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Research Article

## In-Silico Evaluation of *Tiryaaq-E-Wabai*, an Unani Formulation for its Potency against SARS-CoV-2 Spike Glycoprotein and Main Protease

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### Abstract

COVID-19 was originated in Wuhan, China, in December 2019 and has been declared a pandemic disease by WHO. The number of infected cases continues unabated and so far, no specific drug approved for targeted therapy. Hence, there is a need for drug discovery from traditional medicine. *Tiryaaq-e-Wabai* is a well-documented formulation in Unani medicine for its wide use as prophylaxis during epidemics of cholera, plague and other earlier epidemic diseases. The objective of the current study is to generate in-silico evidence and evaluate the potency of *Tiryaaq-e-Wabai* against SARS-CoV-2 spike (S) glycoprotein and main protease (3CLpro). The structures of all phytochemicals used in this study were retrieved from PubChem database and some were built using Marvin Sketch. The protein structure of the SARS-CoV-2 S glycoprotein and 3CLpro was retrieved from the PDB ID: 6LZG and 7BQY respectively. AutoDock Vina was used to predict top ranking poses with best scores. The results of the molecular docking showed that phytochemicals of *Tiryaaq-e-Wabai* exhibited good docking power with spike glycoprotein and 3CLpro. Among tested compounds Crocin from Zafran and Aloin A from Sibr showed strong binding to spike glycoprotein and 3CLpro respectively. Molecular dynamics simulation confirmed the stability of the S glycoprotein-Crocin and 3CLpro-Aloin A complexes. The Unani formulation *Tiryaaq-e-Wabai* has great potential to inhibit the SARS-CoV-2, which have to be substantiated with further *in-vitro* and *in-vivo* studies.

**Keywords:** In-silico study, SARS-CoV-2, *Tiryaaq-e-Wabai*, Unani formulation, Crocin, Aloin A

## INTRODUCTION

COVID-19, the current pandemic is caused by Severe Acute Respiratory Syndrome Corona-Virus 2 (SARS-CoV-2) and originated in Wuhan, China, in December 2019<sup>1</sup>. The virus could infect human with symptoms similar to Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS)<sup>3</sup>. SARS-CoV-2 infection has been reported across 223 countries and India has the largest number of confirmed cases in Asia as per the latest reports<sup>4</sup>. The number of infected cases continues to soar high and effective targeted therapy options for COVID-19 remains limited. A number of research studies are in progress to find out a potent drug to curb the grave situation. Many traditional drugs, being used for millennia in Ayurveda, Unani, Siddha systems, have been reported to possess significant antiviral activities against a wide range of viruses<sup>5</sup>. A recent study demonstrated a high binding affinity of a Siddha formulation with spike protein of SARS-CoV-2<sup>6</sup>. These reports support the hypothesis that traditional drugs may also have a significant potential against SARS-CoV-2.

*Tiryaaq-e-Wabai* is a well-documented Unani formulation for its wide use as prophylaxis and treatment

during epidemics of cholera, plague and other epidemic diseases. It is comprised of 3 ingredients, viz Sibr (*Aloe barbadensis*), Murr Makki (*Commiphora mirrha*) and Zafran (*Crocus sativus*)<sup>7-10</sup>. Zakariya Razi (Rhazes, 865-925 CE) narrates 'whoever has used a mixture of two part of Sibr, one part zafran and one part Murr Makki, remained protected during epidemics'<sup>11</sup>. It is stated that the use of *Tiryaaq-e-Wabai* thrice a week on alternate days in a single dose of 500 mg, with *Arq-e-Gulab* 60 ml or *Arq-e-Badiyan* 120 ml, may protect the individual from infection during epidemics<sup>12-15</sup>. The formulation ingredients fall under the category of *Tiryaaqi Advia* (literally - antidote drugs) and are considered to be very effective in SARS like conditions especially in respiratory distress<sup>7,15,16</sup>. These drugs have been reported for wide-ranging pharmacological activities. The formulation has been reported to possess immune-stimulation activity in immunocompromised elderly persons<sup>17</sup>.

