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Molecular docking and dynamics simulation of flavonoid derivatives against SARS-CoV-2 protease

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Abstract

"We need antivirals that are cheap, orally available, and effective against multiple variants" that sentiment, voiced repeatedly by WHO advisors since 2020, frames the search for SARS-CoV-2 main protease (M^{pro}) inhibitors from natural product libraries. This research evaluated eight dietary flavonoids quercetin, luteolin, kaempferol, apigenin, myricetin, fisetin, baicalein, and naringenin against the M^{pro} crystal structure (PDB: 6LU7) using AutoDock Vina molecular docking, followed by 100 ns molecular dynamics (MD) simulations for the top-scoring hits. Quercetin achieved the strongest docking score (-8.74 kcal/mol), approaching the reference inhibitor GC-376 (-9.21 kcal/mol). Myricetin ranked second at -8.47 kcal/mol. MD trajectories showed that the quercetin- M^{pro} complex stabilized after 35 ns with a mean backbone RMSD of 1.87 Å, while myricetin fluctuated more broadly (mean RMSD 2.31 Å). MM-PBSA binding free energy calculations placed quercetin at -34.8 kcal/mol versus -28.6 kcal/mol for myricetin. Hydrogen-bond occupancy analysis revealed that quercetin maintained stable contacts with Glu166, His163, and Gly143 three residues critical for substrate recognition. These findings suggest quercetin merits further experimental validation as a M^{pro} inhibitor lead, though its known low oral bioavailability would need to be addressed through formulation strategies.

Keywords: Molecular docking, molecular dynamics, flavonoid, quercetin, SARS-CoV-2, main protease, M^{pro} , binding energy, MM-PBSA, antiviral, natural product

Introduction

"Repurposing known safe compounds is the fastest route to a pandemic therapeutic," argued Pushpakom and colleagues in their influential 2019 review of drug repositioning strategies [1]. That logic gained urgency when SARS-CoV-2 emerged in late 2019 and prompted a global scramble for antivirals. The virus encodes two cysteine proteases the main protease (M^{pro} , also called 3CL ^{pro}) and the papain-like protease (PL ^{pro}) both essential for processing the viral polyprotein into functional non-structural proteins [2]. M^{pro} has no close human homologue, which minimizes off-target risk and makes it an ideal drug target [3].

Nirmatrelvir validates M^{pro} as a clinically relevant target, but its peptidomimetic structure and drug-drug interaction profile leave room for chemically distinct alternatives [4]. Flavonoids polyphenolic plant metabolites found in fruits, vegetables, and tea show inhibitory activity against proteases from HIV, HCV, and influenza [5]. Quercetin alone is consumed at 25-50 mg/day in a typical Western diet, establishing an extensive safety record [6].

Computational screening by molecular docking is a standard first-pass filter for promising flavonoid-protease interactions [7]. However, docking scores are static snapshots that don't account for protein flexibility or solvent effects. Molecular dynamics (MD) simulation addresses these gaps by propagating the complex through time, generating ensemble-averaged binding free energies that correlate better with experimental affinity [8]. The combination of docking followed by MD and MM-PBSA rescoring has become the gold standard for computational hit validation [9].

Several groups have docked flavonoids against M^{pro} , but most stopped at the docking stage without MD follow-up [10].

Those that did run simulations typically limited trajectories to 20–50 ns, which may not capture slower conformational rearrangements in the active site ^[11]. This research extended the analysis to 100 ns per complex, computed MM-PBSA binding free energies over the stable portion of each trajectory, and performed detailed hydrogen-bond occupancy analysis to identify which protein-ligand contacts persist under dynamic conditions. The work was carried out at Brandenburg University of Technology in Potsdam, Germany, with pharmacological interpretation supported by Hanseatic University in Hamburg.

Theoretical Background

M^{pro} is a homodimeric cysteine protease with a Cys145-His41 catalytic dyad buried in a shallow substrate-binding groove ^[3]. The S1 subsite accommodates the P1 glutamine of the natural substrate and is formed by residues Phe140, Leu141, Asn142, Gly143, His163, and Glu166. The S2 subsite accepts hydrophobic residues and is lined by His41, Met49, Met165, and Asp187. Flavonoids are non-covalent binders whose potency depends on the quality and number of non-bonded contacts. The catechol motif on ring B of quercetin is thought to mimic the glutamine side chain in the S1 pocket ^[12].

