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Effectiveness of *Desmanthus virgatus* Leaf Ethyl Acetate Extract and Antiviral Drugs as Antirabies Based on In Silico Study

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Abstract: Rabies is a deadly viral disease that has no effective therapy after clinical symptoms appear. This study aims to evaluate the antirabies potential of the dominant compounds in the ethyl acetate extract of *Desmanthus virgatus* leaves, as well as several antiviral drugs, through an in silico approach. The extract was obtained through maceration and partitioning methods using ethyl acetate solvent, then analyzed using LC-MS, which identified 182 secondary metabolite compounds, and ten dominant compounds were selected for further analysis. These compounds, along with seven antiviral drugs, were docked against the rabies virus glycoprotein (PDB ID: 6LGX) using AutoDockTools 4.2.6 software. The docking results were analyzed based on the values of binding affinity, inhibition constant (Ki), and interaction with active amino acid residues. Quercitrin and quercimeritrin were the dominant flavonoid glycosides in the ethyl acetate extract of *D. virgatus* leaves that showed binding affinity values of -8.45 kcal/mol and -8.10 kcal/mol, respectively. In addition, bictegravir and tegobuvir showed binding affinity values of -9.17 kcal/mol and -9.05 kcal/mol, respectively. Four compounds indicated potential as antirabies drugs. Pharmacokinetic feasibility tests using Lipinski parameters showed that most of the dominant compounds violated one or more parameters, especially the number of hydrogen bond donors/acceptors and molecular weight. However, such violations were also found in some antiviral drugs that have been used, such as remdesivir and darunavir. These results suggest that D. virgatus leaf extracts contain compounds with promising potential antirabies activity and deserve further investigation through in vitro and in vivo tests.

Keywords: Antiviral Drugs; *Desmanthus virgatus*; Flavonoid Glycosides; Molecular DockingRabies Virus.

Introduction

Rabies is a disease most feared by humans because its death rate is almost uniform after symptoms appear [1]. This virus belongs to a species of the *Lyssavirus* genus that causes fatal inflammation of the brain and spinal cord (encephalitis) [2]. The rabies virus genome encodes five proteins such as phosphoproteins, glycoproteins, matrix proteins, nucleoproteins, and viral RNA polymerase. Glycoprotein is a major component of the rabies virus that plays an important role in inducing neutralizing antibodies. The virus has a high affinity for the nervous system and is able to replicate within nerve cells. The infection process begins with the attachment of the virus to cell receptors, such as p75 neurotrophin receptor, acetylcholine receptor and neural cell adhesion molecule, which allows the virus to enter the target cell [3]. Rabies is most commonly transmitted through unvaccinated dogs and carnivorous animals. The virus is transmitted from the animal's saliva and enters through bite wounds on the body [4].

Rabies virus infection should be quickly prevented. However, when treatment or vaccines are not available, the virus inoculated into the bite wound can ascend into the neurons of the brain, resulting in inflammation [5]. The treatment of rabies encephalitis has been a major challenge throughout history. In traditional medicine, methods used include poultices, the use of dog hair applied to wounds, and herbal concoctions. Some practitioners also make use of animal horns to neutralize the venom from animal bites [6]. In Roman times, some patients infected with the rabies virus were forced into pools of water to overcome the hydrophobia caused by the viral infection [7]. Without intensive treatment, patients with unvaccinated malignant rabies encephalitis will generally die within days. In contrast, patients with paralytic rabies may survive for several weeks even without optimal treatment [8]. However, no therapy has consistently demonstrated efficacy in treating rabies infection after symptoms develop.

Research on the therapy and effectiveness of drugs as antirabies continues to be developed to reduce the number of deaths due to viral infections [9]. The search for medicinal plants continues because they have fewer toxic effects than synthetic drugs [10]. One of the wild plants as a drug candidate is *Desmanthus virgatus*. *D. virgatus* leaf extract contains various secondary metabolites such as alkaloids, flavonoids, tannins, and steroids [11]. Flavonoid compounds are known to have antiviral activity more effectively than other compounds [12]. In addition, various synthetic antiviral drugs are thought to inhibit rabies virus replication [13]. In silico studies were conducted to determine the mechanism of interaction between ligands and rabies virus proteins. The study results of these interactions are expected to predict compounds as drug candidates in the treatment of rabies virus infection [14].