The medicinal use of Sibr (*Aloe vera*) can be traced thousands year back in the history. It has been used for various diseases in Unani medicine including digestive, respiratory, nervous system disorders and skin disorders. It is mentioned that the use of Sibr in any form; oral intake, fumigation, and spraying has promising effects during

epidemics. Gargle of Sibr in combination with Murr Makki is very effective in shortness of breath during epidemics<sup>16</sup>. *Aloe vera* has been reported to possess antiviral, anti-inflammatory, antimicrobial, antiseptic, immune-stimulating and wound healing activities<sup>18,19</sup>. It exhibits potent inhibitory effects against different viruses including human cytomegalovirus (HCMV), Influenza A virus (IAV), herpes simplex type 2 virus (HSV-2) via a number of mechanisms<sup>20-23</sup>.

Murr, commonly known as myrrh is an aromatic resin produced by *Commiphora myrrha* tree and traditionally used to treat a number of diseases including fever, common cold, chronic cough, diphtheria, tonsillitis, pharyngitis, bronchitis, flu, catarrh, asthma, arthritis, infectious diseases including leprosy and syphilis, septic wounds and other skin disorders<sup>24,25</sup>. It is considered to be very beneficial during epidemics due to its antiseptic property. Murr alone and in combination with other suitable drugs is very effective for asthma and respiratory distress syndrome in different forms *i.e.* oral, fumigation and local application on chest<sup>16</sup>. Murr has been reported for antimicrobial, antifungal, antiviral, antitumor, anticancer, anti-inflammatory, analgesic, antipyretic, antioxidant activities. Moderate antiviral activity of Myrrh essential oil against Newcastle virus (NDV) on chicken embryo has been reported<sup>26</sup>.

The utility of Zafran / Saffron in treating respiratory diseases is well acknowledged by Unani scholars besides its wide range of therapeutic actions. It is highly effective in all kind of altered respiratory functions<sup>16</sup>. Ibn Baitar (1197-1248 CE) has stated that '*saffron invigorates the pneuma and respiratory organs and facilitates respiration*'<sup>27</sup>. Avicenna has also stated that saffron especially its oil facilitates respiration and strengthens the respiratory organs<sup>28</sup>. Saffron has been reported for antiviral, antibacterial, anti-inflammatory, immunomodulatory, antioxidant, smooth muscle relaxant, antitussive, anti-allergic anticancer, anti-genotoxic activities<sup>28,29</sup>. Significant anti-HSV and anti-HIV activity of bio-active components of saffron 'crocin and picrocrocin' has been reported<sup>29</sup>.

SARS-CoV-2 entry into host cells is mediated by the transmembrane spike glycoprotein that forms homotrimers protruding from the viral surface. It binds favourably to the human Angiotensin-converting enzyme 2 (hACE2) receptor, through receptor binding domain (RBD) and delivers the virus particle inside the host. RBD is the most crucial target for finding appropriate inhibitors to stop entry of the virus in the host cell<sup>30</sup>. Similarly, to conquer viral infections the inhibition of proteases essential for proteolytic processing of viral polyproteins, such as main protease or 3-chymotrypsin-like cysteine protease (3CLpro), is an attractive therapeutic target<sup>31</sup>.

Molecular docking and molecular dynamic simulation are efficient tools in the field of drug discovery which could determine the binding energy between ligand and receptor. These tools have extensively been utilized for investigating the binding affinity of drug molecules and blocking SARS-CoV-2 receptors<sup>32-35</sup>. To our knowledge, molecular docking and molecular dynamic simulation between spike glycoprotein and 3CLpro of SARS-CoV-2 and *Tiryq-e-Wabai* ingredients has not yet been explored in previous studies. Hence this study was contemplated to generate in-silico evidence and evaluate the potency of *Tiryq-e-Wabai* against SARS-CoV-2 S glycoprotein and 3CLpro which could further be used for the development of potent drug for COVID-19.

## MATERIAL AND METHODS

### Receptor preparation

The protein structure of SARS-CoV-2 S glycoprotein and 3CLpro was retrieved from the PDB ID: 6LZG and 7BQY respectively. Receptor structures were processed with the Dock Prep module in UCSF (University of California, San Francisco) Chimera software (v1.14)<sup>36</sup>. The Dock Prep wizard adds hydrogen atoms, merges charge, removes non-polar hydrogen and deletes solvents. AM1-BCC charges were computed for the receptor which is included in Chimera. The covalent bond between the Cys145 residue and the crystallized ligand in 7BQY was eliminated. His and Cys residues were protonated and optimized using Chimera software.

### Ligand Preparation

The structures of phytocompounds used in this study were retrieved from PubChem database<sup>37</sup> and some were built using Marvin Sketch of the Marvin (v20.8.0) suite<sup>38</sup>. The 3D structure of the ligands was protonated and assigned AM1-BCC charges using Chimera's Dock Prep module.