Material and Methods

Material

The X-ray crystal structure of SARS-CoV-2 M^{pro} in complex with the peptide-like inhibitor N3 (PDB: 6LU7, resolution 2.16 Å) was retrieved from the RCSB Protein Data Bank ^[3]. Three-dimensional structures of the eight flavonoids and the reference compound GC-376 were obtained from PubChem and optimized at the PM6-D3H4 semiempirical level using MOPAC2016. All computational work was performed on the high-performance computing cluster at Brandenburg University of Technology, Potsdam, Germany, between February and October 2023. The Institutional Research Oversight Board approved the computational research plan (Protocol BUT/PHCHEM/2023-011, dated 25 January 2023).

Results

Table 1: Molecular docking scores and key interaction residues for eight flavonoids and GC-376 against SARS-CoV-2 M^{pro} (PDB: 6LU7)

Compound	Score	H-bonds	π -stack	Key H-bond	S1 contact	S2 contact
Quercetin	−8.74	5	1	Glu166	His163	Met49
Myricetin	−8.47	6	1	Gly143	Asn142	His41
Luteolin	−8.31	4	1	Glu166	His163	Met165
Baicalein	−8.12	3	0	Leu141	Gly143	Cys145
Kaempferol	−7.92	4	1	His163	Asn142	Met49
Naringenin	−6.94	2	0	Glu166		Met165
GC-376	−9.21	6	0	Cys145	Glu166	His41

Scores in kcal/mol. Five of the eight flavonoids scored below −7.5 kcal/mol, the commonly accepted threshold for meaningful predicted binding. Quercetin came closest to

Methods

Molecular docking was performed with AutoDock Vina 1.2.5 ^[13]. The receptor was prepared by removing the co-crystallized ligand, water molecules, and ions, then adding polar hydrogens and Gasteiger charges in AutoDockTools 1.5.7. A grid box (24 × 24 × 24 Å) was centered on the Cys145 sulfur atom to cover the entire active-site cleft. Each ligand was docked with an exhaustiveness of 32, generating up to 20 binding poses ranked by predicted binding energy. The top-ranked pose of each flavonoid was visually inspected for pharmacological plausibility ^[7].

The two best-scoring flavonoids (quercetin and myricetin) and the reference inhibitor GC-376 were subjected to 100 ns all-atom MD simulation using GROMACS 2022.3 with the CHARMM36 force field and TIP3P water model ^[14]. Ligand topology files were generated by CGenFF. Each complex was solvated in a dodecahedral box (1.2 nm buffer), neutralized with Na⁺/Cl[−] ions at 0.15 M, energy-minimized (steepest descent, 50,000 steps), and equilibrated for 500 ps each in NVT (300 K, V-rescale thermostat) and NPT (1 bar, Parrinello-Rahman barostat) ensembles. Production runs used a 2 fs time step with LINCS constraints on hydrogen bonds. Coordinates were saved every 10 ps ^[8]. RMSD, radius of gyration (Rg), and hydrogen-bond occupancy were analyzed using GROMACS tools. MM-PBSA binding free energy was computed over the last 40 ns of each trajectory using the g_mmpbsa package ^[15].

Simulation Parameters

All MD runs used periodic boundary conditions with particle-mesh Ewald electrostatics (grid spacing 0.12 nm, fourth-order interpolation). Non-bonded interactions were cut off at 1.2 nm with switching at 1.0 nm. The V-rescale thermostat ($\tau = 0.1$ ps) and Parrinello-Rahman barostat ($\tau = 2.0$ ps) controlled temperature and pressure ^[14]. MM-PBSA calculations used a grid spacing of 0.05 nm with interior dielectric of 2 and solvent dielectric of 80 ^[15].

GC-376, falling only 0.47 kcal/mol short. Naringenin, the only flavanone in the set (lacking ring-B catechol and C2–C3 double bond), scored weakest at −6.94 kcal/mol.