Based on this, this study aims to analyze the content of secondary metabolite compounds in the ethyl acetate extract of D. virgatus leaves using a liquid chromatography-mass spectrometry instrument. The dominant compounds in

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the ethyl acetate extract of *D. virgatus* leaves and antiviral drugs were further analyzed through the in silico method against rabies virus glycoproteins. Simultaneously, the binding affinity values of the dominant compounds in D. *virgatus* leaf extract were compared with the antiviral drugs. In addition, this study also evaluated the molecular interactions between the dominant compounds of *D. virgatus* extract and antiviral drugs against the active site of rabies virus glycoprotein. The pharmacological properties of the dominant compounds of *D. virgatus* leaves and antiviral drugs were analyzed for their evaluation as drug candidates for further drug development. This analysis can help in creating drug candidate compounds and formulations for curing patients infected with the rabies virus.

Research Methods

D. virgatus leaf samples were collected from Kedungrungkem Village, Benjeng Subdistrict, Gersik District, East Java, located between latitude -7.2918593° and longitude 112.4490716° East, Indonesia, in March 2024. Plant samples were identified at Genbinesia, Gersik, East Java, Indonesia, with Service Order ID: BT-02/035/24. A total of 6.8 Kg of *D. virgatus* leaves were pulverized into powder form and macerated using ethanol solvent in a ratio of 1:3 for three days. The filtrate from maceration was then evaporated so that a concentrated ethanol extract of *D. virgatus* leaves was obtained as much as 478 g. The ethanol extract was then partitioned with ethyl acetate solvent three times in a ratio of 1:1, so that an ethyl acetate filtrate was obtained, which was then evaporated and yielded an ethyl acetate extract of *D. virgatus* leaves of 1.5 grams.

Identification of chemical compounds can be done using an LC system (Shimadzu LCMS-8040 LC/MS). A total of 1 µL of extract was injected into the LC instrument equipped with a Shim Pack FC-ODS column (2 mm × 150 mm, 3 µm particle size) and a column temperature of 35 °C. Separation was performed by isocratic elution with methanol as the mobile phase at a flow rate of 0.5 mL/min. Parameter analysis was performed in negative ion mode as follows: 100°C source temperature, 23 eV cone sampling voltage, 3.0 kV capillary voltage, 350°C desolvation temperature, and 60 mL/h desolvation gas flow. Mass spectra were detected in ESI negative ion mode between m/z 10-1000 with a scan duration of 0.6 s/scan) and a run time of 80 min.

The dominant compounds in the extract (with more than 1% content) and antiviral drugs (bictegravir, darunavir, favipiravir, hydroxychloroquine, remdesivir, ribavirin, and tegobuvir) were pretreated through AutodockTools 4.2.6 software. and added polar hydrogen and Gasteiger charge. Rabies virus protein (PDB ID: 6LGX) was pretreated by removing residual water and adding Kollman Charges. Prepared proteins and ligands were subjected to molecular docking tests with Grid Settings set with a grid spacing of 0.375 Å with x, y, and z dimensions of 126 × 126 × 126 Å and grid centers x, y, and z with values of 42,563, 23,235, and 14,859. The Lamarck Genetic Algorithm method was used to find the smallest binding affinity value by applying minimization to the genetic algorithm by adjusting the gene population. Other parameters were set as follows: Number of GA Runs: 100; Population Size: 150; Maximum Number of Evaluations: medium (2500000); Maximum Number of Generations: 27000; Gene Mutation Rate: 0.02; and Crossover Rate: 0.8. The results of molecular docking then analyzed the pharmacological properties of drug candidate compounds based on Lipinski's five rules including the value of hydrogen donors and acceptors, molecular weight, log P value, and molar refraction of a compound.

Results and Discussion

The results showed that the ethyl acetate extract of *D. virgatus* leaves had various secondary metabolite compounds in LC-MS analysis. In addition, the dominant compounds of *D. virgatus* leaf ethyl acetate extract and antiviral drugs have various active sites by binding through amino acid residues in inhibiting rabies virus glycoproteins.

LC-MS Analysis

The results of LC-MS analysis of ethyl acetate extract of *D. virgatus* leaves obtained 182 secondary metabolite compounds identified using the principle of determining the ratio of mass to charge and mass spectrum fragmentation patterns. The results showed that secondary metabolite compounds were identified through molecular weight, compound abundance, and the presence of peak fragments at certain retention times.