### Receptor-ligand Docking

AutoDock Vina (v1.1.2), which is a molecular docking and virtual screening application, was used to predict top ranking poses with best scores<sup>39</sup>. SARS-CoV-2 protein structures retrieved from PDB ID: 6LZG and 7BQY for S glycoprotein and 3CLpro respectively. Protein and compounds were prepared for docking using UCSF Chimera Dock prep tool. Binding affinities between the protein and compounds were calculated using AutoDock Vina with exhaustiveness parameter at 8. UCSF Chimera was used to elucidate the binding interactions.

The search space for SARS-CoV-2 S glycoprotein was made as wide as the size of the RBD external subdomain (S438-Y505) in a grid box of (x= -37, y= 30.5, z= 6), so that the ligand will likely be docked to all parts of the receptor. In 3CLpro the co-crystallized ligand N3 (PDB ID: 7BQY) within the catalytic site was taken as a reference and the search space with a grid box of (x= -9.5, y= 12, z= 68.5). The results were subsequently analyzed using BIOVIA Discovery Studio Visualizer 2020 (v20.1.0)<sup>40</sup>.

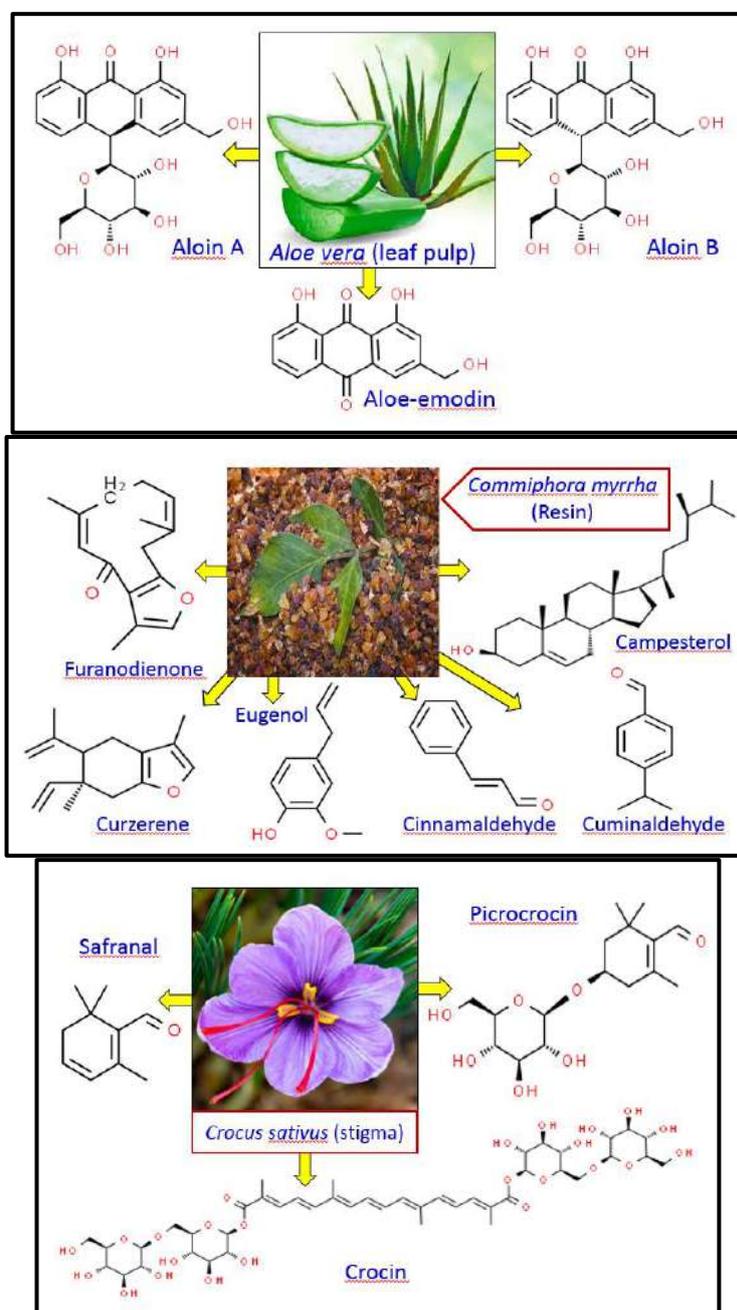
### Molecular Dynamics Simulation

The best two ligand-protein (Crocin & Aloin A) complexes were subjected to molecular dynamics simulation, using GROMACS (Version 2020.5) with OPLS (Optimized Potentials for Liquid Simulations) all-atom force field 2011<sup>41,42</sup>. The complexes were set in an orthorhombic box containing TIP3P water molecules and neutralized by adding salt counter-ions by employing the gmxgromp and gmxgenion commands<sup>43</sup>. The distance between the box wall and complexes was set to greater than 10 Å to avoid direct interactions with its own periodic image. In order to equilibrate the prepared systems, a series of restrained minimizations and MD simulations was carried out. The simulation system was relaxed by the constant NPT (number of atoms N, pressure P and temperature T) ensemble condition for the generation of the simulation data for the post simulation analyses. In order to provide constraints for the atoms and ions, the protein which is involved in the hydrogen bond interaction, the SHAKE algorithm was used<sup>44</sup>. The steepest descent minimization was carried out with the maximum of 2000 steps. The final production MD was carried out for 100ns for the complexes and then analysed using XmGrace tool<sup>45</sup>.

