Geraniin as a potential inhibitor of SARS-CoV-2 3CL\textsuperscript{pro}

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ABSTRACT
Geraniin is a polyphenolic compound first isolated from \textit{Geranium thunbergii}. The major protease (M\textsubscript{pro}), namely 3C-like protease (3CL\textsubscript{pro}), of coronaviruses is considered an attractive drug target as it is essential for the processing and maturation of viral polyproteins. Thus, our primary goal is to explore the efficiency of geraniin on 3CL\textsubscript{pro} of SARS-CoV-2 using the computational biology strategy. In this work, we studied the anti-coronavirus effect of geraniin \textit{in vitro} and its potential inhibitory mode against the 3CL\textsubscript{pro} of SARS-CoV-2. We found that geraniin inhibited HCoV-OC43 coronavirus-infected cells during the attachment and penetration phases. Molecular docking and dynamics simulations exhibited that geraniin had a strong binding affinity and high stable binding to 3CL\textsubscript{pro} of SARS-CoV-2. Geraniin showed a strong inhibitory activity on coronavirus and may be a potential inhibitor of SARS-CoV-2 3CL\textsubscript{pro}.

1. Introduction
The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly spread throughout the world (Cao et al., 2020). Currently, there is an urgent need to identify novel anti-viral properties to impede viral pathogenesis in the host system.

The main protease (M\textsubscript{pro}), namely 3C-like protease (3CL\textsubscript{pro}), is essential for the coronaviruses (e.g. SARS-CoV, SARS-CoV-2, and OC43-CoV) replication cycle and represents a highly conserved molecular target for anti-coronavirus drug design (Zhang et al., 2022).
Geraniin (C_{41}H_{28}O_{27}) is a main polyphenolic compound isolated from *Geranium thunbergii* (Wang et al. 2016). Interestingly, an *in silico* docking analysis revealed that the binding affinity of geraniin to 3CL{\textsuperscript{pro}} of SARS-CoV-2 (−10.60 kcal/mol) was higher than that of remdesivir (−9.50 kcal/mol) (Hiremath et al. 2021). Therefore, it is valuable to investigate the antiviral effect of geraniin and its potential mechanism of targeting the 3CL{\textsuperscript{pro}} of SARS-CoV-2. In this study, we investigated the antiviral activity of geraniin against human coronavirus CoV-OC43 *in vitro* and the mechanism by which it mediates its potential inhibitory effect on M{\textsuperscript{pro}}. Molecular dynamics (MD) simulations and docking studies were performed to examine the stability of the protein-ligand complex. In addition, the pharmacokinetics, and pharmacodynamics of geraniin were also studied (See Supplementary Material for Experimental).

2. Results and discussion

The inhibitory effect of geraniin on the human CoV-OC43 coronavirus was examined *in vitro* (Figure S1). A dose of 2.08–33.3 μg/mL geraniin significantly reduced the CPE caused by infected cells. Geraniin could increase the viability of infected HTC-8 cells in a dose-dependent manner (Figure S1C). Additionally, geraniin could significantly inhibit of M{\textsuperscript{pro}} activity at concentrations of 0.625–10 μg/mL and the 50% effective concentration was 15.85 μg/mL (Figure S1D). The results suggested that geraniin could protect HCT-8 cells from coronavirus infection.

The crystal structure of the 2019-nCoV M{\textsuperscript{pro}} complex with boceprevir (PDB ID: 7BRP) was used as a template for molecular docking to further study the potential binding mechanism between M{\textsuperscript{pro}} and geraniin (Fu et al. 2020). As shown in Table S1 in the Supplementary Material, the docking binding energies for geraniin and boceprevir were −12.1 kcal/mol and −7.8 kcal/mol respectively. This finding indicated that the binding ability between geraniin and 3CL{\textsuperscript{pro}} was higher than that of boceprevir. The contribution of different energies in the docking results were analyzed. Van der Waals, desolvation energy, electrostatic energy, and hydrogen bonds (H-bond) were the main components associated within the interaction of boceprevir, geraniin, and M{\textsuperscript{pro}}, respectively. Among these, a vdW + Hbond energy of −51.95 kcal/mol for geraniin was comparable to that of boceprevir (−54.02 kcal/mol). The electrostatic energy of geraniin (−48.20 kcal/mol), was higher than that of boceprevir (−23.47 kcal/mol) (Table S1). These results indicated that geraniin bound with a high affinity to M{\textsuperscript{pro}}. The interactions between geraniin, boceprevir, and M{\textsuperscript{pro}} active site residues were visualized in 2D and 3D diagrams, respectively (Figures S2 and S3 in the Supplementary Material). An abundance of H-bonds were observed between geraniin and the four residues of 3CL{\textsuperscript{pro}} (Figure S3). The molecular docking results showed that geraniin could interact with residues in the active pocket of 3CL{\textsuperscript{pro}} to form a stable complex that inhibited M{\textsuperscript{pro}} activity.

