Indonesian Journal of Pharmaceutical Science and Technology
Journal Homepage : http://jurnal.unpad.ac.id/ijpst/
Research Article

Flavonoids from Basil Leave as a Potential Inhibitor of SARS-CoV2 Main Protease: an *In Silico* Approach

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Submitted 14 March 2023; Revised 07 May 2023; Accepted 24 June 2023; Published 31 July 2023
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Abstract
SARS-CoV2 a virus that caused the global pandemic Coronavirus 2019 (COVID19) has infected about 659.108.952 peoples, with 6.684.756 deaths in the world as stated by WHO on 23rd December 2022. Basil leaves have antiviral potential, especially in groups of flavonoids. People exposed to the COVID19 virus are given many drugs that cause many side effects. Therefore, it is advisable to consume drugs from natural ingredients low in side effects such as basil leaves. This study aims to predict flavonoid derivative compounds from basil leaves that have the potential as an antiviral drug for COVID19 by in silico, which has high affinity with low side effects. The method used is molecular docking for target analysis, ADME prediction, and toxicity prediction. The results of this study, show there is a naringenin compound that has the highest potential to become an antiviral for COVID19 with a ΔG value of -4.04 kcal/mol and a KI of 1.09 mM. These flavonoid-derived compounds comply with Lipinski rules, so the flavonoid content in basil leaves can help treat COVID19 as a drug derived from natural ingredients that have antiviral potential with low side effects.
Keywords: Basil Leave, Pandemic, COVID19, Main Protease (Mpro).

Flavonoid dari Daun Kemangi sebagai Inhibitor Potensial Protease Utama SARS-CoV2: Pendekatan *In Silico*

Abstrak
SARS–CoV2 menyebabkan pandemi global Coronavirus 2019 (COVID19) telah menginfeksi sekitar 659.108.952 orang, dengan 6.684.756 kematian di dunia sebagaimana disebutkan oleh WHO pada 23 Desember 2022. Daun kemangi memiliki potensi untuk antivirus, terutama pada golongan flavonoid. Orang yang terkena virus COVID19 diberi banyak obat yang menimbulkan banyak efek samping. Maka dari itu, disarankan mengkonsumsi obat dari bahan alam yang rendah efek samping seperti daun kemangi. Penelitian ini bertujuan untuk memprediksi senyawa turunan flavonoid dari daun kemangi yang berpotensi sebagai obat anti-virus COVID19. Secara *in silico*, yang memiliki afinitas tinggi dengan efek samping yang rendah. Metode penelitian ini adalah molecular docking untuk analisis target, prediksi ADME, dan prediksi toksisitas. Hasil dari studi ini, ada senyawa naringenin yang berpotensi paling tinggi untuk menjadi antivirus COVID19 dengan nilai ΔG -4,04 dan Ki 1.09 mM. Senyawa turunan flavonoid ini mematuhi aturan Lipinski, sehingga kandungan flavonoid pada daun kemangi dapat membantu terapi perawatan COVID19 sebagai obat yang berasal dari bahan alam yang memiliki potensi antivirus dengan efek samping yang rendah.
Kata Kunci: Daun Kemangi, Pandemi, COVID19, Protease Utama (Mpro).

1. Introduction

Coronaviruses (CoVs) consist of single-stranded, large, positive-stranded copies of RNA, which can infect humans and animals[1]. In December 2019, a new type of coronavirus caused an increase in lung disease, first in Wuhan, China, and then spread around the world. For SARS-CoV2 infection, bats were found to be responsible for the origin of the virus[2]. The disease caused by SARS-CoV2 is referred to as coronavirus disease 2019 (COVID19)[3,4,5]. CoV is endocytosed in lysosomes and endosomes before fusion with the cell. The absolute sequencing of the genome reveals that the new virus is related to a large family of coronaviruses and is closely related to the previous SARS-CoV. During infection, it encodes for two types of large polyproteins which are then processed by viral proteases, the major or 3-chymotrypsin-like protease, (3CLpro) and the papain-like protease (PLpro)[6,7].

