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BIOINFORMATICS APPROACH: EVALUATING THE ANTIVIRAL POTENTIAL OF FLAVONOID COMPOUNDS FROM *PHYLLANTHUS URINARIA* AGAINST FOOT-AND-MOUTH DISEASE IN LIVESTOCK ANIMALS USING MOLECULAR DOCKING ON RDRP RECEPTOR

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ABSTRACT

Foot-and-mouth disease is a severe problem that must be faced in the livestock sector. This disease has a negative impact on various aspects, especially the economy. One way to develop herbal medicinal compounds is through local Indonesian wild plants, meniran (*Phyllanthus urinaria*). This research aims to determine the potential of the wild plant *P. urinaria* as an antiviral agent for FMD using an in silico approach using molecular docking. The compounds used as ligands are flavonoid compounds in *P. urinaria*, namely, routine, quercetin 7-methyl ether, quercetin 3-*O*-*β*-*D*-glucoside, quercetin, rhamnocitrin, astragalin, and kaempferol. This study used the control drug ribavirin as a comparison. The research stage began with the preparation of the RdRp protein from the FMD virus with Discovery Studio, ligand preparation with the Lipinski druglikeness test and minimization using OpenBabel, followed by docking and visualization. The research results found that the six flavonoid compounds in *P. urinaria* have potential as antiviral FMD by inhibiting RdRp, with the most potent compound being quercetin with -7.9 kcal/mol of binding affinity. Further research is needed, including *in vitro* and *in vivo* testing, to provide confidence in the potential of this wild plant as an antiviral for FMD.

Keywords: *Phyllanthus urinaria*, FMD, FMDV, inhibitor, *in silico*

1. Introduction

The problem of foot and mouth disease is a big challenge in the livestock industry and requires immediate resolution. This disease is attributed to a transmissible viral infection that gives rise to complications in animals leading to mortality [1]. Foot-and-mouth disease (FMD) has significant concerns for fencing due to its high level of contagiousness and the ease with which it may be transmitted by sick animals. This transmission can occur through several means, including contaminated equipment, vehicles, clothing, and feed. Additionally, close contact with domestic predators can also facilitate the spread of FMD. These factors contribute to the severe implications that FMD has for fencing [2]. Moreover, the incidence of this virus is predominantly observed among children who are below the age of five [3]. According to the Ministry of Health, this disease is divided into four stages with different manifestations. Hospitalization and close monitoring in a healthcare facility are necessary for managing severe symptoms associated with the disease, including neurological consequences and mortality rates of grade 2 or above. As per the Ministry of Health, this particular ailment is categorized into four distinct stages, each exhibiting unique signs [4].

The etiological agent responsible for foot and mouth disease (FMD) is the aphthovirus, specifically known as the foot and mouth disease virus. The presence of this particular disease in agriculture has resulted in significant economic and ecological challenges. Infection occurs when viral particles successfully penetrate host cells. Subsequently, subsequent to being compelled to generate a multitude of virus victims, the cell has an explosive event, thereby dispersing newly formed particles into the bloodstream [5]. The genetic composition of this virus exhibits a high degree of diversity [6], [7], this genetic diversity plays a crucial role in enhancing the efficacy of the virus's programming. The transmission of this virus primarily happens via the gastrointestinal system, namely through the presence of saliva, blisters, and fecal matter from individuals who are afflicted with the illness. The onset of viral pathogenicity occurs upon the interaction between the virus and the cellular membrane of the host. Subsequently, the virus undergoes replication facilitated by RdRp [3], [4], [8]. The synthesis of viral genetic material is facilitated by the RdRp enzyme, which accomplishes this task by translating a single polyprotein [8], [9]. One potential approach to prevent infection caused by Foot-and-Mouth Disease Virus (FMDV) involves the inhibition of RdRp receptors.

The pharmaceutical business places significant emphasis on the exploration and advancement of natural medicinal substances due to their inherent advantages, such as limited adverse effects and their natural availability [10]. *Phyllanthus urinaria* is a natural component that can be utilized. Chamber bitter, known as meniran in Indonesian, is a native plant species in Indonesia that is commonly classified as a weed due to its spontaneous and uncontrolled growth [11], [12]. The plant is considered a competitive weed in certain regions due to its prolific seed production, high shade tolerance, and extensive root system [13]. The plant under investigation has a high concentration of secondary metabolites, specifically flavonoids and phenolics [13], [14]. The utilization of this particular plant is infrequent, primarily limited to traditional practices, and it is frequently uprooted due to its propensity for wild growth [15]. The plant under investigation exhibits a wide range of flavonoid compounds, which holds promising prospects for its utilization as an antiviral agent against Foot-and-Mouth Disease (FMD). The primary objective of this investigation is to provide a comprehensive account of the potential of flavonoid compounds found in *P. urinaria* as possible candidates for the treatment of foot-and-mouth disease in livestock. This will be accomplished through the utilization of an in silico methodology.

