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In Silico Inhibitory Potential of Isolated Molecules of *Scoparia dulcis* L. (Scrophulariaceae) on SARS-CoV-2 Main Protease M^{pro}

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Abstract

Background: The Coronavirus disease 2019 (COVID-19) pandemic, caused by the SARS-CoV-2 coronavirus, remains a global threat despite lifting the health emergency. Scientists from all continents have been mobilized to develop vaccines and medicines for prevention and cure. In Burkina Faso, traditional healers proposed using *Scoparia dulcis* L., a medicinal plant, to manage COVID-19. **Method:** *In silico* screening offers a quick drug-likeness evaluation of *Scoparia dulcis* L.-isolated biomolecules toward SARS-CoV-2 targets, such as M^{pro} protease. A review of the literature retrieved 35 biomolecules isolated from *Scoparia dulcis*. The potential interactions of these biomolecules with the amino acid residues of the SARS-CoV-2 M^{pro} protease were visualized. Affinities and probable oral route delivery were assessed using reference molecules such as remdesivir and nelfinavir. **Results:** The screening allowed the retention of 20 hit molecules, which had a better affinity for the target than the reference molecules remdesivir and nelfinavir, and analysis of the results identified height lead molecules with a significant interaction with the M^{pro} protease and being druggable. There are six flavonoids: cirsimarin, cynaroside, hydroxy-tetramethoxyflavone, gossypetin, luteolin, vitexin, one diterpene, glutinol, and one glycoside, eugenyl-glucoside. These molecules interact with methionine 6 and tyrosine 126 of SARS-CoV-2 M^{pro}. These two amino acids are essential for the dimerization of M^{pro} protease. Inhibitory action on M^{pro}

protease can be expected from these biomolecules. **Conclusion:** *Scoparia dulcis* L. could help manage COVID-19 because it contains biomolecules that can inactivate SARS-CoV-2 M^{pro}.

Keywords

COVID-19, Molecular Docking, M^{pro}, Biomolecules, *Scoparia dulcis*

1. Introduction

Coronavirus disease (COVID-19) is an emerging infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that appeared in December 2019. The rapid spread of this disease to the entire planet has made it a pandemic that has strained the capacity of healthcare institutions. This pandemic has affected all areas of human life, with the most devastating consequences affecting health and the economy. [1] [2] The latest data from WHO, on 31 December 2023, mentioned 776,754,317 confirmed cases and 7,073,466 confirmed deaths reported globally. [3] SARS-CoV-2 belongs to the order Nidovirus, family Coronaviridae, subfamily Coronavirinae, and genus Betacoronavirus. [4] It is similar to the severe acute respiratory syndrome coronavirus (SARS) and Middle East Respiratory Syndrome coronavirus (MERS), with RNAs ranging from 26 to 32 kb. The SARS-CoV-2 Main protease, M^{pro}, is an optimally active enzyme in its homodimeric form. Each monomer has enzymatic activity, but it is less than that of the homodimeric form. Each monomer consisted of three domains: I (amino acid residue 8 to 101), II (amino acid residue 102 to 184, and III (amino acid residue 201 to 303). [5] Domain III is a helical domain from which dimerization is initiated. Domains II and III are connected by a long loop region of 185 to 200 amino acid residues. The M^{pro} catalytic site lies between domains I and II and has five pockets. The active site of M^{pro} is a catalytic dyad composed of Cys145 and His41. This dyad requires a water molecule energetically held by His164 and Asp187. [6] In the viral multiplication process, M^{pro} plays an essential role in the replication and transcription of SARS-CoV-2, making it one of the best targets for searching effective therapeutics for treating coronavirus disease. In addition, this protease has no human homologs, which is advantageous for effective and specific M^{pro} inhibitor development. [6] [7]

The lack of treatment during the pandemic has led to the repositioning of some antivirals, such as ensitrelvir, simnotrelvir, and nirmatrelvir. These drugs target M^{pro} and were approved in some countries for the management of COVID-19. [8]-[10] Other drugs such as remdesivir, hydroxychloroquine, and ivermectin have also been used during the pandemic against SARS-CoV-2. [11] None of these drugs gave satisfactory efficacy. Hence, *in silico* methods are positioned as rapid and accessible research and development tools for developing new medicines against SARS-CoV-2. [12] Two methods, target structure-based and ligand-based

computer-aided drug design, are used. X-ray crystallography, Nuclear Magnetic Resonance (NMR), or homology modeling data served the target-based method. The ligand-based method is used for unknown targets while characteristics of the active molecules are available. [13] [14] Chemical libraries, such as PubChem, managed by the National Center for Biotechnology Information (NCBI), a branch of the National Library of Medicine of the United States under the authority of the National Institutes of Health (NIH), provide relevant information on ligands' structures and physicochemical, pharmacological, and toxicological properties. [15]

Molecular docking is fast, inexpensive, and easy to implement and is the most widely used testing method in new drug research and development. [16] Many software packages containing numerous search algorithms and scoring functions (Autodock, Dock, Flex, Fred, Glide, Gold, Surflex-dock, and Pro Leads) have been developed. [17] *In silico* tools allow drug discoveries such as antibiotics (norfloxacin, isoniazid, and dorzolamide), antiretrovirals against HIV (ritonavir, saquinavir, amprenavir, and indinavir), and other molecules such as captopril and flurbiprofen. [18]