## RESULTS

Twelve phytochemicals present in *Tiryag-e-Wabai* viz Aloin A, Aloin B, Aloe-emodin from *Aloe vera*; Curzerene, Campesterol, Furanodienone, Eugenol, Cinnamaldehyde, Cuminaldehyde from Murr; Crocin, Picrocrocin and Safranal from saffron were retrieved from PubChem database or built using Marvin Sketch and depicted in Figure 1. Biological interactions of these compounds with RBD of S glycoprotein were studied in reference to the key contact residues for possible inhibition of the binding of RBD with host cell (Table 1). Table 2 shows the biological interactions of phytochemicals with 3CLpro in reference to the key contact residues. The AutoDock Vina docking scores are used as the key parameters to assess the ligand-protein interactions. A low (negative) energy indicates a stable system and thus a likely binding interaction.

Binding affinities of phytochemicals towards active site of SARS-CoV-2 S glycoprotein was studied in detail and reported in Table 1 and 2D interaction in Table S1 (Supplemental file). The results of the molecular docking showed that all the tested compounds have good docking energies ranging from -7.3 to -4.7 kcal/mol (Table 1; Figure 2). Nelfinavir was taken as a positive control and had binding energy of -7.2 kcal/mol. Three phytoconstituents 'Crocin from Saffron and Aloin A & Aloin B from *Aloe vera*' shown promising docking results with a binding energy of -7.3, -6.8 and -6.8 kcal/mol respectively, and had interactions with amino acid residues Arg403, Glu406, Asp420, Tyr421, Tyr453, Asn460, Tyr473, and Tyr505 that played important role in the formation of H-bond network. The candidate poses significant interactions with the residues of protein are presented in Figure 3.



**Figure 1:** Structural depiction of phytochemicals in *Aloe vera*, *Crocus sativus* and *Commiphora myrrha* for in-silico docking analysis

**Table 1: Amino acid residues of SARS-CoV-2 S glycoprotein (6LZG) participated in H-Bond and hydrophobic interactions with ligands.**

