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Caffeic acid derivatives (CAFDs) as inhibitors of SARS-CoV-2: CAFDs-based functional foods as a potential alternative approach to combat COVID-19

Şevki Adem a, Volkan Eyupoglu a, Iqra Sarfraz b, Azhar Rasul b,*, Ameer Fawad Zahoor c, Muhammad Ali d, Mohnad Abdalla e, Ibrahim M Ibrahim f, Abdo A Elfiky f

a Department of Chemistry, Faculty of Sciences, Çankırı Karatekin University, 18100 Çankırı, Turkey
b Cell and Molecular Biology Lab, Department of Zoology, Faculty of Life Sciences, Government College University Faisalabad, 38000 Faisalabad, Pakistan
c Department of Chemistry, Faculty of Life Sciences, Government College University Faisalabad, 38000 Faisalabad, Pakistan
d Vice Chancellor, Quaid-e-Azam University (QAU), Islamabad
e Key Laboratory of Chemical Biology (Ministry of Education), Department of Pharmaceutics, School of Pharmaceutical Sciences, Cheeloo College of Medicine, Shandong University, 44 Cultural West Road, Shandong Province 250012, PR China
f Biophysics Department, Faculty of Sciences, Cairo University, Giza, 12613, Egypt

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ABSTRACT

Background: SARS-CoV-2, an emerging strain of coronavirus, has affected millions of people from all the continents of the world and received worldwide attention. This emerging health crisis calls for the urgent development of specific therapeutics against COVID-19 to potentially reduce the burden of this emerging pandemic.

Purpose: This study aims to evaluate the anti-viral efficacy of natural bioactive entities against COVID-19 via molecular docking and molecular dynamics simulation.

Methods: A library of 27 caffeic-acid derivatives was screened against 5 proteins of SARS-CoV-2 by using Molegro Virtual Docker 7 to obtain the binding energies and interactions between compounds and SARS-CoV-2 proteins. ADME properties and toxicity profiles were investigated via www.swissadme.ch web tools and Toxtree respectively. Molecular dynamics simulation was performed to determine the stability of the lead-protein interactions.

Results: Our obtained results has uncovered khainaoside C, 6-O-Caffeoylarbutin, khainaoside B, khainaoside C and vitexfolin A as potent modulators of COVID-19 possessing more binding energies than nelfinavir against COVID-19 Mpro, Nsp15, SARS-CoV-2 spike S2 subunit, spike open state and closed state structure respectively. While Calceolarioside B was identified as pan inhibitor, showing strong molecular interactions with all proteins except SARS-CoV-2 spike glycoprotein closed state. The results are supported by 20 ns molecular dynamics simulations of the best complexes.

Conclusion: This study will hopefully pave a way for development of phytonutrients-based antiviral therapeutic for treatment or prevention of COVID-19 and further studies are recommended to evaluate the antiviral effects of these phytochemicals against SARS-CoV-2 in vitro and in vivo models.

Introduction

A severe respiratory coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) has emerged as a pandemic at the end of 2019 (Du et al., 2020; Zhu et al., 2020). By July 2020, SARS-CoV-2 has affected more than 200 territories, infecting more than 13 million individuals and causing more than 0.5 million deaths (WHO, Situation Report–177). COVID-19 has marked the history with third life-threatening coronavirus epidemic into the human population during 21st century (Guo et al., 2020). By 7th January, 2020, Chinese scientists released the sequenced SARS-CoV-2 genome for the identification and development of potential candidates against COVID-19 by computational methods and other therapeutic techniques (Lu et al., 2020).

The SARS-CoV-2 is a β-coronavirus, enveloped and positive sense-RNA virus, with ~30 kb genome (Wu et al., 2020). SARS-CoV-2 genome possesses a complex organization encoding various structural as well as non-structural proteins (Nsps) (Kim et al., 2020a). Majority part of viral genome (replicase ORF1ab encompassing Nsps) is translated into two overlapping polyproteins known as pp1a and pp1ab.
Fig. 1. Chemical structures of caffeic acid and its derivatives.
These polypeptides also code for ~306 amino acid long main protease (M<sub>pro</sub>) (Liu and Wang, 2020), which digests polypeptides at various conserved sites yielding 16 functional viral Nsps possessing multiple enzymatic activities especially in viral replication (Amoretti et al., 2002; Kim et al., 2020a). One of such enzymatic protein, Nsp15, is an endoribonuclease known to be indispensable for protein interference during innate immune response (Kim et al., 2020a). Due to functional importance of M<sub>pro</sub> and Nsp15 in viral replication and survival, both could be potential therapeutic drug targets to combat COVID-19.

