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In silico prediction of the inhibitory effect of phytochemical components extracted from *Knautia sarajevensis* on the main protease of SARS-CoV-2 virus

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Abstract

Essential role in replication and transcription of coronavirus makes the main protease of SARS-CoV-2 a great traget for drug design. The aim of this study was to predict structural interactions of compounds isolated from the Bosnian-Herzegovinian endemic plant *Knautia sarajevensis* (G. Beck) Szabó against the 3CLpro of SARS-CoV-2 virus. The three-dimensional crystal structure of SARS-CoV-2 main protease was retrieved from the RCSB Protein Data Bank and the three-dimensional structures of isolated compounds were obtained from the PubChem database. Active site was predicted using PrankWeb, while the preparation of protease and compounds was performed using AutoDock Tools and OpenBabel. Molecular docking was carried out using AutoDock Vina. Structural interactions are visualised and analyzed using PyMOL, LigPlus and UCSF Chimera. Apigenin, kaempferol, myricetin and quercetin showed the highest binding affinity for SARS-CoV-2 main protease and formed significant hydrogen bonds with the given protein. Results obtained in this study are in accordance with previous studies and showed that these compounds could potentially have antiviral effects against SARS-CoV-2. These findings indicate that *K. sarajevensis* could be potentially utilized as an adjuvant in the treatment of coronavirus disease 2019, but further pharmacological studies are required in order to prove the potential medicinal use of the plant.

Keywords

In silico, Knautia sarajevensis, phenolic compounds, antiviral effects, SARS-CoV-2 main protease

Introduction

Dipsacaceae family counts 10 genera and around 270 species. Six genera from this family are found in Bosnia and Herzegovina (*Knautia* L.; *Cephalaria* Schrad. Ex Roem. & Schult; *Dipsacus* L.; *Scabiosa* L.; *Succisa* Heller; *Succisella* Beck) (Karalija et al., 2014). Research about potential medicinal properties of genus *Knautia* demonstrates results of *Knautia arvensis* acting as a relaxant and blood purifier, a remedy for different skin disorders, and the plant whose flowers and leaves can be used to make tea for different problems with lungs (Grieve 1931, Mattalia et al., 2013). There is also evidence of *Knautia bidens* being a rich source of phenolic compounds (Alali et al., 2007). *Knautia sarajevensis* (G. Beck) Szabó, biannual plant, is an endemic species of wood margins and woodland meadows of the Dinaric Alps. It is one of the endemic plant species included in the Red List of flora of the Federation of B&H. *Knautia sarajevensis* is closely related to *K. arvensis* and its phenolic content was investigated in a few studies (Karalija et al., 2014; Karalija et al., 2017; Karalija et al., 2018; Karalija et al., 2020).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused COVID-19 infection that was declared as a pandemic in March 2020 by the World Health Organization (Kamel et al., 2021). It is a positive-sense single-stranded RNA virus, extremely contagious in humans. Fourteen open reading frames (ORFs) of SARS-CoV-2 encodes 27 proteins (Wu et al., 2020). One of them is main protease (Mpro, 3CLpro, nsp5) that cleaves the overlapping pp1a and pp1ab polyproteins to functional proteins, which makes it an ideal drug target because that step is critical during viral replication (Ullrich and Nitsche 2020). It contains three domains, where domain II has mainly antiparallel β-barrels, while domain III has a helical arrangement. In the cleft between domain I and II the substrate binding site is located. Residues involved in ligand binding are in the long flexible loop that borders the active site and connects domain II and III (Motyan et al., 2022).

Most of the inhibitors bind to the active site of Mpro, and predictions of compounds with new activities is easiest to be made with a computational tool known as protein-ligand docking. Though the results of docking studies should not be used as the only evidence to predict inhibitors of proteins, it could be a valuable starting point that discriminates decoys from potentially active compounds (Macip et al., 2021). The aim of our study was to investigate antiviral potencies of phenolic compounds of *Knautia sarajevensis* against the main protease of SARS-CoV-2 virus using *in silico* docking method.