The docking principle combined the search for conformations of the ligand that can establish an ideal interaction with the biological target and the analysis of the score associated with the estimated interaction energy. Lipinski's rule allows the rapid identification of drug-like molecules based on bioavailability characteristics, such as molecular weight (<500), number of hydrogen bond donors (<5), number of hydrogen bond acceptors (<10), and water/octanol partition coefficient (<5). Veber and Ghose-Viswanadhan-Wendoloski's rules were also used to identify drug-like molecules. [19]-[21] Using a molecular docking tool, we tested the drug-likeness of biomolecules isolated from *Scoparia dulcis* L. (*S. dulcis*) on M^{pro}, the main protease of SARS-COV-2. *Scoparia dulcis* L. is a medicinal herb widely used in Africa, America, and Asia. In traditional Chinese medicine, *S. dulcis* is used to treat colds, fever, coughs with lung heat, sore throats, enteritis, abnormal urination, hunger swelling, eczema, and miliaria. [22] In traditional medicine in Burkina Faso, it is used for gastric ulcers, sore throats, coughs, oral disease, and gingivitis. [23] More than 160 compounds with therapeutic potential have been found in *S. dulcis*. These compounds are divided into alkaloids, flavonoids, diterpenoids, triterpenoids, steroids, phenolics, and other aliphatic compounds. [22] The identified hits from this traditional medicinal plant could trigger further biological investigations to verify its anti-COVID-19 properties.

2. Material and Method

2.1. Ligands

The ligands used in this study were biomolecules isolated from *Scoparia dulcis* L., a medicinal plant widely used in several continents, such as Asia, Africa, and America. More than 160 active biomolecules have been extracted from that plant. Traditional Chinese medicine uses it to treat colds, fever, sore throats, tiredness,

enteritis, urinary tract pathologies, and eczema. [22] [24] *Scoparia dulcis* has several pharmacological properties, including anti-diabetic, anti-atherosclerotic, anti-hyperlipidemic, hepatoprotective, antioxidant, anti-inflammatory, anti-arthritic, and anti-urolithiasis properties. [22] Several biomolecules, such as scoparic acid A, scoparic acid B, scopadulcic acid A and B, scopadulciol, and scopadulin, have antiviral, antitumor, and antimicrobial properties. [25]

2.2. Ligands' Selection

The biomolecules of *Scoparia dulcis* L. were selected through a literature search of articles published in PubMed, Science Direct, and Google Scholar, describing the extraction and identification processes.

Compounds isolated from polar extracts (water and alcohol) were used in this study. Indeed, the plant is used as a decoction to treat the most well-known COVID-19 symptoms (cough, fever, and fatigue). [24] The three-dimensional structures of the selected biomolecules were collected from the PubChem database in Structure Data File (SDF) format. [26]

2.3. Ligands' Preparation

Autodock Tools were used to optimize the selected ligands. Open Babel was used to convert the Structure Data File (SDF) format to Protein Data Bank (PDB) format. Ligands were prepared by adding partial loads according to the Gasteiger method, polar hydrogens, and adjusting the number of rotatable bonds. The prepared ligands were registered in our compound library in the 3D Protein Data Bank, Partial Charge (Q), and Atom Type (T) (PDBQT) format.

2.4. M^{pro} Protease Preparation

The main protease of SARS-CoV-2 in its mature form, from Noske *et al.*, with 7KPH as its Protein Data Bank (PDB) unique code, has been used as a receptor target for *Scoparia dulcis* biomolecules, in PDB format. [27] [28] The protein preparation consisted of removing water molecules and adding fillers and polar hydrogens using the AutoDock Tools® software.

2.5. Ligand-M^{pro} Interactions Determination

All molecular docking experiments were performed using AutoDock Vina® software. Computational docking generated several promising ligand orientations and conformations within the binding sites. Since the target protein is not coupled to an inhibitor, we performed semi-flexible blind docking, in which the flexible ligand traverses the rigid conformational space of the target protein. The fixed center of the grid (X = 14.24 Y = 2.14 Z = 3.49) was large enough to encompass all possible ligand-protein complexes.

The compounds were individually evaluated during docking and before their first interaction, and the classical molecular mechanics (MM2) force field was applied to optimize the structures of these small molecules, thus ensuring the rigidity

of their active sites. Finally, ligand-protein complex interactions were studied using Biovia Discovery Studio 4.5 program. This analysis produces a result describing the different low-energy interactions, namely hydrogen bonds, hydrophobic bonds, and Van Der Waals bonds, which are hydrophobic bonds of deficient energy, and the bond length of each pose. According to the degree of flexibility of the molecules, there are three categories of docking: rigid docking or static docking, semiflexible docking, and flexible docking or dynamic docking. [29]

2.6. Ligands' Drug-Likeness Evaluation

Lipinski's rule of five indicates the probability that a molecule will be delivered orally. The pharmacological effectiveness of ligands depends on an adequate plasma concentration and enough at the site of action. This rule is based on the physicochemical properties of drugs taken orally and is called Lipinski's Rule of Five. It is a standardized parameter that evaluates the suitability of biomolecules for development as oral drugs. The rule of five is based on four physicochemical parameters that must be present for the molecule to have adequate pharmacokinetic properties such as: Molecular weight (<500 g/mol), Water/octanol partition coefficient Log P (Log P < 5), Number of hydrogen bond donors (<5), and Number of hydrogen bond acceptors (<10).

The pharmacokinetic parameters of the selected biomolecules were studied using the SwissADME server. [30] After being converted to the Simplified Molecular-Input Line Entry System (SMILES) format, the structures of the molecules are transmitted to the online server for calculation. Molecules that satisfy at least three parameters are likely to exhibit good bioavailability.

3. Results

Two reference molecules, nelfinavir and remdesivir (**Figure 1**), and 35 biomolecules isolated from polar extracts of different parts of *Scoparia dulcis* L. (**Table 1**) were collected from the literature. These biomolecules were distinguished into 19 flavonoids (**Figure 2**), seven diterpenes (**Figure 3**), four triterpenes, two glycosides, one alkaloid, one steroid, and one vitamin (**Figure 4**).