In addition to Nsps, SARS-CoV-2 genome also consists of structural protein encoding genes including S (spike) gene, E gene (viral envelope protein), and N (nucleo-capsid protein) gene (Khan et al., 2020). Viral spike proteins possess strong affinity with the human ACE2 (angiotensin-converting enzyme 2) receptor by which virus fuses with target membrane to gain entry into human cells (Hussain et al., 2020). SARS-CoV-2 fusion potential and ACE2 affinity is much greater as compared to SARS-CoV, suggesting that the SARS-CoV-2 fusion machinery is a novel target for coronavirus fusion inhibitors. S protein binds ACE2 receptor via its S1 subunit, while its S2 subunit interacts to form fusion core, which brings viral and target cell membranes into proximity for efficient fusion and subsequent infection (Xia et al., 2020). Thus, SARS-CoV-2 S2 subunit could be a potential target for coronavirus fusion inhibitors. Moreover, S1 trimeric crowns removal or opening is expected to be essential for exposure of receptor binding domain (RBD) to ACE2 receptor and S2 conformational changes which enable binding and membrane fusion (Walls et al., 2020b) suggesting that SARS-CoV-2 spike ectodomain structure and SARS-CoV-2 spike closed state glycoprotein structure might be novel therapeutic targets to develop anti-COVID-19 drugs.

Growing evidences have established the worth of polyphenols as lead compounds for drug discovery against various human diseases (Dos Santos et al., 2018). Recent studies reported that polyphenol have potential to combat with COVID-19 (Adem et al., 2020). Caffeic acids are one of the abundant plant-based polyphenols possessing 2 phenolic hydroxyl moieties and commonly found in coffee, fruits and vegetables (Magnani et al., 2014). Caffeic acids have been reported for their potent virucidal activity against herpes simplex virus (Langland et al., 2018), SFTS (severe fever with thrombocytopenia syndrome) virus (Ogawa et al., 2018), and influenza virus (Usunomiya et al., 2014). Based upon these results, we have screened a library of caffeic acid derivatives (CAFDs) (Fig. 1) for the identification of novel natural anti-COVID-19 compounds against various SARS-CoV-2 drug targets including COVID-19 M<sub>pro</sub> (6LU7), SARS-CoV-2 S2 subunit (6LXT), Nsp15 endoribonuclease (6VWW), SARS-CoV-2 spike ectodomain open state structure (6VYB), and SARS-CoV-2 spike closed state glycoprotein structure (6VXX). Our results present in silico-based identification of khainaoside C, 6-O-Caffeoylarbutin, khainaoisde B, khainaoisde C and vitexol A as potent modulators of COVID-19 M<sub>pro</sub>, Nsp15, coronavirus fusion protein, spike open state and closed state structure respectively. Our findings will provide valuable data for exploration and development of caffeic acid-derivatives as lead structures, novel therapeutic and prophylaxis agents against COVID-19 in the near future.

**Methods**

To obtain binding interactions between CAFDs and binding pockets of 5 different proteins of SARS-CoV-2, five independent docking analyses were performed by using Molegro Virtual Docker (MVD) software in a computer cluster system provided by EXPER, model-PQC-01266 running Intel Core i3-2100 CPU @3.10GHz Processor, 64 BIT, 4 GB RAM, 1TB hard disk, and NVIDIA GeForce GT 630 Graphic card. The crystal structures of the following SARS-CoV-2 proteins were retrieved from the protein data bank web site (http://www.rcsb.org/pdb): SARS-CoV-2 M<sub>pro</sub> (PDB ID: 6LU7: Resolution 2.16 Å) (Jin et al., 2020), Nsp15 endoribonuclease (PDB ID: 6VWW) (Kim et al., 2020b), coronavirus...
Fig. 2. Docking poses of caffeic acid derivatives with COVID-19 virus M\textsuperscript{pro} (A) Hydrogen bonding interactions of khionaoside C, scrophuloside B, vitexfolin A, calceolarioside B and calceolarioside C with amino acid residues of COVID-19 virus M\textsuperscript{pro}, (B) 2D view of interaction types of khionaoside C, scrophuloside B, vitexfolin A, calceolarioside B and calceolarioside C with surrounding amino acids of COVID-19 virus M\textsuperscript{pro}.
Table 2