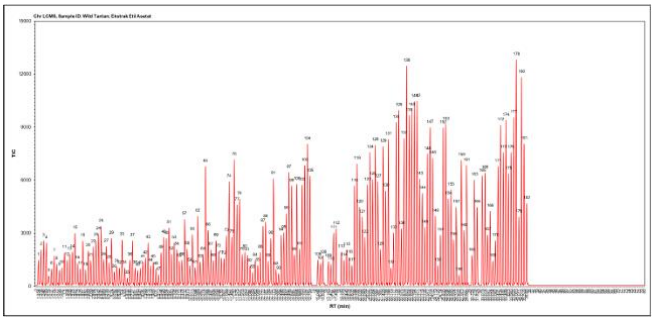

Figure 1. LC-MS Chromatogram of Ethyl Acetate Extract of *D. virgatus* Leaf

Table 1. Dominant Compound of Ethyl Acetate Extract of *D. virgatus* Leaf

Compound Name	RT (minute)	Composition (%)
Trifolin	22.178	1.367
Kaempferol-5-glucoside	22.185	1.469
Astragalin	22.623	1.838
Cynaroside	22.628	1.471
Quercitrin	23.194	1.542
Quercimeritrin	23.311	1.544
Procyanidin B3	33,499	1.385
Kaempferol-3-O-neohesperidoside	33.625	1.408
Kaempferol-7-rhamnoside-4'-glucoside	34.003	1.890
Kaempferol-3-feruloylapioside	34.016	1.743

A total of 182 compounds were detected in the ethyl acetate extract of *D. virgatus* leaves, and 10 dominant compounds were selected based on the highest composition obtained from the LC-MS analysis results (Table 1). The dominant compounds in the extract have the potential to affect living organisms, either positively or negatively

depending on the type of compound, dosage, and bioavailability. The abundant content of these compounds increases the chances of involvement in biological processes and the effectiveness of their biological activities [15].

The dominant compound contained in the ethyl acetate extract of *D. virgatus* leaves is a flavonoid glycoside group compound. Flavonoid glycosides are known to have a variety of biological activities, including as anti-inflammatory, antibacterial, antiviral, antiallergic, cytotoxic, antitumor, and vasodilator agents [16]. Flavonoid glycosides are able to modulate the behavior of cell systems and have a positive effect on the body [17]. Flavonoid glycoside class compounds can affect enzyme activity through inhibition of lipid peroxidation processes as well as cyclooxygenase and lipoxygenase enzyme activities that contribute to suppressing NADH oxidase activity and maintaining reactive oxygen balance, platelet aggregation, and stabilizing capillary permeability [18].

Molecular Docking Analysis

The analysis was then continued by conducting molecular docking between the dominant compounds and antiviral drugs against rabies virus glycoproteins. In this study, one target protein was used, namely the rabies virus glycoprotein (PDB ID: 6LGX), which is a crystal structure in a prefusion form under alkaline pH conditions with a resolution of 3.10 Å. The glycoprotein was chosen because it plays a crucial role in the early stages of viral infection of host cells. The structure of the rabies virus glycoprotein is considered biologically most relevant as it represents the active conformation prior to membrane fusion. Additionally, the use of a single target protein was employed in the early stages of the in silico study to ensure analytical focus, particularly when the target structure is experimentally available with sufficient quality. Validation of docking results was not performed because the structure of the rabies virus glycoprotein (PDB ID: 6LGX) does not contain a bound ligand in its crystal data and is categorized as an apo protein, i.e., a ligand-free state [19]. The native ligand of the protein is generally used as a positive control because it is supported by experimental data such as IC_{50} or EC_{50}, which can be found on protein database websites. In molecular docking, the native ligand serves as a benchmark or standard for evaluating the effectiveness of the test ligand. However, since the rabies virus glycoprotein structure lacks an inherent ligand, a positive control is not directly available, and conventional docking validation cannot be performed [20], [21].

The results of molecular docking were analyzed based on binding affinity values, inhibition constants, and molecular interactions between ligands and target proteins. The binding affinity value shows how strongly the ligand of the drug candidate compound binds to the receptor. The smaller the binding affinity value of the drug candidate compound to the receptor, the stronger the bond that occurs, which causes the bond of the drug candidate compound to the receptor to be stable [22]. The inhibition constant plays a role in determining the amount of concentration of drug candidate compounds needed to inhibit rabies virus receptors. The smaller the Ki value, the stronger the binding affinity and the less concentration of the drug candidate compound needed to inhibit the receptor activity [23]. The results of the binding affinity and inhibition constant values produce molecular interactions in the form of non-covalent bonds such as hydrogen bonds, ionic bonds, Van Der Waals bonds, and hydrophobic interactions between the ligand and the target protein [24]. Data from the binding affinity, Ki, and molecular interaction values between the ligand and the target protein can be seen in Tables 2 and 3.

