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In silico preliminary evaluation of bioactive compounds from five Unani drugs as potential SARS-CoV-2 inhibitors

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COVID-19, although on the decline currently with the role of vaccines, still impose public health challenges due to the quick evolution of SARS-CoV-2 into several variants and periodic surge in cases. Identifying therapeutic interventions that can effectively target virulence, boost immune response, and protect target organs is of paramount importance. Behi dana (*Cydonia oblonga*), Unnab (*Zizyphusjujuba*), Sapistan (*Cordia myxa*), Banafsha (*Viola odorata*), and Aslassus (*Glycyrrhiza glabra*) are the most commonly prescribed drugs in Unani Medicine for upper respiratory tract infections and have been shown to have antiviral, antimicrobial, immunomodulatory, anti-inflammatory, and antioxidant activities. The current study investigated the inhibitory response of phytocompounds contained in these drugs on putative SARS-CoV-2 drug targets. Phytocompounds structures were retrieved from PubChem database, with some being constructed using Marvin Sketch. 3CLpro and SARS-CoV-2 S glycoprotein were chosen as the target proteins. To determine the binding affinities and predict top-ranking poses with the highest scores, AutoDock Vina was utilized. The results of molecular docking indicated that the phytoconstituents of these drugs interacted well with 3CLpro and S glycoprotein with strong binding affinities. Zijusesquilignan A, Zijusesquilignan B, Emetine, Glycyrrhizin and 3,4-Dicaffeoylquinic acid, Vicenin-2, Isoschaftoside, Schaftoside, Zijusesquilignan A & C, Emetine, Glycyrrhizin were shown to be intriguing candidates with the capability of interacting with spike glycoprotein and 3CLpro, respectively and preventing the virus from replicating and infecting the host. Molecular simulation results showed the structural stability of the docked complexes. To conclude, the combination of these drugs may be useful in the development of novel remedial candidates for COVID-19; however, additional *in vitro* and *in vivo* investigations are required to ascertain this claim.

Keywords: Emetine, Glycyrrhizin, Isoschaftoside, Molecular docking, SARS-CoV-2, Unani medicine

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The COVID-19 pandemic caused by the SARS-CoV-2 virus has had a considerable negative influence on almost everyone's quality of life worldwide. It has seriously impeded the worldwide economic, academic, and public healthcare infrastructure and turned into a threat to global health[1,2]. The pace of the disease transmission and lethality is slowed down currently by the advent of various vaccines; however, periodic surge in cases due to the quick evolution of SARS-CoV-2 into several variants continues posing serious public health challenges across the globe. As per the World Health Organization (WHO) report, over 1.1 million new cases and over 8700 new fatalities have been reported worldwide in 28 days from 11 December 2023 to 7 January 2024. This represents a 4% increase in new cases and 26% decrease in deaths, compared to the previous 28 days. Over 774 million confirmed cases and over 7 million fatalities have been reported globally as of 7 January 2024[3]. Several global initiatives have been taken and various drugs such as remdesivir, ritonavir, lopinavir, steroids, interferons, monoclonal antibodies, Chloroquine, and hydroxychloroquine have been recommended to curtail the situation, however, targeted therapy options remain limited for this dreadful condition[4,5].

Indian traditional systems of medicine including Ayurveda, Unani, and Siddha offer compelling approaches to treating infectious and epidemic diseases and may play a conclusive role in the redressal of current and future pandemics by targeting the virulence factors and augmenting the immune

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resilience of the host[6]. Unani scholars have recommended a number of single drugs and compound formulations to prevent and treat infectious diseases and epidemics[7-10]. Behi dana (*Cydonia oblonga*), Unnab (*Zizyphus jujuba*), Sapistan (*Cordia myxa*), Banafsha (*Viola odorata*), and Aslassus (*Glycyrrhiza glabra*) are the five most commonly prescribed drugs in Unani Medicine for the treatment of upper respiratory tract infections. These drugs are extensively being used and claimed to be very effective in relieving symptoms of COVID-19 patients exhibiting *i.e.,* fever, body ache, cold and coryza, cough, sore throat, and difficulty in breathing[11-15]. It has been reported that these drugs exhibit wide-ranging biological activities.

The first three ingredients *i.e.,* Behi dana (*Cydonia oblonga*), Unnab (*Zizyphus jujuba*), and Sapistan (*Cordia myxa*) are the ingredients of well-known decoction prescribed frequently in Unani Medicine for the treatment of bronchial asthma, pleurisy, pneumonia, and other respiratory afflictions. Owing to its great potential, the decoction was advocated by the Ministry of Ayush, Govt. of India for mild cases of COVID-19 during the pandemic. These ingredients have been reported to have antioxidant, anti-SARS-CoV-2, anti-viral, anti-bacterial, anti-inflammatory, anti-allergic, anti-asthmatic, cardioprotective, neuroprotective, nephroprotective, anti-diarrhoeal, immune-modulatory, anti-thrombotic, hemopoietic, hepatoprotective and gastro-protective activities[16]. Aslussoos (*Glycyrrhiza glabra* Linn) has long been utilized in Unani and other Ayush systems of medicine as an excellent remedy for peptic ulcer, skin diseases, cold (Catarrh), cough, pharyngitis, hoarseness of voice, and other respiratory problems. Clinical and experimental studies suggest that it possesses of several pharmacological activities including antiviral, antimicrobial, anti-inflammatory, antitussive, immunomodulatory, antioxidative, gastroprotective, hepatoprotective, and cardioprotective effects[17,18].

Banafsha (*Viola odorata*) commonly known as sweet violet has been used medicinally in Unani as well as in other traditional systems of medicine since antiquity. The whole plant acts as an anti-inflammatory, expectorant, emollient, diuretic, diaphoretic, and laxative and is known to be effective in bronchitis, respiratory catarrh, cough, asthma, and breast cancer. Flowers act as demulcent and emollient and are used in the treatment of biliousness and respiratory disorders. The drug is mainly used for cough, cold, and catarrh but it has also been used for treating urinary tract infections, rheumatism, cancer, whooping cough, and skin conditions. Banafsha has been reported for numerous pharmacological activities such as cough suppressant, antipyretic, anti-viral, anti-microbial, bactericidal, anti-inflammatory, anti-oxidant, antitubercular, hepatoprotective, antihypertensive, dyslipidemic, hypnotic, diuretic activities[19-21].

The SARS-CoV2 spike glycoprotein which constructs homotrimers and protrudes from the viral surface, mediates SARS-CoV-2 entrance and delivers viral particles into host cells, through the receptor binding domain (RBD). Similarly, the main protease (Mpro/3CLpro) which mediates the proteolytic processing of viral proteins is thought to be crucial to the process of virus replication. Hence RBD and 3CLpro are considered to be the most crucial druggable targets[2]. For more than three decades, the development of therapeutically crucial molecules has been greatly helped by computer-aided drug discovery and design techniques[22]. Molecular docking has been exploited considerably to examine the binding affinities of both conventional and traditional drug molecules and predicting their possible biological activities against SARS-CoV-2[2]. To the best of our knowledge, the effects of bioactive compounds contained in *C. oblonga*, *Z. jujuba*, *C. myxa*, *V. odorata*, and *G. glabra* against SARS-CoV-2 have not yet been investigated. The objective of the current investigation was to find possible multitarget drug candidates and further develop effective COVID-19 medications by examining the inhibitory potential of phytocompounds found in these drugs on possibleSARS-CoV-2 therapeutic targets.