| Ligand                          | MolDock Score | Protein-Ligand Interactions | Internal Ligand Interactions | H-Bond Score |
|--------------------------------|---------------|----------------------------|-----------------------------|--------------|
| Nelfinavir                      | -148.413      | -176.918                   | 28.5042                     | -6.24452     |
| 6-O-Caffeoylbutin               | -171.541      | -192.585                   | 21.0444                     | -21.774      |
| Dodecanoside A                  | -168.82       | -189.321                   | 20.5011                     | -20.0539     |
| Calceolarioside B               | -164.77       | -178.002                   | 13.2237                     | -16.1947     |
| Scrophuloside B                 | -163.023      | -188.728                   | 25.7045                     | -17.7        |
| Calceolarioside A               | -157.557      | -191.866                   | 34.3089                     | -22.6471     |
| Calceolarioside D               | -157.531      | -176.714                   | 19.1827                     | -13.5793     |
| Robustaside D                   | -152.212      | -166.649                   | 14.4366                     | -13.1528     |
| Vitefoxin A                     | -151.664      | -174.554                   | 22.8901                     | -14.0706     |
| Eutigoside A                    | -150.42       | -173.682                   | 23.2626                     | -14.3851     |
| Robustaside E                   | -150.175      | -181.359                   | 31.1841                     | -20.7424     |
| Chicoric acid                   | -149.662      | -165.153                   | 15.4907                     | -9.06476     |
| Prenyl caffeate acid            | -148.42       | -160.41                    | 11.9901                     | -11.2875     |
| Methyl (2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enyl]oxypropanoate | | | | |
| Khaínoside B                    | -146.216      | -159.431                   | 13.2149                     | -18.838      |
| Khaínoside C                    | -144.449      | -169.295                   | 24.8469                     | -14.338      |
| Calceolarioside C               | -143.258      | -190.295                   | 47.0315                     | -26.9485     |
| Methyl (2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enyl]oxypropanoate | | | | |
| Cynarin                         | -136.239      | -168.638                   | 32.3994                     | -9.84515     |
| Dactylic acid                   | -124.395      | -135.853                   | 11.4856                     | -11.8072     |
| Chlorogenic acid                | -117.22       | -146.224                   | 29.004                      | -19.2892     |
| Fertaric acid                   | -115.811      | -131.072                   | 15.2609                     | -10.3567     |
| Neochlorogenic acid (NCHL)      | -114.913      | -140.215                   | 25.3047                     | -13.8222     |
| Prenyl caffeate acid            | -113.339      | -118.376                   | 5.03646                     | -9.94435     |
| Costaric acid                   | -112.946      | -123.273                   | 10.3279                     | -9.20319     |
| Caftaric acid                   | -110.286      | -130.278                   | 19.9917                     | -13.6044     |
| p-Coumaric acid                 | -90.4353      | -98.5634                   | 8.12807                     | -7.41811     |
| Caffeic acid                    | -86.1121      | -92.4587                   | 6.34661                     | -7.61903     |
| Ferulic acid                    | -78.5357      | -83.0275                   | 4.49178                     | -4.65339     |

Results of the docking of CAFDs on the crystal structure of Nsp15 endoribo nuclease (6VWW).

The docking x y z positions of the proteins were identified as-10.87 15 68.21 for 6LU7 (radius 15 Å) -68.51 29.06 29 for 6VWW (radius 14 Å), -20 19 -25 for 6LXT (radius 22 Å), 217 195 265 for 6VXX (radius 24 Å) and 231 185 168 for 6VYB (radius 21 Å). MolDock Score was selected at the scoring function and the search algorithm. After docking, energy minimization and H-bond optimizations were performed. The docking simulation was repeated for each ligand 20 times. The top binding scores were utilized for further analysis. Also, Discovery Studio Visualizer 2020 was used for in-depth analysis of docking results.

The chemical structures of selected compounds were received at 3D SDF conformer from the PubChem site. PubChem IDs of examined compounds are given respectively; Khainaoside C (44606078), Calceolarioside B (5273567), Vitefoxin A (10458788), Calceolarioside C (45360240), Scrophuloside B (11712581), Cynarin (CYN) (5281769), Eutigoside A (10026568), Calceolarioside D (14015431), Robustaside D (38358972), Chicoric acid (CHA) (5281764), Robustaside E (50994836), Dodecanoside A (44513070), 6-O-Caffeoylbutin (15689808), Khainaoside B (44606238), Calceolarioside A (5273566), Propyl 3-(3,4-dihydroxyphenyl)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enyl]oxypropanoate (11956645), Dactylic acid (6124136), Neochlorogenic acid (NCHL) (5280633), Fertaric acid (FTA) (22298372), Prenyl caffeate acid (5281790), Caftaric acid (CF) (6440397), Chlorogenic acid (CHL) (1794427), Cau taric acid (CTA) (5751924), Caffeic acid (689043), p-Coumaric acid (637542), Ferulic acid (4458584). 3D SDF structures were prepared with ChemBio3D or MarvinSketch for simple molecules.

**ADME and toxicity prediction**

In silico ADME analysis was conducted to investigate physicochemical properties of potent hits, such as water solubility, lipophilicity and pharmacokinetics by using following website http://www.swissadme.ch (Daina et al., 2017). Absorption (% ABS) of potent hits from intestine was evaluated by: % ABS = 109 × (0.345xTPSA). Toxicity analysis was performed using offline software Toxtree 3.1 application (Zhao et al., 2002).

**Molecular dynamics study**

The structures of the best-docked complex for each protein are selected for in-depth molecular dynamics simulation (MDS) study for a period of 20 ns. NAMD software was utilized to conduct the MDS with CHARMM 36 force field (Huang and MacKerell, 2013; Phillips et al., 2005). VMD is used to prepare the complexes for the MDS (Humphrey et al., 1996). Complexes are subjected to equilibration using the CHARMM GUI web server after that a production run for 20 ns is performed on Shaheen supercomputer of King Abdullah University of Science and Technology (KAUST) under the project number k1482 (Jo et al., 2008). The equilibration is done on the protein-small molecule solvated in the TIP3P water model and 0.154 M NaCl solution at 310 K temperature and pH 7 (Mark and Nilsson, 2001). VMD is utilized in trajectories analysis, while the Chimera software of UCSF is used for cluster analysis (Mark and Nilsson, 2001; Pettersen et al., 2004). After trajectory clustering, the five most populous clusters are represented by a conformation and tested for its binding to the protein. AutoDock Vina software is used in the binding energy calculations using 40 Å × 40 Å × 40 Å box dimensions (Morris et al., 2009; Trott and Olson, 2010).
Fig. 3. Docking poses of caffeic acid derivatives with COVID-19 virus Nsp15 endoribonuclease (A) Hydrogen bonding interactions of 6-O-Caffeoylarbutin, calceolarioside B, dodegranoside A, calceolarioside A and scrophuloside B with amino acid residues of virus Nsp15 endoribonuclease (B) 2D view of interaction types of 6-O-Caffeoylarbutin, calceolarioside B, dodegranoside A, calceolarioside A and scrophuloside B with surrounding amino acids of COVID-19 virus Nsp15 endoribonuclease.
Results