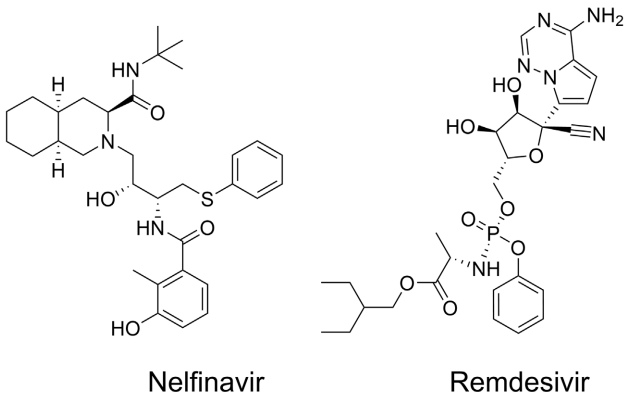

Figure 1. 2D structure of reference molecules.

Table 1. Chemical molecules isolated from *Scoparia dulcis* L.

N°	Molecules	Used part of the plant	References
1	Acacetin	Whole plant	[31] [32]
2	Apigenin	Whole plant, leaves	[31] [33]
3	Betulic acid	Roots, whole plant	[34] [35]
4	Cirsiliol	Leaves	[33]
5	Cirsimarin	Whole plant, leaves	[31] [33]
6	Cirsimaritin	Leaves	[33]
7	Coixol	Aerial parts, whole plant	[34] [36]
8	Cynaroside	Whole plant	[31] [37]
9	Dihydroxy-dimethoxyflavone	leaves	[33]
10	Eugenyl-glucoside	Aerial parts	[38]
11	Friedellin	Whole plant	[34] [35]
12	Glutinol	Whole plant	[32] [35]
13	Glutinone	Whole plant	[39]
14	Gossypetin	Whole plant	[37]
15	Hispidulin	Aerial parts, whole plant	[40] [41]
16	Hispitulin 7 glucuronide	Leaves	[33]
17	Hydroxy-tetramethoxyflavone	Leaves	[33]
18	Isovitexin	leaves	[31] [33]
19	Luteolin	Whole plant, leaves	[33] [40]
20	Nevadensin	Leaves	[33]
21	Nicotinic acid	Leaves	[33]
22	Salvigenin	Leaves	[33]
23	Scopadulcic acid A	Aerial parts, whole plant	[42] [43]
24	Scopadulcic acid B	Aerial parts, whole plant	[42] [43]
25	Scopadulciol	Aerial parts, whole plant	[42] [43]
26	Scopadulin	Whole plant	[43] [44]
27	Scoparic acid A	Aerial parts, whole plant	[43] [45]
28	Scoparic acid B	Aerial parts, whole plant	[43] [45]
29	Scoparic acid C	Whole plant	[43] [45]
30	Scutellarein	Whole plant	[37] [40]
31	Scutellarin	Whole plant	[37] [46]
32	Sitosterol	Whole plant	[41] [46]
33	Tuberonic acid glucoside	Leaves	[33]
34	Vicenin 2	Whole plant, leaves	[31] [33]
35	Vitexin	Leaves	[31] [33]

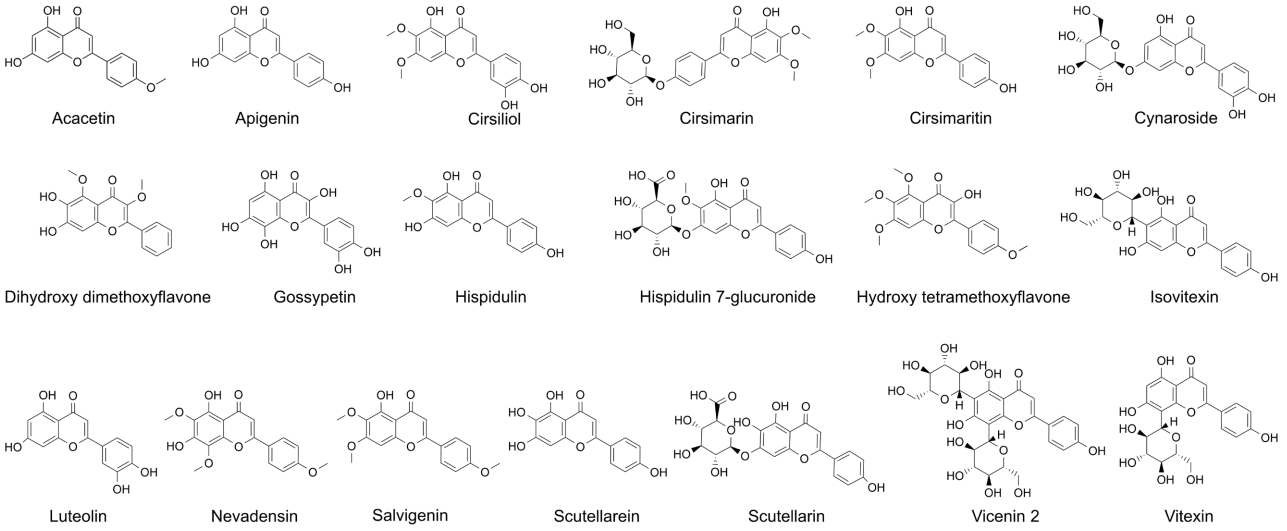

Figure 2. 2D structure of flavonoids isolated from *S. dulcis*.