Table 2. Bioactivity of Dominant Compounds of *D. virgatus* Leaf Extract

Compound Name	Binding Affinity (kcal/mol)	Inhibition Constant	Molecular Interactions	Amino Acid Residue
Dominant Compound of Ethyl Acetate Extract of *D. virgatus* Leaf				
Trifolin	-6.47	18.00 µM	Hydrogen bond	ASN57, TRP14, HIS352, GLU10, PRO353, HIS354
			Carbon-hydrogen bond	PRO13, SER15, PRO16
			Pi-alkyl	LEU11
Kaempferol-5-glucoside	-6.58	15.11 µM	Hydrogen bond	TYR50, ASN57, TRP14, GLY49, ASP326
			Pi-cation	LYS55
			Pi-alkyl	PRO13
			Carbon hydrogen	SER15
Astragalin	-7.12	6.05 µM	Hydrogen bond	SER151, VAL139, ASP141, PRO137
			Pi-cation	HIS21
			Pi-anion	ASP18
			Pi-alkyl	PRO16
Cynaroside	-7.68	2.33 µM	Hydrogen bond	ARG280, SER302, GLU282, ARG299
			Pi-cation	LYS299
			Pi-sigma	LEU307
			Pi-Pi stacked	HIS303
Quercitrin	-8.45	642.03 nM	Hydrogen bond	HIS150, ASP141, ASP18
			Pi-Cation	HIS21

			Pi-lone pair	VAL139
			Pi-alkyl	VAL153
			Pi-anion	ASP18
			Pi-Pi T-shaped	VAL139
Quercimeritrin	-8.10	1.16 µM	Hydrogen bond	LYS294, LYS320, LUE322, PRO16, VAL139, ASP18
			Carbon-hydrogen bond	SER138
			Pi-alkyl	ILE19
Procyanidin B3	-6.89	8.83 µM	Hydrogen bond	PHE154, LYS342, ASP369, GLY343, HIS352, ASN172
			Pi-donor hydrogen bond	SER341
			Pi-alkyl	LEU365, CYS351
			Pi-cation	HIS354
Kaempferol-3-O-neohesperidoside	-6.50	17.09 µM	Hydrogen bond	CYS24, ASN26, PHE311, LYS306, LEU304, LYS313, HIS21
			Pi-sigma	PRO25
Kaempferol-7-rhamnoside-4'-glucoside	-6.00	40.04 µM	Hydrogen bond	LEU365, ASN319, LYS320, VAL296
			Pi-sigma	VAL358
			Pi-alkyl	ILE364, ILE317
Kaempferol-3-feruloylapioside	-7.41	3.69 µM	Hydrogen bond	PHE154, ASN172, GLY156, HIS20, ARG152, CYS351, LEU284
			Pi-cation	ARG350
			Pi-sulfur	MET291
			Alkyl	LYS342
			Pi-alkyl	LEU287

Table 3. Bioactivity of Antiviral Drugs

Compound Name	Binding Affinity (kcal/mol)	Inhibition Constant	Molecular Interactions	Amino Acid Residue
Bictegravir	-9.17	191.36 nM	Halogen (fluorine)	LYS220, SER218
			Carbon-hydrogen bond	ASP237
			Pi-donor hydrogen bond	LEU28, PRO309
			Pi-alkyl	VAL308, VAL29, VAL30, LEU235
			Pi-sigma	LEU215
Darunavir	-6.28	25.02 µM	Hydrogen bond	ARG280, ASN26, CYS24, SER52
			Carbon-hydrogen bond	GLY312
			Pi-Pi T-shaped	PHE311
Favipiravir	-6.09	34.46 µM	Hydrogen bond	LEU28, LYS306, PRO309, PHE311, ASP237
			Halogen (fluorine)	TYR216
Hydroxychloroquine	-5.29	57.22 µM	Hydrogen bond	ILE17, LYS320, VAL139
			Salt bridge	ASP18
			Alkyl	PRO16, VAL296, ILE19
Remdesivir	-6.79	10.61 µM	Hydrogen bond	TRP14, LEU11, VAL48, GLU288
			Alkyl	MET323, PHE318
			Pi-alkyl	VAL355, PRO13
Ribavirin	-5.84	52.27 µM	Hydrogen bond	ASP141, SER15, PRO16, PRO137, ASP18
			Carbon hydrogen	VAL139, HIS21, LYS55
Tegobuvir	-9.05	230.84 nM	Hydrogen bond	LYS279, ASN27, PRO25

Halogen (fluorine)	ASN26
Pi-sigma	LEU307
Pi-Pi stacked	HIS303
Pi-alkyl	ARG280, CYS283