Material and Methods

Receptor preparation

The SARS-CoV-2 S glycoprotein and 3CLpro protein structures were retrieved from the PDB ID: 6LZG and 7BQY, respectively. The Dock Prep module of the Chimera program (v1.14) from UCSF (University of California, San Francisco) was utilized to process the receptor structures[23]. The Dock Prep wizard eliminates solvent molecule, removes non-polar hydrogens, assigns charges, and adds hydrogen atoms. The charges for AM1-BCC were figured for the receptor that is included in Chimera. In 7BQY, the covalent bond formation between the crystallized ligand and the Cys145 residue was detached. Chimera

software was used to protonate and optimize the His and Cys residues.

Ligand preparation

Lead compounds of each 5 plant, with promising biological activities such as antiviral, antimicrobial, antioxidant, and or immunomodulatory potential, were searched and selected for the study based on the previous published reports available on leading science research databases, such as ScienceDirect, PubMed, Springer, and Google Scholar. The structures of all the selected phytocompounds investigated in this study were recovered from PubChem database[24], with some being constructed utilizing Marvin Sketch of the Marvin (v20.8.0) suite[25]. Phytoconstituents of these five drugs retrieved or built are depicted in Table 1. The Dock Prep module of the chimera was used to protonate the ligands' 3D structure and assign AM1-BCC charges.

Receptor-ligand docking

Top-ranking poses with the highest scores were predicted using AutoDock Vina (v1.1.2), an efficient molecular docking tool[26]. It determines the most advantageous binding interactions and forecasts binding affinities (kcal/mol). The SARS-CoV-2 protein structures for S glycoprotein and 3CLpro were retrieved from PDB ID: 6LZG and 7BQY, respectively. The UCSF Chimera Dock prep tool was utilized to prepare proteins and compounds for docking. AutoDock Vina with an exhaustiveness parameter set to 8, was used to compute the binding affinities between the protein and compounds. Binding interactions were elucidated using UCSF Chimera. To increase the likelihood that the ligand will be docked to every portion of the receptor, the search area for SARS-CoV-2 S glycoprotein was expanded to match the size of the RBD external subdomain (S438-Y505) in a grid box of (x= -37, y= 30.5, z= 6). The co-crystallized ligand N3 (PDB ID: 7BQY) in the catalytic site was used as a reference in 3CLpro, and a grid box with the dimensions of (x=-9.5, y=12, z=68.5) was used as the search space. BIOVIA Discovery Studio Visualizer 2020 (v20.1.0) was then used to analyze the results[27].

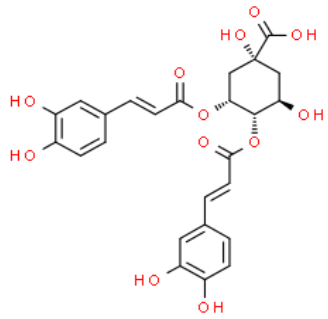
Molecular dynamics

Gromacs (v.2022.4) software was employed to perform the MD simulation to demonstrate the potential of the selected compounds through molecular docking and grooms 96 53 a 6 force field was utilized for preparing protein topology. The SPC water model was chosen for solvating complexes in cubic box size, followed by adding ions to neutralize. Online server PRODRG was used to prepare ligand topology[28]. Prior to the MD simulations production run, the complex minimized its energy using heating and equilibrium processes (NVT and NPT). Additionally, the system normalized in an equilibrium condition at 10000000 steps, with a time step of 100 ps. MD simulation was performed for 20 ns with a time step of 10000 ps.

Results

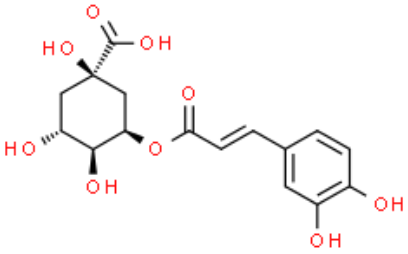
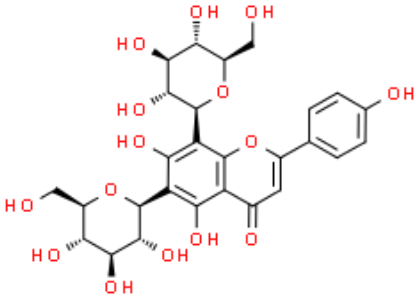
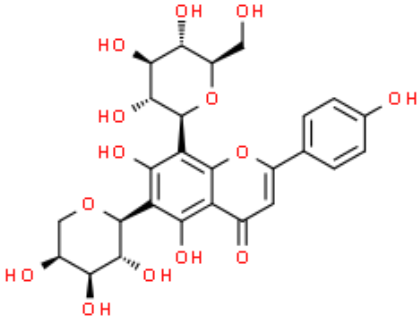
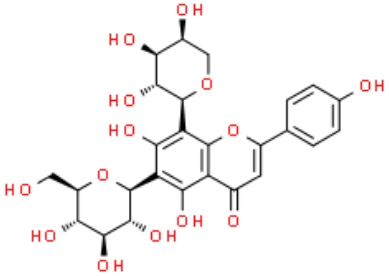
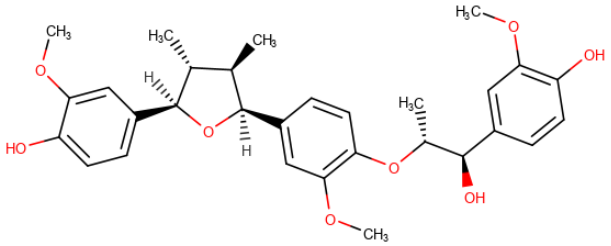
The phytocompounds that were either obtained from PubChem database or constructed using Marvin Sketch include 3,4-Dicaffeoylquinic acid, 5-O-Caffeoylquinic acid, Vicenin-2, Isoschaftoside and Schaftoside from *C. oblonga*; Zijusesquilignan A, B & C from *Z. jujuba*; Bornyl acetate and α-terpineol from *C. dichotama*; Triacetonamine and Emetine from *V. odarata*; Glycyrrhizin from *G. glabra* (Table 1). In order to explore the possible inhibitory

Table1 — Phytocompounds from five Unani drugs

	Botanical Name	*Cydonia oblonga* Mill.
	Unani Name	Behi-dana
1	3,4-Dicaffeoylquinic acid	

...Contd.

Table1 — Phytocompounds from five Unani drugs (Contd.)

	Botanical Name	*Cydonia oblonga* Mill.
	Unani Name	Behi-dana
2	5-O-Caffeoylquinic acid	
3	Vicenin-2	
4	Isoschaftoside	
5	Schaftoside	
	Botanical Name	*Zizyphus jujuba* Lam.
	Unani Name	Unnab
6	Zijusesquilignan A	

…Contd.

Table1 — Phytocompounds from five Unani drugs (Contd.)

