome P2D
CYP3A4 : Cytochrome 3A4; Renal OCT2: Renal Organic Cation Transporter

In this study, Protox-II was used to evaluate the toxicity profiles of Cynarine, and it was reported that Cynarine was predicted to exhibit no cytotoxicities. Moreover, Lipinski's rule of five was used as a preliminary screening tool to evaluate the absorption and permeability properties of cynarine, suggesting that it may have suboptimal absorption and permeability characteristics.

Overall, Lipinski's rule of five, absorption parameters, distribution parameters, metabolism effects on CYP enzymes, and excretion profiles are important factors to consider when evaluating the potential of phytoconstituents as inhibitors of the SARS-CoV-2 TMPRSS.

## Discussion

Since ancient times, Indonesians have used boiled-up bidara upas tubers to treat tuberculosis. In addition, bidara upas is a plant known for its pharmacological effects that can prevent the multiplication of HIV, H1N1, and *Mycobacterium tuberculosis* (Purwitasari *et al*., 2020; Agil *et al*., 2021; Purwitasari *et al*., 2023).

Molecular docking analysis has identified 6 compounds from this plant that have an activity to inhibit the TMPRSS2 of SARS-CoV-2. Three of the compounds, such as cynarine, phellodensin F and gemixanthone A, were flavonoids that may interact with the substrate-binding and catalytic site amino acids of TMPRSS2 (Hussain *et al*., 2020). Cynarine was known to have strong inhibitory activity against SARS-CoV-2's main protease (3CLpro or Mpro), but this compound is quickly metabolised after oral administration (Jo *et al*., 2022). Gemixanthone A was also known to have an activity to inhibit the SARS-CoV-2 spike protein with MMGBSA value -60.2985. It has hydrogen bonds with SER 47, ASH 350, TYR 385, and PHE 390 (Purwitasari *et al*., 2023). This inhibition will prevent the early step of SARS-CoV2 infection. Furthermore, cynarine has strong activity and showed no toxicity.

## Conclusion

Molecular docking was successfully used to identify the potential compounds from bidara upas that can inhibit TMPRSS2 activation. Three flavonoid compounds found in Bidara upas which are cynarine, phellodensin F and gemixanthone A. Cynarine showed promising results to be developed as anti-SARS-CoV2 in the entry step of infection. Further purification and NMR characterisation are needed. Also, further *in vitro* and *in vivo* experiments may be necessary to confirm the absence of cytotoxicity and to assess other potential toxic effects of Cynarine.

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