The MolDock Scores obtained from the docking studies of CAFDs and 6LU7 are summarized in Table 1. Based on these in-silico results, khaınaosıde C, calceolarioside B, vitexfolin A, calceolarioside C and scrophuloside B exhibited best binding potential with COVID-19 virus M<sup>pro</sup> having MolDock scores of -191.5990, -191.2950, -186.2820, -178.5540 and -177.7990 respectively. Khaınaosıde C showed the highest binding affinity at the active site. It forms hydrogen bond interactions with His 41, His 163, Met 165, Arg 188, Glu 192, Gly 193, Arg 187, Glu 192, Thr 190, Arg 188, Glu 192, Cys 145, Gly 143, Ser 144, Leu 141 (Fig. 2A). Calceolarioside B binds to SARS-CoV-2 M<sup>pro</sup> by forming hydrogen bonds with His 41, His 163, His 164, Met 165, Asp 187, Glu 192, Thr 190, Arg 188, Glu 192, Gly 193, Arg 188, Glu 192, Cys 145, Gly 143, Ser 144, Leu 141 (Fig. 2A). Vitexfolin A and calceolarioside C also showed good binding affinities to the active sites of COVID-19 virus M<sup>pro</sup> via interacting with Met 49, Tyr 54, Cys 44, His 163, His 164, Arg 188, Glu 192, Gly 193, Arg 188, Glu 192, Cys 145, Ser 144, Leu 141, Phe 140 (Fig. 2A) and His 41, Tyr 54, Thr 26, His 163, His 164, Met 165, Asp 187, Glu 192, Thr 190, Arg 188, Glu 192, Gly 193, Arg 188, Glu 192, Cys 145, Gly 143, Ser 144, Leu 141 respectively (Fig. 2A). According to the obtained results, khaınaosıde C and calceolarioside B affinities with COVID-19 virus M<sup>pro</sup> and their scores are significantly higher than Nelfinavir. Ligand plot in Fig. 2B represents residual wise van der Waals interactions, pi-alkyl interactions, pi-pi interactions and pi-sulfur interactions of potent hits with M<sup>pro</sup>.

The MolDock Scores obtained from the docking studies of CAFDs and 6VWW are provided in Table 2. 6-O-Caffeoylarbutin, dodegranoside A, calceolarioside B, scrophuloside B and calceolarioside A possess significantly good binding potential to Nsp15 endoribonuclease with MolDock Scores -171.541, -168.82, -164.77, -163.023 and -157.557 respectively as compared to Nelfinavir which possess MolDock score of -148.413. The interactions of these compounds with amino acid residues of target protein are shown in Fig. 3A. Fig. 3B represents residual wise van der Waals interaction, pi-alkyl interactions, pi-cation and pi-anion interactions are presented in Fig. 3B.

Table 3 presents MolDock Score, interactions and H-Bonds obtained from the docking studies of CAFDs with 6LXT. The results show highest binding potential of khaınaosıde B (-150.44), followed by choric acid (-150.017), vitexfolin A (-149.558) 6-O-Caffeoylarbutin (-146.12) and calceolarioside C (-148.747). The amino acid residues of spike protein participating in interactions with these compounds are presented in Fig. 4A. According to the obtained results, khaınaosıde C, calceolarioside B, scrophuloside B and calceolarioside A possess significantly good binding potential to COVID-19 virus M<sup>pro</sup> via interacting with Tyr 49, Tyr 54, Cys 44, His 163, His 164, Arg 188, Glu 192, Gly 193, Arg 188, Glu 192, Cys 145, Gly 143, Ser 144, Leu 141 respectively (Fig. 2A). The binding interactions of potent compounds with S2 subunit of fusion protein are shown in Fig. 4A. Fig. 4B shows residual wise van der Waals interactions, pi-alkyl interactions, pi-pi interactions, amide-pi-stacked interactions and pi-anion interactions of potent hits with 6LXT.

Table 4 shows the obtained results from the docking studies of CAFDs with crystal structure 6VYB. The results show highest binding potential of khaınaosıde C (-166.448), followed by khaınaosıde B (-165.435), and calceolarioside B (-153.135), calceolarioside C (-151.284), and calceolarioside D (-149.841) with comparison to reference drug Nelfinavir (-148.747). The amino acid residues of spike glycoproteins that interact with target protein via van der Waals interactions, pi-alkyl interactions, pi-pi interactions, amide-pi-stacked interactions and pi-sigma interactions (Fig. 5B).