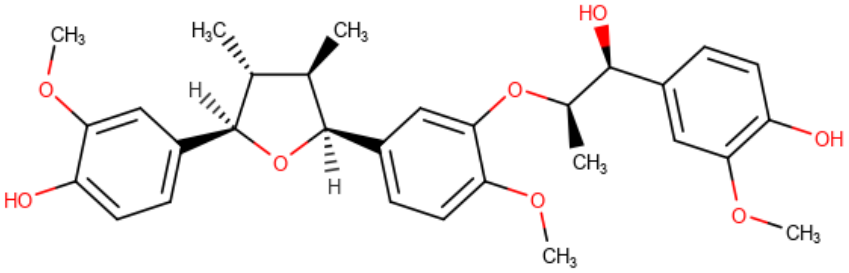
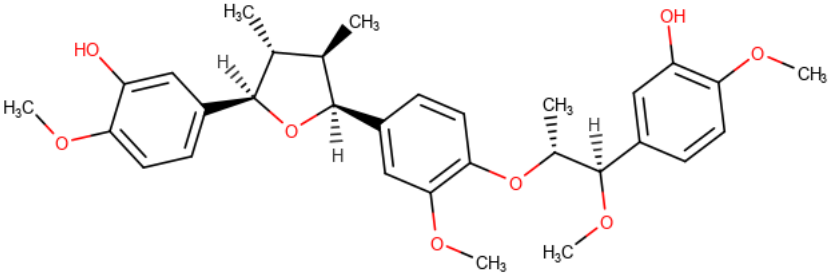
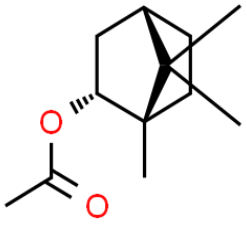
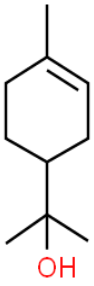
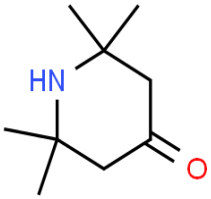
	Botanical Name	*Zizyphus jujuba* Lam.
	Unani Name	Unnab
7	Zijusesquilignan B	
8	Zijusesquilignan C	
	Botanical Name	*Cordia dichotama*Forst. F.
	Unani Name	Sapistan
9	Bornyl acetate	
10	α-terpineol	
	Botanical Name	*Viola odarata* L.
	Unani Name	Banafsha
11	Triacetonamine	

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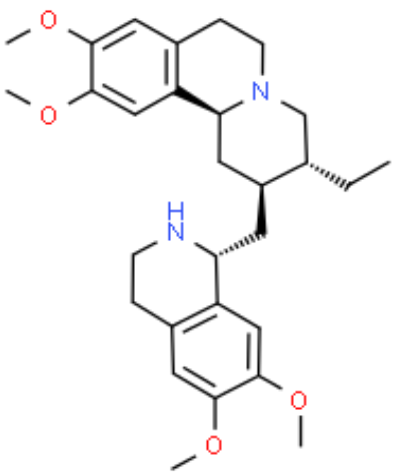
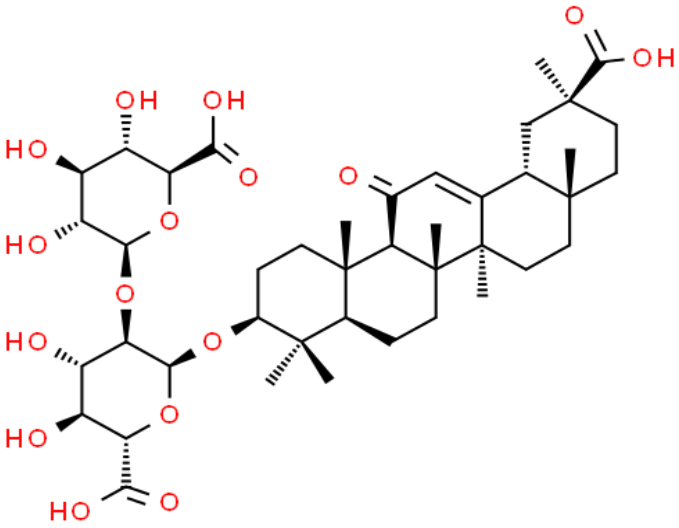
Table1 — Phytocompounds from five Unani drugs (Contd.)

	Botanical Name	*Viola odarata* L.
	Unani Name	Banafsha
12	Emetine	
	Botanical Name	*Glycyrrhiza glabra* L.
	Unani Name	Asl-us-Soos
13	Glycyrrhizin	

effect of these 13 phytocompounds on the binding of the S glycoprotein RBD with the host cell, their interactions with the external subdomain of S glycoprotein were examined with respect to the major contact residues. The primary metrics employed to evaluate the ligand-protein interactions were the AutoDock Vina docking scores. A stable system and therefore a potential binding interaction are indicated by a low (negative) energy.

S glycoprotein

The structure of SARS-CoV-2 S glycoprotein in the external subdomain (S438-Y505) interacts with hACE2 subdomains I and II[29]. The key contact resides in S glycoprotein external subdomain include Lys417, Tyr453, Ala475, Asn487, Gly496, Gln498, Tyr499, Thr500, Asn501, and Gly502. Docking scores and amino acid interactions were obtained using AutoDock Vina. Nelfinavir, with a binding energy of -7.2, served as the positive control, demonstrated H-bonding with Glu406, Gly496, Tyr453, and exhibited Pi-Alkyl hydrophobic interactions with Tyr505 and Leu455. The results of the molecular docking revealed that the phytocompounds tested in this study had good docking energies ranged from -7.6 to -4.6 kcal/mol. The orientation of binding for each

compound having the best binding affinity with the target protein was analyzed using BIOVIA Discovery Studio Visualizer 2020. Table 2 and Supplementary Table S1 present the results of the detailed study on the binding affinities and 2D interactions of the tested compounds towards the active site of S glycoprotein.

Biological interaction analysis of phytoconstituents was carried out with respect to main contact residues with human ACE2 subdomains I and II. All the tested compounds had efficient binding interactions with S glycoprotein and it was found that every docked ligands interacted well with the identical amino acid residues as hACE2 (Fig. 1). Phytoconstituents *i.e.*, Glycyrrhizin, Emetine, Zijusesquilignan A and B had showed binding energy of -7.6, -7.3, -7.2 and -7.2, respectively. Glycyrrhizin showed interactions with Arg403, Tyr453, Ser494, Gly496, Gln498, and Asn501 amino acid residues that were crucial to the formation of the H-bond network. Based on the analysis of the ligand-molecules interactions and energy values comparison, it is predicted that phytocompound Glycyrrhizin- a triterpene glycoside and Zijusesquilignan A- a sesquilignan, both effectively obstruct the binding of spike protein to the host cell. Figure 2 displays the interactions between candidate and the protein residues.

