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VIRTUAL SCREENING AND MOLECULAR DOCKING STUDIES OF SEVERAL PLANT COMPOUNDS WITH THE PRIMARY PROTEASE OF THE COVID-9 VIRUS

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ABSTRACT

Researchers have tried to find a compound that can inhibit the replication of the SARS-CoV-2 virus since the outbreak of the Covid-19 pandemic. The present study evaluates the bioactive compounds found in several plants using a molecular binding approach to inhibit the primary protease of SARS-CoV-2. This study investigated 40 different herbal compounds with the 6Y2F protein of coronavirus. Auto Dock Vina 1.5.6 software was used to evaluate molecular binding. Validation was performed in PyMol software. The results were also analyzed by Biovia Discovery Studio 4.5. The best protein-ligand complex compound was selected by determining the binding score that had the highest affinity (the most negative ΔG Gibbs binding free energy). Among 40 herbal compounds, 21 herbal compounds showed a high energy of -8.0 kJ/mol. Based on the results of binding energy and RMSD value, among the dockings performed, 8 compounds including Ganoderic acid C2, Ursolic Acid, Lupeol, Kuwanon B, Emodin-8-glucoside, Adonitoxin, Kuwanon E, and Isohemiphloin are recommended for further studies in the invivo and invitro sections.

Keywords: Coronavirus, SARS-CoV-2, Herbal compounds, Molecular binding, Auto Dock Vina

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INTRODUCTION

A new respiratory viral disease was reported in Wuhan, China, in December 2019. It was an infectious disease caused by severe acute respiratory syndrome of the SARS-CoV-2 virus [1]. This disease spread worldwide, leading to an ongoing epidemic [2]. Different types of this virus have appeared in many countries until now. The most dangerous types are the alpha, beta, gamma, delta, and omicron strains [3]. About 551 million patients and more than 6 million deaths from this virus were confirmed up to July 2022, making this disease one of the deadliest in history [4]. Although investigations have revealed that various drugs are effective against viruses belonging to the same group, none of them has shown the same potential as a treatment for COVID-19. The primary protease of the COVID-19 virus is considered an attractive target for the study of antiviral drugs against the SARS-CoV-2 virus and other coronavirus infections. Covid-19 symptoms vary but often include fever, cough, headache, fatigue, breathing problems, and loss of smell and taste [5, 6]. This complicated situation has resulted in searching for new treatments and rapid practical measures to treat the disease and reduce its prevalence. Hence, understanding how this virus works and spreads is vital for developing a vaccine.

Further studies are still needed to find effective drugs to inhibit the virus and specific treatment regimens to overcome morbidity and mortality since COVID-19 is a new disease with severe health problems. Covid-19 is very similar to the SARS-CoV-2 virus. There are primary five therapeutic protein targets for SARS-CoV-2, including angiotensin-converting enzyme 2 (ACE2), spike protein, major protease (M pro), RNA-dependent RNA polymerase (RdRp), and papain-like protease. In microscopic imaging, SARS-CoV-2 with its crown-like surface protrusion appears to belong to the family of beta-coronaviruses, which have encapsulated and single-stranded RNA. They primarily infect host lung cells by binding to the angiotensin-converting enzyme 2 (ACE2) receptor [7]. The viral genome is translated as mRNA by the host cell machinery and produces enzymes necessary for RNA synthesis, including RNA-dependent RNA polymerase [8]. Then, these polyproteins are divided into structural proteins such as RdRp, and PL pro. Inhibition can block the synthesis of viral proteins. Thus, it plays a vital role in the viral life cycle [9].

Plants are one of the sources of active medicinal compounds used extensively in the treatment of diseases [10-11]. Many reported bioactive plant compounds have antifungal, antibacterial, and antiviral activities [12]. Plants and plant-derived products have advantages such as simplicity, greater safety, less toxicity, lower cost, higher speed of action, and compatibility with the environment compared to conventional treatment methods [13]. Drugs were extracted from natural sources in the past. These natural sources are still a major source of conductor compounds and new drugs. Nowadays, much attention is paid to pre-studies in drug design using bioinformatics methods to reduce cost and time in drug production. The use of bioinformatics tools and calculation methods that predict the effectiveness of medicinal compounds and their possible toxicity with a high confidence factor has been much considered in recent years [14]. Molecular docking, simulation, determining target point, and chemical stability studies are among the most significant bioinformatics methods used in drug design. In this regard, molecular docking plays a unique role. By considering the different states of the desired molecules in the three-dimensional space and predicting how the protein (receptor) interacts with bioactive compounds (ligand), it is possible in this technique to examine their interactions and the effective factors in the interaction and determine the more stable and important action in terms of drug identification [15].

