Supplementary Materials

Anti-Influenza A Virus Activity of *Rhododendron brachycarpum* Extract and Identification of Hyperoside as the Active Constituent

Yung Hun Park[1, †], Soo Yong Shin[1, †], Hayeong Choi[1], Jae hyeok Lee[1], You Jin Kim[1], Seong Ji Woo[1] Wonkyun Ronny Im[1], Sung Ho Jeon[1,*]

[1] Department of Life Science and Multidisciplinary Genome Institute, Hallym University, Chuncheon 24252, Republic of Korea;

[*] Correspondence: sjeon@hallym.ac.kr (S.H.J.)

[†] These authors contributed equally to this work.

Binding Site Prediction

To identify potential ligand-binding sites on the influenza A virus hemagglutinin (HA) protein, we used the FTSite server (https://ftsite.bu.edu/), a computational tool for accurate detection of ligand binding hot spots based on structural and energetic criteria (Ngan et al., 2012; Kozakov et al., 2015). The 3D structure of HA (PDB ID: 1RU7) was uploaded to FTSite, and the top-ranked binding pockets were analyzed based on conserved residues and druggability scores. These predicted binding sites were used as guides for docking grid definition and pose interpretation.

The FTSite predictions were cross-validated using FTMap for fragment-based hotspot analysis (Brenke et al., 2009; Jones et al., 2022), confirming the presence of a prominent binding pocket corresponding to the receptor-binding domain (RBD) of HA.

In silico molecular docking analysis

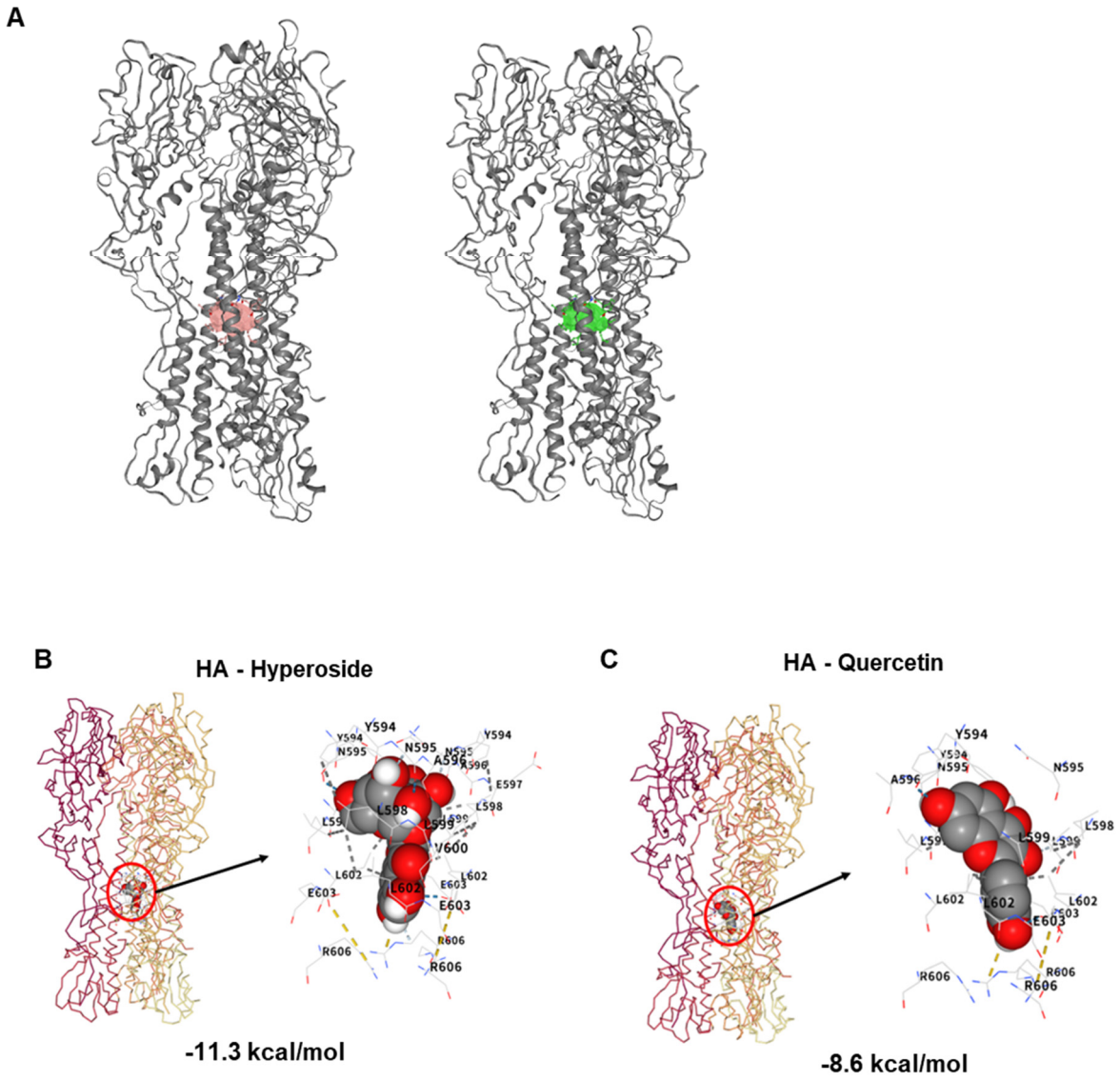
To predict the interaction between hyperoside (PubChem CID: 5281643) and the influenza A virus hemagglutinin (HA) protein, *in silico* molecular docking was performed using CB-Dock2 and FitDock servers. The 3D structure of the HA protein (PDB ID: 1RU7) was retrieved from the Protein Data Bank.

Ligand structures of hyperoside and quercetin were obtained from the PubChem database and converted to 3D conformations using Open Babel. The docking process was conducted using CB-Dock2, which integrates cavity detection, blind docking via AutoDock Vina, and homologous template fitting to improve prediction accuracy (Liu et al., 2022). To validate and cross-check binding predictions, FitDock was also used, which applies a template-based docking algorithm optimized for flavonoid-like structures (Yang et al., 2022).

Among the top five docking results ranked by binding affinity, the best and second-best poses for both compounds were selected for further interaction analysis. Docked poses were

visualized and annotated using PyMOL and LigPlot+. Interaction types, including hydrogen bonds, hydrophobic interactions, and ionic contacts, were analyzed and color-coded (blue, gray, and yellow, respectively).

Docking results were interpreted based on binding energy (kcal/mol) and proximity to the receptor-binding domain of HA, with comparisons made between hyperoside and quercetin.



Supplementary Figure 1. Predicted binding site and additional docking poses for hyperoside and quercetin.

(A) Predicted ligand-binding pocket on the surface of influenza A virus hemagglutinin (HA) protein, identified using FTSite. The binding site corresponds to the receptor-binding domain (RBD), which was used as the docking region in subsequent molecular docking simulations.

(B) Second-best docking pose of hyperoside showing stable interaction with the predicted HA binding pocket. The compound forms multiple hydrogen bonds (blue), hydrophobic interactions (gray), and ionic interactions (yellow) within the RBD.

(C) Second-best docking pose of quercetin within the same predicted binding site. Interaction patterns are visualized using the same color scheme: hydrogen bonds (blue), hydrophobic interactions (gray), and ionic interactions (yellow)