The development of new, cost-effective, and specific antiviral COVID19 drugs is a major focus of current medical research[8]. Exploration of new bioactive compounds with antiviral COVID19 therapeutic properties must be targeted directly. Various scientific research works have revealed the enormous therapeutic antiviral potential of several medicinal plants and algae[9,10]. Indeed, infection by SARS-CoV2 can be limited by different strategies. One could consider blocking viral entry into the host cell, by targeting the S2 spike protein or the ACE2 protein of the plasma membrane where the virus binds. However, another strategy is to prevent the formation of viral RNA. Blocking viral proteases can prevent them from cutting the viral polyproteins synthesized by infected cells. Virus particles cannot aggregate inside cells, which will stop infection[11,12,13,14].

Ocimum Sanctum, an aromatic herb that belongs to the family Lamiaceae and the subfamily Nepetoideae[15], has been used in traditional medicine for the treatment of gastrointestinal and respiratory ailments. This species has also been reported to have beneficial effects on kidney damage, warts, and worm infestations[16].

Based on Lipinski's rules in the development and discovery of a candidate drug substance that is used orally, it must fulfill five conditions known as the "Rule of Five"[17]. If two or more of these requirements are not met then there is a high probability that the compound has low oral activity and low bioavailability[18]. If two or more of these requirements are not met then there is a high probability that the compound has low oral activity and low bioavailability[19].

2. Method

2.1. Tools

Hardware: ASUS N76V Laptop with Intel® Core™ i7-3630QM CPU @ 2.40 GHz, Windows 10 64-Bit Operating System. Software: Chem 3D 19.0, PubChem [https://pubchem.ncbi.nlm.nih.gov/], ProTox-II [https://toxnew.charite.de/protox_II/], SwissADME [http://www .swissadme.ch/] and PKCSM [https://biosig.lab.uq.edu.au/pkcsm/].

2.2. Materials

The materials used were 3CL protease receptors (3CLpro) and 10 flavonoid derivative compounds from basil leaves, namely: apigenin, captopril, catechin, cirsimaritin, epicatechin, isothymusin, kaemferol, luteolin, naringenin, and quercetin.

2.3. Procedure

2.3.1. Prediction of Physicochemical Properties

The molecular structures of the test compounds were downloaded via PubChem in 2D SDF format. The test compounds were then converted to SMILES using SwissADME. After that, the physicochemical properties of the test compounds were predicted online on the SwissADME, PKCSM, and ProTox-II websites.

2.3.2. Lipinski Rule of Five

The molecular structures of the test compounds were downloaded via PubChem in SDF 3D format. The test compounds were then converted to PDB using Chem 3D 19.0. After that, the test compounds were analyzed

online on the Lipinski Rule of Five sites.

2.3.3. Molecular Docking

3CL Protease Enzyme taken from PDB (Protein Data Bank) with PDB ID: 6M2N. Then separate the receptor and ligand using the BIOVIA Discovery Studio software and save each in the format (.pdb). Validation done. Macromolecules were optimized with AutoDockTools-1.5.6 software. The 3D structure of the macromolecule is added by hydrogen atoms and then the charge is repaired by adding a Gasteiger partial charge and giving a force field.

3. Result and Discussion

The 3CLpro structure of SARS-CoV2 was found on the PDB RCSB server with the PDB identifier 6M2N. The docking protocol was validated by docking coordinated ligands from the 3CLpro COVID19 structure (PDB ID: 6M2N) into the same binding pocket. The docking results showed that the RMSD value was 1.656 Å (figure 1), and the binding affinity was -3.05 kcal/mol. The results show that the docking protocol used in this study is reliable because the RMSD value is less than the required value of 2.0 which is set to evaluate reliability, the results show that the docking protocol used is accurate. The residue found in the 3CLpro binding pocket was targeted in this investigation to block viral activity.

This study used molecular docking analysis to identify the antiviral potential of natural compounds derived from medicinal plants and other synthetic molecules such as flavonoid derivatives from basil leaves. The docking analysis of 3CLpro revealed that among the 10 test compounds obtained from the literature, 8 test ligand compounds had low energy and therefore had good interactions with SARS-CoV2 (table 1).

