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Exploring the Anti-Sars-Cov-2 Potential of Passiflora Flavonoids: An In-Silico Investigation of Main Protease (Mpro) Inhibition

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Abstract
Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), remains a global health concern for which broadly effective oral antivirals are still limited. The viral main protease (Mpro, also called 3CLpro) is an essential, highly conserved enzyme that mediates the maturation of viral polyproteins, making it one of the most attractive druggable targets. In this study we used molecular docking to investigate the inhibitory potential of ten flavonoids characteristic of the genus Passiflora — the C-glycosyl flavones isoorientin, isovitexin, vitexin and orientin, the O-glycosides hyperoside and astragalin, and the aglycones quercetin, luteolin, kaempferol and chrysin against the crystal structure of SARS-CoV-2 Mpro (PDB ID: 6LU7). The co-crystallised peptidomimetic inhibitor N3 (S = -9.7822 kcal/mol) and the repurposed drug chloroquine (S = -5.1753 kcal/mol) were used as references. All ten Passiflora compounds outperformed chloroquine. Hyperoside (-8.3874 kcal/mol) and astragalin (-8.2122 kcal/mol) showed the most favourable scores, followed by the C-glycosyl flavones isoorientin, vitexin, orientin and isovitexin (-7.4 to -7.9 kcal/mol). The top ligands engaged key substrate-binding residues of the active site, including the catalytic His41, the oxyanion-hole region around Asn142/Gly143/Cys145, and the anchoring residues Glu166, Gln189 and Thr190. ADME/drug-likeness analysis (SwissADME) indicated that aglycones such as quercetin, luteolin, kaempferol and chrysin fully satisfy Lipinski's rule of five, whereas the more potent glycosides violate one to two criteria owing to their high polarity. These computational results support the traditional and nutritional interest of Passiflora and identify its flavonoids particularly the glycosylated derivatives as promising natural scaffolds for the design of Mpro inhibitors, warranting further in-vitro and in-vivo validation.
KEYWORDS: COVID-19; SARS-CoV-2; main protease (Mpro); molecular docking; Passiflora; flavonoids; hyperoside; astragalin; C-glycosyl flavones.

1. INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported in late 2019 and rapidly evolved into a global health emergency that has resulted in hundreds of millions of infections worldwide [1,2]. Although vaccines and a small number of direct-acting antivirals have profoundly changed the clinical landscape, the continued emergence of variants and the limitations of existing therapies sustain the search for new, safe and broadly active inhibitors [3].

Among the viral proteins, the main protease (Mpro, also designated 3C-like protease, 3CLpro) is widely regarded as one of the most promising drug targets. Mpro is a cysteine protease that operates through a non-canonical catalytic dyad formed by His41 and Cys145 and is responsible for cleaving the viral polyproteins pp1a and pp1ab at no fewer than eleven conserved sites, a step that is indispensable for viral replication and transcription [4,5]. Because no human protease shares the same cleavage specificity (Mpro cleaves preferentially after a glutamine residue), selective inhibition of this enzyme is expected to produce few off-target effects [6]. The active site is organised into well-defined subsites (S1', S1, S2, S3, S4) that anchor the peptide substrate; residues such as Thr26, Asn142, Gly143, Ser144, Cys145, His163, His164, Glu166, Gln189 and Thr190 form the hydrogen-bond network that orients the substrate towards the catalytic dyad [5,7].

Natural products, and flavonoids in particular, have repeatedly emerged from screening campaigns as credible Mpro binders [8–11]. The genus Passiflora (Passifloraceae), comprising more than 500 species, is a rich source of such compounds. Its phytochemistry is dominated by C-glycosyl flavones principally, isoorientin, isovitexin, vitexin and orientin, which almost invariably co-occur, together with O-glycosides (hyperoside, astragalin) and the corresponding aglycones apigenin, luteolin, quercetin, kaempferol and chrysin [12–14]. These metabolites underlie the traditional sedative and anxiolytic use of Passiflora and have documented antioxidant, anti-inflammatory and antiviral activities [14,15]. An earlier docking study already suggested that several Passiflora constituents bind SARS-CoV-2 Mpro with energies below -8 kcal/mol [8], motivating a focused re-examination of its principal flavonoids.