The binding energies obtained from the docking analysis of 6VXX with ligands are presented in Table 5. Vitexfolin A, choric acid, eutıgosıde A exhibited the best potential against spike glycoproteins of SARS-CoV-2. According to the results of docking analysis, the compounds, which have a better affinity to bind with spike glycoproteins than Nelfinavir (Table 5). The ligand-protein interactions are shown in Fig. 6A. Amino acid residues of 6VXX interact with CAFDs via van der Waals interaction, pi-alkyl interactions, pi-pi interactions and pi-sigma interactions are (Fig. 6B).

Among all the screened compounds, only calceolarioside B possess good binding affinities with four out of five selected targets of SARS-CoV-2 (COVID-19 virus M<sup>pro</sup> and Spike S2 subunit, spike ectodomain (open state), and Nsp15 endoribonuclease) while vitexfolin A exhibits...
Fig. 4. Docking poses of caffeic acid derivatives with COVID-19 virus fusion protein S2 subunit (A) Hydrogen bonding interactions of khaınösıde B, chicoric acid, vitexfolin A, 6-O-Caffeoylarbutin, and calceolarioside B with amino acid residues of fusion protein S2 subunit (B) 2D view of interaction types of khaınösıde B, chicoric acid, vitexfolin A, 6-O-Caffeoylarbutin, and calceolarioside B with surrounding amino acids of COVID-19 virus fusion protein S2 subunit.
Table 4

Results of the docking of CAFDs on the crystal structure of SARS-CoV-2 spike ectodomain structure (open state) (6VYB).

| Ligand Name                        | MolDock Score | Protein-Ligand Interactions | Internal Ligand Interactions | H-Bond Score |
|------------------------------------|---------------|-----------------------------|-----------------------------|---------------|
| Nelfinavir                         | -148.747      | -176.89                     | 28.1432                     | -2.49896      |
| Khaanaoside C                      | -166.448      | -161.803                    | -4.64496                    | -8.71808      |
| Khaanaoside B                      | -165.435      | -193.843                    | 28.408                      | -9.1829       |
| Calceolariside B                   | -153.135      | -177.198                    | 24.0631                     | -11.6422      |
| Calceolariside C                   | -151.284      | -203.133                    | 51.8465                     | -15.5073      |
| Calceolariside D                   | -149.841      | -171.435                    | 21.5938                     | -6.20592      |
| Eutigoside A                       | -144.298      | -150.757                    | 6.45875                     | -8.94751      |
| Dodegranoside A                    | -142.831      | -161.943                    | 19.1118                     | -6.51745      |
| Vitexofolin A                      | -142.604      | -173.882                    | 31.278                      | -12.4266      |
| Chicoric acid                      | -141.566      | -164.796                    | 23.2302                     | -6.02961      |
| Propyl 3-(3,4-dihydroxyphenyl)-2-[(E)-3-(3,4-dihydroxyphenyl)-prop-2-enoyl]oxypropanoate | -139.154 | -149.85 | 10.6965 | -6.13396 |
| Robustaside E                      | -132.278      | -154.031                    | 21.7532                     | -4.63369      |
| Calceolarioside C                  | -133.035      | -170.659                    | 37.6243                     | -13.4663      |
| Robustaside D                      | -133.688      | -170.659                    | 37.6243                     | -13.4663      |
| Calceolarioside B                  | -133.964      | -180.052                    | 46.0882                     | -3.61955      |
| Methyl (2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-(3,4-dihydroxyphenyl)-prop-2-enoyl]oxypropanoate | -129.274 | -159.233 | 29.959 | -5.75544 |
| Neochlorogenic acid                | -116.183      | -137.163                    | 20.9802                     | -8.85752      |
| Dactylifric acid                   | -114.666      | -128.192                    | 15.3263                     | -5.90671      |
| Ferratic acid                      | -111.217      | -123.587                    | 12.3705                     | -2.59398      |
| Cafaric acid                       | -109.539      | -124.99                     | 15.4504                     | -9.02851      |
| Premyl caffeate acid               | -109.296      | -118.722                    | 9.42623                     | -3.47987      |
| Chlorogenic acid                   | -108.993      | -132.887                    | 23.8942                     | -8.6906       |
| Costaric acid                      | -103.861      | -113.724                    | 9.86261                     | -2.97925      |
| p-Coumaric acid                    | -87.9961      | -96.8163                    | 8.82013                     | -4.60597      |
| Caffeic acid                       | -85.3883      | -91.7692                    | 6.38084                     | -7.06512      |
| Ferulic acid                       | -83.9251      | -89.969                     | 6.04391                     | -2.71179      |

Molecular dynamics simulation

Fig. 9A shows the Root Mean Square Deviation (RMSD) in Å, Radius of Gyration (RoG) in Å, and Surface Accessible Surface Area (SASA) in Å². The values of the three parameters indicate the equilibration of the systems during the 20 ns MDS. On the other hand, Fig. 9B shows the stability of the residues during the simulation through per residue Root Mean Square Fluctuation (RMSF) in Å. Dashed-red lines indicate the positions of the active site residues. Fig. 9C shows the average binding energies obtained for the representative conformation for each cluster after the MDS run for 20 ns on the protein-ligand complexes.