The compounds apigenin, captopril, catechin, epicatechin, kaemferol, luteolin, naringenin, and quercetin have the lowest binding energy with the lowest naringenin compound, namely -4.04 kcal/mol (Table 1). These compounds can bind directly to the 3CL Pro receptor with high affinity indicating competition with SARS-CoV2.

Table 1. Interactions of Amino Acid Residues

Compound	ΔG (Kcal/mol)	Ki (mM)	Amino Acid Residue Interactions	
			Hydrogen Bond	Van Der Waals Bond
5,6,7-trihydroxy-2-phenyl-4H-Chromen-4-One	-3.05	5.84	GLU A:166, LEU A:141, SER A:144, CYS A:145, ASN A:142	ASP A:187, TYR A:54, HIS A:164, MET A:49, GLY A:143, HIS A:163, PHE A:140
Apigenin	-3.9	1.39	LYS A:137, GLU A:290	VAL A:125, SER A:139, GLY A:138, GLN A:127, ILE A:136, ALA A:129
Captopril	-4.01	1.16	GLN A:127, LYS A:5, GLU A:290	TYR A:126, GLY A:138, ALA A:129
Catechin	-3.73	1.84	GLU A:290, ALA A:129, LYS A:5, SER A:139	ARG A:131, THR A:135, MET A:130, ILE A:136, GLY A:138, GLN A:127
Cirsimaritin	-1.61	65.63	LYS A:5, CYS A:128, ALA A:129	SER A:139, GLY A:138, GLN A:127, ILE A:136, MET A:130, THR A:135, ARG A:131
Epicatechin	-3.96	1.26	LYS A:137, GLU A:290, ALA A:129	TYR A:126, GLN A:127, ILE A:136, GLY A:138, ARG A:131
Isothymusin	-1.01	182.24	LYS A:5, LYS A:137, ALA A:129	SER A:139, GLY A:138, GLN A:127, ILE A:136, MET A:130, THR A:13, ARG A:131
Kaemferol	-3.49	2.76	GLU A:290	VAL A:125, SER A:139, GLY A:138, GLN A:127, ILE A:136, ALA A:129
Luteolin	-3.34	3.56	ALA A:129, GLN A:127, ARG A:131	ILE A:136, TYR A:126
Naringenin	-4.04	1.09	GLU A:290	SER A:139, VAL A:125, GLY A:138, GLN A:127, ILE A:136, ALA A:129
Quercetin	-3.23	4.29	GLU A:290, LYS A:137, ALA A:129	ARG A:131, ILE A:136, GLY A:138, GLN A:127, TYR A:126

The interaction of the tested ligand compound lower than the standard ligand, namely -3.05 kcal/mol, showed a high affinity, therefore this test ligand compound could be used as a potential candidate to inhibit SARS-CoV2.

Structural study of the main protease enzyme CYS145 surrounded by amino acids ASN142, GLU166, HIS163, LEU141, SER144, PHE140, GLY143, and MET165. CYS145 and HIS41 residues act as strong catalysts with 3CLpro. According to the results of the docking, the Native ligand binds strongly to the main protease enzyme of SARS-CoV2, forming interactions with the side chains of HIS41, GLY143, CYS145, GLU166, and MET165 amino acid residues which can enhance inhibition of SARS-CoV2. The oxygen atom of the CO group forms a hydrogen bond interaction with the CYS145 residue (figure 1a).

The essential amino acid residues completely encapsulate the selected compound in the active binding pocket. In this study, we manually investigate the yield of docked molecules and their interactions with target proteins. Then the location of the compound is determined in the binding pocket, with its structure reaching the appropriate components. As a result, the potential compounds form extensive interactions with the amino acid residues that build up the binding pocket.

The interactions formed include hydrophobic van der Walls, Pi-Cations, T-sigma, Pi-Alkyl, and hydrogen bonds. 10 compounds derived from flavonoids from basil leaves could not bind to the catalytic site, because there were no CYS145 and HIS41 amino acid residues like native ligands. The highest potency of the tested ligands was the naringenin compound with a value of -4.04 kcal/mol but did not bind to a pocket site. So structural modifications must be made to bind amino acid residues with side chain modifications. This modification aims to allow the test compound to bind to the 3CLpro receptor.

