Beyond the Magic of *Moringa oleifera*: Its Potential to Control Indonesian Serotype of Foot-and-Mouth-Disease Virus Replication through Inhibition of 3-Cysteine Protease

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Abstract. Foot-and-Mouth Disease (FMD) poses a significant threat to livestock worldwide, necessitating innovative approaches to combat its causative agent, the FMD virus (FMDV). On the other hand, *Moringa oleifera* is a feed alternative for cattles with numerous bioactive compounds. This paper delves into the captivating realm of *Moringa oleifera* (MO) bioactives and their potential in thwarting FMDV replication by targeting the essential enzyme, 3C Protease (3CP). To elucidate the inhibitory potential of these bioactives, a rigorous investigation involving molecular docking and molecular dynamics simulations was conducted. Specifically, the 3CP was modeled based on the amino acid sequence of FMDV Indonesian Serotype. Results showed that most of the compounds from MO outperformed Ribavirin as the standard therapy for FMD. Among them, Baicalin, Chlorogenic Acid, and Rutin have binding affinity -9.1, -8.1, and -8.1 kcal/mol, respectively. Those compounds also formed more hydrogen bonds than Ribavirin through their binding sites. Molecular dynamics simulation also revealed that interaction of 3CP with those compounds had minor influence on its structural stability. The conformation of those compounds is also more stable than Ribavirin, supported by more hydrogen bonds. In summary, this research highlighted the potential mechanism of MO bioactives in preventing severe FMDV infection through inhibition of viral replication.

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1 Introduction

Foot-and-mouth disease (FMD) is a disease that primarily affects livestock with cloven hooves. The FMD causes a significant loss in livestock production as it causes the loss of weight, reproductive disturbances, and in the worst case, death [1]. In 2022, Indonesia had a FMD outbreak after 35 years of free cases of FMD [2]. The outbreak was first reported in East Java and then spread in other regions [3]. With the contagious nature of the disease and no specific drug available for this disease [4], viral inhibitors could be the alternative solution [5]. The genetic diversity of Foot-and-Mouth Disease Virus (FMDV), characterized by different serotypes [6], hinders effective control through vaccination due to antigenic variations [7,8]. To address this, an alternative approach is to target the virus's non-structural proteins (NSPs), as these can offer a promising avenue for FMDV control, in contrast to traditional vaccines that primarily focus on structural proteins [9,10].

The NSPs, frequently serving as enzymatic catalysts, play an integral role in orchestrating the assembly of viral architectures [11]. Following the onset of FMDV infection, the virus expeditiously engages in transcription and translation processes, resulting in the synthesis of both structural and NSPs and, ultimately, the genesis of new viral particles [12]. Among several NSPs, there is a 3-Chymotrypsin-like Cysteine Protease (3CP), an enzyme responsible for processing and maturing the polyproteins that are translated from the viral RNA [13]. Moreover, this enzyme also plays a role in attenuating the host's immune response via the blockage of the host type-I interferon (IFN) responses [14]. Due to its indispensable role in augmenting viral replication and harbouring host's immune response, pharmaceutical agents have been designed to target 3CP [9,15]. Moreover, Indonesian serotype is very exclusive phylogenetically compared to another O serotype across Asiatic region [2]. With that uniqueness along with no information about the effect of natural compounds in inhibiting FMDV replication, particularly Indonesian serotype, underscores an avenue for exploration that could potentially enhance the arsenal against FMDV infections.

Moringa oleifera is an alternative of livestock feed that has high nutritional value. It is commonly used as a milk production booster by replacing a proportion of hay in goat, ewe, and cow [16–18]. This plant also has several bioactive compounds such as carotenoids, alkaloids, flavonoids, phenolic compounds, and many others [19]. The previous studies found that bioactive compounds from *M. oleifera* had antiviral properties [20,21]. The antiviral properties of *M. oleifera* were effective against several viruses like HIV, HSV, HBV, EBV, and even FMDV [22]. Previous study reported that *M. oleifera* extract exhibited a potent antiviral property against FMDV at a low concentration [23]. However, a detailed mechanism about that activity remains unknown. Hence, this study will explore the potential mechanism of *M. oleifera*'s bioactives against FMDV, particularly Indonesian serotype, by inhibition of 3CP through computational simulation.