Discussion

Polyphenols possess various beneficial properties against viral diseases such as modulation of immune system (Jasso-Miranda et al., 2019), inhibiting viral replication (Lalani and Poh, 2020) and reduction of viral uptake by target membrane (Vazquez-Calvo et al., 2017). CAFDs are polyphenolic compounds which possess prominent antiviral activity especially against hepatitis B virus (HBV) (Zhang et al., 2014) and HIV (Pluymers et al., 2000). Keeping in view the potential of CAFDs against viral diseases (Fig. 1), 27 CAFDs were docked with 5 proteins of SARS-CoV-2 to evaluate their binding energies against these targets. Out of 27 CAFDs, 19 of them showed greater MD score (ranging between -191 to -159, Table 1) against COVID-19 virus M\(^{pro}\), Fusion S2 subunit and spike protein. Based upon these findings, calceolariside B could be regarded as pan inhibitor of SARS-CoV-2 proteins.

Relative assessment of MolDock scores were performed with (β), of CAFDs against SARS-CoV-2 proteins encoded by PDB IDs as 6LU7, 6VWW, 6TLX, 6VYB, and 6VXX (Fig. 7). We normalized all MolDock scores using equation as described below

\[
\beta_i = \frac{\beta_i}{\beta_{\text{max}}}
\]

Where; \(\beta_i\), \(\beta_{\text{max}}\) represent the normalized MolDock score, MolDock score of a compound for any drug target protein and maximum MolDock score among all compounds of any drug target protein.

ADME profiling of potent caffeic acid derivatives

The eight compounds with high-binding affinity against COVID-19 were analysed for ADME properties using SwissADME web tool. The results of eight compounds with high activity potential are presented in Fig. 8. Eutigoside A meets all criteria for oral use. Physicochemical, pharmacokinetics and drug-likeness properties of potent hits are presented in Table 6. All of the compounds are water soluble. They do not cross the blood brain barrier, do not interact with interaction of main enzymes of Cytochromes P450, and have P-gp substrate properties. Toxicity assessment according to the chemical structure was carried out using Toxtree software.
Fig. 5. Docking poses of caffeic acid derivatives with SARS-CoV-2 spike ectodomain (A) Hydrogen bonding interactions of khinaoside C, khinaoside B, calceolarioside B, calceolarioside C, calceolarioside D with amino acid residues of SARS-CoV-2 spike ectodomain (open state) (B) 2D view of interaction types of khinaoside C, khinaoside B, calceolarioside B, calceolarioside C, calceolarioside D with surrounding amino acids of SARS-CoV-2 spike ectodomain (open state).
Table 5

| Ligand Name                  | MolDock Score | Protein-ligand interactions | Internal ligand interactions | H-Bond score |
|------------------------------|---------------|----------------------------|-----------------------------|--------------|
| Nelfinavir                   | -133.655      | -160.117                   | 26.4619                     | -7.62916     |
| Vitexofolin A                | -158.443      | -177.854                   | 19.4111                     | -15.769      |
| Choricic acid                | -141.781      | -166.842                   | 25.0606                     | -7.55396     |
| Eugenoside A                 | -137.834      | -167.189                   | 29.3553                     | -11.5249     |
| Scrophuloside B              | -136.54       | -169.158                   | 32.6181                     | -10.2562     |
| Cynarin                      | -136.457      | -176.339                   | 39.8825                     | -11.3919     |
| Calceolariside D             | -135.748      | -145.865                   | 10.1171                     | -7.33425     |
| Khaínoside B                 | -134.98       | -141.167                   | 6.18667                     | -14.3158     |
| Robustaside D                | -130.586      | -154.384                   | 23.7941                     | -12.9145     |
| Robustaside E                | -126.92       | -126.404                   | -0.5195                     | -14.472      |
| Khaínoside C                 | -126.895      | -124.812                   | 2.0832                      | -10.5752     |
| Propyl 3-(3,4-dihydroxyphenyl)-2-[(E)-3-(3,4-dihydroxyphenyl)]-prop-2-enoyl | -125.712 | -153.822 | 28.1099 | -11.792 |
| Calceolariside A             | -123.256      | -152.379                   | 29.1231                     | -13.9537     |
| Calceolariside B             | -122.232      | -149.1                     | 26.6864                     | -13.6966     |
| 6-O-Caffeoylarbutin          | -121.921      | -116.367                   | 5.5538                      | -8.70194     |
| Dodegranoside A              | -119.728      | -144.494                   | 24.7467                     | -9.47601     |
| Methyl (2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-(3,4-dihydroxyphenyl)]-prop-2-enoyl oxycoproproanate | -116.478 | -176.585 | 60.1073 | -21.2389 |
| Dactylicric acid             | -111.795      | -132.804                   | 21.0096                     | -3.32111     |
| Cafftaric acid               | -106.167      | -121.402                   | 15.2351                     | -12.1219     |
| Guotaric acid                | -105.763      | -118.798                   | 13.0344                     | -11.9847     |
| Fertaric acid                | -104.402      | -115.523                   | 11.1211                     | -9.01248     |
| Neochlorogenic acid          | -102.203      | -130.877                   | 28.6741                     | -9.99063     |
| Premyl caffeic acid          | -100.523      | -107.187                   | 6.66386                     | 2.5          |
| Chlorogenic acid             | -92.1219      | -117.747                   | 25.6247                     | -17.3432     |
| Ferulic acid                 | -78.2151      | -82.4019                   | 4.19678                     | -0.5326      |
| Caffeic acid                 | -75.6669      | -83.0829                   | 7.41603                     | -0.37606     |
| p-Coumaric acid             | -73.8693      | -82.9404                   | 9.07116                     | -6.05487     |