A computational binding approach using various molecular binding software such as Auto Dock [16] provides the opportunity to identify and evaluate the bindings and efficiency of various inhibitors of natural and synthetic origin. The appropriateness of the drug can be determined by analyzing the medicinal properties after evaluating the efficient inhibitors. Although potential therapeutic agents can only be validated after experimental testing, computational binding could be a gateway to faster development of effective drugs against diseases such as COVID-19. The present study aims to find potential inhibitors of coronavirus among several selected herbal

compounds that have antitussive, antipyretic, anti-viral, anti-inflammatory, antioxidant, etc. impacts using molecular docking studies. It also aims to answer the questions of whether the 6Y2F protease of Covid-19 can be the target of selected herbal compounds in clinical trials and what are the interactions between this protein and the compounds.

METHODS

First, the primary sequence of the 6Y2F protein of Covid-19 was extracted from the PDB database (Table 1). Dimethyl Sulfoxide and ~{tert}-butyl ~{N}-[1-[(2~{S})-3-cyclopropyl-1-oxidanylidene-1-[[(2~{S},3~{R})-3-oxidanyl-4-oxidanylidene-1-[(3~{S})-2 oxidanylidenepyrrolidin-3-yl]-4-[(phenylmethyl)amino]butan-2-yl]amino]propan-2-yl]-2 oxidanylidene-pyridin-3-yl]carbamate in 6Y2F protein was removed from the protein using Discovery Studio 4.5 software [17]. The three-dimensional structure of 40 plant compounds that had antiviral, antitussive, antipyretic, and anti-inflammatory effects were extracted from Pubchem and ChemSpider databases. Table 2 shows the characteristics of plant compounds. Then, docking was performed by Auto Dock Vina software [18]. Table 3 presents the Grid Box dimensions and its coordinates for docking operations. Kollman Charges were used to determine the overall load. Docking results were analyzed by Biovia Discovery Studio 4.5 software. The validity of the docking operation was confirmed by RMSD determination in PyMol software.

Table 1- Receptor examined in this study

Receptor	Specifications
6Y2F	Crystal structure (monoclinic form) of the complex resulting from the reaction between SARS-CoV-2 (2019-nCoV) main protease and tert-butyl (1-((S)-1-(((S)-4-(benzylamino)-3,4-dioxo-1-((S)-2-oxopyrrolidin-3-yl)butan-2-yl)amino)-3-cyclopropyl-1-oxopropan-2-yl)-2-oxo-1,2-dihydropyridin-3-yl)carbamate (alpha-ketoamide 13b)[19].

Table 2- Specifications of ligands studied in this study

	Plant Name	Compound Name	Molecular Formula	CID	MW
	Arachis hypogaea	Soyasaponin I	$C_{48}H_{78}O_{18}$	108898*	943.1
	Water lily	Nupharin A	$C_{41}H_{30}O_{26}$	8709251*	938.7
	Lady's glove	Digoxin	$C_{41}H_{64}O_{14}$	2006532*	780.9
	Cissampelos	Warifteine	$C_{36}H_{38}Cl_2N_2O_6$	170074	665.6
	Forsythiae fructus	Forsythiaside A	$C_{29}H_{36}O_{15}$	5281773	624.6
	Mint	Hesperidin	$C_{28}H_{34}O_{15}$	10621	610.6
	Water pepper	Rutoside	$C_{27}H_{30}O_{16}$	5280805	610.5