In the present work we apply molecular docking a fundamental tool of computer-aided drug discovery used to predict ligand–receptor binding geometries and affinities [16,19]. to evaluate ten representative Passiflora flavonoids against the crystal structure of SARS-CoV-2 Mpro (PDB ID: 6LU7), using the co-crystallised inhibitor N3 and the repurposed drug chloroquine as references. Drug-likeness was subsequently assessed with SwissADME.

2. MATERIAL AND METHODS

2.1. Selection and preparation of ligands

Ten flavonoids representative of the chemical profile of the genus Passiflora were selected from the published phytochemical literature [12–14]: the C-glycosyl flavones isoorientin (luteolin-6-C-glucoside), isovitexin (apigenin-6-C-glucoside), vitexin (apigenin-8-C-glucoside) and orientin (luteolin-8-C-glucoside); the flavonol O-glycosides hyperoside (quercetin-3-O-galactoside) and astragalin (kaempferol-3-O-glucoside); and the aglycones quercetin, luteolin, kaempferol and chrysin. Three-dimensional structures were retrieved from the PubChem database. Each ligand was energy-minimised with the Molecular Operating Environment (MOE) software using the MMFF94x force field to obtain the lowest-energy conformer, and the optimised molecules were assembled into a database for docking. The reference antimalarial drug chloroquine was optimised by the same protocol.

2.2. Receptor preparation

The three-dimensional crystal structure of SARS-CoV-2 main protease in complex with the peptidomimetic inhibitor N3 was downloaded from the RCSB Protein Data Bank under accession code 6LU7 (resolution 2.16 Å) [4]. Water molecules were removed, hydrogen atoms were added and the structure was protonated at physiological pH. The largest cavity of the enzyme the substrate-binding cleft between domains I and II that contains the His41–Cys145 catalytic dyad was identified with the MOE “Site Finder” module and defined as the docking site.

2.3. Docking protocol and validation

Docking was performed in MOE under default conditions (300 K, pH 7), and binding affinity was quantified through the scoring function ΔG (S, in kcal/mol), which sums the electrostatic and van der Waals contributions of the ligand–enzyme complex [16,17]. Candidate poses were ranked by score, and the lowest-energy pose of each ligand was retained for interaction analysis. The protocol was validated by re-docking the co-crystallised N3 ligand; superposition of the experimental and re-docked poses gave a root-mean-square deviation (RMSD) below the accepted 2 Å threshold, confirming the reliability of the procedure. The co-crystallised inhibitor N3 and the repurposed drug chloroquine served as benchmarks for comparison.

2.4. ADME and drug-likeness prediction

The physicochemical and pharmacokinetic profiles of the ligands were predicted with the open-access SwissADME web tool [20]. Compliance with the extended Lipinski rule of five was assessed using the thresholds: molecular weight ≤ 500 g/mol, lipophilicity (Log P) ≤ 5, hydrogen-bond donors ≤ 5, hydrogen-bond acceptors ≤ 10 and topological polar surface area (TPSA) ≤ 140 Å^2. A molecule is generally considered orally drug-like when it satisfies most of these criteria, although exceptions are well documented [21].