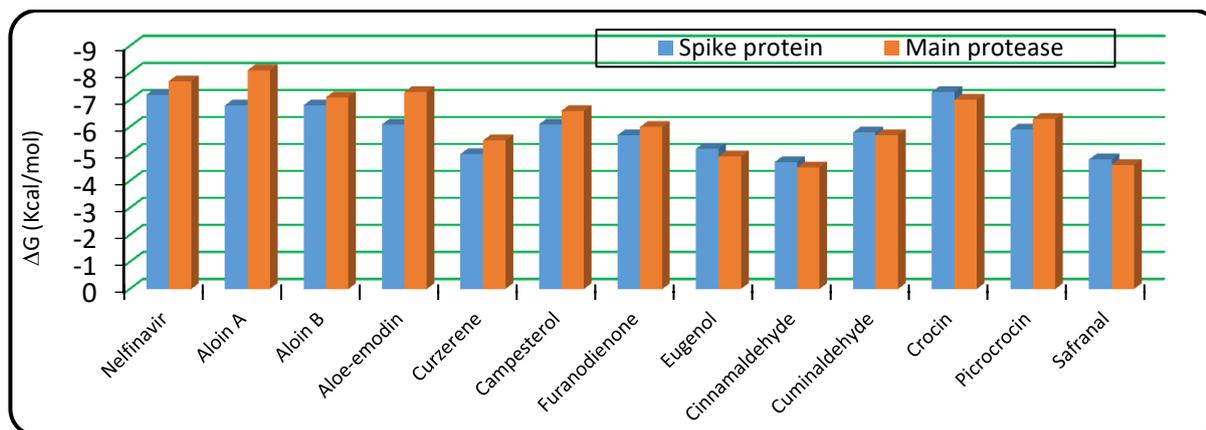
| Compound       | Binding Energy (Kcal/mol) | Interactions   |  |
|----------------|---------------------------|--|--|
|                |                           | H-Bonding  | Hydrophobic                            |
| Nelfinavir     | -7.2                      | Glu406, Tyr453, Gly496   | Leu455, Tyr505                         |
| Aloin A        | -6.8                      | Tyr453, Ser494, Gly496, Gln498, Asn501                         | Tyr505                                 |
| Aloin B        | -6.8                      | Tyr453, Gly496, Asn501, Tyr505                                 | Arg403                                 |
| Aloe-emodin    | -6.1                      | Tyr449, Tyr453, Ser494   | Gly496                                 |
| Curzerene      | -5.0                      | NHB  | Arg403, Tyr495, Gly496, Phe497, Tyr505 |
| Campesterol    | -6.1                      | NHB  | Tyr505                                 |
| Furanodienone  | -5.7                      | NHB  | Gly496                                 |
| Eugenol        | -5.2                      | Gly496   | Arg403, Tyr453, Tyr495, Tyr505         |
| Cinnamaldehyde | -4.7                      | Asn501   | Tyr505                                 |
| Cuminaldehyde  | -5.8                      | Tyr453, Ser494   | Arg403, Tyr495, Phe497, Tyr505         |
| Crocin         | -7.3                      | Arg403, Glu406, Asp420, Tyr421, Tyr453, Asn460, Tyr473, Tyr505 | Leu455, Phe456, Tyr495                 |
| Picrocrocin    | -5.9                      | Tyr453, Tyr449   |  |
| Safranal       | -4.8                      | NHB  | Tyr505                                 |

NHB: No Hydrogen Bond Interactions.

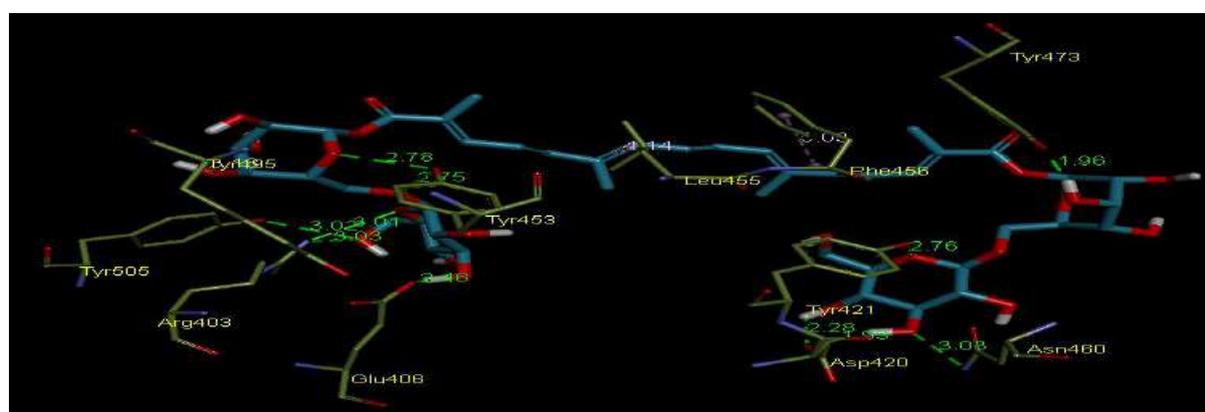
**Table 2: Amino acid residues of SARS-CoV-2 main protease 3CLpro (7BQY) participated in H-Bond and hydrophobic interactions with ligands.**