Main Protease (3CLpro)

The 3CLpro of SARS-CoV-2 is a cysteine protease that at its active site includes His41, and Cys145, the

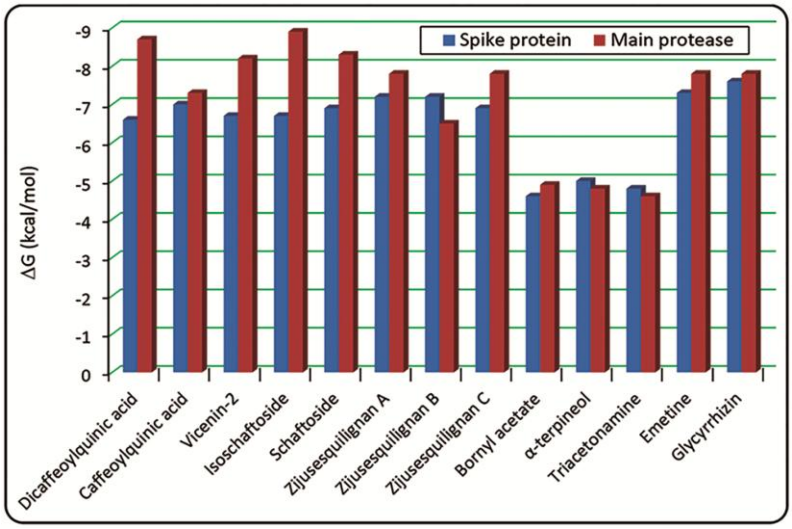


Fig. 1 — Histogram showing the energy binding value of ΔG (kcal/mol) of S protein and Main protease with phytocompounds of Unani drugs

Table 2 — Amino acid residues of SARS-CoV-2 S glycoprotein (6LZG) participated in H-Bond and hydrophobic interactions with ligands.

Compound	Binding Energy (Kcal/mol)	Interactions	
		H-Bonding	Hydrophobic
Nelfinavir	-7.2	Glu406, Tyr453, Gly496	Leu455, Tyr505
3,4-Dicaffeoylquinic acid	-6.6	Arg403, Arg408, Gln492, Gly496	Tyr495
5-O-Caffeoylquinic acid	-7.0	Tyr453,Tyr505	Arg403, Asn501,Tyr505
Vicenin-2	-6.7	Arg403, Tyr505	Lys417, Leu455
Isoschaftoside	-6.7	Glu406, Tyr453, Gln493, Ser494	Lys417, Leu455
Schaftoside	-6.9	Tyr453, Gln493, Gly496	Leu455
Zijusesquilignan A	-7.2	Arg403, Asp405,Arg408,	Glu406, Tyr449, Tyr453, Glu498
Zijusesquilignan B	-7.2	Tyr453, Gly496	Try505
Zijusesquilignan C	-6.9	Gln409, Lys417, Gln493, Asn501	Asp405, Glu406, Tyr453, Gly496, Tyr505
Bornyl acetate	-4.6	Gly496	-
α-terpineol	-5.0	NHB	Tyr495, Tyr505
Triacetonamine	-4.8	Gly496	-
Emetine	-7.3	NHB	Glu406, Tyr449, Tyr453, Ser494, Tyr495, Glu498, Tyr505,
Glycyrrhizin	-7.6	Arg403, Tyr453, Ser494, Gly496, Gln498, Asn501	Tyr495

NHB: No Hydrogen Bond Interactions.

catalytic dyad residues. The interaction appears to be largely mediated by Glu166, Gln189, Thr190, Phe140, and His164[30]. Hence, for the molecular docking computations and binding interactions, these residues were mainly targeted as the centre of attention. With a binding energy of -7.7, Nelfinavir showed H-bonding with His41, Glu166, Gln189 residues and hydrophobic interactions with His41, Cys145, and Met165 residues for Pi-Pi, Pi-Alkyl, and Pi-donor, respectively.

The molecular docking results showed that the phytocompounds tested in this study had good docking energies ranged from -8.9 to -4.6 kcal/mol. Isoschaftoside- a C-glycosyl apigenin and 3,4-Dicaffeoylquinic acid - a polyphenol with diverse biological activities showed docking energy -8.9 and -8.7 kcal/mol, respectively as shown in Table 3. In the protease receptor site, the flavonoid group formed an expanded Hydrogen-bonds network with residues Phe140, Glu166, and Thr190 and exhibited hydrophobic interactions with the residues Gln189, Met165, and Pro168. Figure 3 illustrates the best-docked poses with 3CLpro as represented by the BIOVIA Discovery Studio Visualizer. The OH atom of the apigenin group in isoschaftoside, as demonstrated, established H-bond with Thr190 (bond