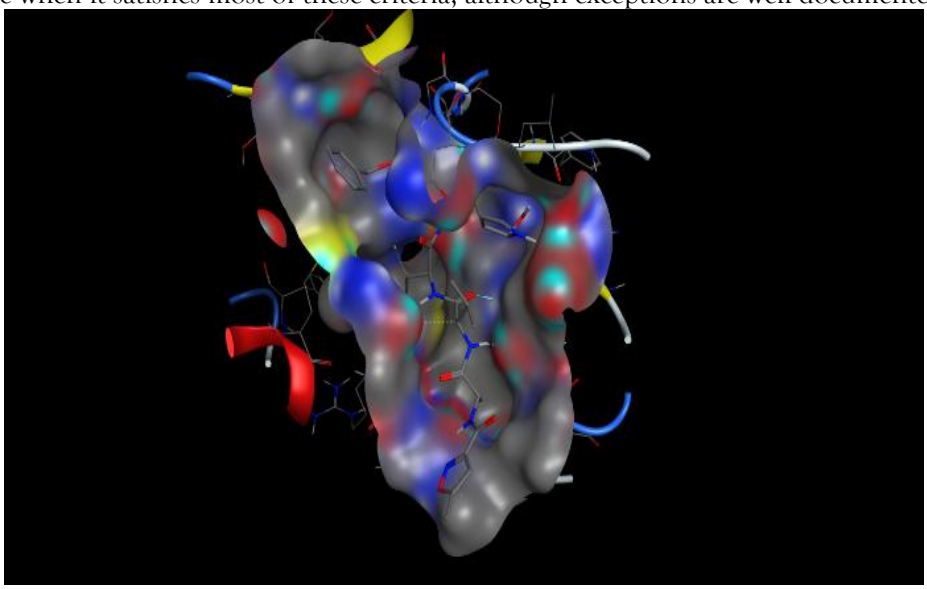

Figure 1. The simplified SARS-CoV-2 main protease (Mpro, PDB ID 6LU7) showing the substrate-binding pocket (molecular surface) into which the ligands were docked.

3. RESULTS AND DISCUSSION

3.1. Docking scores

The docking scores of the ten Passiflora flavonoids and of the two references are summarised in Table 1 and illustrated in Figure 2. All ten flavonoids displayed more favourable (more negative) scores than chloroquine (-5.1753 kcal/mol), indicating a stronger predicted affinity for Mpro than the repurposed drug. The two flavonol O-glycosides ranked highest: hyperoside reached -8.3874 kcal/mol and astragalin -8.2122 kcal/mol, both within roughly 1.4 kcal/mol of the co-crystallised peptidomimetic inhibitor N3 (-9.7822 kcal/mol), a far larger and specifically optimised molecule. The C-glycosyl flavones followed closely (isoorientin -7.8762, vitexin -7.7872, orientin -7.6485 and isovitexin -7.4777 kcal/mol), while the aglycones quercetin, luteolin, chrysin and kaempferol occupied the -6.0 to -6.7 kcal/mol range.

A clear structure–activity trend emerges: glycosylation enhances the predicted binding. The pendant sugar provides additional hydroxyl groups that form extra hydrogen bonds with the polar anchoring residues lining the S1 and S4 subsites, consistent with previous reports that glycosylated flavonoids are among the strongest in-silico Mpro binders [9]. The superiority of the catechol-bearing scaffolds (quercetin, luteolin and their glycosides) over the mono-hydroxylated B-ring scaffolds (kaempferol, chrysin) further underlines the contribution of the 3',4'-dihydroxy motif to hydrogen bonding.

Table 1. Docking scores of Passiflora flavonoids and reference ligands against SARS-CoV-2 main protease (PDB ID 6LU7).

Compound (common name)	Class / type	Score S (kcal/mol)
Co-crystallised reference ligand (N3)	Peptidomimetic inhibitor	**-9.7822**
Hyperoside (quercetin-3-O-galactoside)	Flavonol O-glycoside	-8.3874
Astragalin (kaempferol-3-O-glucoside)	Flavonol O-glycoside	-8.2122

Compound (common name)	Class / type	Score S (kcal/mol)
Isoorientin (luteolin-6-C-glucoside)	Flavone C-glycoside	-7.8762
Vitexin (apigenin-8-C-glucoside)	Flavone C-glycoside	-7.7872
Orientin (luteolin-8-C-glucoside)	Flavone C-glycoside	-7.6485
Isovitexin (apigenin-6-C-glucoside)	Flavone C-glycoside	-7.4777
Quercetin	Flavonol aglycone	-6.7231
Luteolin	Flavone aglycone	-6.3791
Chrysin	Flavone aglycone	-6.0555
Kaempferol	Flavonol aglycone	-5.9671
Chloroquine	Reference antimalarial drug	**-5.1753**