Van der Waals interactions and hydrogen bonds play a major role in the binding process. Van der Waals interactions are the weakest intermolecular attractions between two molecules. Even though it is the weakest bond between two bodies, many Van der Waals forces can make the interaction very strong[24]. In protein-ligand interactions, hydrogen bonds help the ligands to stabilize but there are also other interactions like hydrophobic or Van der Wall interactions which help in the stabilization of nonpolar ligands as well. Analysis of ligand structures and docking poses is usually helpful in understanding the interactions and bond energies that determine which compounds are important. Based on these bases, molecular docking programs are the most widely used tool in drug discovery because the results show potential drug compounds based on the lowest binding energy[25]. A comparison

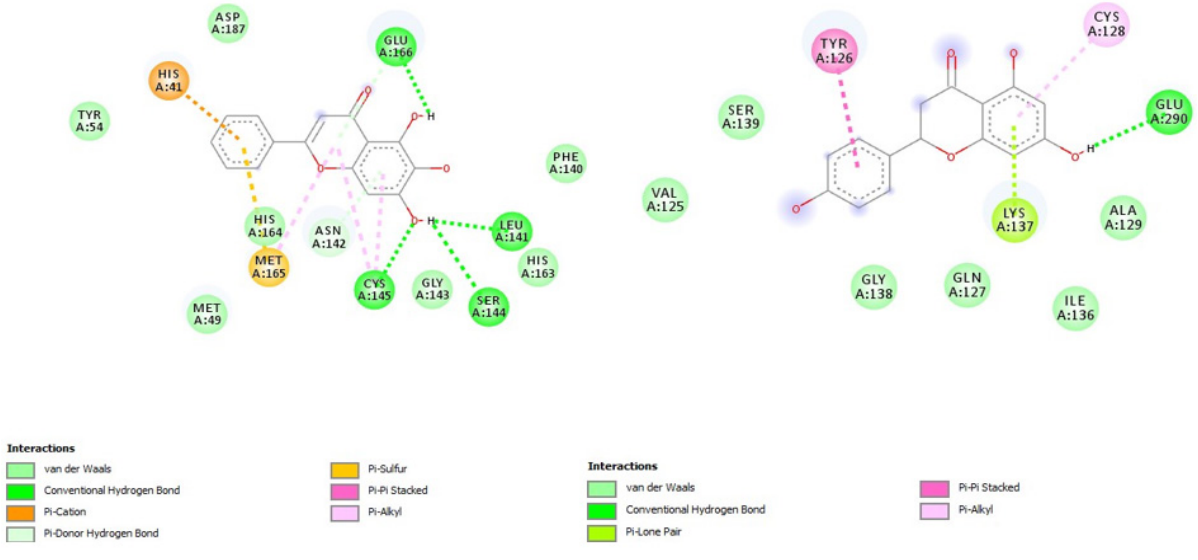

Figure 1. Visualization of Amino Acid Residue Interactions native ligand and naringerin

Table 2. ADMET Prediction

Compound	absorption		Distribution	Toxicity				
	HIA (%)	P-gp Substrate	BBB Permeability (log BB)	Hepatotoxicity	Carcinogen	Immunotoxicity	Mutagen	Cytotoxicity
Apigenin	92	Yes	-0.957	inactive	inactive	inactive	inactive	inactive
Captopril	75.898	No	-0.211	inactive	Active	inactive	inactive	inactive
Catechin	73.172	Yes	-1.094	inactive	inactive	inactive	inactive	inactive
Cirsimaritin	93.656	No	-0.588	inactive	inactive	Active	inactive	inactive
Epicatechin	73.172	Yes	-1.094	inactive	inactive	inactive	inactive	inactive
Isothymusin	64.554	Yes	-1.154	inactive	inactive	Active	inactive	inactive
Kaemferol	75.481	Yes	-1.223	inactive	inactive	inactive	inactive	inactive
Luteolin	84.492	Yes	-1.171	inactive	inactive	inactive	inactive	inactive
Naringenin	90.508	Yes	-0.969	inactive	inactive	inactive	inactive	inactive
Quercetin	75.335	Yes	-1.377	inactive	inactive	inactive	inactive	inactive