Material and methods

The three-dimensional crystal structure of the target protein SARS-CoV-2 $3CL^{pro}$ (PDB ID: 7LZT) was obtained in PDB format from the RCSB Protein Data Bank (Berman et al., 2000; https://www.rcsb.org/). This protein was present in a complex with an inhibitor, therefore the small molecules were first removed and then the main protease structure was used for further analysis. Protein structure was submitted to PrankWeb (Jendele et al., 2019) for the prediction of potential active sites. PrankWeb displayed multiple pockets of which one with the highest score and probability was selected. The best ranked pocket had a score of 25.54 with the highest probability 0.888 and it included the following amino acid residues: Thr24, Thr25, Thr26, Leu27, His41, Cys44, Met49, Phe140, Leu141, Asn142, Gly143, Ser144, Cys145, His163, His164, Met165, Glu166, His172, Asp187, Arg188 and Gln189. To further validate the active sites a review of the

literature was performed and four residues from two highly conserved areas named oxyanion hole (Gly143, Ser144 and Cys145) and catalytic dyad (Cys145 - His41) were determined as active sites (Kumar et al., 2016; Ramos-Guzmán et al., 2021; Selvaraj et al., 2021; Zhou et al., 2021). Preparation of the protein was performed using AutoDock Tools 1.5.6 (Morris et al., 2009), and it included removal of water molecules, addition of polar hydrogen atoms and assignment of Kollman charges. The prepared protein was then converted from PDB to PDBQT format. The gridbox dimensions were 18 x 20 x 18 Å, centered at 7.000, 6.225 and 22.648 and they were defined using AutoDock Tools 1.5.6 for the most accurate capture of the oxyanion hole and catalytic dyad of SARS-CoV-2 $3CL^{pro}$. The value of spacing (ångstrom) was set to 1.0. The 3D structures of 17 compounds and three positive controls were retrieved from PubChem database (Kim et al., 2021; https://pubchem.ncbi.nlm.nih.gov/) in the SDF format and then were prepared and converted to the PDBQT format using OpenBabel 3.1.1 (O'Boyle et al., 2011). Compounds were selected on the basis of the review of a literature about the HPLC analysis of *Knautia sarajevensis* (Karalija et al., 2014; Karalija et al., 2017; Karalija et al., 2018; Karalija et al., 2020). Compounds and their PubChem CIDs used in this study are shown in table 1. Molnupiravir (145996610), nirmatrelvir (155903259) and remdesivir (121304016) were selected as positive controls due to their proven antiviral activity against the SARS-CoV-2 virus (Vangeel et al., 2022). All molecular docking simulations were performed using AutoDock Vina 1.1.2, with an energy range value of 4 and exhaustiveness value of 8 (Trott and Olson, 2010). Intermolecular interactions between the receptor and the selected compounds were analyzed and visualized using UCSF Chimera 1.16, LigPlot+ 2.2.4 and PyMOL 2.5.2 (Pettersen et al., 2004; Laskowski and Swindells, 2011; The PyMOL, 2022).

Results and Discussion

Results of the molecular docking analysis with corresponding binding affinities for each compound and positive controls are shown in table 1. The highest binding affinities for the SARS-CoV-2 main protease were obtained for apigenin (-8.0 kcal/mol), kaempferol (-7.9 kcal/mol), myricetin (-7.9 kcal/mol) and quercetin (-7.9 kcal/mol), so these compounds are selected to analyze the interactions and type of bonds. Binding affinities of positive controls molnupiravir, nirmatrelvir and remdesivir were -7.3, -8.0 and -7.7 kcal/mol, respectively. Visualization of interaction was conducted using PyMOL, LigPlot+ and UCSF Chimera. PyMOL showed the best results and it recorded the largest number of bonds. The results obtained with PyMOL are mostly confirmed using the other two software.