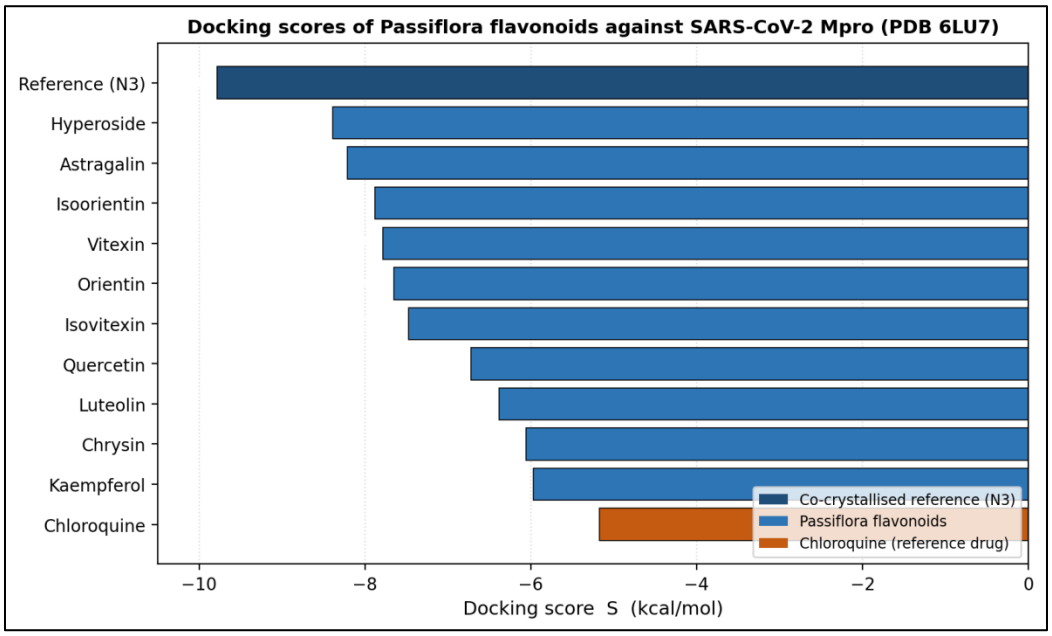

Figure 2. Comparative docking scores of the ten Passiflora flavonoids against SARS-CoV-2 Mpro, ranked against the co-crystallised reference inhibitor N3 and the repurposed drug chloroquine. More negative values indicate stronger predicted binding.

3.2. Binding interactions with the active site

Analysis of the lowest-energy poses (Table 3) shows that the Passiflora flavonoids consistently occupy the substrate-binding cleft and engage the residues known to anchor the natural peptide substrate. Recurrent contacts were observed with Glu166, Asn142, His164, Gln189 and Thr190 the same anchoring residues that orient the substrate towards the catalytic centre [5,7] as well as with the catalytic His41 itself. The fact that the most potent compounds reproduce the interaction pattern of the co-crystallised N3 inhibitor (which spans the S1–S4 subsites and contacts His41/Cys145; Figure 7) strengthens confidence in the docking poses. Hyperoside (Figure 3) anchors its galactoside moiety and B-ring catechol through a hydrogen-bond network involving Glu166, Asn142, His164 and Gln189, with additional contacts towards His41 and Met49 of the catalytic pocket. Astragalin (Figure 4) similarly orients its 3-O-glucoside and chromone carbonyl towards the oxyanion-hole region (Asn142–Gly143–Cys145) and the catalytic dyad, consistent with its high score. Among

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the C-glycosides, isoorientin (Figure 5) forms hydrogen bonds with Thr26, Thr190, Glu166, Asn142 and His164, distributing its sugar and catechol hydroxyls across the S1 and S4 anchoring subsites, while vitexin (Figure 6) engages Glu166, Gln189, Thr190, Asn142 and His164 through its C-glucoside and 4'-OH groups. Less-hydroxylated aglycones such as chrysin and kaempferol still reach the catalytic pocket (contacts with His41, His164 and Glu166) but form fewer hydrogen bonds, in line with their weaker scores. Taken together, the interaction data indicate that Passiflora flavonoids act as competitive, substrate-mimicking binders that could hinder access of the polyprotein to the His41–Cys145 dyad.