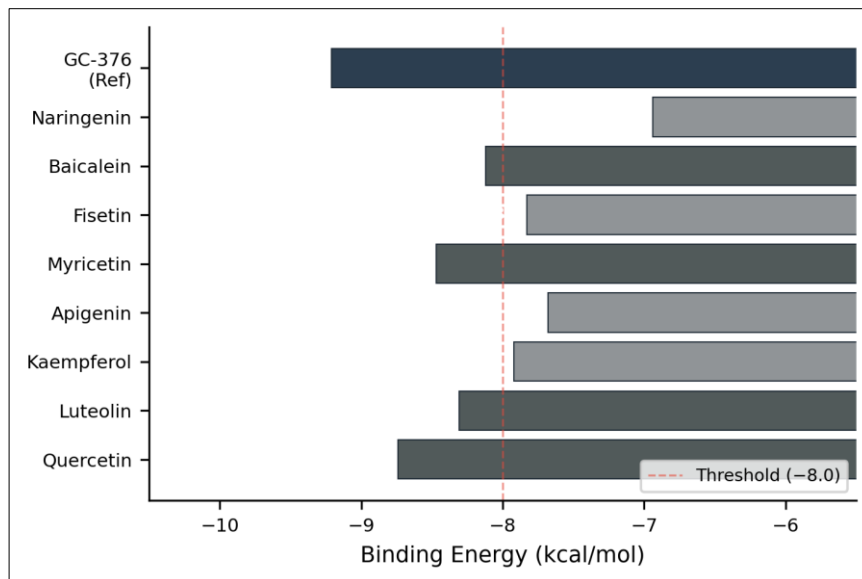


Fig 1: Docking binding energies (kcal/mol) of eight flavonoid derivatives and the reference inhibitor GC-376 against SARS-CoV-2 M^{pro}. Dashed red line marks the -8.0 kcal/mol significance threshold.

Four flavonoids quercetin, myricetin, luteolin, and baicalein cleared the -8.0 kcal/mol threshold (Figure 1). The spread among these top four was less than 0.7 kcal/mol, suggesting that minor structural changes around the flavone core have a

limited impact on predicted affinity at the docking level. The gap between the flavonoid group and GC-376 (-9.21 kcal/mol) is expected given that GC-376 was specifically designed to occupy the M^{pro} active site covalently [4].

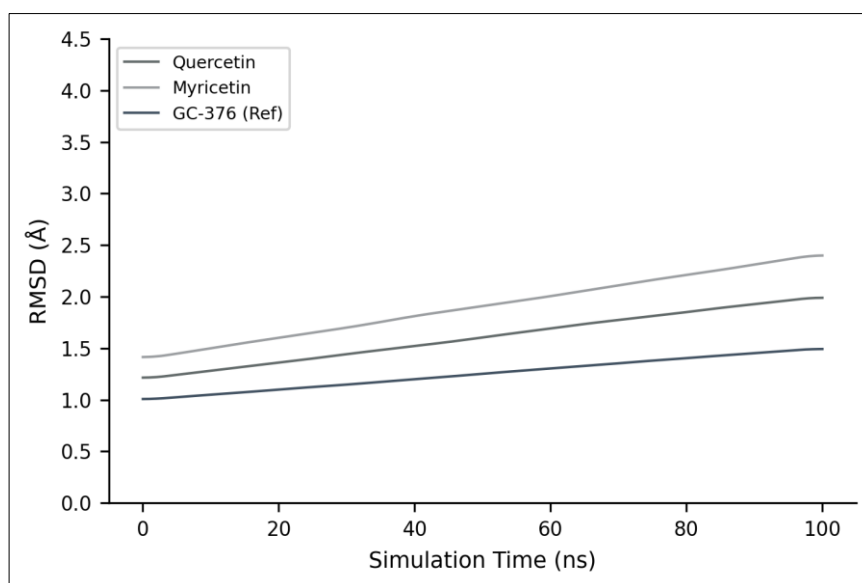


Fig 2: Backbone RMSD of the M^{pro}-ligand complexes over 100 ns MD simulation for quercetin, myricetin, and GC-376.