PyMOL results showed that apigenin reacted with Tyr54, Phe140, Leu141, Ser144, His163, His164, Glu166, Asp187 and Arg188, forming three good (yellow) hydrogen bonds with Tyr54 (2.8 Å), Leu141 (2.2 Å) and His163 (2.4 Å). In the same software, kaempferol showed interactions with Tyr54, Phe140, Leu141, Ser144, His163, His164, Glu166, Asp187, Arg188 and Gln189, forming four solid hydrogen bonds with Tyr54 (2.9 Å), Leu141 (2.1 Å), His163 (2.4 Å) and Gln189 (2.1 Å). Myricetin reacted with Leu141, Asn142, Gly143, Ser144, Cys145, His163, His164, Glu166, Arg188, Gln189 and Thr190, forming nine yellow bonds with Leu141 (2.3 Å), Gly143 (2.4 Å and 2.8 Å), Ser144 (2.2 Å), Cys145 (2.5 Å), His163 (2.3 Å), His164 (2.3 Å), Arg188 (3.8 Å) and Gln189 (4.0 Å). Results for quercetin showed that it

Table 1. Best molecular docking results of selected compounds isolated from *Knautia sarajevensis* and positive controls against SARS-CoV-2 main protease

Compound	PubChem CID	Binding affinity (kcal/mol)	Distance from best mode	
			Rmsd l.b.	Rmsd u.b.
4-Hydroxybenzoic acid	15007	-4.9	0.000	0.000
Apigenin	5280443	-8.0	0.000	0.000
Caffeic acid	689043	-5.9	0.000	0.000
Chlorogenic acid	1794427	-7.4	0.000	0.000
Chrysin	5281607	-7.7	0.000	0.000
Ferulic acid	445858	-6.0	0.000	0.000
Galangin	5281616	-7.6	0.000	0.000
Kaempferol	5280863	-7.9	0.000	0.000
Myricetin	5281672	-7.9	0.000	0.000
Naringenin	932	-7.8	0.000	0.000
Pinocembrin	68071	-7.5	0.000	0.000
Quercetin	5280343	-7.9	0.000	0.000
Rosmarinic acid	5281792	-7.0	0.000	0.000
Salicylic acid	338	-4.8	0.000	0.000
Sinapic acid	637775	-6.0	0.000	0.000
Syringic acid	10742	-5.5	0.000	0.000
Vanillic acid	8468	-5.3	0.000	0.000

reacted with Asn142, Gly143, Ser144, Cys145, His163, His164m Glu166, Arg188, Gln189 and Thr190, forming seven good hydrogen bonds with Gly143 (2.4 Å), Ser144 (2.2 Å), Cys145 (2.5 Å), His163 (2.4 Å), Arg188 (3.8 Å), Gln189 (4.0 Å) and Thr190 (2.7 Å). Mentioned interactions for all four components are shown in figure 1.

Taking into acount the active sites, apigenin and kaempferol showed a significant interaction with Ser144, which is one of the three aminoacids that form the oxyanion hole. Besides Ser144, myricetin and quercetin also interacted with Gly143 and Cys145, which are the other two residues of the oxyanion hole, while the amino acid Cys145 is also part of the catalytic dyad (Cys145 - His41).

LigPlot+ results showed that apigenin reacts with Ser144 (2.77 Å) and His163 (3.25 Å), while UCSF Chimera results showed apigenin interaction with Cys44, Cys145 and His163. Kaempferol reacted with Ser144 (2.70 Å), His163 (3.24 Å) and Gln189 (2.96 Å) in LigPlot+ and with Cys44, Cys145 and His163 in UCSF Chimera. Results for myricetin showed that it reacted with Leu141 (2.67 Å and 3.18 Å), Gly143 (2.97 Å), Ser144 (2.87 Å, 2.96 Å and 3.18 Å), Cys145 (3.30 Å) and His163 (3.11 Å). In Chimera, myricetin reacted with Leu141, Ser144, Cys145, His163 and His164. In LigPlot+ quercetin reacted with Leu141 (2.69 Å and 3.16 Å), Gly143 (2.96 Å), Ser144 (2.83 Å, 2.97 Å and 3.22 Å), Cys145 (3.31 Å) and His163 (3.14 Å). In Chimera interactions were with Cys145 and Thr190. Results obtained using LigPlot+ and UCSF Chimera software for four selected compounds are shown in figures 2-5.