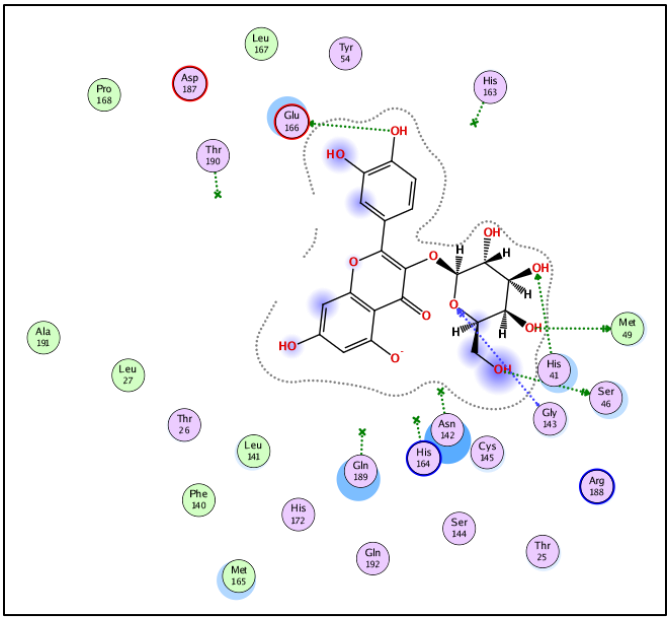

Figure 3. 2D interaction diagram of hyperoside (top-ranked ligand, -8.3874 kcal/mol) in the active site of SARS-CoV-2 Mpro (PDB ID 6LU7).

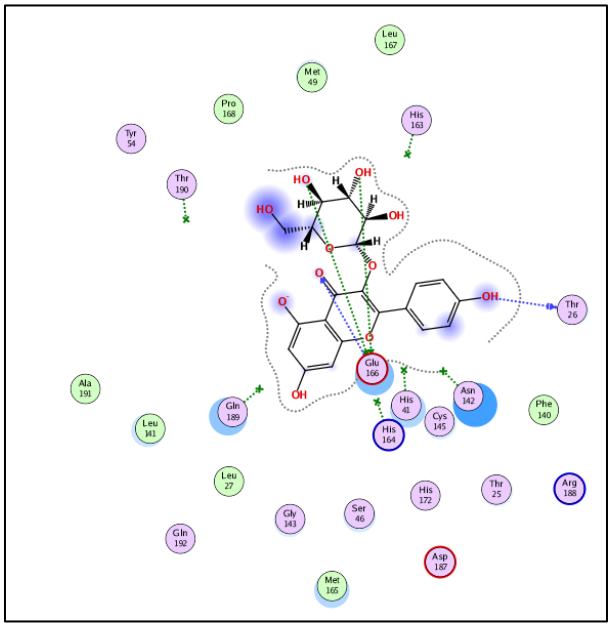

Figure 4. 2D interaction diagram of astragalin (-8.2122 kcal/mol) in the active site of SARS-CoV-2 Mpro (PDB ID 6LU7).

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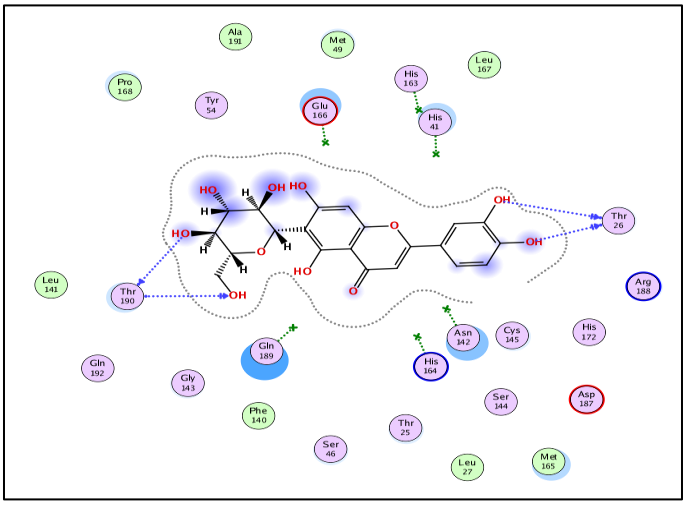

Figure 5. 2D interaction diagram of isoorientin (-7.8762 kcal/mol) in the active site of SARS-CoV-2 Mpro (PDB ID 6LU7).