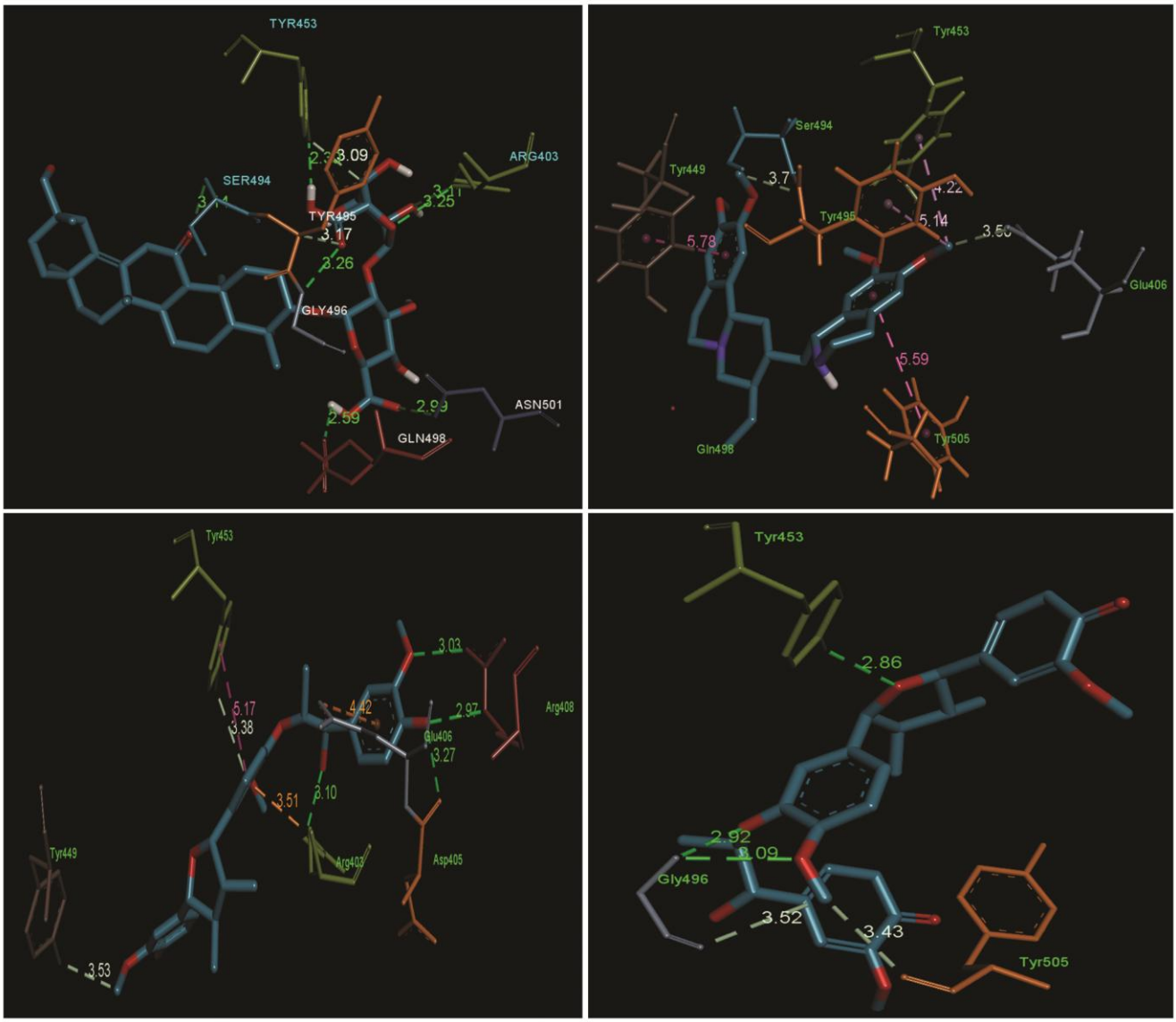


Fig. 2 — Interaction profile of phytocompounds and active site residues of SARS-CoV-2 S glycoprotein. Each color of amino acid residues and interaction markers indicates different types of interaction. Green represents a conventional H-bonding, Yellow indicates π-SH interaction, Pink denotes π-amide interaction and the rest of them represents weak van der Waals interaction

length: 2.35Å) and Arg188 (bond length: 2.28Å). In addition, hydrophobic interactions of Pi-Sulfur and Pi-Alkyl with Met49, Met165, Cys145, and Glu166 established six H-bonds with Phe140, Ser144, Leu141, His163, Arg188 and Thr190 amino acids, with a bond length of 1.99Å, 2.48Å, 2.44Å, 2.07Å, 2.05Å and 2.09Å respectively. Also, Pi-Sulfur and Pi-Alkyl hydrophobic interactions with amino acidsMet49, Cys145, Asn142, Met165, and Glu166 formed H-bond interactions with Leu141, Arg188, and Thr190 amino acids.

Molecular dynamic simulation

On the basis of the docking score, four protein-ligand complexes were selected namely, SARS-CoV-2 S-Glycyrihizin, SARS-CoV-2 S-Zijusesquilignan A, 3CLpro-Isochaftoside, and 3CLpro-3,4 Dicaffeolquininc acid to perform the MD simulation to analyze their structural conformation, conformational stability and dynamics variations for a 20 ns period. This study investigates the stability of the secondary structure of the complexes by Root Mean Square Deviation (RMSD) and Root Mean

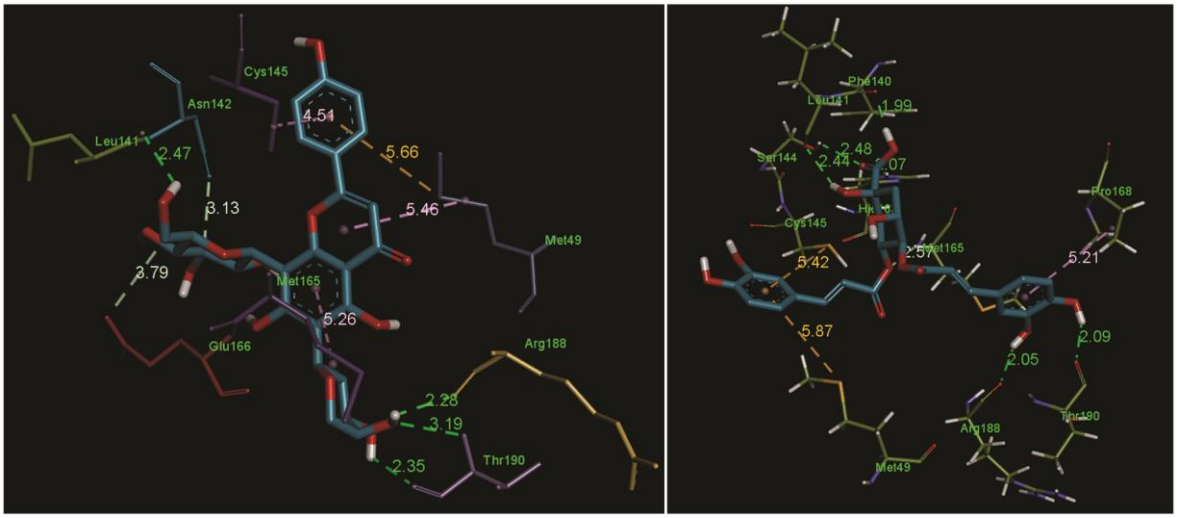


Fig. 3 — Interaction profile of phytocompounds and active site residues of SARS-CoV-2 main protease 3CLpro. Each color of amino acid residues and interaction markers indicates different types of interaction. Green represents a conventional H-bonding, Yellow indicates π-SH interaction, Pink denotes π-amide interaction and the rest of them represents weak van der Waals interaction

Table 3 — Amino acid residues of SARS-CoV-2 main protease 3CLpro (7BQY) participated in H-Bond and hydrophobic interactions with ligands.

Compound	Binding Energy (Kcal/mol)	Interactions	
		H-Bonding	Hydrophobic
Nelfinavir	-7.7	His41, Glu166, Gln189	His41, Cys145, Met165
3,4-Dicaffeoylquinic acid	-8.7	Phe140, Leu141, Ser144, His163, Arg188, Thr190	Met49, Cys145, Met165, Pro168
5-O-Caffeoylquinic acid	-7.3	Asn142, Ser144, Cys145, His163, Gln189, Thr190	Met165
Vicenin-2	-8.2	Tyr54, Asn142, Gly143, Ser144, Cys145, Pro168	His41, Leu141, His163, Met165, Glu166,
Isoschaftoside	-8.9	Leu141, Arg188, Thr190	Met49, Cys145, Asn142, Met165, Glu166
Schaftoside	-8.3	Asn142, Ser144, Glu166, Leu167	His163, Pro168, Ala191
Zijusesquilignan A	-7.8	Thr24, Thr26, Asn119, Asn192	Leu27, Thr25, His41, Gly143, Met165, Asp187
Zijusesquilignan B	-6.5	Thr190	Met165
Zijusesquilignan C	-7.8	Thr45, Ser46, Met165	Thr25, Met49, Cys145
Bornyl acetate	-4.9	Cys145	Leu27
α-terpineol	-4.8	His164	His41, Met165
Triacetonamine	-4.6	Asn142, His163	His172
Emetine	-7.8	NHB	His41, Met49, Pro168, Asp187, Gln189
Glycyrrhizin	-7.8	Phe140, His164, Glu166, Ala191	Asn142, Thr190

NHB: No Hydrogen Bond Interactions.

Square Fluctuations (RMSF) by extracting the data from the trajectories. RMSD explains the displacement of backbone atoms of proteins and the conformational shifts in ligand binding pockets throughout various time scales. The RMSF explains the average residual deviance from its initial structure over time.