The results of molecular docking analysis show that quercitrin, quercimitrin, bictegravir, and tegobuvir have the most negative binding energy compared to other compounds. Quercitrin and quercimeritrin showed more negative binding affinity values than the dominant compounds in the ethyl acetate extract of *D. virgatus* leaves. Quercitrin, with a binding affinity value of -8.45 kcal/mol, interacts with rabies virus glycoproteins through hydrogen bonding with amino acid residues HIS150, ASP141, and ASP18. Ring A on quercitrin forms a pi-cation interaction with residue HIS21, while ring C forms a pi-anion bond with residue ASP18. In addition, residue VAL139 interacts with ring C through a pi-lone pair bond, while ring B binds to VAL153 through a pi-alkyl interaction. A pi-pi T-shaped interaction was also formed between ring C and residue VAL139. Quercimeritrin shows a binding affinity value of -8.10 kcal/mol and interacts with rabies virus glycoproteins through hydrogen bonds with amino acid residues LYS294, LYS320, LEU322, PRO16, VAL139, and ASP18. The B ring of quercimeritrin forms a pi-alkyl interaction with residue ILE19, while the oxygen atom on the glycoside group forms a carbon-hydrogen bond with residue SER138.

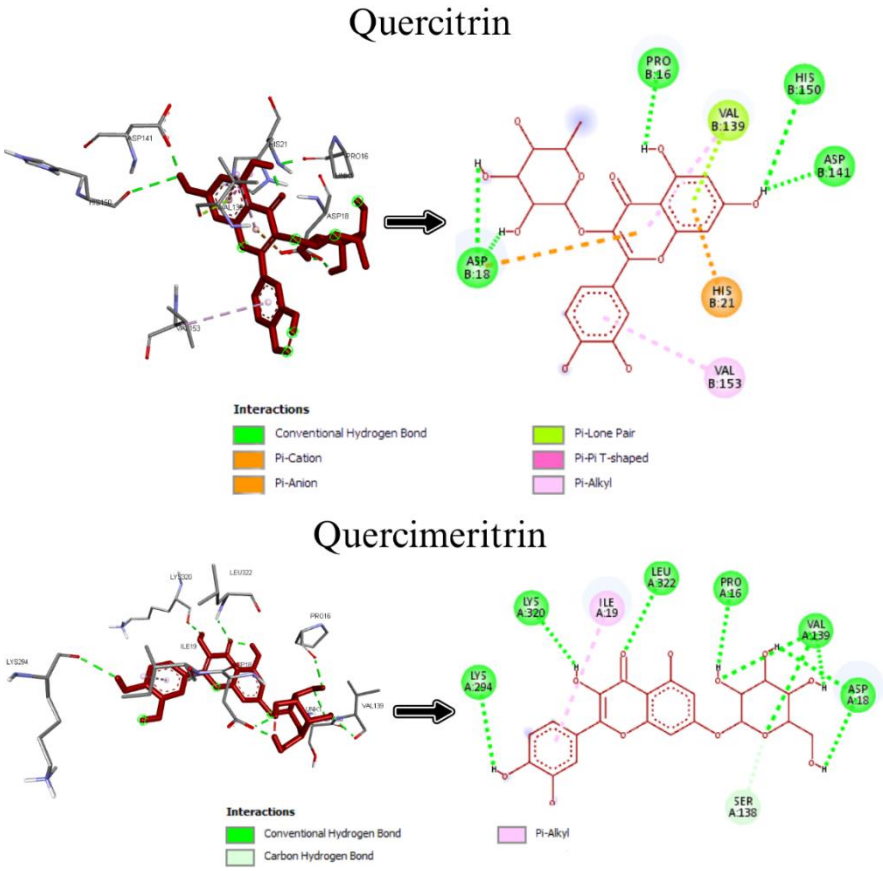

Figure 2. Visualization of the Interaction between Quercitrin and Quercimitrin Compounds and Rabies Virus Glycoprotein

Some antiviral drugs have very negative binding affinity values, such as tegobuvir (-9.05 kcal/mol) and bictegravir (-9.17 kcal/mol), which indicates the stability of the bond formed between the ligand and the receptor. Tegobuvir is an antiviral drug with a clinical trial phase for the treatment of chronic hepatitis C [25]. The tegobuvir compound interacts with rabies virus glycoproteins through hydrogen bonds with amino acid residues LYS279, ASN27, and PRO25. The imidazole ring on tegobuvir forms pi-sigma interactions with residue LEU307, pi-pi stacking with residue HIS303, and pi-alkyl with residues ARG280 and CYS283. In addition, the fluorine group on tegobuvir forms a halogen bond with residue ASN26. Bictegravir is an antiretroviral drug that is used to treat HIV infection by targeting the viral integrase [26]. The molecular docking results of bictegravir with rabies virus glycoprotein showed a halogen bond between the fluorine group and amino acid residues LYS220 and SER218. A carbon-hydrogen interaction was formed between the alkyl group on the benzene ring and residue ASP237. A pi-donor hydrogen bond occurs between the dihydropyridine ring and residues LEU28 and PRO309, and a pi-sigma bond with residue LEU215. In addition, the rings of bictegravir interact via pi-alkyl bonds with residues VAL308, VAL29, VAL30, and LEU235.