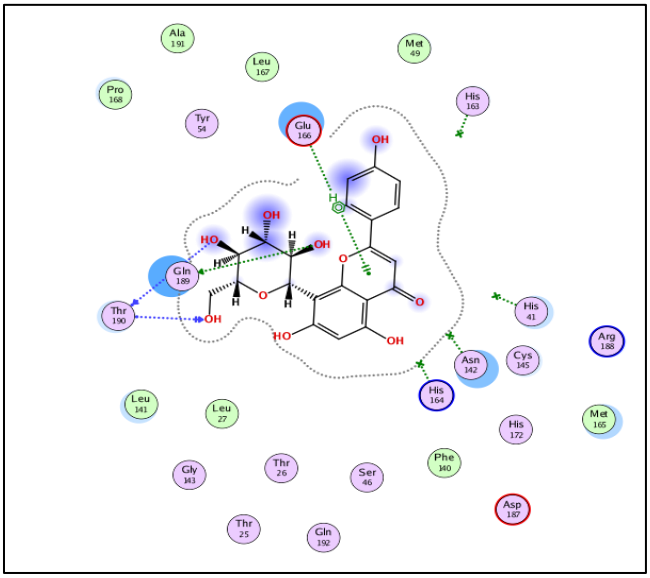

Figure 6. 2D interaction diagram of vitexin (-7.7872 kcal/mol) in the active site of SARS-CoV-2 Mpro (PDB ID 6LU7).

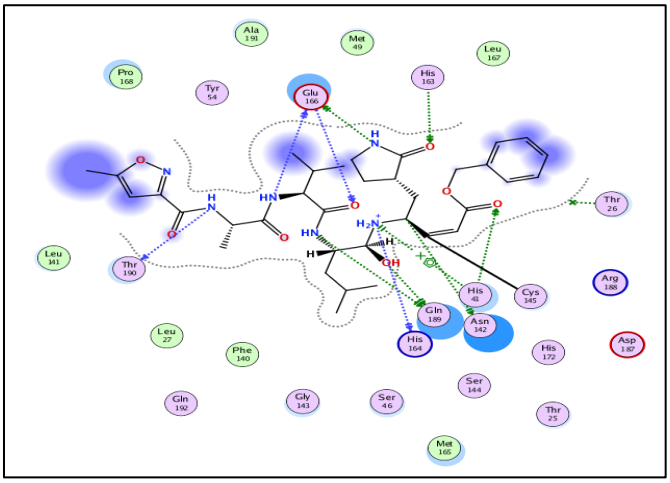

Figure 7. 2D interaction diagram of the co-crystallised reference inhibitor N3 (-9.7822 kcal/mol) in the active site of SARS-CoV-2 Mpro (PDB ID 6LU7), spanning the S1–S4 subsites and contacting the His41/Cys145 catalytic dyad.

Table 3. Docking scores and main interactions of the studied ligands within the active site of SARS-CoV-2 Mpro (PDB ID 6LU7).

Ligand	Score (kcal/mol)	Active-site residues involved	Interaction type	Catalytic / anchor subsite
Hyperoside	-8.3874	Glu166, Asn142, His164, Gln189, Met49, His41	H-bonds (B-ring catechol & sugar OH), hydrophobic	S1/S2 anchor + catalytic pocket
Astragalin	-8.2122	Glu166, Asn142, His164, His41, Cys145, Gln189	H-bonds (3-O-glucoside & ketone), π-contacts	Oxyanion hole / catalytic dyad vicinity
Isoorientin	-7.8762	Thr26, Thr190, Glu166, Asn142, His164	H-bonds (sugar & catechol OH)	S1 anchor + S4 (Thr190/Gln189)
Vitexin	-7.7872	Glu166, Gln189, Thr190, Asn142, His164	H-bonds (C-glucoside & 4'-OH)	S1/S4 anchor subsites
Orientin	-7.6485	Glu166, Gln189, Thr190, Asn142, His164, His41	H-bonds (catechol & sugar OH)	S1/S2 anchor + catalytic pocket
Isovitexin	-7.4777	Glu166, Asn142, His164, Thr190	H-bonds (C-glucoside OH), hydrophobic	S1 anchor subsite
Quercetin	-6.7231	Glu166, His41, Asn142, His164	H-bonds (B-ring catechol & 3-OH)	Catalytic dyad vicinity (His41)
Luteolin	-6.3791	Thr190, His41, Gln189, Glu166	H-bonds (catechol OH), π-contacts	S2 (His41) + S4 anchor
Chrysin	-6.0555	Glu166, Met165, His164, His41, Arg188, Gln189	H-bonds (5-/7-OH), π-contacts	S1/S2 + catalytic pocket
Kaempferol	-5.9671	Glu166, His41, Asn142, His164	H-bonds (3-/5-OH & ketone)	Catalytic dyad vicinity
Reference (N3)	-9.7822	His41, Cys145, Glu166, Gln189, Thr190, Thr26, Asn142, His164	Peptidomimetic H-bond network spanning S1–S4	Full S1–S4 occupancy + catalytic dyad