In LigPlot+ all four selected compounds interacted with Ser144, while myricetin and quercetin

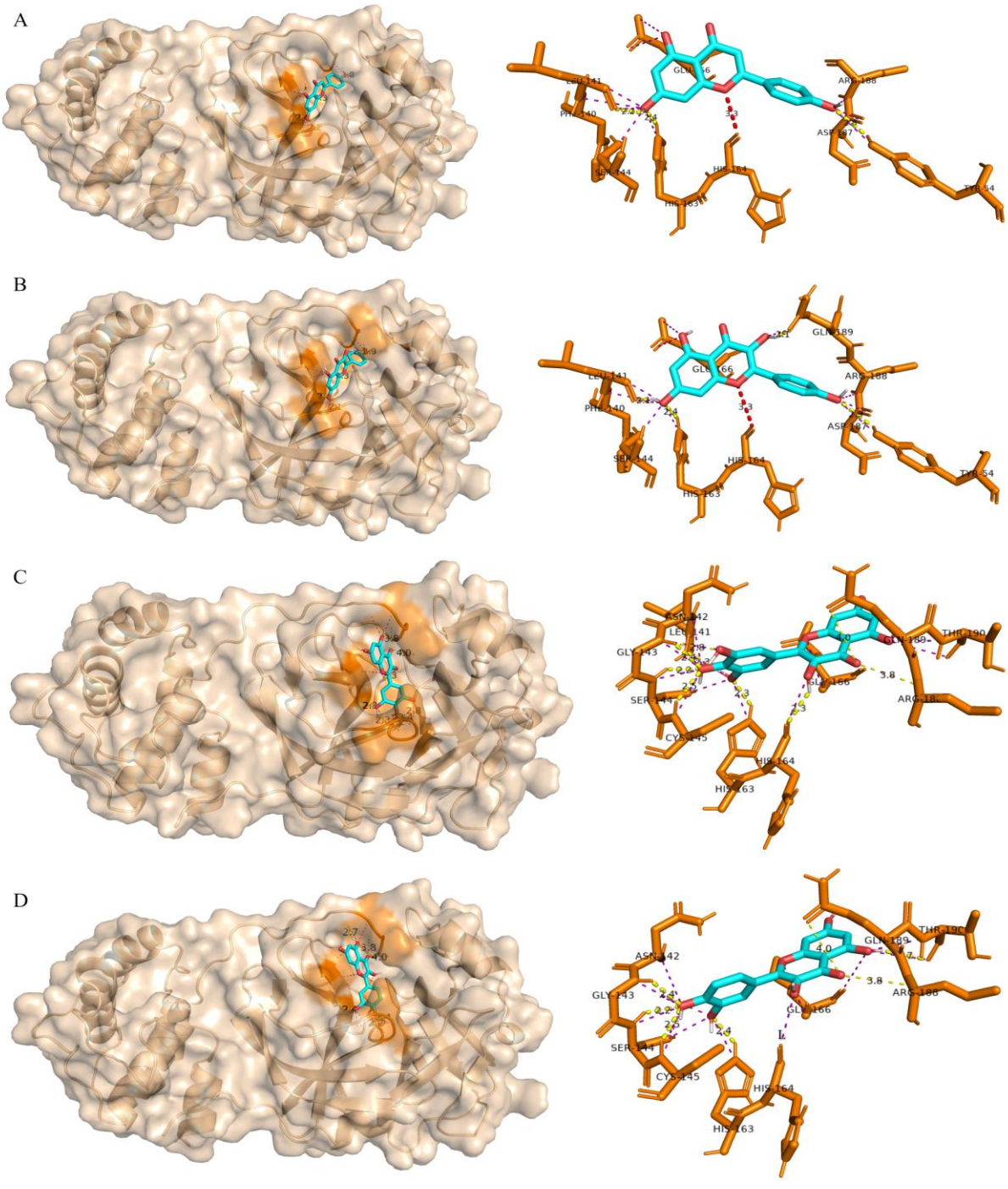

Figure 1. Interactions between SARS-CoV-2 main protease and apigenin (A), kaempferol (B), myricetin (C), and quercetin (D) using PyMOL software. Left – surface view; right – closer look of the interactions

achieved additional interactions with Gly143 and Cys145. In contrast to PyMOL and LigPlot+, by using UCSF Chimera it was shown that all four selected compounds formed significant bonds with Cys145, and in addition, myricetin also interacted with Ser144. In the comparison of the results obtained using PyMOL and LigPlot+, considering the active sites, identical interactions and bonds

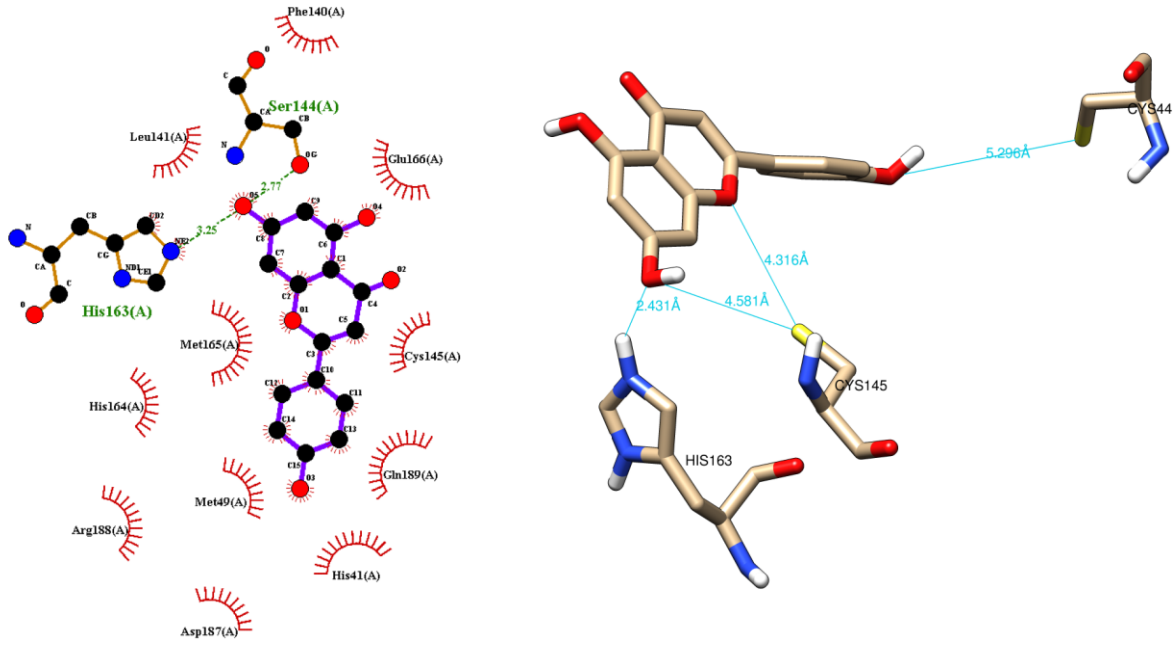

Figure 2. Interactions between SARS-CoV-2 main protease and apigenin. Left – LigPlot+ results; right – UCSF Chimera results

were established. The results of both software showed excellent contacts of myricetin and quercetin with the main protease, where by using PyMOL four hydrogen bonds of myricetin and quercetin with Ser144 were observed, while there were three and two bonds with Gly143, respectively. Using LigPlot+, three hydrogen bonds of myricetin and quercetin were observed with Ser144, and one each for both compounds with Gly143 and Cys145. On the other hand, by using UCSF Chimera, interactions with Cys145 were observed for all four selected components, where it is important to mention that apigenin and kaempferol formed a double bond, while myricetin also formed a bond with Ser144.