2. Materials and Methods

2.1. Protein Preparation

In this investigation, the RNA-dependent RNA polymerase (RdRp) protein derived from the Foot-and-Mouth Disease Virus (FMDV). The information can be accessed through the official website of the RCSB (Research Collaboratory for Structural Bioinformatics), which is rcsb.org. The protein structures of RNA-dependent RNA polymerase (RdRp) with PDB ID: 2E9R were created using the Discovery Studio program in order to ascertain the docking position and remove extraneous components from the protein [16].

2.2. Ligand Preparation

The ligand molecules employed in this investigation consisted of the primary flavonoid compounds derived from *P. urinaria*, specifically rutin, astragalin [17], quercetin 7-methyl ether, quercetin 3-*O*-β-D-glucoside [18], quercetin [19], rhamnocitrin [20], and kaempferol [21], [22]. The current investigation provides evidence that ribavirin, a pharmacological agent utilized as a control measure, possesses antiviral characteristics and is utilized in the therapeutic management of foot-and-mouth disease (FMD). The 3-dimensional configuration of the ligand was obtained using the PubChem web service (pubchem.ncbi.nlm.nih.gov). Subsequently, the ligand was submitted to minimization using OpenBabel within the PyRx software [23], [24].

2.3. Druglikeness Lipinski

In this study, the druglikeness of flavonoid compounds from *P. urinaria* was assessed by employing Lipinski's rule of five (Ro5) as a criterion for ligand selection. The criterion encompasses a molar mass below 500 Daltons; a maximum of five hydrogen bond donors; a maximum of ten hydrogen bond acceptors; a lipophilicity value (log P) below 5; and a molar refraction within the range of 40 to 130 [25], [26]. The investigation was carried out via the ScfBio webserver, accessible at scfbio-iitd.res.in/software/drugdesign/lipinski. The compounds that met at least three of Lipinski's rules were utilized for molecular docking analysis with the RdRp protein obtained from the Foot-and-Mouth Disease Virus (FMDV) [26], [27].

2.4. Molecular docking and Visualization

The molecular docking analysis was performed using the VinaWizard tool in PyRx [28] software, employing the receptors and ligands that meet with Lipinski's rule (Ro5). The docking study was performed using a grid box measuring X=25.00, Y=25.00, and Z=25.00, with a central point positioned at X=17.514310, Y=24.712483, and Z=18.011483. Compounds with binding affinity values lower than those of the ribavirin controls were visualized utilizing Discovery Studio and PyMOL software to visualize the sites and types of interactions produced [16], [29].

3. Result and Discussion

P. urinaria is a local Indonesian plant known as a wild plant. This plant often grows irregularly, so people often think of this plant as a weed [12]. Despite the presence of elevated concentrations of secondary metabolites, notably flavonoids, the primary objective of this research is to assess the medicinal potential of the predominant flavonoid compounds derived from this plant in treating foot and mouth disease in livestock, which is caused by the FMD virus. This evaluation will be conducted through an in silico approach utilizing molecular docking. The study will focus on the RdRp (RNA-dependent RNA-polymerase) receptors, as the crucial components in the FMD virus replication process [8], [30]. The structure of the ligands used in this study is presented in Figure 1, senyawa sebagai ligan antara lain rutin, quercetin 7-methyl ether, quercetin 3-*O*-β-D-glucoside, quercetin, rhamnocitrin, astragalin, and kaempferol [22].