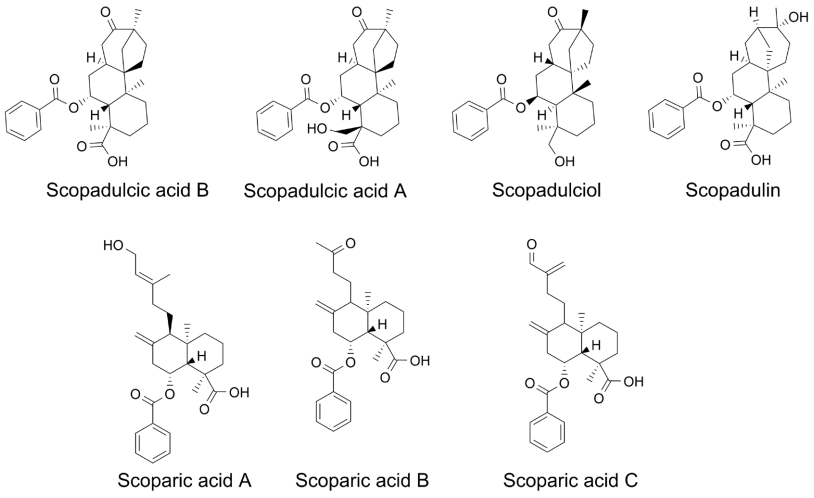

Figure 3. 2D structure of diterpenes isolated from *S. dulcis*.

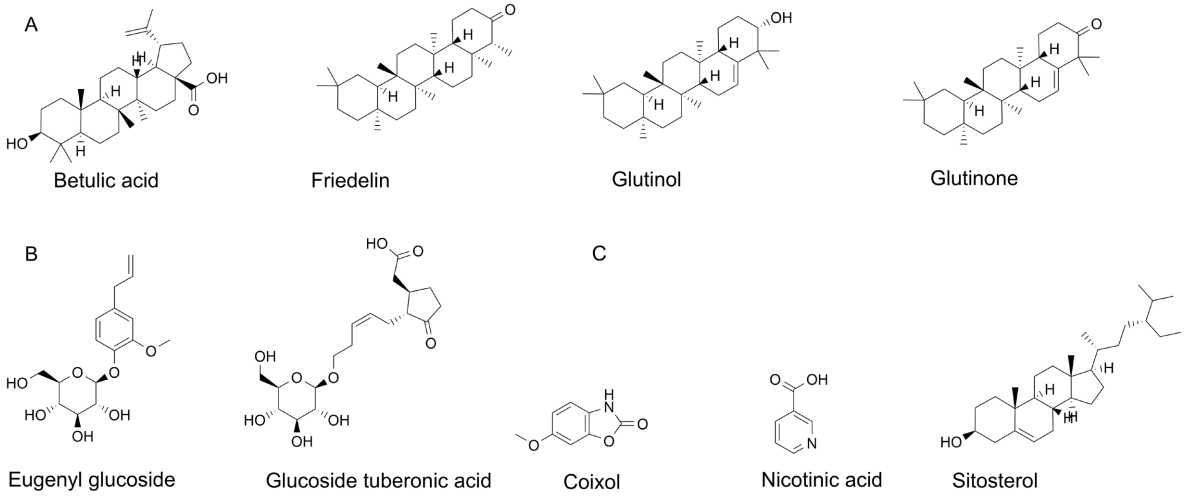

Figure 4. 2D structure of triterpenes (a) glycosides (b), divers compounds (steroids, alkaloids, vitamins) isolated from *S. dulcis*.

3.1. Molecular Docking

3.1.1. Bond Energy

The binding energies obtained during molecular docking tests of biomolecules with M^{pro} protease showed energy values between −5.7 and −8.8 Kcal/Mol (Table 2). Scopadulcic acid B had the lowest interaction energy (−8.8 Kcal/Mol) among the studied biomolecules. The reference molecules had binding energies, −6.8 and −7.5 kcal, respectively, for nelfinavir and remdesivir (Table 2).

The binding energy showed that 20 of the 35 biomolecules had lower interaction energies (<−7.5 kcal/mol) with the M^{pro} protease than the two reference molecules. The studied biomolecules are listed according to their chemical family, and their binding energies in ascending order in the table below according to their binding energy (Table 2).

Table 2. Energy bounds calculation of studied biomolecules.

Group	Molecules	Affinity (Kcal/Mol)
Reference molecules	Nelfinavir	−6.8
	Remdesivir	−7.5
Flavonoids	Isovitexin	−6.1
	Dihydroxy-dimethoxyflavone	−6.1
	Salvigenin	−6.8
	Cirsiliol	−7.1
	Cirsimaritin	−7.1
	Hispidulin	−7.1
	Nevadensin	−7.1
	Vicenin 2	−7.4
	Apigenin	−7.4
	Acacetin	−7.5
	Cirsimarin	−7.7
	Hispitulin 7 glucuronide	−7.8
	Scutellarin	−7.9
	Vitexin	−8.0
	Hydroxy-tetramethoxyflavone	−8.0
	Scutellarein	−8.0
	Gossypetin	−8.0
	Cynaroside	−8.1
	Luteolin	−8.4

Continued

Glucosides	Tuberonic acid glucoside	−7.2
	Eugenyl-glucoside	−8.0
Diterpenes	Scoparic acid A	−7.6
	Scoparic acid B	−7.6
	Scoparic acid C	−7.9
	Scopadulin	−7.9
	Scopadulciol	−8.0
	Scopadulcic acid A	−8.2
	Scopadulcic acid B	−8.8
Triterpenes	Glutinone	−7.3
	Betulic acid	−7.8
	Friedelin	−8.5
	Glutinol	−8.7
Vitamin	Nicotinic acid	−5.7
Alkaloid	Coixol	−6.2
Steroids	β-Sitosterol-β-D-glucoside	−7.0

3.1.2. Interactions' Visualization

Visualization of the interactions of ligand-enzyme complexes showed the types of interactions formed and the amino acids engaged with the reference molecules and *S. dulcis* biomolecules. In our study, all biomolecules exhibited measurable interactions with the active protease residues. The amino acid residues of the protease involved in interactions with ligands were Ser113, Gln127, Tyr126, Phe112, Lys5, Cys128, Met6, Ala7, Val125, Thr111, Val114, Tyr128, Phe291, and Phe8. Figure 5 to Figure 8 highlight the interactions between ligands and M^{pro}.