Protein-ligand docking is widely used tool in pharmaceutical industry that predicts the position and orientation of a ligand when it interacts with protein receptor. Using molecular docking techniques, large databases of chemicals could be screened virtualy with the purpose of drug candidates selection (Adeoye et al, 2019). Natural medicines are shown to prevent SARS-CoV-2 infection and to improve the recovery of infected patients (Farhat et al, 2022). Results of many studies showed that green tea polyphenols, natural coumarin derivates and many other phytochemicals have higher affinity for Mpro than some drugs (Xiao et al, 2021).

Medicinal properties of Dipsacaceae family and plants from genus *Knautia* have been demonstrated. *Knautia sarajevensis* is rich source of phenolic compounds and in some cases its in vitro shoots has higher antioxidant potential than some species of *Salvia*, *Rosmarinus* and *Echium* genera (Karalija et al.,2017, Karalija et al., 2018). Using literature data on phenolic compounds of *K. sarajevensis*, we identified 17 compounds and used them to perform molecular docking analysis. The best score of binding affinities for the SARS-CoV-2 main protease were for apigenin, kaempferol, myricetin and quercetin.

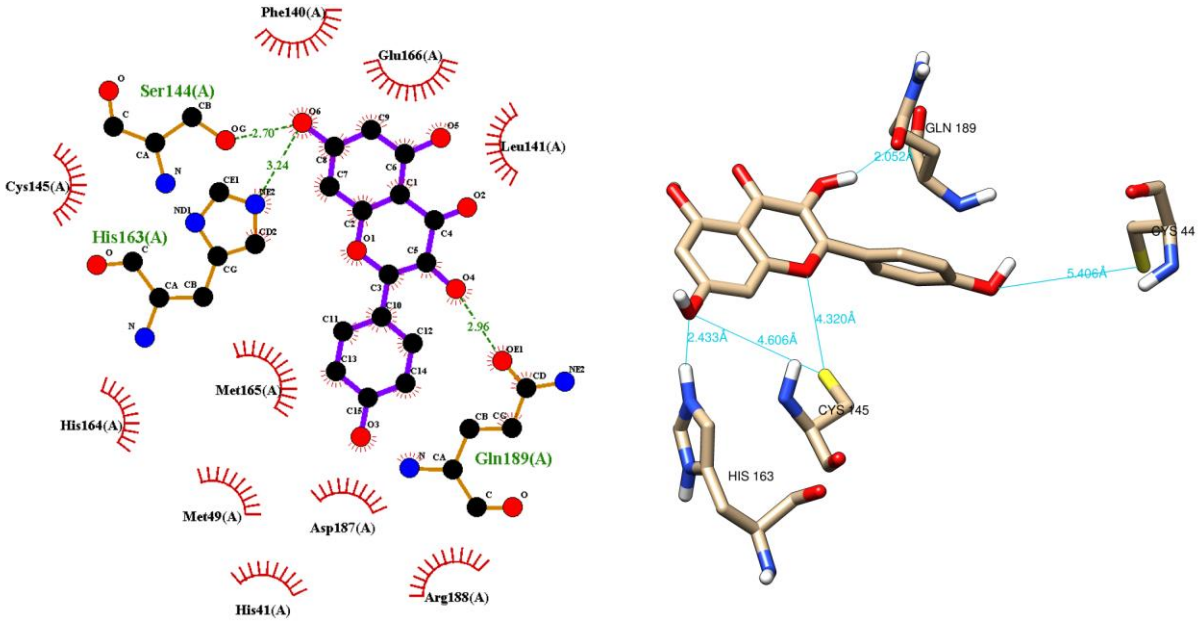

Figure 3. Interactions between SARS-CoV-2 main protease and kaempferol. Left – LigPlot+ results; right – UCSF Chimera results