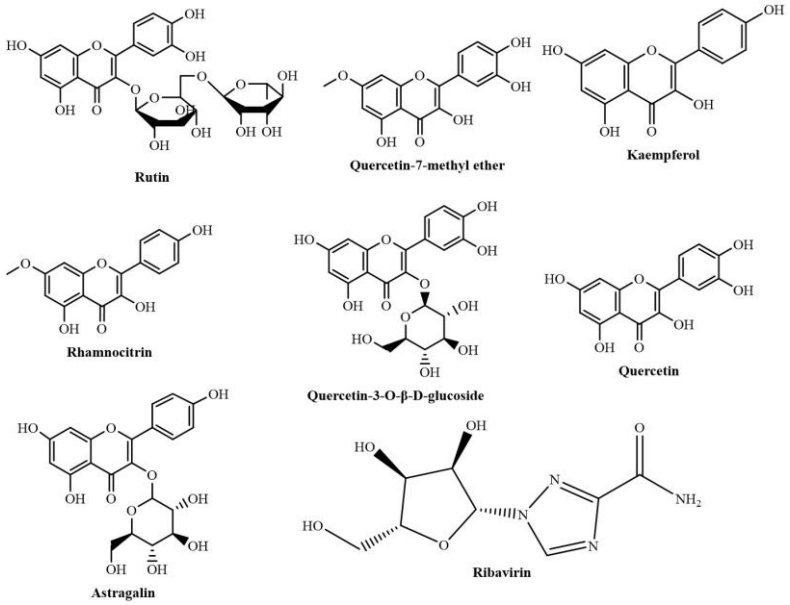

Figure 1. Ligand Structure for Docking Study

The druglikeness analysis was conducted on the seven flavonoid compounds found in *P. urinaria*, which serve as the ligands for the first selection process. The objective of druglikeness research is to assess the suitability of a chemical as an oral drug candidate by evaluating its adherence to Lipinski's five characteristics. The application of Lipinski's rule of five (Ro5) is advantageous in assessing the oral bioavailability of a chemical. This rule encompasses several factors, namely molar mass, number of hydrogen acceptors, number of hydrogen donors, lipophilicity, and molar refractivity, which collectively aid in understanding the molecule's distribution upon oral consumption [25], [26]. The druglikeness analysis results, as presented in Table 1, indicate that among the seven flavonoid compounds found in *P. urinaria*, six compounds satisfy a minimum of three Lipinski rules (Ro5), whereas one molecule (Rutin) fails to adhere to the Ro5 criterion. The criteria for this rule encompass several factors, molar mass below 500 Daltons; a maximum of five hydrogen bond donors; a maximum of ten hydrogen bond acceptors; a lipophilicity value (log P) below 5; and a molar refraction within the range of 40 to 130.

Table 1. Druglikeness and Molecular Docking Result

Ligand	Druglikeness Lipinski						Binding Affinity (kcal/mol)
	MM[1]	HA[2]	HD[3]	MR[4]	MLOGP[5]	Note	
Ribavirin (Control drug)	-	-	-	-	-	-	-6.9
Rutin	610.52*	16*	10*	141.38*	-3.89	No	Not docked
Quercetin 7-methyl ether	316.26	7*	4	82.5	-0.31	Yes	-7.7
Quercetin 3-*O*-β-D-glucoside	463.37	12*	7*	108.27	-2.59	Yes	-7.8
Quercetin	302.24	7*	5	78.03	-0.56	Yes	-7.9
Rhamnocitrin	300.26	6*	3	80.48	0.22	Yes	-7.4
Astragalin	448.38	11*	7*	108.13	-2.1	Yes	-7.7
Kaempferol	286.24	6*	4	76.01	-0.03	Yes	-7.7

Note: MM[1]= molecular mass; HA[2]=hydrogen acceptor; HD[3]=hydrogen bond; MR[4]=molar refractivity; MLOGP[5]=lipophilicity; *= does not comply with Lipinski's rules

The findings of Lipinski's analysis yielded six compounds that exhibited the ability to bind to the FMD virus RdRp receptor at its active site. The RNA-dependent RNA polymerase (RdRp) plays a crucial function in the replication cycle, thereby necessitating its inhibition [8]. The present investigation employed the control medication ribavirin, an antiviral agent commonly prescribed for the management of respiratory syncytial virus (RSV), hepatitis C, and dengue infections [31], [32]. Ribavirin is an antiviral agent that typically exerts inhibitory effects on the production of the RNA-dependent RNA polymerase (RdRp) of various viruses [33]. The findings from the molecular docking analysis, as presented in Table 1, indicate that the six flavonoid compounds found in *P. urinaria* possess promising antiviral activity against foot-and-mouth disease (FMD) by inhibiting the RdRp enzyme. This conclusion is drawn based on their comparatively lower binding affinity values in comparison to the reference drug ribavirin (-6.9 kcal/mol). Among these compounds, quercetin exhibits the highest potency, as evidenced by its most negative binding affinity value [34]. The binding affinity refers to the stability of the complex that is established between the receptor and the ligand. A lower binding affinity number indicates a higher level of stability for the created complex. The inhibitory activity of the ligand is positively correlated with the stability of the complex. A higher level of stability in the complex corresponds to a greater degree of inhibitory activity [16], [35].