	Ginsen radix	Panasenoside	$C_{27}H_{30}O_{16}$	9986191	610.5
	Ziziphi Spinosae Semen	Spinosin	$C_{28}H_{32}O_{15}$	155692	608.6
	Mint	Eriocitrin	$C_{27}H_{32}O_{15}$	83489	596.5
	Eriobotryae folium	-	$C_{27}H_{32}O_{14}$	10507459	580.5
	Citrus reticulata	Naringin	$C_{27}H_{32}O_{14}$	442428	580.5
	Chrysantbemi flos	Isorhoifolin	$C_{27}H_{30}O_{14}$	9851181	578.5
	Boldo	-	$C_{18}H_{16}N_8O_7S_3^{-2}$	2656	552.6
	Pheasant's eye	Adonitoxin	$C_{29}H_{42}O_{10}$	441838	550.6
	Ganoderma	Ganoderic acid C2	$C_{30}H_{46}O_7$	57396771	518.7
	Hedysarum multijugum	-	$C_{23}H_{24}O_{11}$	46899140	476.4
	Chrysanthemi flos	Thermopsoside	$C_{22}H_{22}O_{11}$	11294177	462.4
	Currant	-	$C_{22}H_{18}O_{11}$	65064	458.4
	Illicium Difengpi KLB Et KIM	Betulinic Acid	$C_{30}H_{48}O_3$	64971	456.7
	Perilla Frutescens	Ursolic Acid	$C_{30}H_{48}O_3$	64945	456.7
	Spinach	Vitamin K	$C_{31}H_{46}O_2$	5280483	450.7
	Ginsen radix	Kaempferol	$C_{21}H_{20}O_{11}$	5318755	448.4
	Eriobotryae folium	Isohemiphloin	$C_{21}H_{22}O_{10}$	42607891	434.4
	Myrrh	-	$C_{20}H_{18}O_{11}$	5317847	434.3
	Sennae Folium	Emodin-8-glucoside	$C_{21}H_{20}O_{10}$	99649	432.4
	Fritillaria pallidiflora	Imperialine	$C_{27}H_{43}NO_3$	442977	429.6
	Ricinus	Lupeol	$C_{30}H_{50}O$	259846	426.7
	Farfarae flos	Taraxasterol	$C_{30}H_{50}O$	115250	426.7
	Mori cortex	Kuwanon E	$C_{25}H_{28}O_6$	6440408	424.5

	Gossampini flos	Mangiferin	$C_{19}H_{18}O_{11}$	5281647	422.3
	Mori cortex	Kuwanon B	$C_{25}H_{24}O_6$	44258295	420.5
	zingiberis	beta-sitosterol	$C_{29}H_{50}O$	222284	414.7
	Soja semen nigrum	Stigmasterin	$C_{29}H_{48}O$	5280794	412.7
	Pennyroyal	Cleomiscosin A	$C_{20}H_{18}O_8$	442510	386.4
	Elder	(+)-Bicuculline	$C_{20}H_{17}NO_6$	10237	367.4
	Tripterygii radix	Triptolide	$C_{20}H_{24}O_6$	107985	360.4
	Viper's-buglosses	-	$C_{20}H_{22}O_6$	12309637	358.4
	Papaveris pericarpium	-	$C_{22}H_{29}NO_3$	21287385	355.5
	Knotweed	-	$C_{22}H_{20}O_4$	11163864	348.4

*The codes are chemspider.

Table 3- The dimensions and size of the studied protein space

	center_x	center_y	center_z	size_x	size_y	size_z
6Y2F	-4.73	-2.885	12.052	126	126	126

RESULTS

Among the 40 dockings performed, 21 plant compounds with the 6Y2F receptor of the coronavirus have energy above -8.0. Table 4 shows the results whose minimum free energy is less than -8.0 Kcal/mol. It also presents the target proteins and minimum free energy changes (GΔ) and amino acids involved in hydrogen bonding and the RMSD value of each.

Table 4- Interactions and energy results of complexes higher than ΔG = -8.0

	Plant Name		ΔG (Kcal/mol)	H - Bond	RMSD (A°)
	Lady's glove	Digoxin	-9.6	TYR A:239_LEU A:271_LEU A:272_LYS A:137	3.463
	Cissampelos	Warifteine	-9.3	-	0.001
	Mint	Hesperidin	-9.1	PHE A:140_CYS A:145_HIS A:163	1.326