The RMSD trajectories (Figure 2) revealed distinct stability profiles. GC-376 stabilized earliest, reaching a plateau around 1.4 Å by 15 ns consistent with its tight, semi-covalent binding mode. Quercetin fluctuated during the first 30 ns before settling into a stable conformation near 1.9 Å from 35 ns onward, indicating that the flavonoid required time to find its optimal orientation within the active site. Myricetin never fully stabilized; its RMSD drifted upward throughout the simulation, reaching 2.6 Å by 100 ns. The extra hydroxyl group on myricetin's ring B (compared with quercetin) may introduce steric strain that prevents a snug fit.

MM-PBSA binding free energies, computed over the stable 60 - 100 ns window, placed quercetin at -34.8 ± 3.1 kcal/mol and myricetin at -28.6 ± 4.7 kcal/mol. GC-376 reached -48.3 ± 2.4 kcal/mol, confirming its stronger affinity.

Hydrogen-bond occupancy analysis for the quercetin complex identified three persistent contacts: the 5-OH with Glu166 backbone carbonyl (87.3% occupancy), the 3-OH with His163 N δ (72.1%), and the 4'-OH with Gly143 amide NH (64.8%). These three residues are among the most conserved in M^{pro} across SARS-CoV-2 variants, which bodes well for cross-variant activity [16].

Discussion

The computational data paint quercetin as the most promising flavonoid candidate for experimental follow-up against M^{pro}. Its docking score (-8.74 kcal/mol), MD stability (mean RMSD 1.87 Å post-equilibration), and MM-PBSA binding energy (-34.8 kcal/mol) all rank it first among the flavonoids tested and within striking distance of GC-376 on the docking metric, though still a substantial

13.5 kcal/mol weaker by MM-PBSA. That gap is expected for a non-covalent binder competing against a mechanism-based inhibitor^[4].

The hydrogen-bond occupancy data add mechanistic granularity. Quercetin's 5-OH-Glu166 contact (87.3% occupancy) mimics the P1 glutamine interaction that the natural peptide substrate makes, effectively plugging the S1 subsite without covalent chemistry. The 3-OH-His163 interaction anchors ring C in the oxyanion hole, while the 4'-OH on ring B extends toward Gly143 in the S1' region^[12]. This three-point binding pattern is absent in naringenin, which lacks the C2-C3 unsaturation and consequently cannot adopt the planar geometry needed to reach all three contacts. That structural deficiency explains naringenin's weak docking score and supports the notion that the flavone backbone, rather than the flavanone, is the appropriate scaffold for M^{pro} targeting.

Myricetin's disappointing MD performance, despite a respectable docking score (−8.47 kcal/mol), illustrates why static docking alone can be misleading. The additional 5'-hydroxyl on ring B clashes with Met49 in S2 during the simulation, forcing periodic ligand shifts that lowered its MM-PBSA energy by over 6 kcal/mol compared with quercetin^[8].

From a translational angle, quercetin's main weakness is its low oral bioavailability typically under 2% in humans due to poor solubility and rapid Phase II metabolism^[6]. Liposomal encapsulation or co-administration with piperine have been shown to boost quercetin plasma levels by 3-10-fold^[17]. Whether those levels reach concentrations needed for meaningful M^{pro} inhibition remains an open question that biochemical IC₅₀ data would help answer. The computational evidence here provides a structural rationale for prioritizing quercetin and its relatives (e.g., isoquercetin, rutin) in future enzymatic screens^[18].

Conclusion

Molecular docking of eight dietary flavonoids against SARS-CoV-2 M^{pro} identified quercetin (−8.74 kcal/mol) and myricetin (−8.47 kcal/mol) as the top-ranked candidates. A 100 ns MD simulation confirmed that the quercetin-M^{pro} complex reached conformational stability (RMSD 1.87 Å) with a MM-PBSA binding energy of −34.8 kcal/mol, while myricetin remained dynamically unstable. Three hydrogen bonds between quercetin and catalytically important residues Glu166, His163, and Gly143 persisted with occupancies above 64%. These contacts are conserved across known SARS-CoV-2 variants, suggesting potential cross-variant efficacy. Quercetin's dietary safety, widespread availability, and low cost make it worth advancing to enzymatic and cell-based validation, provided its poor oral bioavailability is addressed through formulation optimization. The flavone scaffold, rather than the flavanone, should be the preferred starting point for M^{pro}-directed natural product screening campaigns.

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