2 Materials and Methods

2.1 Homology Modelling and Structure Assessment

The 3C protease protein sequence from FMD Indonesia was obtained from the NCBI database with AAT0176.1 as protein ID [6]. Homology modelling was carried out using the SWISS Model webserver [24] using the 2j92 as a template [25]. The quality of the 3D protein structure was analysed using the Ramachandran plot. The model with the lowest Ramachandran outliers value was selected.

2.2 Molecular Docking

The active compounds contained in *M. oleifera* were obtained from a previous study [26]. The three-dimensional structures of active compounds were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [27]. Compounds were prepared by optimizing their conformations using Open Babel in PyRx 0.8 [28]. The inhibitor compound used as a positive control is Ribavirin, which is a drug that targets the 3C protease protein [29]. Molecular docking was performed using AutoDock Vina on PyRx 0.8 interface [30,31] with the protein treated as rigid structure and the compounds as the flexible entity [32]. The molecular docking results were saved in .pdb format and visualized using Biovia Discovery Studio 2019.

2.3 Molecular Dynamic Simulation

The three complexes with the most negative binding affinity values were subjected to molecular dynamic simulation (MDS). MDS was performed in YASARA software [33] under system parameters adjusted to mammalian cell conditions [34], i.e., pH 7.4, temperature 310 °K, NaCl content 0.9%, 1 bar pressure, 0.997 g/mL water density for 20 ns. Simulations were carried out under AMBER14 forcefield [35]. The Root-Mean-Square Deviation (RMSD) of atomic position, Root-Mean-Square Fluctuation (RMSF), and number of hydrogen bonds were analyzed using the md_analyze macro.

3 Results and Discussion

A total of eleven compounds of *M. oleifera* were obtained according to the previous study [26]. Based on binding affinity with the target protein 3CP, *M. oleifera*'s bioactives exhibited a low energy affinity, ranging from -9.1 to -5.2 kcal/mol (Table 1). Several compounds also displayed lower binding affinities compared to Ribavirin, a standard therapy to inhibit FMDV replication [29]. Among all of the best performing compounds, Baicalin, Chlorogenic Acid, and Rutin served as the top three compounds with low binding affinity values at -9.1, -8.1, and -8.1 kcal/mol, respectively (Table 1). Thus, these three compounds are then considered for future analysis related to the amino acid interaction chemistry and molecular dynamics analyses.

Further investigation focused on determining the three compound interactions with the active site of 3C Protease. This analysis is a crucial part of assessing the potential of the compound to inhibit 3C Protease protein. Since the docking step was aimed at the active site of 3CP, the position of the selected compounds was similar (figure 1A). Based on the interaction compound-protein, Baicalin had the same residues as control of hydrogen bonds, namely Thr 178 and Ser 81. By creating two hydrogen bond interactions in the same residue as the control, such as Ser 180 and Gly 148, chlorogenic acid is bound to the active site of 3c Protease. Rutin created three hydrogen bonds with Gly 148, Phe 150, and Ser 81, respectively (Figure 1B). Hydrogen bond plays a crucial role in maintaining a stable complex stability [36]. However, other interactions such as CH–π interaction and van der Waals also determine and facilitate the stability of protein-ligand interaction [37,38]. With the chemical structure owned by those screened compounds (Figure 2), Baicalin, Chlorogenic Acid, and Rutin may perform better through their aromatic rings to induce the CH–π interactions. Nevertheless, MDS analysis is still required to further comprehend the complex stability upon interacting with these compounds.