	Chrysanthemi flos	Isorhoifolin	-9.1	PHE A:140_GLU A:166_LEU A:167_GLN A:192_CYS A:145	0.729
	Farfarae flos	Taraxasterol	-9.1	-	0.001
	Arachis hypogaea	Soyasaponin I	-9.0	ASN A:238_LYS A:137_THR A:169_ASN A:133	1.415
	water lily	Nupharin A	-9.0	GLN A:189_MET A:49_VAL A:186_ARG A:188_GLY A:143	1.897
	Mori cortex	Kuwanon E	-8.8	ILE A:152_GLN A:110	1.117
	Chrysanthemi flos	Thermopsoside	-8.7	HIS A:163_CYS A:145_PHE A:140	1.386
	Ganoderma	Ganoderic acid C2	-8.6	ASN A:203_THR A:111_ASN A:151	0.000
	Mint	Eriocitrin	-8.5	GLU A:166_LEU A:167_THR A:190_CYS A:145	2.846
	Citrus reticulata	Naringin	-8.5	LEU A:271_ASN A:238_LEU A:287_ASP A:197_ARG A:131_ASP A:289	2.797
	Perilla Frutescens	Ursolic Acid	-8.4	ASP A:289_AA:131	0.000
	Ricinus	Lupeol	-8.4	ASN A:203	0.000
	Elder	(+)-Bicuculline	-8.4	ASN A:151_GLN A:110	1.928
	Mori cortex	Kuwanon B	-8.3	ASN A:238_TYR A:239_ASP A:197_THR A:198_ARG A:131	0.113
	Forsythiae fructus	Forsythiaside A	-8.2	ASN A:238_LYS A:137_ TYR A:239_LEU A:287_THR A:199	1.260

	Sennae Folium	Emodin-8-glucoside	-8.2	CYS A:145_GLN A:192	0.386
	zingiberis	beta-sitosterol	-8.2	-	0.000
	Eriobotryae folium	Isohemiphloin	-8.1	CYS A:145_GLN A:189	1.137
	Pheasant's eye	Adonitoxin	-8.0	THR A:198_LYS A:137_MET A:276_ALA A:285_LEU A:271	0.063

A minimum number of hydrogen bonds must be formed for the pseudo-drug to affect the receptor [20] and the RMSD value is used to confirm the binding protocol. Thus, results without hydrogen bonding and RMSD values above 1.5 angstroms in Table 4 were omitted, despite having the highest **G**Δ values. Table 4 presents 21 compounds. Seven compounds among them showed the best RMSD results, indicating the reliability of the data of this study. Digoxin compound in binding with 6Y2F receptor did not have good RMSD value despite the best binding energy. Ganoderic acid C2, Ursolic Acid, and Lupeol compounds caused protein instability by binding to 6Y2F, which can be seen in RMSD analysis. The RMSD obtained from this compound is zero A° (Figures 1, 2, and 3), indicating the validation of the binding protocol.

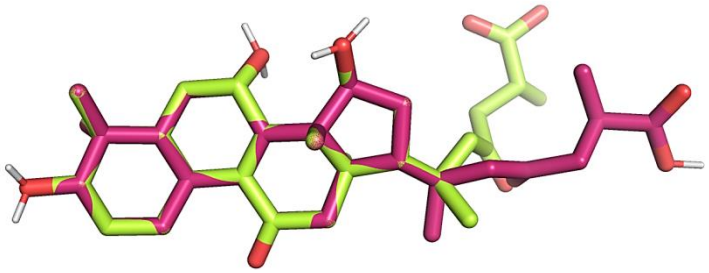

Figure 1- RMSD of 0.0 A° for Ganoderic acid C2 compound of Ganoderic acid C2 by PyMol software

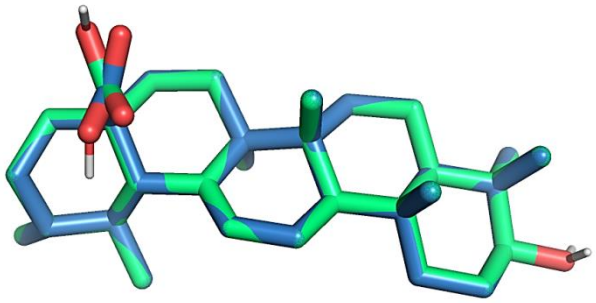

Figure 2- RMSD of 0.0 A° for Ursolic Acid compound of Perilla Frutescens by PyMol software