| Compound       | Binding Energy (Kcal/mol) | Interactions                                   |                       |
|----------------|---------------------------|--|-----------------------|
|                |                           | H-Bonding                                      | Hydrophobic           |
| Nelfinavir     | -7.7                      | His41, Glu166, Gln189                          | His41, Cys145, Met165 |
| Aloin A        | -8.1                      | Arg188, His164                                 | Met165, Glu166        |
| Aloin B        | -7.1                      | Leu141, Cys145, His163, Met165, Glu166, Asp187 | His41, Met49          |
| Aloe-emodin    | -7.3                      | Glu166   | His41, Arg188, Met165 |
| Curzerene      | -5.5                      | NHB  | Met49, Met165         |
| Campesterol    | -6.6                      | NHB  | Pro168                |
| Furanodienone  | -6.0                      | Gly143, Ser144, Cys145                         | Met49                 |
| Eugenol        | -4.9                      | Glu166   | Met49, Met165, Arg188 |
| Cinnamaldehyde | -4.5                      | Glu166   | Met49                 |
| Cuminaldehyde  | -5.7                      | Cys44, Tyr54                                   | His41, Met49, Met165  |
| Crocin         | -7.0                      | Thr25, Ser46, Asn119, Gln189                   | Cys145                |
| Picrocrocin    | -6.3                      | Thr26, Gly143, Ser144, Cys145, His163, Glu166  | Thr25, Met165         |
| Safranal       | -4.6                      | NHB  | His41, Met49          |

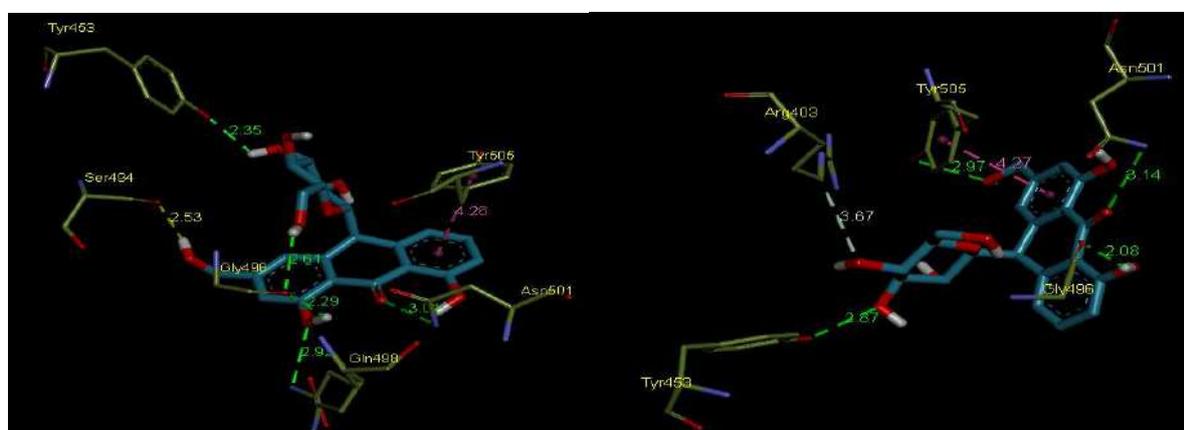
NHB: No Hydrogen Bond Interactions.



**Figure 2:** Histogram showing the energy binding value of  $\Delta G$  (kcal/mol) of S glycoprotein and main protease 3CLpro with *Triyaq-e-Wabai* phytocompounds.



**Crocin  $\Delta G$  -7.3 kcal/mol**



**Aloin A  $\Delta G$  -6.8 kcal/mol**

**Aloin B  $\Delta G$  -6.8 kcal/mol**

**Figure 3:** Interaction profile of *Triyaq-e-Wabai* 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 conventional H-bonding, Yellow indicates  $\pi$ -SH interaction, Pink denotes  $\pi$ -amide interaction and rest of them represents weak van der Waals interaction.