The RMSD value for the Protein-Ligand complexes ranges from 0.1 to 0.3 nm. Deviation occurred in the above graph from 5000- 15000 ps for Gycyrhizin (Glycyrr) and Zijusesquilignan A (Zijuses), and finally, they converged at the end. RMSF graph exhibited the highest residual fluctuation throughout the Process, in which Zijuses showed more stable conformation than Glycerr (Fig. 4a). The hydrogen bond diagram of the SARS-CoV-2 ligand complex showed that the number of hydrogen bonds is higher for Glycyrr (Glycyrrhizin) than for Zijuses (Zijusesquilignan A). The radius of gyration (ROG) ranged from 1.8 to 1.9 (nm), indicating that Glycyrr varied more than Zijuses. Zijuses showed stability at the end from 15000 ps to 20000 ps. Comparing Zijuses with Glycyrr, Zijuses show a more stable ROG (Fig. 4b).

The RMSD value for both the complexes of Protein-Ligand ranges from 0.1 to 0.3 nm, in which 3,4 Dicaffeolquininc acid (3,4 Dicaff) showed a higher RMSD than Isochaftoside (Isochaff). The RMSF graph showed that various locations exhibited fluctuations, with the C- and N-terminal regions showing the highest

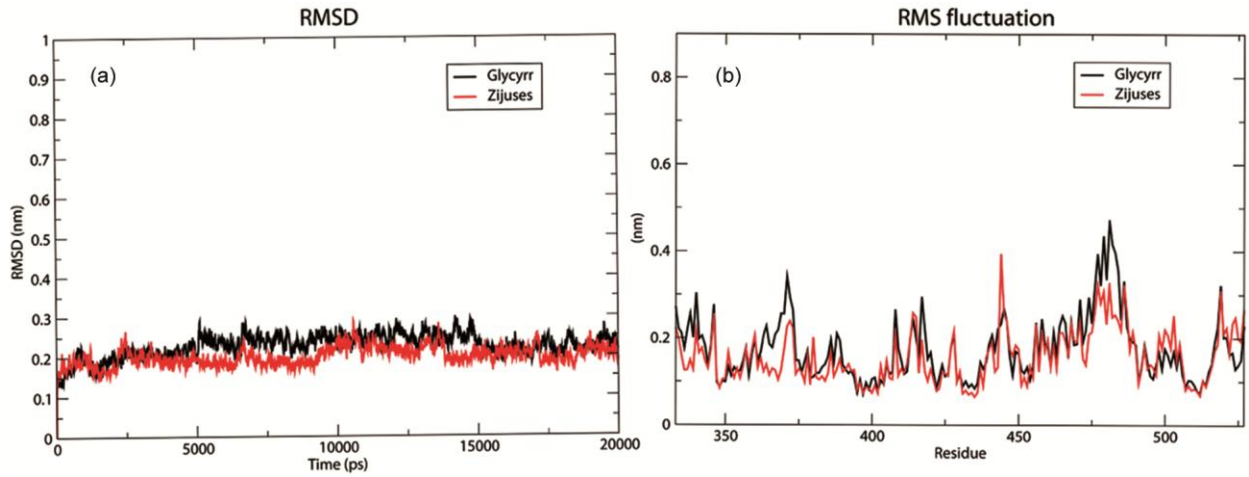


Fig. 4(a) — Molecular Dynamics Simulation for SARS-CoV-2-Ligand complex (20ns). A) RMSD and B) RMSF (Black represents the 6W41-Glycyrr complex, and red represents the 6W41-Zijuses complex)

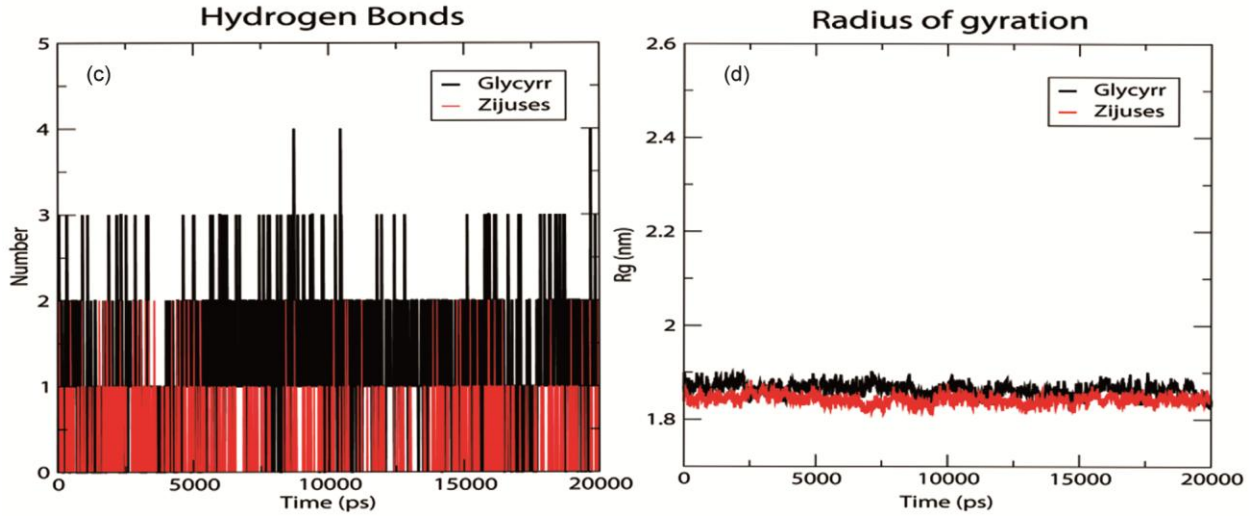


Fig. 4 (b) — Molecular dynamics simulation for SARS-CoV-2-Ligand complex (20ns). C) Hydrogen Bond for Protein-Ligand complex (black represents 6W41-Glycyrr (Glycyrrhizin) and the red represents 6W41-Zijuses (Zijusesquilignan A). D) Radius of Gyration for Protein-ligand complex (black represents Glycyrr, red represents Zijuses)

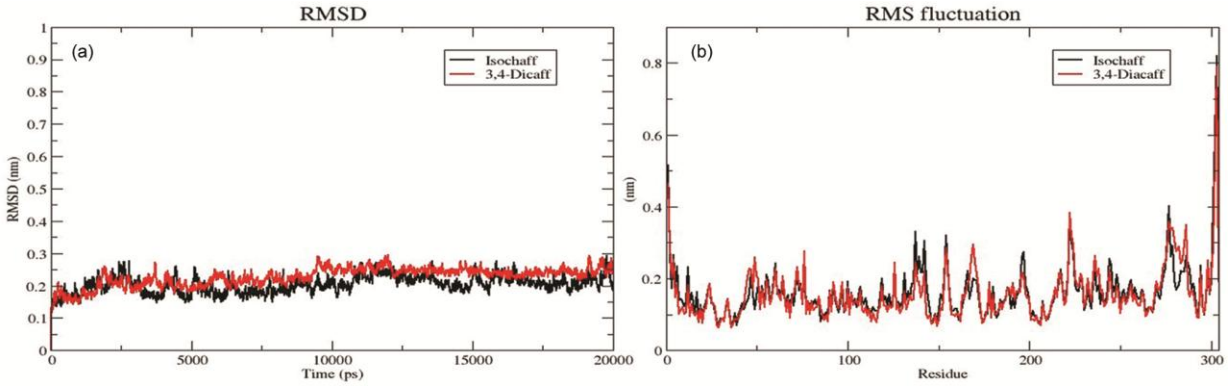


Fig. 5 (a) — Molecular dynamics simulation for 3CL pro-Ligand complex (20ns). A) RMSD and B) RMSF; Black) represents 7WO3-Isochaftoside (Isochaff) complex and Red) represents 7WO3 -3,4 Dicaffeolquininc acid (3,4 Dicaff) complex