Each system show equilibration, while SASA and RoG both show stabilities during the MDS. The per-residues RMSF profiles in Fig. 9B indicate the stability of the binding since the interacting residues (H41, C149 in Mpro, K90, T167 in NSP15, R185 in the post-fusion core, N343, Y380, G431 in spike ectodomain, and F342, T430, N437 in spike closed state) have low RMSF values in all the complexes. Additionally, the average binding energies (Fig. 9C) calculated for the different clusters during the MDS runs show that the binding is stable. Hence, the MDS study supports previous docking scores values.

Most of the CAFDs possessing good binding energies with various proteins of SARS-CoV-2 have been isolated from traditionally used medicinal plants such as: khainoside B and khainoside C isolated from Thai medicinal plant Vitex glabrata which is traditionally used to support lactation (Luecha et al., 2009) and possess anti-diabetic potential (Somtimuang et al., 2018). Vitexofolin A is an important constituent of Vitex rotundifolia and possesses strong analgesic effect (Okuyama et al., 1998). Scrophuloside B has been identified in traditional Chinese medicinal plants including Picrophisa scrophulariiflora (Wang et al., 2013) and Radix scrophulariae (Jing et al., 2011). Calceolariside B has been isolated from Fraxinus sieboldiana and Forsythia suspensa plants both of which possess anti-inflammatory and anti-viral properties (Kim et al., 2002; Wang et al., 2009). While some of potent CAFDs are found in vegetables and fruits such as: Lettuce (Lactuca sativa), a widely consumed leafy vegetable, is found to be enriched with various CAFDs such as chicoric acid and chlorogenic acid (Abu-Reidah et al., 2013). Chicoric acid has been long known as potent anti-viral agent against HIV (Lin et al., 1999) and its mechanism of action involves deactivation of HIV-1 integrase, increased T-lymphoblastoid viability, and down-regulation of reverse transcriptase of HIV-1 (Peng et al., 2019). 6′-O-cafeoylarbutin is abundantly found in Vaccinium dunaliamus. V. dunaliamus is a commonly cultivated blueberry species in China and its dried leaf buds as herbal tea while its leaves are used as folk medicine (Li et al., 2016; Luo et al., 2015). Sunflower (Helianthus annuus) sprouts (nutritious sunflower lettuce) found to be enriched with cynarin (Sun et al., 2012), recommending these foods as beneficial food choice for COVID-19 patients.

Among all these potent compounds, calceolariside B is identified as pan-inhibitor of SARS-CoV-2 having potential to target SARS-CoV-2 M^\text{pro}, NSp15, coronavirus fusion protein, as well as spike ectodomain which brought excitement about calceolariside B’s potential against COVID-19. Interestingly, calceolariside B is also an important constituent of Akebia trifoliata fruit which is popularized in Asia due to its nutritional values as well as delicious taste and also used as dietary supplement for its various health benefits including anti-microbial and anti-inflammatory (Wang et al., 2019), suggesting that Akebia trifoliata fruit could potentially help COVID-19 patients to fight this disease. Calceolariside B has potential to inhibit gp41 transmembrane glycoproteins of HIV (human immunodeficiency virus) (Kim et al., 2002). These glycoproteins play critical role in virus cell fusion with target membrane to enable viral entry into the host cell (Buzon et al., 2010). As calceolariside B is identified as potent binder of SARS-CoV-2 spike glycoprotein during our study, the results from both of these studies propose calceolariside B as viral and target membrane fusion inhibitor further strengthening its role against COVID-19.

In addition, calceolariside B exhibits good anti-RSV (respiratory syncytial virus) effects (Dong et al., 2017) which recommend that calceolariside B rich foods could be a potential alternative approach for the prevention and treatment of COVID-19. In addition to its anti-viral properties, it has potential to inhibit IL-6 production to exert its anti-inflammatory properties (Jin et al., 2014). COVID-19 patients suffer with severe inflammatory response in the later stages of infection, thus, dual anti-viral/anti-inflammatory properties of this compound makes it ideal candidate for drug development for prevention and treatment of COVID-19. As coronaviruses mutations make it difficult to develop vaccine, so does the single-target drugs. Single-target drugs might encounter low efficacy as the virus mutates. Thus, complex diseases like...
Fig. 6. Docking poses of caffeic acid derivatives with SARS-CoV-2 spike glycoprotein (closed state) (A) Hydrogen bonding interactions of scrophuloside B, chicoric acid, vitexfolin A, cynarin and eutıgosıde A with amino acid residues of SARS-CoV-2 spike glycoprotein (closed state) (B) 2D view of interaction types of scrophuloside B, chicoric acid, vitexfolin A, cynarin and eutıgosıde A with surrounding amino acids of spike glycoprotein (closed state).
**Docked Compound Number/Name**