Potential inhibitory effects of these components are also investigated in other studies. Apigenin and its analogues have been investigated againts SARS-CoV-2 main protease and it is reported that the apigenin 7-glucoside-4'-p-coumarate has the best binding energy and good results are also predicted for its toxicity properties (Farhat et al., 2022). Computational chemistry methods predicted good therapeutic profile of apigenin's druggability, and toxicity predictions showed that it is safe (Matondo et al., 2021). Khan et al. (2021) reported that docking predictions of kaempferol confirmed its interaction with M^{pro}active site, even in different conformations. Their experiment

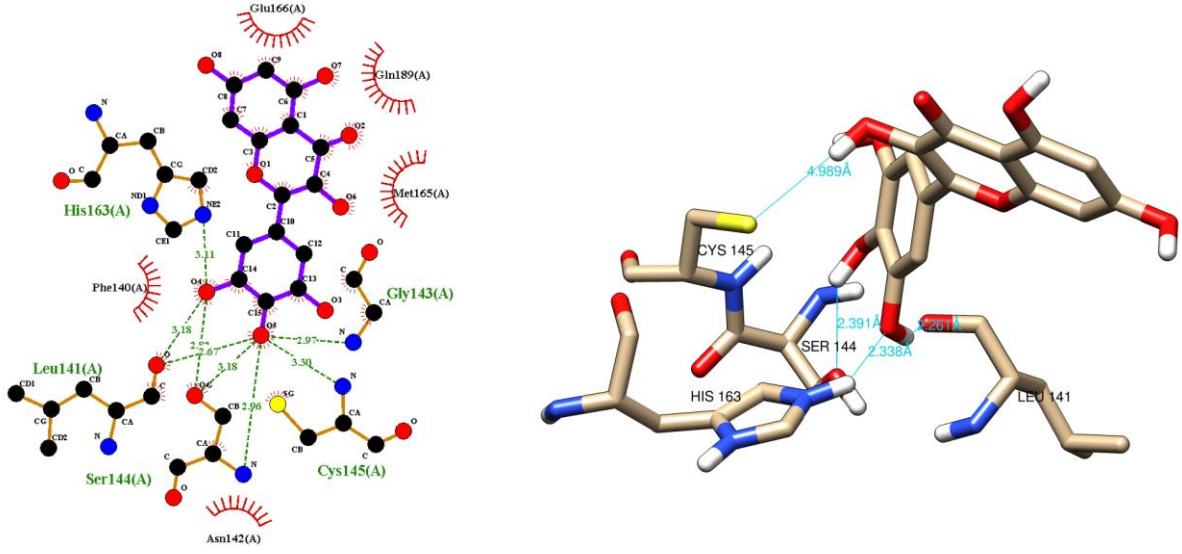

Figure 4. Interactions between SARS-CoV-2 main protease and myricetin. Left – LigPlot+ results; right – UCSF Chimera results