The six candidate compounds were subsequently subjected to interaction analysis using PyMOL and visualized using Discovery Studio software in order to ascertain the specific location and nature of the interactions established within the complex. In the realm of interactions, it is commonly observed that two distinct categories can be discerned: pleasant interactions and unfavorable interactions [36], [37]. Favorable interactions refer to attractive interactions occurring between ligands and receptors that contribute to the stability of the complex. Conversely, unfavorable interactions denote repulsive interactions between ligands and receptors, leading to decreased stability of the complex [38] . The stability of the complex is supported by the interactions that have been established. The stability of a complex is directly proportional to the number of beneficial interactions it possesses. The present study elucidates the positive connections established, including hydrogen bonds, hydrophobic bonds, electrostatic bond, and Van der Waals forces, while also highlighting the formation of unfavorable interactions, namely unfavorable bonds. Hydrogen bond is robust interactions that occur between hydrogen atoms and atoms with high electronegativity, such as fluorine, nitrogen, and oxygen [39]. The utilization of this connection is frequently observed within the realm of pharmaceutical research and development [40]. Hydrophobic bond is established through the interaction of hydrophobic groups present in ligands and receptors [41]. Electrostatic bonds are robust interactions that arise from disparities in charge [31]. Van der Waals forces refer to relatively weak intermolecular interactions that occur between atoms [42]. These interactions are notably influenced by the spatial separation between the amino acid residues of the receptor and the atoms constituting the ligand. In contrast, an unfavorable bond refers to a repulsive interaction occurring between the ligand and the receptor, primarily resulting from steric hindrances or the presence of like charges, hence inducing repulsion [38], [43]. A high-quality complex is characterized by a limited number of unfavorable bonds [44].

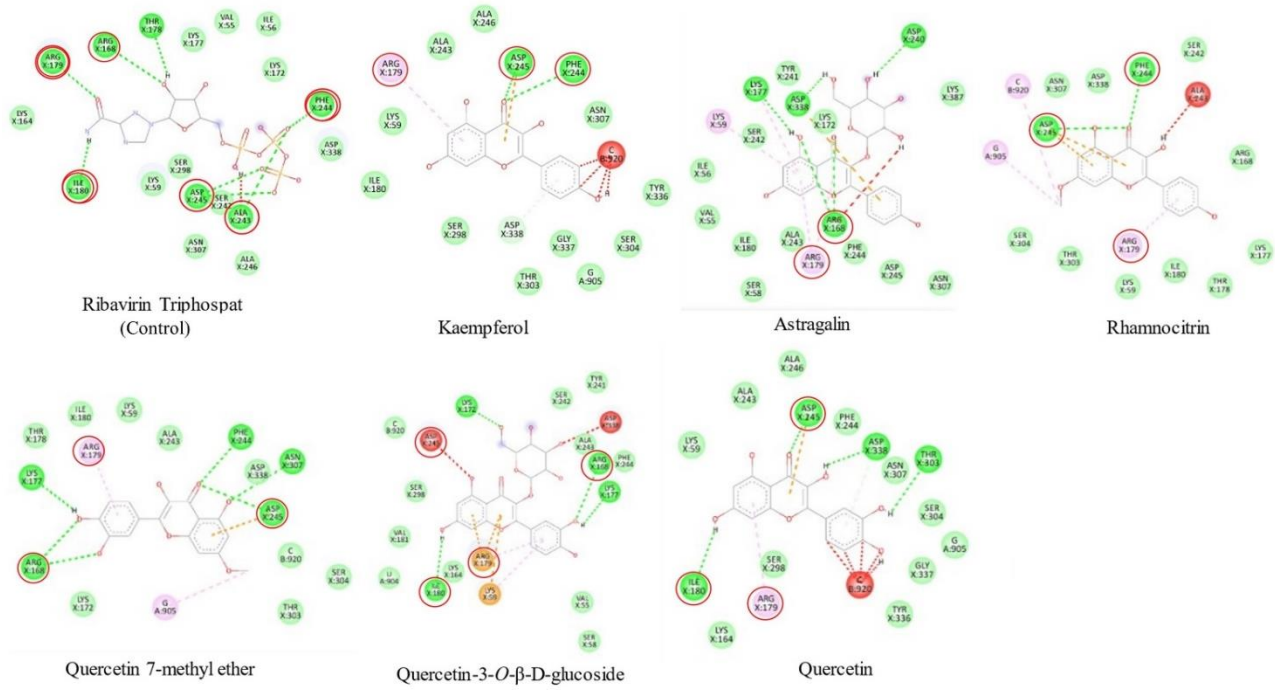

Figure 2. Visualization of Control and Potential Compound on RdRp Complex (Note: Red circles are identical amino acid residues to drug control)