Table 1. Binding affinity between compounds from *Moringa oleifera* and 3C Protease.

Compound	CID	Binding Affinity (kcal/mol)
Baicalin	64982	-9.1
Chlorogenic Acid	1794427	-8.1
Rutin	5280805	-8.1
Isoquercetin	5280804	-7.6
Quercitrin	5280459	-7.5
Galogen	5281855	-7.4
Quercetin	5280343	-7.3
Kaempferide	5281666	-7.1
Ferulic acid	445858	-6.4
Gallic acid	370	-5.8
Vanillin	1183	-5.2
Ribavirin (Control)	37542	-6.8

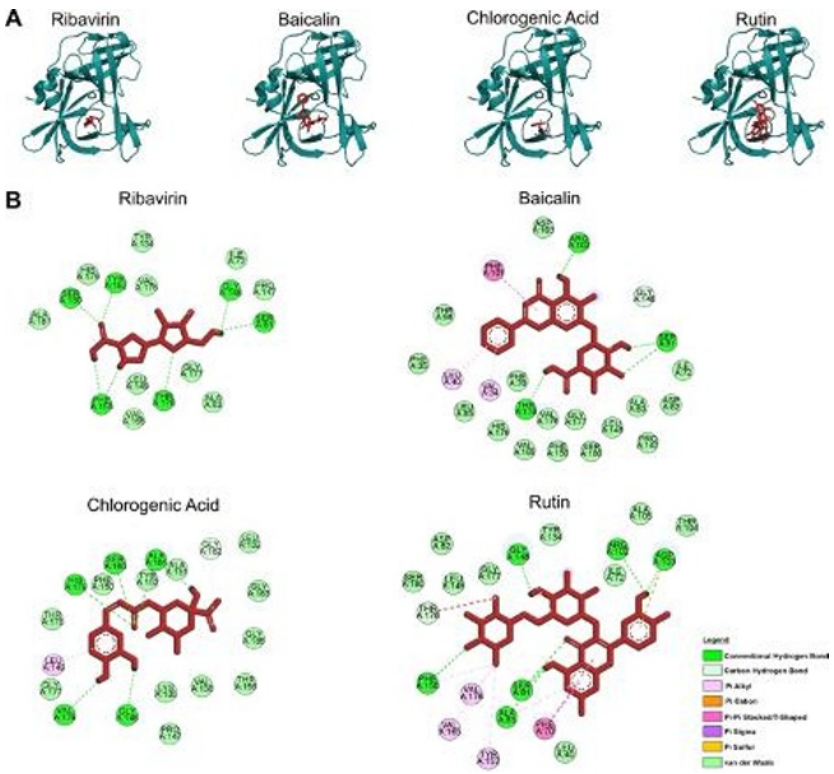

Fig. 1. Interaction between 3C Protease and compounds from *Moringa oleifera*. (A) 3D representation of docked interaction compounds-protein (3C Protease presented in blue cyan structure and ligands

presented as red). (B) The compounds-protein molecular interaction is obtained from the docked complexes.

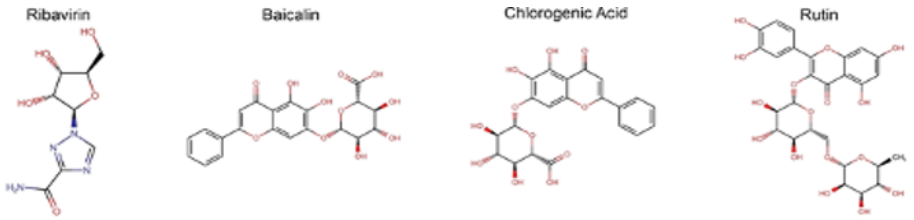

Fig. 2. The chemical structure of Ribavin and the three compounds that achieved the lowest binding energy