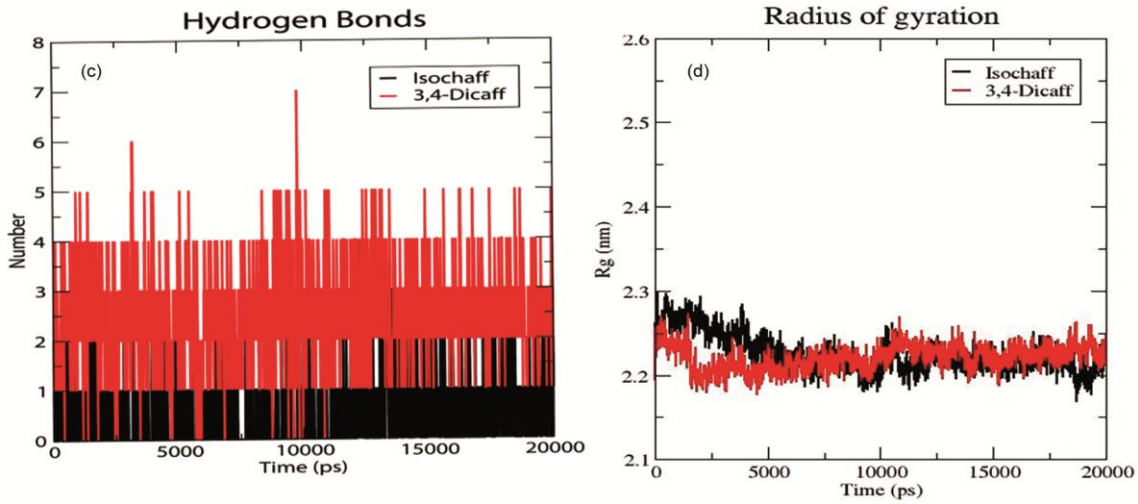


Fig. 5 (b) — Molecular dynamics simulation for 3CL pro-Ligand complex (20ns). C) Hydrogen Bond for Protein-Ligand complex; D) Radius of Gyration for protein-ligand complex; Black) represents 7WO3-Isochaftoside (Isochaff) and Red) represents 7WO3 -3,4 Dicaffeolquininc acid (3,4 Dicaff)

changes levels. The RMSF showed that Isochaff and 3,4 Dicaff has almost similar flexibility, but residues within the amino acid range 140-220 showed significant changes (Fig. 5a). The number of hydrogen bonds determines protein stability. Figure 5(b) depicts the intermolecular hydrogen bond formation between protein-ligand complexes where 3,4 Dicaff has more hydrogen bonds than Isochaff. The ROG measures the compactness of protein. The ROG ranged from 2.2 to 2.3 (nm), depicting that Isochaff has fluctuated more in the initial stage than 3,4 Dicaff, up to 5000 ps, and at the end, slight fluctuations have been observed. The graph stabilized between the ranges of 11 to 17 ns (Fig. 5b).

Discussion

The use of plants as medicine has long existed, and it has garnered significant prominence over the past few decades as a potential source of medications for a variety of illnesses including respiratory conditions[31]. It has been demonstrated that certain medications that have been utilized for centuries in traditional medical systems have strong antiviral activity against a wide range of viruses. Computational studies have revealed significant effects of numerous phytocompounds against various active targets of SARS-CoV-2, suggesting their potential in limiting disease transmission and infectivity[2]. The present study investigated the binding affinity of 13 compounds with SARS-CoV-2 S-glycoprotein and main protease by utilizing computational tools. Docking scores have determined that the most potential inhibitors of S-glycoprotein are Glycyrrhizin, Emetine, Zijusesquilignan A, and B, as they exhibited significant binding energy, comparable to the positive control, nelfinavir. It is inferred from the analysis of

ligand molecule interactions and energy values comparison that the Glycyrrhizin, Emetine, Zijusesquilignan A, and B effectively inhibit the binding of spike protein to host cells, suggesting their potential role in limiting virus entry into the host. Additionally, the phytoconstituents 5-O-Caffeoylquinic acid, Zijusesquilignan C, Schaftoside, Isoschaftoside, Vicenin-2, and 3,4-Dicaffeoylquinic acid have also demonstrated good binding affinity with spike glycoprotein, but slightly lower than the standard reference antiviral drug. These compounds may also play a vital role in preventing viruses from entry into the host.

The phytocompounds Isoschaftoside, 3,4-Dicaffeoylquinic acid, Schaftoside, Vicenin-2, Zijusesquilignan A and C, Emetine, and Glycyrrhizin showed significant binding affinity with 3CLpro. When compared to nelfinavir, a recognized protease inhibitor, Isoschaftoside, 3,4-Dicaffeoylquinic acid, Schaftoside, and Vicenin-2 had greater binding energy to 3CLpro, whereas, Zijusesquilignan A and C, Emetine, and Glycyrrhizin had binding energy comparable to the standard drug. The extensive network of H-bonds formed by these phytocompounds within the active site of protease receptor is noteworthy. It suggests that 3,4-Dicaffeoylquinic acid, Isoschaftosid, Schaftoside, Vicenin-2, Zijusesquilignan A and C, Emetine, and Glycyrrhizin may be able to limit the proteolytic processing and prevent viral transcription and replication. The remaining three phytocompounds Bornyl acetate, α-terpineol, Triacetonamine showed significantly lower binding affinity with both spike glycoprotein and 3CLpro, when compared to the standard reference drug. It may be inferred that while these compounds might not be able to directly interfere with the virus entry into the host cell and their transcription and replication, they might act synergistically in concert with other potential S-glycoprotein and 3CLpro inhibitors reported in this study to complement the repressing effects of these phytocompounds.

Molecular dynamics simulation demonstrates the conformational changes, dynamic equilibration, binding strength, and stability of native proteins with their protein-ligand docked complexes[2]. The MDS results revealed significant structural stability of the docked complexes in this study. Among four protein-ligand docked complexes, SARS-CoV-2 S-Zijusesquilignan A, and 3CLpro, 3,4-Dicaffeolquininc acid showed higher RMSD value and stable conformation when compared with their other counterparts, indicating their accelerated rigidity and stability upon binding to their respective native protein.

The solidity, compactness, and stability of docked complexes were confirmed by the RMSF pattern, and ROG analysis, suggesting the phytocompounds, Zijusesquilignan A and 3,4 Dicaffeolquininc acid, as potent therapeutic candidates against the SARS-CoV-2 S-glycoprotein and main protease, respectively.