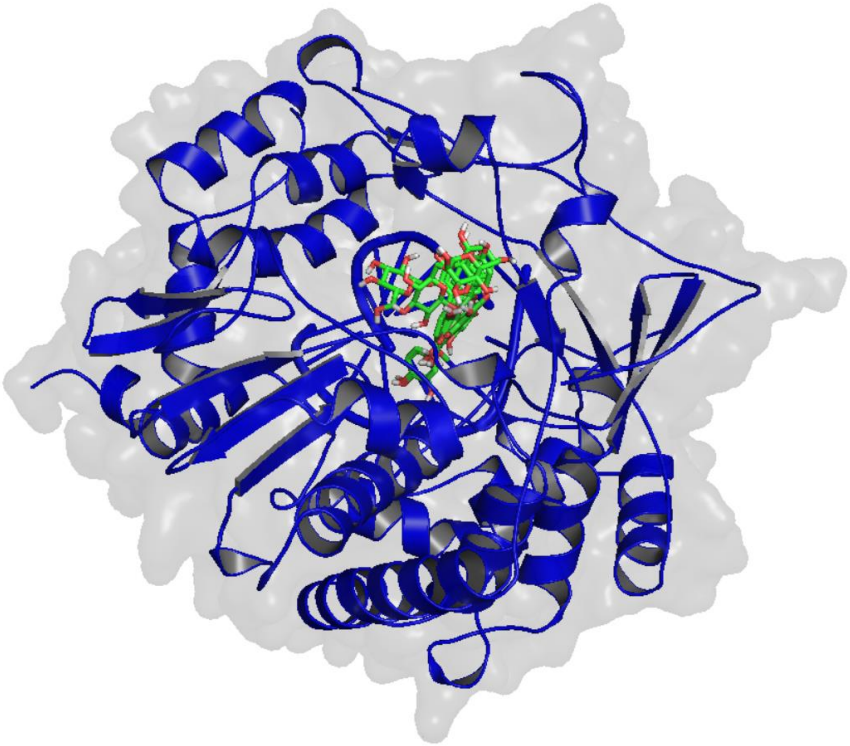

Figure 3. 3D Visualization (Surface Area Interaction) of RdRp-Ligand Complex in Active Site

The obtained visualization results, as depicted in Figure 2 and 3, illustrate the interaction between each possible chemical and the RdRp receptor of the FMD virus, along with the control ribavirin. The complex involving prospective chemicals exhibits interactions with amino acid residues that are identical to those seen in the control. The observed similarity in the locations of these residues indicates that the potential compounds exhibit a comparable inhibitory position to the drug control compounds. Consequently, the degree of similarity in the amino acid residues to the control compounds directly correlates with the level of similarity in their inhibitory activity [29], [44]. The findings indicate that kaempferol exhibits three analogous amino acid residues at positions Arg 179, Asp 245, and Phe 244. Astragalin, on the other hand, demonstrates two similarities at positions Arg 168 and Arg 179. Rhamnocitrin displays three similarities at positions Phe 244, Asp 245, and Arg 179. Quercetin 7-methyl ether showcases three similarities at positions Arg 179, Arg 168, and Asp 25. Quecetin 3-*O*-β-D-glucoside reveals three favorable interactions at positions Arg 179, Ile 180, and Arg 168, along with one unfavorable interaction at position Asp 245. Lastly, quercetin shares three common positions, namely Asp 245, Arg 179, and Ile 180. The degree of similarity between the inhibitory position and the control compound directly correlates with the level of similarity in inhibitory action exhibited by the drug [41].

4. Conclusion

The research findings indicate that *P. urinaria* exhibits promise as an antiviral agent against Foot-and-Mouth Disease (FMD) through the inhibition of RdRp. Among the compounds tested, quercetin shown the highest potency with a binding energy of -7.9 kcal/mol. Additional investigation is warranted, encompassing in vitro and in vivo experimentation, in order to establish a higher level of certainty regarding the potential of this indigenous botanical specimen as an antiviral agent against Foot-and-Mouth Disease (FMD).

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