The stability analysis of the interaction between the 3CP protein and the three compounds was carried out using MDS. The RMSD of the backbone atom and RMSF are frequently used to assess the stability of the complex or the receptor protein in protein-ligand complex [39–42]. On the other hand, the RMSD of ligand conformation will describe the stability of ligand's structure during its interaction with the protein and number of hydrogen bonds will give an insight about the protein-ligand interaction stability [39–41]. During the simulation period, almost all compound protein-ligand complexes had RMSD values less than 3Å, which indicated that this complex is stable [43] and only chlorogenic acid has a different trend, which increased at the end of the simulation (Figure 3A). The RMSF value of each amino acid residue on the 3C protease-ligand indicated low flexibility, which means it tends to be stable (Figure 3B). All ligand conformation showed that the stability of the RMSD value was below 3Å. Interestingly, the compounds from *M. oleifera* showed more stable conformation than Ribavirin (Figure 3C). The stable conformation of those compounds will facilitate the stable complex formation by incurring entropic penalty during complex formation [44]. Hydrogen bonds are important in the stability of protein conformation and protein-ligand interactions through contribution in the aforementioned entropic penalty [44]. Once again, *M. oleifera*'s compounds outperformed 3CPRibavirin in terms of hydrogen bond number, indicating a more stable complex stability than 3CP-Ribavirin (Figure 3D). Overall, Baicalin, Chlorogenic Acid, and Rutin have a promising chance to be a FMDV viral replication inhibitor through inhibition of the 3CP.

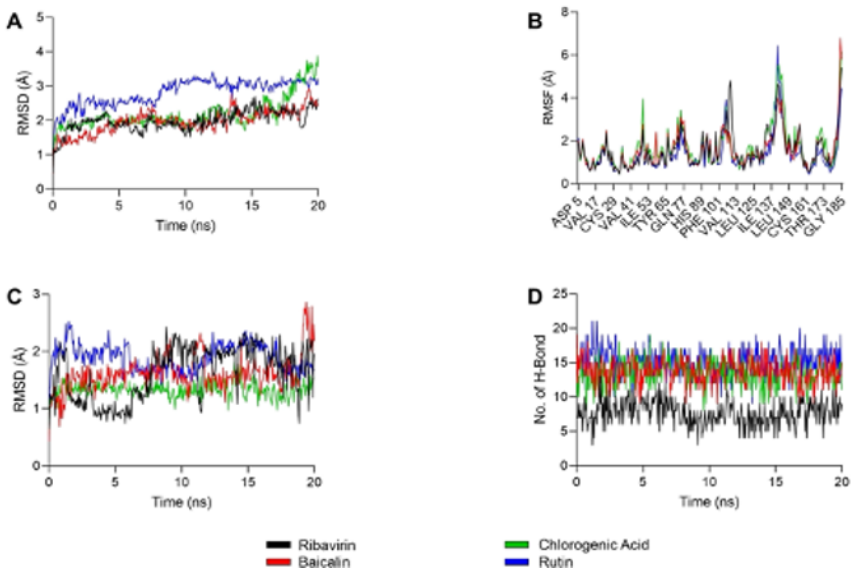

Fig. 3. The molecular dynamic simulation of protein-ligand stability (A) The RMSD value of backbone atoms, (B) The RMSF value of each amino acid residue, (C) The RMSD value of ligand conformation, (D) The number of hydrogen bonds during the simulation.

4 Conclusion

The bioactive compounds found in *M. oleifera* exhibit exceptional inhibitory potential against 3CP, surpassing the performance of Ribavirin. Baicalin, Chlorogenic Acid, and Rutin have shown remarkable binding affinity to the 3CP active site, forming stable complexes crucial for inhibitory action. These findings pave the way for *M. oleifera* to emerge as a promising functional feed additive in the fight against FMDV replication. However, further research is imperative to validate these promising results, with a particular focus on comprehending the bovine immune response to FMDV infection. Exciting possibilities lie ahead in the quest to combat this devastating virus.

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