An MD simulation of geraniin with M{\textsuperscript{pro}} was studied to further investigate the stability of complexes (Figure S4 in the Supplementary Material). A 100 ns of simulation trajectory was analyzed for the geraniin, boceprevir, and M{\textsuperscript{pro}} complex, respectively (Figure S5 in the Supplementary Material). The RMSD values of the geraniin, boceprevir, and 3CL{\textsuperscript{pro}} complex did not substantially fluctuate after the 10 ns and 2 ns MD
simulation trajectory, respectively, which tended to stabilize to reach equilibrium (Figure S5A). The root mean square fluctuation (RMSF) curves displayed fluctuations in amino acid residues in the loop region of $M^{\text{pro}}$ with ligands (Figure S5C and D). In addition, no significant conformational fluctuation was observed in the $M^{\text{pro}}$ active site, and the residues bound to geraniin and boceprevir, respectively, were highly stable. The radius of gyration (Rg) was extremely stable during the course of 100 ns by maintaining a highly stable compact (folded) form after geraniin bound to $M^{\text{pro}}$ (Figure S6). Extremely stable and continuous hydrogen bond interactions were formed between geraniin and $M^{\text{pro}}$, which played a crucial role in the stability of the complex (Figure S7). Although the existence of ligands stabilized the secondary structure of 3CL$^{\text{pro}}$, it also led to changes in the secondary structure of residues (Figure S8). Moreover, the interaction between geraniin and $M^{\text{pro}}$ had little effect on the solvent accessible surface area (SASA) of the protein structure (Figure S9). The binding free energy of 3CL$^{\text{pro}}$ with geraniin was $-32.57$ kcal/mol (Table S2 in the Supplementary Material). The entire ligand (geraniin) was found to be trapped like a key in a cavity, consisting of GLU166, GLN192, ALA191, THR169, PRO168, HIS163, THR190, ASN142, MET165, CYS145, SER144 and other factors (Figure S10). During the MD of complex of geraniin-3CL$^{\text{pro}}$, the average short-range coulombic interaction energy (Coul-SR) was $-133.0 \pm 5.7$ kJ/mol and the short-range Lennard-Jones energy (LJ-SR) was $-164.9 \pm 1.4$ kJ/mol. The total interaction energy was $-297.9$ kJ/mol, which was equivalent to that of boceprevir ($-296.6$ kJ/mol). The absolute binding free energy ($\Delta G_b$) of geraniin-$M^{\text{pro}}$ was $-66.55 \pm 0.98$ kJ/mol, a higher value compared to that of boceprevir-$M^{\text{pro}}$ ($-26.41 \pm 1.05$ kJ/mol) (Table S3 in the Supplementary Material). These results demonstrated that the interaction between geraniin and 3CL$^{\text{pro}}$ was extremely stable and strong (Figure S11). The MD simulation results revealed that geraniin and 3CL$^{\text{pro}}$ formed a stable complex. The RMSD, RMSF, SASA, Rg, and secondary protein structures displayed small fluctuations, forming a state of dynamic equilibrium with low energy. Van der Waals energy, nonpolar dissolution, and electrostatic interaction energy played a vital catalytic role in the combination of geraniin and 3CL$^{\text{pro}}$.

Geraniin exhibited poor intestinal absorption and water solubility, whereas blood-brain barrier permeability and plasma protein binding were low. Although geraniin did not exhibit inhibitory effect on the CYP2D6 enzyme, it was toxic to hepatocytes. Geraniin also exhibited low permeability to the BBB and PPB (Tables S4 and S5 in the Supplementary Material). Geraniin had low toxicity and carcinogenicity and did not show signs of skin allergy, but was associated with moderate irritation to the skin and eyes (Table S5). Geraniin did not show any skin sensitization, but had slight irritation to the skin and eyes (Table S5). Therefore, geraniin possessed low toxicity and carcinogenicity. In addition, the results showed that geraniin did not meet 3 of the 5 criteria of Lipinski’s law and have no drug-likeness (Table S6 in the Supplementary Material). Furthermore, the pharmacokinetic prediction results indicated that geraniin had good biological safety and could be used as a potential drug for the treatment of SARS-CoV-2.

COVID-19 pneumonia is characterized by acute pneumonia, and a cytokine storm (i.e. the over-production of interleukin-1, 6, and tumor necrosis factor-$\alpha$) is the main factor leading to the damage and aggravation of local lung inflammation (Fatma et al.)
Geraniin exhibits well-known anti-inflammatory effects in LPS-induced macrophages by inhibiting the NF-κB and Nrf2 signaling pathways (Wang et al. 2016) and inhibitory effect on the entry of SARS-CoV-2 by blocking the interaction between Spike protein RBD (Arokiyaraj et al. 2020). Combined with the above, our findings suggest that geraniin may have a better protective effect against COVID-19.

3. Conclusions
Geraniin exhibited a strong inhibitory activity on coronavirus and may be a potential inhibitor of SARS-CoV-2 3CLpro.

Disclosure statement
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