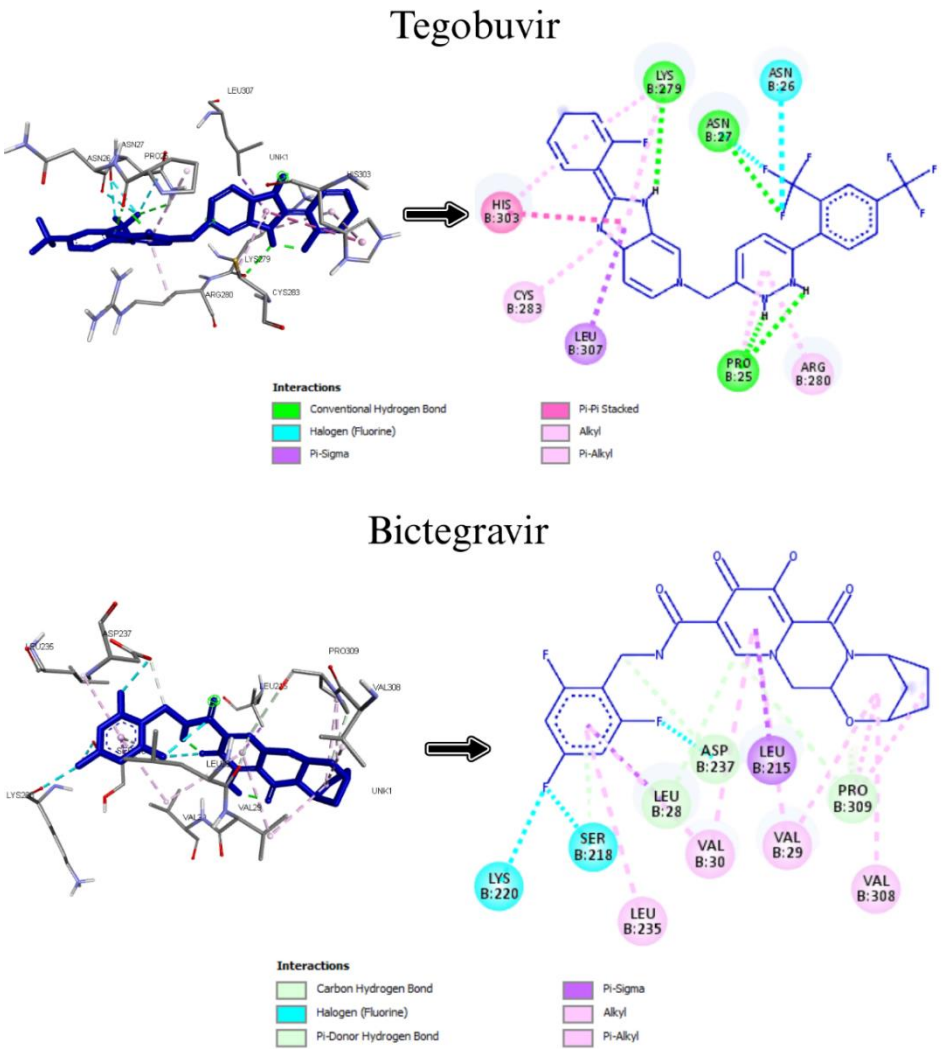

Figure 3. Visualization of the Interaction between Tegobuvir and Bictegravir Compounds and Rabies Virus Glycoprotein

The antiviral drugs tegobuvir and bictegravir have more negative binding affinities than quercitrin and quercimetrin. This is due to the presence of fluorine halogen bonds in both antiviral molecules, which can strengthen the interaction between the ligand and the rabies virus glycoprotein receptor. Halogen bonds exhibit highly directed, specific interactions that act analogously to hydrogen bonds. These interactions form between covalently bonded halogen atoms (e.g., C–X, where X = Cl, Br, or I, as the XB donor) and a nucleophile or Lewis base acting as the acceptor. Due to the anisotropy in the distribution of charge on halogen atoms, an electropositive region known as a σ-hole is formed on the extension of the C-X bond. This region allows for electrostatic attraction with nucleophiles [27]. Meanwhile, quercitrin and quercimetrin compounds have slightly higher binding affinities than tegobuvir and bictegravir, influenced by the number of hydrogen bonds in their glycoside groups. The hydrogen bonds formed play a role in the stability of the complex structure. The more hydrogen bonds formed, the more complex the structure becomes [28]. In addition to hydrogen bonds, electrostatic interactions, and hydrophobic interactions in antiviral drugs and the dominant compounds of *D. virgatus* also influence the stability of the ligand toward the receptor [29]. These interactions can significantly impact the biological activity, physicochemical properties, and pharmacokinetics of drugs, making hydrogen bonds crucial in drug discovery [30].