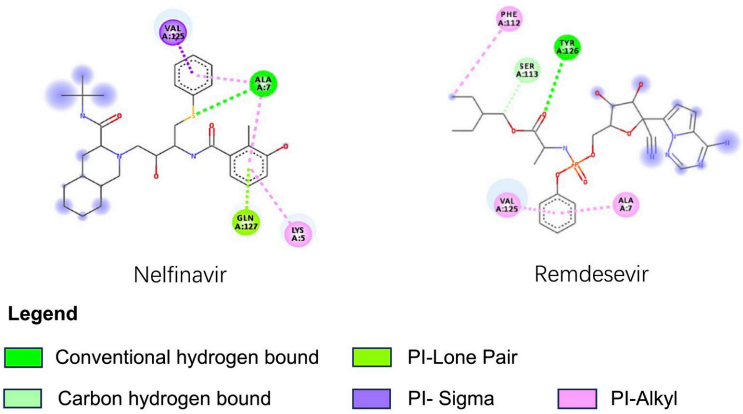

Figure 5. Interactions M^{pro}-reference molecules.

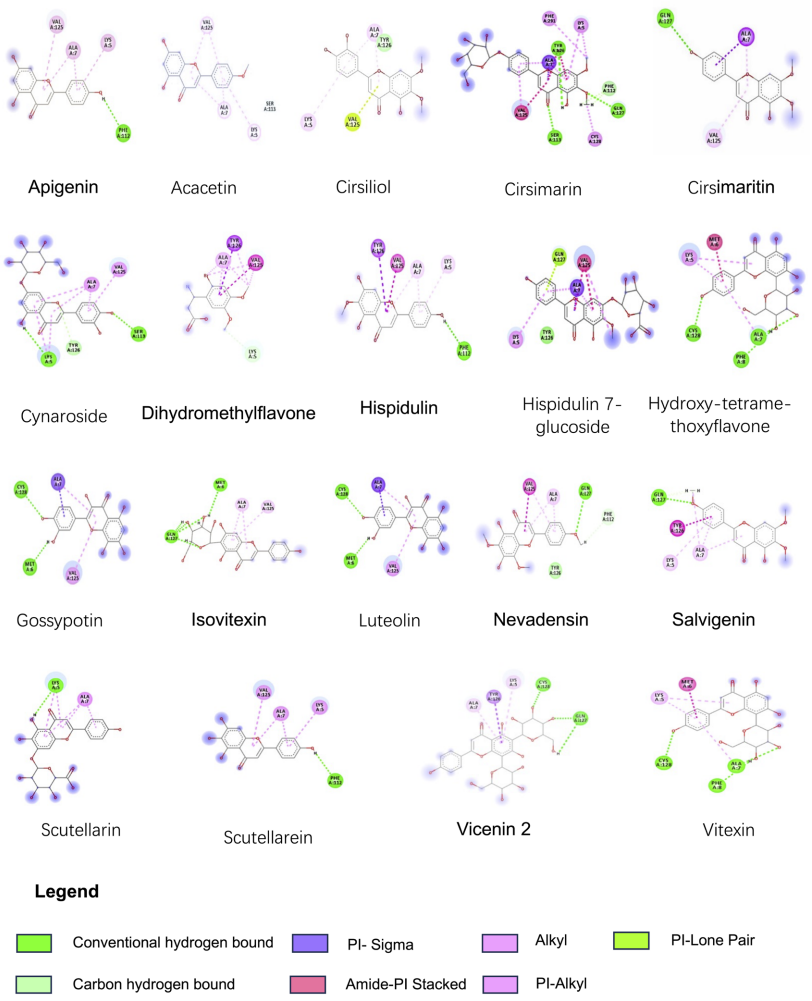

Figure 6. Interactions M^{pro}-flavonoids biomolecules isolated from *S. dulcis.*

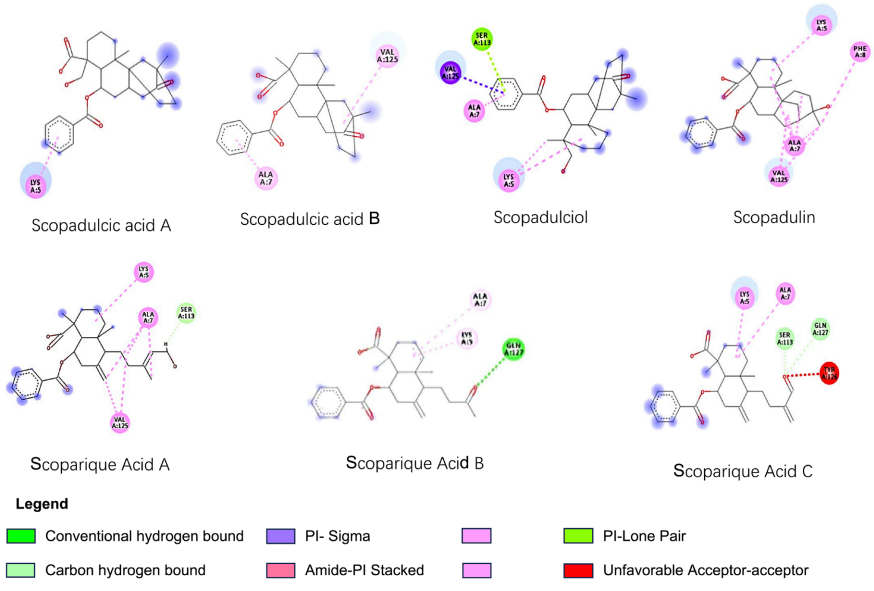

Figure 7. Interactions M^{pro}-diterpenoids biomolecules isolated from *S. dulcis.*