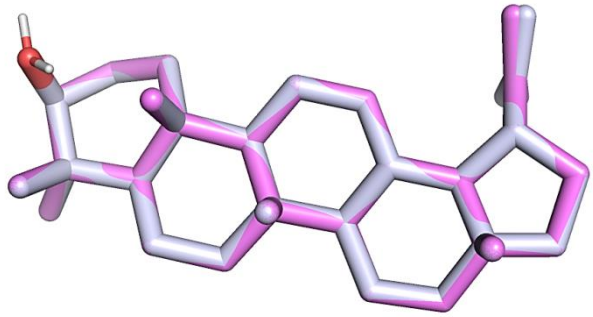

Figure 3- RMSD of 0.0 A° for lupeol compound by Pymol software

Glycoside compounds such as flavonol glycoside have therapeutic properties including treating headache, and fever, and helping to treat cough, bronchitis, and infectious diseases [21]. The binding scores of glycosidic compounds of digoxin, eriocitrin, hesperidin, naringin, Adonitoxin, and Emodin-8-glucoside with 6Y2F protein were -6.9, -5.8, -1.9, -5.8, -0.8, and -2.8, respectively. The results of a study by Otomo et al. [22], Chen et al. [23], and Adam et al. [24] confirm the results of Hesperidin and Naringin. Based on Lin et al.'s experiments, Emodin-8-glucoside compound has very good water solubility and shows almost no cytotoxicity [25]. Animals that consume plants containing cardiac glycosides, including adonitoxin, usually suffer from fatal digestive and cardiac disorders despite the antioxidant, antimicrobial, anti-inflammatory, cardioprotective, neuroprotective, and antiallergic properties of glycosidic compounds [26]. However, recent studies indicate that using a low dose of this compound has therapeutic properties. In short, phytochemical and pharmacological studies of the Adonis L. genus have received much attention [26-27]. Extracts enriched in cardiac glycosides have been made and the active compounds have been isolated and proven to provide cardioprotective activity. However, plants of this genus should be further investigated and developed with special attention to resource conservation and clinical trials.

Terpenoids are active compounds found in plants. The binding score of Soyasaponin I against the original protease was -9.0. This compound has anti-inflammatory activity in addition to being known as an anti-herpes simplex virus [28] and [29]. However, some studies indicate that it can increase the pathogenicity of the virus to the host [30-31]. Warifteine and Nupharin A are alkaloid compounds with binding scores of -9.3 and -9.0, respectively. They have the properties to treat asthma, inflammatory disorders, bronchitis, antiplatelet, and anticoagulants [32]. Several different classes of bioactive molecules isolated from many plants have antiviral activity [33-34]. One of the methods in determining whether a compound has medicinal potential is to follow Lipinski's rule of five (RO5) [35]. Based on this rule, orally active drugs should not violate more than one of the established criteria [36]. Thus, it was examined whether each docking matched Lipinski's RO5 (Table 5).

Table 5- Review of Lipinski's rules

Compound Name	mass	hydrogen bond donor	hydrogen bond acceptors	Log P
Isorhoifolin	556	8	14	0.85
Ganoderic acid C2	476	4	7	0.0
Ursolic Acid	410	2	3	1.74
Lupeol	337	1	1	0.0
Kuwanon B	399	3	6	0.0
Emodin-8-glucoside	418	6	10	-0.67

Adonitoxin	513	5	10	0.0
Hesperidin	584	8	15	1.093
Soyasaponin I	875	11	18	1.99
Kuwanon E	400	4	6	0.0
Thermopsoside	446	6	11	-0.28
Forsythiaside A	597	9	15	-0.34
Isohemiphloin	419	7	10	-3.0

CONCLUSION

This study revealed that the natural compounds Digoxin and Warifteine among the selected plant compounds have better binding free energies with the 6Y2F protein of SARS-CoV-2. Although the molecular binding results of Ganoderic acid C2, Ursolic Acid, Lupeol, Kuwanon B, Emodin-8-glucoside, Adonitoxin, Kuwanon E, and Isohemiphloin are lower than the first two compounds, the analysis of RMSD parameters, interactions, number of hydrogen bonds, and RO5 criteria and their non-toxic properties showed better performance. These compounds have a better potential as antiviral plant chemicals and to solve respiratory, inflammatory, infectious, and coagulation problems, which may prevent the proliferation of the virus or help to treat this disease. These 9 inhibitors are appropriate candidates as drugs for inhibiting the activity of the primary enzyme of the SARS-CoV-2 coronavirus for clinical and laboratory studies. However, the conducted studies are theoretical. Experimental work is required to ensure the accuracy of the data, and the results of this research alone cannot claim that the introduced compounds can inhibit the COVID-19 protease.

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