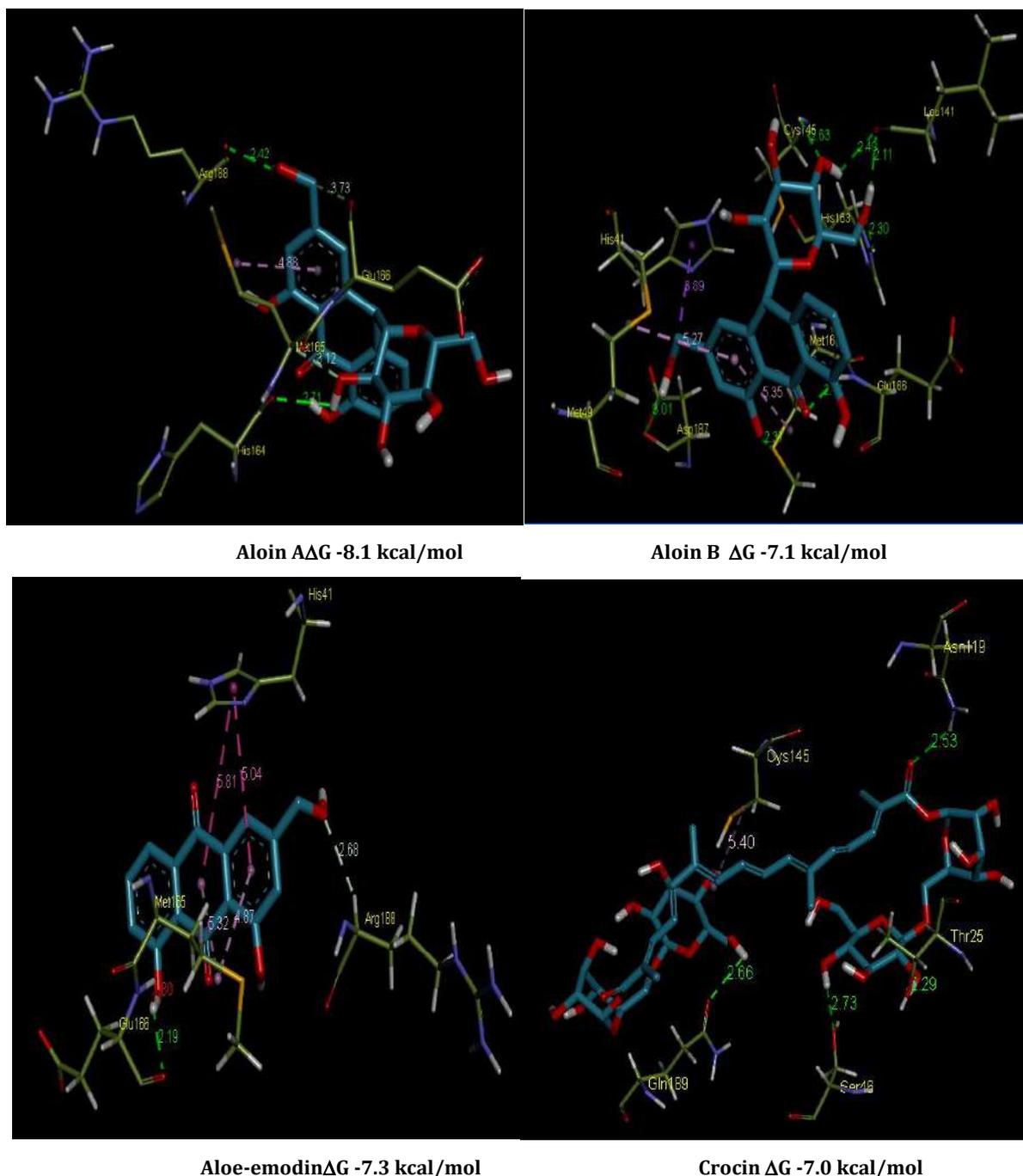
Interactions and binding affinities of phytocompounds with 3CLpro was studied in detail and depicted in Table 2 and 2D interaction in Table S1 (Supplemental file). The results of the molecular docking predicted that all the tested compounds showed docking energies ranging from -8.1 to -4.6 kcal/mol, as depicted in Table 2 and Figure 2. Nelfinavir had a binding energy of -7.7 and had H-bonding with His41, Glu166, Gln189 and hydrophobic interactions of Pi-Pi, Pi-Alkyl and Pi-donor with

His41, Cys145 and Met165 respectively (Table 2; Table S1). Aloin A, Aloin B, Aloe-emodin from *Aloe vera* and Crocin from saffron showed docking energies -8.1, -7.1, -7.3 and -7.0 kcal/mol respectively as shown in Table 2. The hydroxyl groups in anthraquinone form an extensive network of H-bonds within the protease receptor site with His41, Met165, and Glu166 residues.