The findings of the current study are consistent with those of previous reports. Glycyrrhizin and related terpenoids derived from the rhizomes of *Glycyrrhiza* have already been reported for diverse biological properties. Previous studies have demonstrated potent inhibitory effects of glycyrrhizin and its derivatives against SARS-CoV viral adsorption, penetration, and replication. Glycyrrhizin has also been reported to interfere with various potential biomolecular targets that are associated with the replication cycle of SARS-CoV-2[32]. Zijusesquilignan A-C, are fruit-derived compounds, known for anticancer and anti-inflammatory activities[33]. Emetine, a plant-derived alkaloid has been known for wide-ranging activities including antiprotozoal, emetic, anticancer, antiviral and anti-inflammatory activities. Recent studies have shown the inhibitory effects of emetine against SARS-CoV-2 by binding to several biomolecular targets, including main protease (Mpro), papain-like protease (PLpro), and others, suggesting emetine as one among the most potent anti-SARS-CoV-2 agents[34]. Schaftoside and isoschaftoside, a pair of flavonoid di-C-glycosides, are important plant defense compounds present in many cereal corps and medicinal plants including licorice root and quince. They exhibit a number of biological activities including anti-viral, anti-inflammatory, anti-oxidant, hepatoprotective, anti-hypertensive and anti-diabetic[35]. Recently, isoschaftoside has been identified as an effective 3CLpro inhibitors, suggesting its promising candidature for developing a potent anti-SARS-CoV-2 drug[36]. 3,4-Dicaffeoylquinic acid and 5-O-Caffeoylquinic acid also known as chlorogenic acids are the phenolic compound produced by numerous plants. It has been shown to exhibit various biological activities including anti-inflammatory, antioxidant, anti-bacterial, nephroprotective, hepatoprotective, anti-tumor[37]. 3,4-Dicaffeoylquinic acid has also

exhibited significant anti-viral effects against influenza A virus[38] and enterovirus A-71, showing a broad spectrum of inhibitory effects against several EV-A71 genotypes[39]. It has also been reported to have a significant inhibitory effect against SARS-CoV-2 M^{pro} protein[40]. Vicenin-2, a known flavonoid glycoside belonging to the apigenin group of phenolics has been shown to exhibit significant anti-inflammatory, antioxidant, antidiabetic, and wound healing activities[41]. Further, it has also been reported as potential SARS-CoV-2 3CLpro inhibitor[36].

These findings suggest that these 5 drugs may have great potential to inhibit both the SARS-CoV-2 Spike glycoprotein and main protease 3CLpro, and may help ameliorate the disease by targeting multiple pathological conditions. Ayush medical systems including Unani, Ayurveda, and Siddha lay great emphasis on holistic nature of medicinal plants and their products. However, a reductionist approach is primarily implicated in the current drug development strategies, and most studies look into the biological and toxicological potential of isolated compounds from medicinal plants. This leads to the disclosure of incomplete and perhaps inaccurate data regarding their legitimate potential. It is well-recognized that a single plant produces a broad range of diverse phytocompounds with wide-ranging pharmacological activities. The complex mixture of phytocompounds in the plant yields synergistic and multi-targeted effects with potentially improved biological activities[6,42]. Further, it may also limit microorganisms to evolve resistance to multi-sided attacks[6]. The development of dynamic techniques and effective scientific tools that are capable of examining several biological compounds acting simultaneously on possibly various targets is of utmost importance to preserve the holistic essence of traditional medicinal plants. Owing to the multi-targeted effects of diverse biological compounds present in these five drugs, it is pertinent to state that these drugs may prove valuable in the design and development of novel remedies for SARS-CoV-2 with greater potential than monotherapy options addressing only a single target. Besides direct inhibitory effects of phytocompounds reported in this study against SARS-CoV-2, the immunomodulators, antioxidants and anti-inflammatory compounds exist in these five drugs may also help in reversing the destructive processes and limiting the virulence and disease progression by augmenting the immune resilience of the individual.

Nevertheless, there are few limitations to this study. Firstly, the analysis of all the phytocompounds present in *C. oblonga*, *Z. jujuba*, *C. myxa*, *V. odorata*, and *G. glabra*, was not taken into account, as it was beyond the scope of the study. These 5 five plants have been found to contain over 700 phytoconstituents. However, only 13 lead compounds that have been previously documented to exhibit antiviral, antibacterial, antioxidant, and/or immunomodulatory effects were chosen for this study. The remaining phytoconstituents in these plants may also have the capability of limiting the virulence and infectivity of SARS-Co-2, but more thorough research is required to determine the precise potential of each plant by examining a variety of phytoconstituents. Considering only four protein-ligand docked complexes for MDS analysis is another limitation of the present study. Protein-ligand complexes of other compounds having good binding affinity with spike glycoprotein and 3CLpro may also exhibit accelerated rigidity, compactness and structural stability, indicating their potential as SARS-CoV-2 inhibitors. In order to fully investigate the aptitude of these five medicinal plants, it will be imperative to propose a more comprehensive and systemic strategy in future research. The current study has another limitation that the analysis of drug-likeness and ADMET (pharmacokinetics and toxicity) properties of compounds was not taken into account, which is regarded as a crucial step in the drug development process. Among various sets of guidelines and rules, Lipinski's rule, usually referred to as the rule of five (RO5), is a frequently used tool that aids in distinguishing between drug-like structures and non-drug-like structures. The chemical compounds considered to be the potential inhibitors need to adhere to the ADMET properties in additions to RO5 and other rules. As per Lipinski's rule, the chemical compounds that comply with the RO5 will only be regarded as active drug intended for oral administration and will have a greater probability of being commercialized. However, it is frequently observed in virtual screening that a number of approved drugs including remdesivir, the most commonly utilized medicine during the COVID-19 pandemic, do not fully comply with all screening rules, breaching the RO5 and other drug-likeness standards[43]. It is very likely that the phytocompounds that have been identified in this study as possible inhibitors may comply with all

of these rules, while some may defy some rules while validating others. Hence, future studies will be required to evaluate the drug-likeness and ADMET properties. Nevertheless, this study lays the groundwork for computational drug discovery of novel Unani therapeutics to curtail the SARS-CoV-2 transmission and infectivity.

Conclusion

The present study illustrates valuable insight into the inhibitory effects of 13 phytocompounds from the five most commonly used Unani drugs for the treatment of upper respiratory tract infections. The study demonstrated significant inhibitory effects of these phytocompounds against the potential SARS-CoV-2 drug targets, Spike glycoprotein, and main protease. The present study identifies Glycyrrhizin, Zijusesquilignan A, and Emetine as the most potential inhibitors for S glycoprotein and Isoschaftoside and 3,4-Dicaffeoylquinic acid as potent inhibitors of3CLpro. These findings suggest the great potential of traditional medicinal plants with their bioactive components in developing novel therapeutics for inhibiting viral adsorption, and replication and limiting the virulence of SARS-Co-2. However, further *in-vitro* and *in-vivo* investigations are required to substantiate this claim.

Supplementary Data

Supplementary data associated with this article is available in the electronic form at https://nopr.niscpr.res.in/jinfo/ijtk/IJTK_23(03)(2024)247-261SupplData.pdf

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Conflict of Interest

Authors declare that they do not have any conflict of interest.

Authors' Contributions

All the authors have equally contributed in conceiving the study, acquisition, analysis and data interpretation for the manuscript.

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