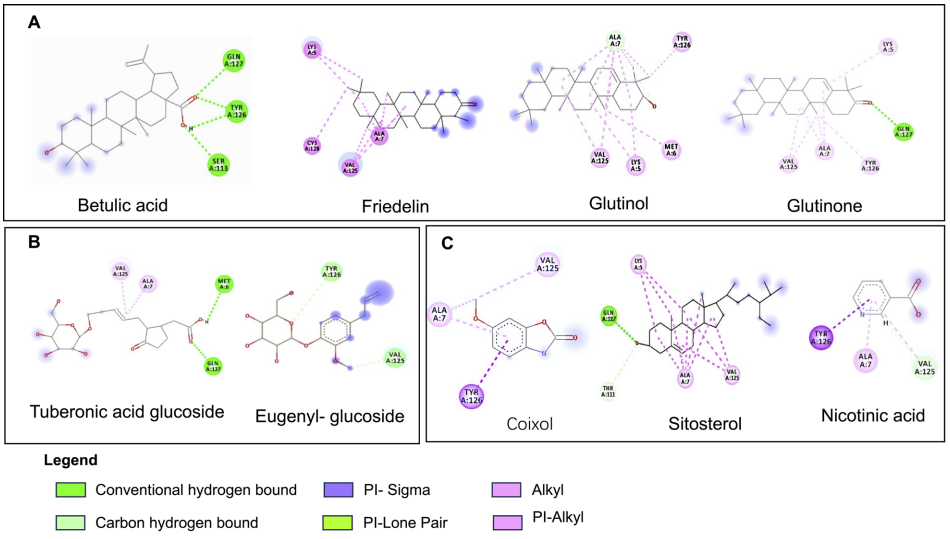

Figure 8. Interactions M^{pro}-triterpenes (A), glycosides (B) and divers (C) biomolecules isolated from *S. dulcis.*

3.2. Drug-Likeness

The drug-likeness evaluation of the biomolecules studied by Lipinski's rule showed that four out of 35 biomolecules do not satisfy this rule. There are cynaroside, hispidulin 7-glucuronide, scutellarin and vicenin 2. These biomolecules met only one or two physicochemical criteria (Table 3). Some other molecules, such as cirsimarin, cymaroside, and isovitexin, have hydrogen bonding donors and hydrogen bond acceptor numbers slightly higher than Lipinski's rule of five. Scopadulcic acid B has a partition coefficient somewhat higher than Lipinski's recommended value.

Table 3. Biomolecules physicochemical criteria.

Biomolecules	MW (<500 g/mol)	Hydrogen bonding donors (<5)	Hydrogen bond acceptors (<10)	Log P partition coefficient (<5)	Number of violations	Suitability to criteria
Acacetin	284.26	2	5	2.10	0	Yes
Apigenin	270.24	3	5	1.70	0	Yes
Betulic acid	456.70	2	3	8.20	1	Yes
Circimartin	314.14	2	6	2	0	Yes
Cirsiliol	330.29	3	7	2.60	0	Yes
Cirsimarin	476.40	5	11	1.20	1	Yes
Coixol	165.15	1	3	1.10	0	Yes
Cynaroside	448.38	7	11	1.76	2	**No**
Dihydroxy-dimethoxyflavone	304.14	2	5	1.30	0	Yes
Eugenyl-glucoside	326.34	4	7	0	0	Yes

Continued

Friedelin	426.70	0	1	9.80	1	Yes
Glutinol	426.70	1	1	9.2	1	Yes
Glutinone	424.70	0	1	8.9	1	Yes
Gossypetin	318.23	6	8	1.80	1	Yes
Hispidolin 7-Glucuronide	476.40	6	12	1.10	2	**No**
Hispidulin	300.26	3	6	1.70	0	Yes
Hydroxytetra-methoxyflavone	358.30	1	7	2.90	0	Yes
Isovitexin	432.40	7	10	0.20	1	Yes
Luteolin	286.24	4	6	1.40	0	Yes
Nevadensin	344.30	2	7	2.90	0	Yes
Nicotinic acid	123.11	1	3	0.40	0	Yes
Salvigenin	328.30	1	6	2.40	0	Yes
Scopaduciol	426.60	2	4	5.70	0	Yes
Scopadulcic acid A	454.60	2	6	4	0	Yes
Scopadulcic acid B	438.60	1	5	5.20	1	Yes
Scopadulin	440.60	2	5	5.20	0	Yes
Scoparic acid A	440.60	2	5	5.70	0	Yes
Scoparic acid B	412.50	1	5	4.40	0	Yes
Scoparic acid C	424.50	1	5	5.30	0	Yes
Scutellarein	286.24	3	6	1.70	0	Yes
Scutellarin	462.40	7	12	0.80	2	**No**
Sitosterol	414.70	1	1	9.30	1	Yes
Tuberonic acid glucoside	388.40	5	9	1.20	0	Yes
Vicenin 2	594.50	11	15	2.30	3	**No**
Vitexin	432.40	7	10	0.20	1	Yes

Table 4. SWISS ADME supplement analysis of 8 lead biomolecules.

Characteristics	Cirsimarin	Cynaroside	Eugenyl Glucoside	Glutinol	Gossypetin	Hydroxy-tetramethox-yflavone	Luteolin	Vitexin
				Water Solubility				
Log S (ESOL) [47]	−4.08 (Moderately soluble)	−3.65 (Soluble)	−1.68 (Very soluble)	−8.25 (Poorly soluble)	−3.40 (Soluble)	−4.03 (Moderately soluble)	−3.71 (Soluble)	−2.84 (Soluble)

Continued

Pharmacokinetics								
Gastrointestinal absorption	Low	Low	High	Low	Low	High	High	Low
Blood-Brain Barrier permeant	No	No	No	No	No	No	No	No
P-glycoprotein substrate	Yes	Yes	Yes	Yes	No	No	No	No
CYP1A2 inhibitor	No	No	No	No	Yes	Yes	Yes	No
CYP2C19 inhibitor	No	No	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	Yes	No	No
CYP2D6 inhibitor	No	No	No	No	Yes	Yes	Yes	No
CYP3A4 inhibitor	Yes	No	No	No	Yes	Yes	Yes	No

4. Discussion

Bioinformatics has been widely used to improve drug design and predict drug interactions with macromolecules. Docking involves searching for interaction sites on the entire macromolecular surface. Therefore, the mature form of the SARS-CoV-2 M^{pro} protease, 7KPH, which is not coupled to a specific inhibitor, was used for the blinded analysis of certain bioactive substances of the medicinal plant *Scoparia dulcis*.