of estimated binding free energy (ΔG) or affinity concluded that naringenin has the lowest affinity value, namely -4.04 (table 1). Bond energy analysis shows that 8 out of 10 compounds have bond energy values between -3.23 to -4.04 kcal/mol (table 1) and 2D interactions (figure 1) illustrate that the ligands of the tested compounds have lots of hydrogen and van der Waals bonds indicating that the ligand is stabilized in the complex.

In this study, structure-based in silico drug design was used to accelerate the discovery of SARS-CoV2 antiviral compounds using a rational and inexpensive approach[26,27]. According to the above findings, the flavonoids from basil leaves exhibit high affinity and good binding interaction for 3CLpro with PDB code: 6M2N. Therefore, this compound can inhibit the activity of 3CLpro. As a result, we recommend this compound as a strong protease inhibitor for COVID19.

ADMET stands for Absorption, Distribution, Metabolism, Excretion, and Toxicity. The prediction of ADMET properties plays an important role in drug discovery and development. Drug absorption depends on membrane permeability, intestinal absorption, degree of skin permeability, and P-glycoprotein substrate or inhibitor. Drug distribution depends on factors including a blood-brain barrier (logBB), CNS permeability, and volume of distribution (VDSs). Metabolism was predicted based on CYP models for substrate or inhibition (CYP2D6, CYP3A4, CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4). Excretion was estimated based on a total clearance model and renal OCT2 substrates. Drug toxicity was predicted based on AMES toxicity, hERG inhibition, hepatotoxicity, and skin sensitization. Properties such as ADMET compounds were determined using SWISS ADME [http://www.swissadme.ch/] and PKCSM [https://biosig.lab.uq.edu.au/pkcsm/]. The distribution of compounds through various body compartments is accessed using a penetrating Blood-brain barrier (BBB)[28]. Among all the flavonoid derivatives, the compounds apigenin, captopril, Cirimaritin, and Naringenin are predicted to have blood-brain barrier penetration. Glycoprotein P is a drug transporter that has broad substrate specificity. Drugs for which P-gp substrates are subject to low intestinal absorption, low permeability of the blood-brain barrier, and face the risk of increased metabolism in intestinal cells. Glycoprotein P is a drug transporter that has wide substrate specificity. Drugs for which P-gp substrates are subject to poor intestinal absorption, low permeability of the blood-brain barrier, and face the risk of increased metabolism in intestinal cells. It is estimated that the compounds apigenin, catechin, epicatechin, isothymucin, kaemferol, luteolin, naringenin, and quercetin have an affinity for p-glycoprotein. The results show that

other flavonoids can act as non-substrates of p-glycoprotein[29]. Metabolism mainly depends on CYP450 enzymes and, namely CYP 3A4, 2D6, 1A2, 2C9, and 2C19. This enzyme is responsible for detoxifying drugs that pass through the liver. Therefore, any compound that blocks CYP450 can cause toxicity. Of the 10 flavonoid-derived compounds, the compounds apigenin, luteolin, naringenin, and quercetin block enzymes responsible for detoxification and can therefore be responsible for toxicity. Intestinal absorption is the main site for drug absorption from solutions administered orally. This method was built to predict the compounds absorbed by the human intestine.

Analysis of candidate drug compounds was carried out based on drug-likeness and their Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) profiles. ADMET prediction can provide information regarding oral bioavailability, cell permeation, metabolism, elimination, and toxicity which are the characteristics of the pharmacokinetics and pharmacodynamics of a drug molecule.