Although tegobuvir and bictegravir exhibit more negative binding affinity compared to quercitrin and quercimitrin compounds, the effectiveness of a compound as an antirabies drug candidate is not only determined by the strength of the interaction between the ligand and the receptor, but also depends on target selectivity, potential toxicity, and pharmacodynamic relevance. Tegobuvir and bictegravir exhibit highly negative binding affinity toward rabies virus glycoprotein. However, these two drugs were primarily developed for the target viruses HCV and HIV. Despite their high binding affinity, their potential selectivity toward rabies virus glycoprotein may be lower, leading to non-specific interactions that could trigger off-target toxicity. Meanwhile, quercitrin and quercimeritrin interact with the rabies virus glycoprotein through ASP18, HIS150, and VAL139. The diverse binding patterns formed with the rabies target indicate higher selectivity potential. This means the compounds are more likely to act specifically against the rabies virus with lower side effects [31].

In addition to quercitrin, quercimeritrin, tegobuvir, and bictegravir, which showed more negative binding affinity values to rabies virus glycoproteins, there were several other dominant compounds and antiviral drugs that had less negative binding affinity values. This reflects that

the strength of the interaction between these ligands and glycoproteins is relatively weak, so it is likely that their effectiveness in inhibiting rabies virus glycoprotein function is also lower. The interactions formed are generally limited to hydrophobic bonds or hydrogen bonds, which are less stable than the complex bonds exhibited by high-affinity compounds. Therefore, although these compounds were detected as dominant components or are known antivirals, their therapeutic potential against the rabies virus through the mechanism of glycoprotein inhibition is still low and requires structural optimisation or compound combination approaches to increase their effectiveness.

Bioavailability Test

Determination of the bioavailability of a drug candidate compound is an important parameter to determine the amount and speed at which the drug is absorbed in the body [32]. Drug bioavailability is influenced by several factors, including the physicochemical properties of the drug, dose of administration, excretion, and hepatic metabolism [33]. In determining the bioavailability test of a drug candidate compound, Lipinski's Rule of Five parameters are used to evaluate the feasibility of the compound as a drug candidate. Lipinski's rule of five parameters includes molecular weight (≤500 Da), molar refractivity (40-130), log P (≤5), hydrogen acceptor (≤10), and hydrogen donor (≤5) [34]. The results of Lipinski's rule of five analysis of the dominant compounds of ethyl acetate extract of *D. virgatus* leaves and antiviral drugs can be seen in Tables 4 and 5.

Table 3. ADME Analysis of Dominant Compounds of *D. virgatus* Leaf Ethyl Acetate Extract

Compound	ADME Properties				
	Molecular Weight	Hydrogen Donor	Hydrogen Acceptor	Log P	Molar Refractivity
Trifolin	448	7	11	-0.43	104.60
Kaempferol-5-glucoside	448	7	11	-0.22	105.11
Astragalin	448	7	11	-0.43	104.60
Cynaroside	448	7	11	-0.40	105.20
Quercitrin	448	7	11	0.29	104.86
Quercimeritrin	464	8	12	-0.51	106.78
Procyanidin B3	578	10	12	2.99	143.38
Kaempferol-3-O-neohesperidoside	594	9	15	-1.58	135.83
Kaempferol-7-rhamnoside-4'-glucoside	594	9	15	-1.72	136.43
Kaempferol-3-feruloylapioside	594	6	13	2.18	146.19

Table 3. ADME Analysis of Antiviral Drugs

Compound	ADME Properties				
	Molecular Weight	Hydrogen Donor	Hydrogen Acceptor	Log P	Molar Refractivity
Bictegravir	449	2	8	1.37	100.75
Darunavir	547	4	10	3.45	141.63
Favipiravir	157	3	5	-1.18	33.96
Hydroxychloroquine	335	2	4	3.78	98.27
Remdesivir	602	5	13	2.31	149.83
Ribavirin	244	5	8	-3.01	51.54
Tegobuvir	517	0	4	6.59	118.83

Hydrogen bonding is an important interaction in the design of a drug because this bond can affect structural stability, partitioning, drug permeability, and enzyme catalysis [35]. All dominant compounds of the ethyl acetate extract of *D. virgatus* leaves have hydrogen bond donors and acceptors exceeding Lipinski's rule parameters. While the category of antiviral drugs, remdesivir, has a hydrogen bond acceptor value exceeding the parameters of Lipinski's rule. The functional groups of the dominant compounds of *D. virgatus* leaf extract and antiviral drugs that are able to form hydrogen bonds can affect important interactions with their receptor targets, so they have strong selectivity and binding. However, too many hydrogen bond donors and acceptors have low effectiveness on drug partitioning and permeability, thus decreasing the binding affinity to hydrophobic membrane regions [36].