1. Khainoside C  
2. Calceolarioside B  
3. Vitexfolin A  
4. Calceolarioside C  
5. Scrophuloside B  
6. Cynarin (CYN)  
7. Eutigoside A  
8. Calceolarioside D  
9. Robustaside D  
10. Chicoric acid (CHA)  
11. Robustaside E  
12. Dodegranoside A  
13. 6-O-Caffeoylshanzusin  
14. KHAINAOSIDE B  
15. Calceolarioside A  
16. Propyl 3-(3,4-dihydroxyphenyl)-2-[E]-3-(3,4-dihydroxyphenyl)prop-2-yn-1-yl]oxypropanoate  
17. Methyl (2R)-3-(3,4-dihydroxyphenyl)-2-[E]-3-(3,4-dihydroxyphenyl)prop-2-enyloxypropanoate  
18. Dactylic acid  
19. Neochlorogenic acid (NCHL)  
20. Fertaric acid (FTA)  
21. Prenyl caffeate acid  
22. Caftaric acid (CFT)  
23. Chlorogenic acid (CHL)  
24. Coutaric acid (CTA)  
25. Caffeic acid  
26. p-Coumaric acid  
27. Ferulic

**Fig. 7.** MolDock Score comparison among 27 compounds versus active sites SARS-CoV-2 6LU7, 6VWW, 6LXT, 6VYB, and 6VXX.
COVID-19 are more likely to be alleviated or healed though simultaneous modulation of multiple targets. Based on the above discussion, in our personal opinion, efficacy of CAFDs-enriched foods against COVID-19 as well as safety suggests that their adoption in daily diets might help prevent the onset of COVID-19 in an alternative and non-pharmacological approach.

Conclusively, this study provides scientific basis for the possible utilization of CAFDs as drug leads to develop anti-COVID-19 therapeutics. Since there is an urgent and timely need to find out effective and specific anti-viral treatment for COVID-19, this study will hopefully lay the foundation to work forward on small scale studies for the determination of 1) efficacy of CAFDs in reducing viral load and shortening the infectious period, 2) optimal dosing regimen based on impact on viremia, 3) impact on antibody production, inflammatory signaling and oxidative stress in COVID-19 patients. It seems that CAFDs have better absorption and good safety profiles as many studies pointed out the better absorption of CAFDs and CAFDs are important constituent of dietary foods. However, it would be worthwhile to conduct pharmacological studies to determine whether the bioavailability of CAFDs is good enough or it is better to administer in combinations. Once we

Fig. 8. Bioavailability Radar related to physicochemical properties of molecules (Criteria: Lipophilicity: \(-0.7 < \text{XLOGP3} < +5.0\), Size: 150 MW 500 g/mol, Polarity: \(20 < \text{TPSA} < 130 \AA^2\), Insolubility: \(0 < \log S < 6\), Insaturation, Flexibility: \(0.25 < \text{rotatable bonds} < 9\)).

| Table 6 ADME profiling of potent caffeic acid derivatives. |
|-----------------------------------------------------------|
| **Compound name** | **Ghose** | **TPSA** | **Absorption (% ABS)** | **Water solubility Log S (ESOL)** | **BBB permeant** | **P-gp substrate** | **CYP isoform interact** |
| Khainasıde C | No | 175.37 | 48.49 | -3.12 | Soluble | No | Yes | No |
| Calceolarioside B | Yes | 186.37 | 44.70 | -2.85 | Soluble | No | Yes | No |
| Vitexolín A | No | 183.21 | 45.79 | -3.01 | Soluble | No | Yes | No |
| 6-O-Caffeoylarbutin | Yes | 166.14 | 51.68 | -2.86 | Soluble | No | Yes | No |
| Khainasıde B | No | 201.67 | 39.42 | -3.14 | Soluble | No | Yes | No |
| Dodegranoside A | Yes | 166.14 | 51.68 | -2.86 | Soluble | No | Yes | No |
| Chicoric acid | Yes | 208.12 | 37.19 | -3.58 | Soluble | No | Yes | No |
| Eutingıse A | Yes | 145.91 | 58.66 | -3.11 | Soluble | No | Yes | No |
| Nelfinavir | No | 127.20 | 65.18 | -6.36 | Poorly soluble | No | Yes | 1A2, 2C19, 3A4 |
Fig. 9. Post-dynamics analysis: (A) Root Mean Square Deviation (RMSD) (blue line), Radius of Gyration (RoG) (orange line), and surface accessible surface area (SASA) (gray line) for the best complex for each viral protein during 20 ns MDS run. (B) Per-residue Root Mean Square Fluctuation (RMSF) for the same complexes with dashed lines represents the active residues. (C) Post-dynamics average binding energies (in kcal/mol) calculated for the complexes using AutoDock Vina. Error bars represent the standard deviations.
understand the best way to deal with the CAFDs, it will be a reasonable initial step for therapeutic interventions based on the pharmacokinetic and pharmacodynamic studies on CAFDs. Despite excitement about CAFD’s potential from antiviral and anti-inflammatory researches, there is an urgent and timely need to fill the room of knowledge for validation of CAFDs potential against COVID-19.

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