In silico assays showed that any molecule is likely to be a suitable inhibitor if it has the lowest binding energy (good affinity) and considerable interactions with the committed amino acid residues of the biological target. Affinity measures the attraction of the ligand to the target or the easiness of binding to the target. Ligand affinity contributes to its potency, which also depends on the ability of the ligand to produce a biological response. [48]

Remdesivir and nelfinavir (**Figure 5**) were used as references to exploit the molecular docking results. Previous studies have shown that these two molecules are effective during *in silico* studies and clinical trials against SARS-CoV-2. Remdesivir was the first Food and Drug Administration-approved antiviral drug in the United States to manage hospitalized patients over 12 years old. [49] Ohashi *et al.* demonstrated the *in vitro* efficacy of nelfinavir in decreasing the viral load of SARS-CoV-2. [50] Studying the energies of the biomolecules of *Scoparia dulcis* L. at 7KPH revealed that 20 biomolecules showed better activity than remdesivir and nelfinavir. These 20 biomolecules are scopadulcic Acid B, glutinol, friedelin, luteolin, scopadulcic acid A, cynaroside, gossypetin, scutellarein, eugenyl glucoside, scopadulciol, hydroxy-tetramethoxyflavone, vitexin, scutellarin, scopadulin, scoparic acid C, betulic acid, hispidulin-7-glucuronide, cirsimarin, scoparic acid B, and scoparic acid A (**Table 2**). These biomolecules exhibit significant interactions with M^{pro} 7KPH through hydrogen bonds and hydrophobic interactions. Tyr126, Met6,

Ala7, Val125, Lys5, Cys128, Ser113, Phe8, Phe112, Phe291, and Gln127 are the amino acid residues involved in the interaction between the 20 biomolecules and M^{pro}. The dimerization interface of M^{pro} 7KPH shows that residues Tyr126, Ala7, and Val125 are involved in the interaction between the two monomers of M^{pro}. These 20 biomolecules can then be considered potential inhibitors of SARS-Cov-2 M^{pro}.

The design of an anti-SARS-CoV-2 drug is fundamentally based on inhibiting the virus's entry into the host cell by targeting Angiotensin-converting enzyme-2 receptors and inhibiting the catalytic activity of the main protease M^{pro}, focusing on the catalytic dyad or sub-S1-S5. Many reports on designing inhibitors for SARS-CoV-2 M^{pro} primarily concentrate on targeting the substrate-binding pocket to block catalytic activity. However, allosteric sites can also be utilized to develop anti-SARS-CoV-2 inhibitors. The mechanism of allosteric inhibition of M^{pro} occurs at the distal or dimerization sites.

The distal site was distant from the catalytic site in domains I and II. It consists of five pockets: Arg131-Thr199, Arg131-Asp289, Pro132-Thr196, Asp197, Thr198-Asn238, and Tyr239-Leu287, which are essential for protease activity. [51]

The dimerization site consists of several vital residues in the dimerization mechanism: Arg4, Ser10, Gly11, Glu14, Asn28, Ser139, Phe140, Ser147, Glu290, and Arg298. These residues work cooperatively to control dimerization and maintain the integrity of the dimer interface. [48] [52]

In addition, the N-terminus of the protease (residues 1 - 7) plays an essential role in dimerization. [53] The most critical residues of this chain are Arg4 and Met6; they ensure dimerization stability. [11] [12] [54] [55] Knelle *et al.* revealed by crystallography that the residues Gly2, Tyr126, Arg4, Lys137, and Leu286 are involved in interactions essential for dimerization. [56]

All these allosteric sites of SARS-CoV-2 M^{pro} constitute an alternative inhibitor development that is less prone to the emergence of resistance than the active site. [57]

The results of the intermolecular interactions between the biomolecules and 7KPH M^{pro} demonstrated no interaction with the catalytic dyad (Cys145 and His41) or with the active subunits of the S1-S5 protease. Thus, the biomolecules studied were not inhibitors of the catalytic activity of M^{pro} protease. Nevertheless, these biomolecules significantly interacted with the critical dimerization residues, Tyr126 and Met6. In our study, the biomolecules that inhibited dimerization by interfering with Tyr126 included cirsimarin, cynaroside, hispidulin 7-glucuronide (**Figure 6**), and eugenyl glucoside (**Figure 7**). Gossypetin, luteolin, hydroxy-tetramethoxyflavone, vitexin (**Figure 6**), and glutinol (**Figure 8**) are the biomolecules that exhibited measurable interactions with Met6. All nine biomolecules mentioned displayed measurable interactions with Ala7 and Val125, which are involved in the hydrophobic interaction between the two monomers of M^{pro} 7KPH.

The docked molecules underwent ADME analysis using Lipinski's rule. We identified four molecules with low bioavailability: scutellarin, vicenin, hispidulin

7-glucuronide; and cynaroside. Analyzing their structures, these four molecules contain a glucoside moiety with more than five hydroxyl groups, which increases their hydrosolubility. Consequently, these four molecules cannot pass through membrane barriers *in vivo*. Transforming the hydroxy groups to methoxy can help improve their bioavailability.