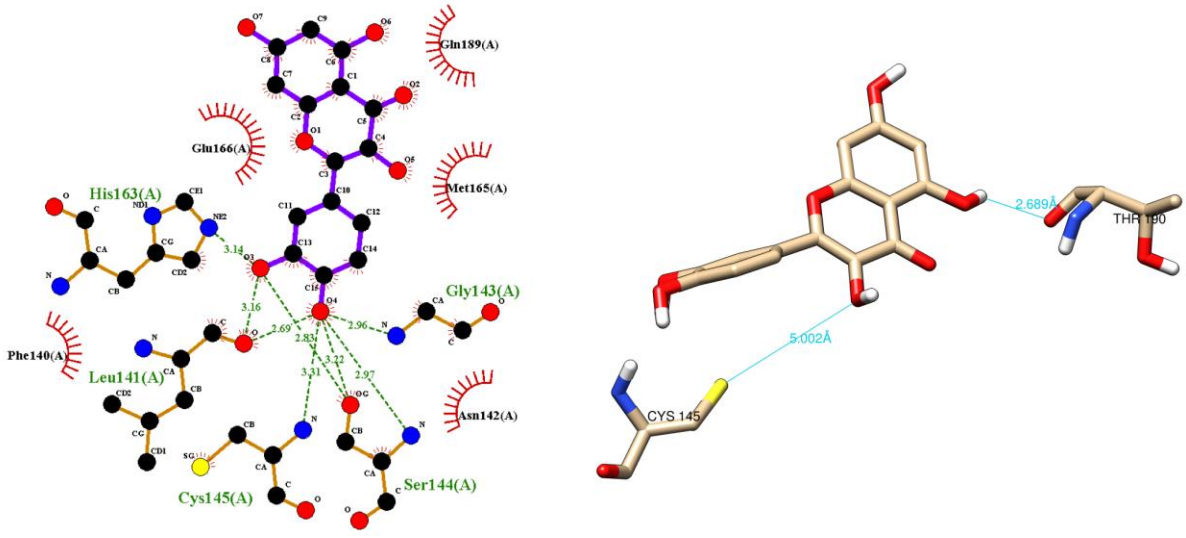

Figure 5. Interactions between SARS-CoV-2 main protease and quercetin. Left – LigPlot+ results; right – UCSF Chimera results

with Vero E6 cells have shown kaempferol's ability to protect cells from virus-induced cell death. Also, favorable ADMET profile and no carcinogenic, mutagenic, cytotoxic or hepatotoxic tendency were also predicted using computational techniques (Johnson et al., 2022). Myricetin was identified as a potential inhibitor by molecular docking, but also with enzymatic assay in the study of Xiao et al. (2021). They also reported potent antiinflammation effect of myricetin on bleomycin-treated mice. Antiviral activities of quercetin are shown against rauscher murine leukemia virus (RLV), human immunodeficiency virus (HIV), rhinoviruses (RV) and many other viruses. It is reported that there are research and clinical trials that investigate quercetin as a potential drug against SARS-CoV-2 (Saakre et al., 2021). Rehman et al. (2021) found that quercetin and kaempferol interact at the substrate binding site of the main protease, located at the interface of domains I and II. Kaempferol with a binding affinity of -7.8 kcal/mol formed two hydrogen bonds with Leu141 and Gln189, one Pi-donor hydrogen bond and two hydrophobic interactions. Quercetin with a binding affinity of -7.5 kcal/mol formed three hydrogen bonds with Ser144, His163 and Gln198, one Pi-Donor hydrogen bond and two hydrophobic interactions. Also, they reported that kaempferol and quercetin are the most potential natural compounds that can inhibit the activity of the main protease, because of their interactions (Rehman et al., 2021). Panchariya et al. (2021) reported that in the presence of quercetin, there is an enhanced antiviral effect of Zn+2 ions, where quercetin in twice the molar concentration of zinc acetate results in more than twice the antiviral effect.

Limited bioavailability of flavonoids and tendency to aggregate limit their therapeutic usage (Kaul et al. 2021). Micropropagation protocols and elicitation for enhancement of metabolite production in *K. sarajevensis* are already done (Karalija et al., 2017, Karalija et al., 2018, Karalija et al., 2020) and it raises the possibility of survival of this endemic plant.

Conclusion

High binding affinity with significant bonds of apigenin, kaempferol, myricetin and quercetin isolated from *K. sarajevensis* against main protease of SARS-CoV-2 virus demonstrated the

potential therapeutic use of this plant. Further evaluation using *in vitro* and *in vivo*, consequently also clinical studies, are needed to prove the potential medicinal use of the plant in the treatment of infected patients.

Conflict of interest

Authors declare no conflict of interest.

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