Molecular weight represents the molecular size of a compound. The larger the molecular size of a compound, the greater the absorption through biological membranes [37]. The dominant compounds of *D. virgatus* extract that do not pass this criterion are kaempferol-3-O-neohesperidoside, kaempferol-7-rhamnoside-4'-glucoside, and kaempferol-3-feruloylapioside. While in the category of antiviral drugs, darunavir, tegobuvir, and remdesivir also do not meet the criteria of Lipinski's rule. Molecular weights exceeding 500

Da have low promiscuity, thus reducing interactions with receptor targets [38].

The log P value is the partition coefficient of solute between water and octanol, in almost infinite dilutions. LogP is widely used in drug discovery and development as an indicator of a compound's potential as a drug, with one of the criteria being that the LogP value is in the range of 0 to 5 [39]. The results of the log P value of antiviral drugs show that favipiravir has a negative log P value, and tegobuvir has a log P value exceeding the parameter limit. While in the dominant compounds of *D. virgatus* extract, only quercitrin, procyanidin B3, and kaempferol-3-feruloylapioside meet the log P value criteria. A negative log P value indicates that a compound prefers to dissolve in water rather than fat, which results in the compound not being able to enter the cell membrane. While the log P value that exceeds 5 in a compound indicates that the compound has hydrophobic and less hydrophilic properties, which can have a toxic effect because the compound will be retained longer in the lipid bilayer [40].

Molar refraction is associated with refractive index and polarizability, assessed according to how much it varies and contributes to the biological activity of compounds, especially in predictive modelling using topological indices to assess drug effectiveness [41]. The compounds procyanidin B3 and kaempferol-3-feruloylapioside in *D. virgatus* leaf extract have molar refraction values exceeding 140 cm^3/mol. In addition, darunavir and remdesivir compounds also did not meet the molar refraction criteria. An acceptable molar refraction value, combined with the number of hydrogen bonds, indicates that a compound has adequate intestinal absorption and oral bioavailability [42].

Although some of the dominant compounds in the ethyl acetate extract of *D. virgatus* leaves and antiviral drugs violate one or more Lipinski parameters, this does not hinder their potential for development as drug candidates. Compounds that violate Lipinski parameters can still be reformulated using a lipid-based drug delivery approach. Lipid-based delivery systems are formulated using plant and animal oils that exhibit higher biocompatibility and lower toxicity. All lipid-based systems can enhance the half-life and bioavailability of the loaded drug and prolong therapeutic efficacy due to controlled drug release [43]. Additionally, both hydrophilic and hydrophobic compounds can be formulated using encapsulation methods. Drug encapsulation is a crucial strategy for compounds that are poorly soluble, fragile, or aggressive, enabling stronger therapeutic effects with minimized toxic effects [44]. Therefore, advanced formulation approaches such as lipid-based delivery systems and encapsulation play a crucial role in overcoming bioavailability limitations caused by violations of Lipinski's rules.

Conclusion

Phytochemical screening of dominant compounds of ethyl acetate extract of *D. virgatus* leaves and antiviruys drugs as antirabies based on in silico studies allows as drug candidates based on their binding affinity. Based on the results of molecular docking, the dominant compounds in *D. virgatus* leaf extract have antirabies properties, namely quercitrin and quercimeritrin. In addition, antiviral drugs suspected as antirabies drug candidates are bictegravir and tegobuvir. The bioactivity of a drug candidate compound is influenced by the interaction of functional groups bound between receptors through amino acid residues in each ring of the drug candidate compound, so that it has more stable binding activity and can be further investigated as an antirabies drug candidate. Therefore, validation through in vitro and in vivo studies is needed to confirm the anti-rabies activity and evaluate the pharmacokinetic and toxicity aspects of the compound, so that it can support its development as a viable anti-rabies therapy candidate.

Author's Contribution

Syahrul Lerry Hendrawan: contributed to drafting the research concept, developing the research methodology, collecting data, analyzing data, and writing the manuscript. Cindy Ambarwati: contributed to the drafting of the research concept, data collection, and data analysis. Tukiran Tukiran: contributed to drafting the research concept and evaluating the authors in writing the manuscript.

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