Based on docking analysis, we hypothesize that cirsimarin, cynaroside, eugenyl-glucoside, gossypetin, glutinol, hydroxy-tetramethoxyflavone, luteolin, and vitexin (**Figure 9**) are potential inhibitors of SARS-CoV-2 by inhibiting the dimerization activity of the main protease M^{pro}. Considering the Estimating aqueous Solubility directly from molecular structure (ESOL), [47] glutinol (**Table 4**) is poorly soluble, cirsimarin and hydroxy-tetramethoxyflavone (**Table 4**) are moderately soluble, eugenyl-glucoside, gossypetin, glutinol, luteolin, and vitexin (**Table 4**) are considered soluble. Regarding gastrointestinal absorption, we found three molecules with high absorption levels, such as eugenyl-glucoside, hydroxy-tetramethoxyflavone, and luteolin (**Table 4**). Cirsimarin, cynaroside, gossypetin, glutinol, and vitexin (**Table 4**) have low absorption levels. Related to distribution, none of these 8 molecules pass the blood-brain barrier; this is somehow good news because the blood-brain barrier's role is to protect the brain from external substances. Cirsimarin, cynaroside, eugenyl-glucoside, and glutinol (**Table 4**) are P-glycoprotein substrates, and the others are not. Coming to metabolism cirsimarin, gossypetin, hydroxy-tetramethoxyflavone, and luteolin (**Table 4**) inhibit some cytochromes. That can be a source of drug-drug interaction.

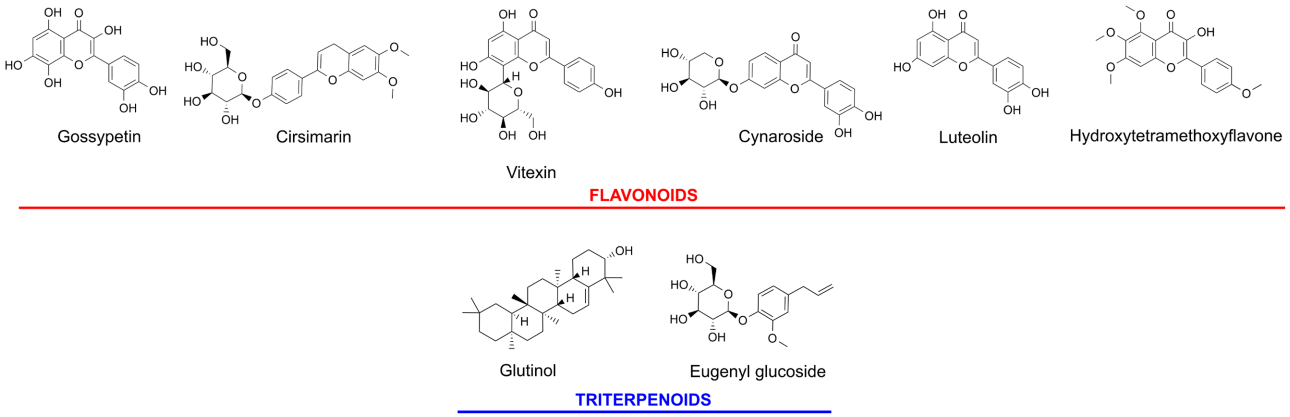

Figure 9. Height Leads, potential allosteric inhibitors of SARS-Cov-2 M^{pro}.

Finely, cynaroside and eugenyl glucoside seem to be the most interesting drug-like molecules.

It has been shown that the aqueous, alcoholic, and hydroalcoholic extract of *Scoparia dulcis* L. contains many biomolecules and has different exciting activities such as anti-diabetic, anti-atherogenic, anti-hypertension, and antioxidant. [22]

Thus, using aqueous, alcoholic, or hydroalcoholic extract of *Scoparia dulcis* L. can be planned from preclinical and clinical perspectives to treat COVID-19.

Our study agrees with other studies that demonstrated the inhibitory potential

of some of the molecules studied on SARS-CoV-2 M^{pro}. For example, during a virtual screening assay, Naik *et al.* demonstrated that vitexin inhibits SARS-Cov-2 M^{pro}. [58] According to Giguet *et al.*, gossypetin is a potential inhibitor of SARS-CoV-2 in a computational study. [59] *In silico* research has confirmed luteolin as a potential M^{pro} inhibitor. [60] Luteolin is an active ingredient of *Artemisia annua*, a plant used to treat COVID-19. [61]

5. Conclusion

To contribute to searching for new drugs against COVID-19, particularly those derived from medicinal plants, we conducted a molecular docking assay of biomolecules isolated from *Scoparia dulcis* L. on the main protease of SARS-CoV-2. The 7KPH protease has been recognized as a key target for inhibiting the novel coronavirus. Among the biomolecules studied, 20 exhibited a higher affinity for 7KPH M^{pro} compared to the reference molecules Remdesivir and nelfinavir. Considering drug-likeness, intermolecular interactions, and binding affinities, the highlighted leads indicated allosteric inhibitory potential against protease M^{pro}. A dynamic molecular analysis is crucial to enhance the semi-flexible docking results. Moreover, *in vivo* tests are essential to validate the drug-likeness properties obtained through Swiss ADME by assessing absorption, distribution, metabolism, excretion, and toxicity. *Scoparia dulcis* contains biomolecules with preclinical and clinical potential regarding SARS-CoV-2 and related diseases.

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Author Contributions

Conceptualization, O.M. O.W.P., Y W.H, and T.I.T. Writing—original draft preparation, O.M., Y W.H., and O.W.P; writing—review and editing, O.M., Y W.H., and O.W.P Read and corrections: O.M. O.W.P., Y W.H, and T.I.T., B.R., N.O., S.R., and O.S. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

Any supplementary information can be obtained on a request addressed to the corresponding author.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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