In general, drugs that are not bound by plasma protein bonds will be free to penetrate the cell membrane and interact with pharmacological targets, therefore this will give rise to pharmacological targets. Drugs that are not bound to plasma proteins will affect the body because the more free fractions, the more harmful it is to the body[20]. Furthermore, a predictable test through ADMET prediction is Blood-Brain Barrier Penetration which aims to avoid side effects on the central nervous system. A compound is said to be able to penetrate the blood-brain barrier well if it has a Log BB > 0.3, and cannot be distributed properly if it has a Log BB < -1[21].

The molecular docking method can be used to predict the activity of bioactive compounds in medicinal plants. This method is more efficient in terms of time and cost before conducting in vivo and in vitro studies[22]. A toxicity test is a test used to observe the pharmacological activity of a compound that occurs in a short time after administration in a certain dose. The principle of toxicity test is that bioactive components are always toxic when given in high doses and become drugs when the dose is low[23].

For 10 flavonoid derivative compounds that have high intestinal absorption values, apigenin (91.502%), captopril (75.898%), catechin (73.172%), cirsimaritin (93.656%), epicatechin (73.172%), isothymusin (65.554%), kaemferol (75.481%), luteolin (84.492%), naringenin (90.508%), and quercetin (75.335%). Compounds that have an intestinal absorption value of more than 30% can be absorbed by the intestine and these drugs pass to be used as oral drugs. The parameters mentioned above for all of these compounds are within the ranges found for successful drug molecules.

Toxicity prediction of the test compounds was carried out using ProTox-II. This prediction includes toxicity to hepatotoxicity, carcinogenicity, immunogenicity, mutagenicity, and cytotoxicity. All test compounds from these flavonoid derivatives are predicted to be non-toxic so they can be recommended as alternative drugs that can inhibit SARS-CoV2 Virus. Lipinski's five rules are a rule of thumb for evaluating drug similarity or determining whether a chemical compound with a particular pharmacological or biological activity has the chemical and physical properties that will make it an active drug in humans (for ingested drugs).

Predicting the absorption or permeation of a drug. The solubility and permeability of a compound play an important role in considering further drug development. This is done to prevent the failure of a drug caused by low absorption or permeation[30]. The Lipinski rule can determine the physicochemical properties of a ligand to determine the hydrophobic/ hydrophilic character of a compound to be able to enter the cell membrane through passive diffusion[31]. Hydrogen bonding as measured by the number of rotatable bonds and the total number of hydrogen bonds (number of donors and acceptors) was found to be important The results of molecular docking showed that the lowest Gibbs energy was produced by naringenin (-4.04 kcal/mol), lower than the

Table 3. Lipinski Rule of Five

Compound	BM (< 500 Da)	Log P (< 5)	H Donor (< 5)	H Acceptor (< 10)
Apigenin	270	2.419599	3	5
Captopril	217	0.6279	1	4
Catechin	290	1.5461	5	6
Cirsimaritin	314	2.731199	2	6
Epicatechin	290	1.5461	5	6
Isothymusin	330	2.436799	3	7
Kaemferol	286	2.305299	4	6
Luteolin	286	2.125199	4	6
Naringenin	272	2.509899	3	5
Quercetin	302	2.0109	5	7

Gibbs energy of standard ligands (-3.05 kcal/mol).

The ligand compounds derived from flavonoids from basil leaves showed high affinity. Based on the research that has been done, it can be concluded that the tested ligand compounds do not have CYS145 and HIS41 amino acid residues, so it is a necessary predictor of good oral bioavailability whereas the number of hydrogen bonds tends to increase with molecular weight to predict oral bioavailability[22]. Based on the results of the analysis, based on the Lipinski Rule of Five, it is known that 10 flavonoid derivative compounds used as ligands in basil leaves, namely apigenin, captopril, catechin, cirsimaritin, epicatechin, isothymusin, kaemferol, luteolin, naringenin, and quercetin meet Lipinski Rules which have been determined so that the compound can passively diffuse into the cells and has good absorption to be taken orally.

4. Conclusion

From the ADMET and Lipinski tests, the flavonoid derivative compounds from basil leaves obtained results that met the requirements, so it was predicted that they could be administered orally. All of these test compounds are predicted to be non-toxic and can be recommended as alternative drugs that can inhibit the Covid-19 virus with high affinity and low side effects.

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