3.3. ADME and drug-likeness

The predicted physicochemical and drug-likeness properties of the ligands are presented in Table 2. The aglycones quercetin, luteolin, kaempferol and chrysin fully satisfy Lipinski's rule of five (zero violations), with molecular weights below 305 g/mol and TPSA values ≤ 131 Å², consistent with good predicted oral absorption. By contrast, the more potent glycosides hyperoside, astragalin, isoorientin, orientin, vitexin and isovitexin violate one or two criteria — essentially through an excess of hydrogen-bond donors/acceptors and high TPSA (181–231 Å²) arising from the sugar moieties which is expected to limit passive gastrointestinal absorption. This reflects the classical tension between binding affinity and oral bioavailability frequently encountered with glycosylated natural products [10,20]. None of the compounds raised toxicity alerts in the SwissADME analysis, and the high polarity that penalises oral absorption may be addressed through

formulation strategies or aglycone/prodrug approaches. The aglycones therefore represent attractive drug-like leads, while the glycosides offer superior predicted potency as starting points for optimisation.

Table 2. Main physicochemical and drug-likeness properties (SwissADME) of the studied Passiflora flavonoids.

Compound	MW (g/mol)	iLogP*	Log S	HBD	HBA	TPSA (Å²)	Lipinski (violations)
Hyperoside	464.38	n.a.	-2.68	8	12	210.51	2 (NorO>10, NHorOH>5)
Astragalin	448.38	n.a.	-2.81	7	11	190.28	2 (NorO>10, NHorOH>5)
Isoorientin	480.38	n.a.	-1.65	9	13	230.74	2 (NorO>10, NHorOH>5)
Vitexin	432.38	n.a.	-2.84	7	10	181.05	1 (NHorOH>5)
Orientin	448.38	n.a.	-1.65	8	11	201.28	2 (NorO>10, NHorOH>5)
Isovitexin	432.38	n.a.	-2.42	7	10	181.05	1 (NHorOH>5)
Quercetin	302.24	1.63	-3.16	5	7	131.36	0
Luteolin	286.24	1.86	-3.07	4	6	111.13	0
Chrysin	254.24	2.08	-3.38	2	4	70.67	0
Kaempferol	286.24	1.70	-3.33	4	6	111.13	0

*iLogP not available (n.a.) for several glycosides in the SwissADME output; Log S values are ESOL predictions. Glycoside descriptors correspond to the SwissADME panels generated in this study; aglycone descriptors are consistent with standard SwissADME outputs.

4. CONCLUSION

In this in-silico study, molecular docking against the SARS-CoV-2 main protease (PDB ID 6LU7) showed that ten flavonoids representative of the genus Passiflora bind the enzyme more favourably than the repurposed drug chloroquine, with several approaching the score of the optimised co-crystallised inhibitor N3. The flavonol O-glycosides hyperoside (-8.3874 kcal/mol) and astragalin (-8.2122 kcal/mol) emerged as the most promising binders, followed by the characteristic C-glycosyl flavones isoorientin, vitexin, orientin and isovitexin. The top ligands engaged the catalytic His41 and the substrate-anchoring residues Glu166, Asn142, His164, Gln189 and Thr190, reproducing the binding pattern of the reference inhibitor and suggesting a competitive, substrate-mimicking mode of inhibition. ADME analysis indicated that the aglycones are fully drug-like, whereas the more potent glycosides trade oral bioavailability for affinity. Collectively, these findings provide computational support for the antiviral interest of Passiflora flavonoids and identify them particularly the glycosylated derivatives as natural scaffolds worth pursuing as Mpro inhibitors.

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