The BIOVIA Discovery Studio Visualizer representations of the best docked poses with 3CLpro are shown in Figure 4. As

shown, the OH atom of anthraquinone group in Aloin A, formed H-bond with carbonyl group of Arg188 with a bond length 2.42 Å and His164 with bond length 2.71 Å. Aloin B formed six H-bonding interactions with six different amino acids (Leu141, Cys145, His163, Met165, Glu166, Asp187), in addition to hydrophobic interactions of Pi-Sigma and Pi-Alkyl in His41 and Met49 respectively. Aloe-emodin showed

three hydrophobic interactions of Pi-Pi, Pi-Alkyl and Pi-donor with His41, Met165 and Arg188 respectively along with H-bond with carbonyl group of Glu16 with a bond length 2.19 Å. Crocin formed H-bond with Thr25, Ser46, Asn119, Gln189 and showed hydrophobic interactions with Cys145.



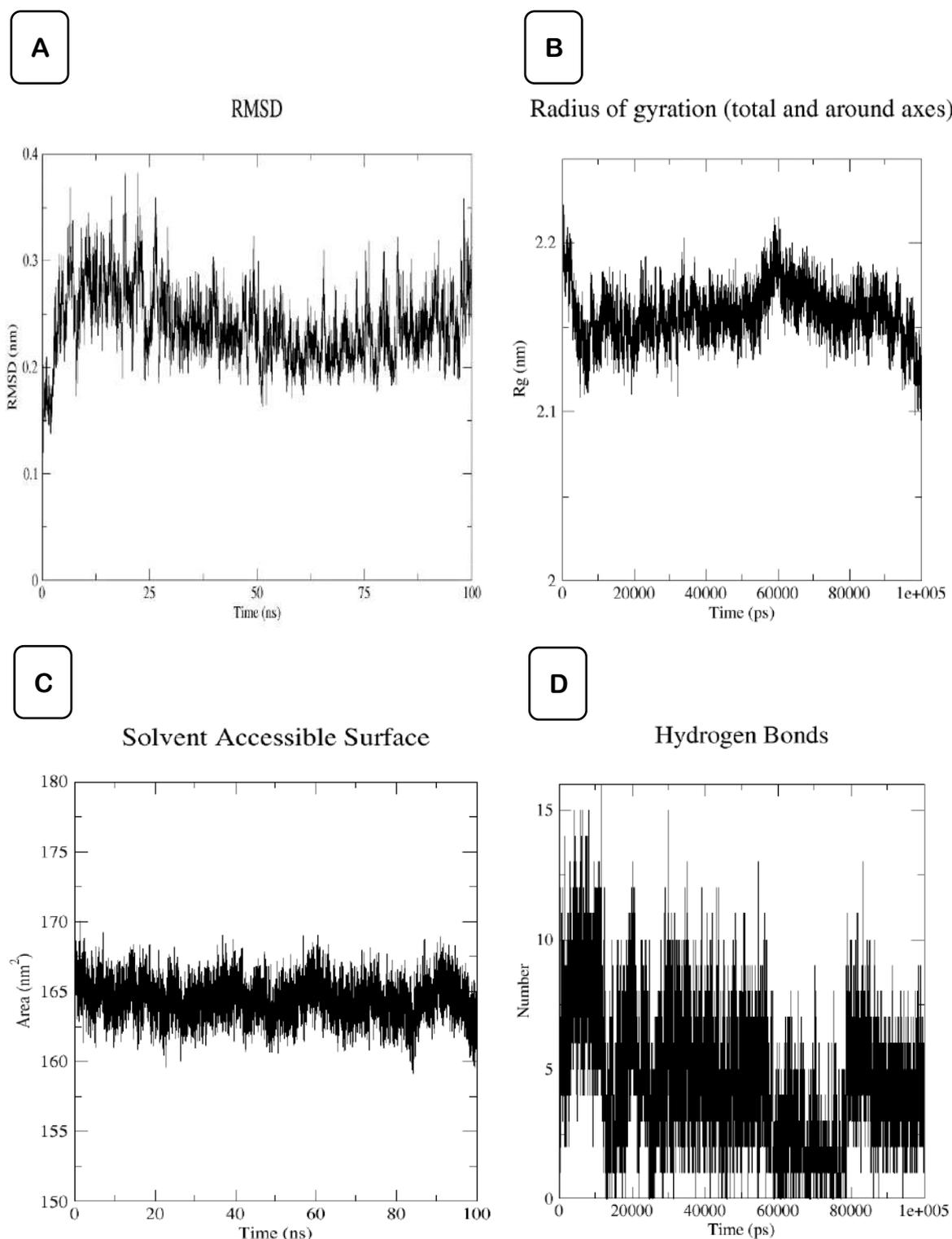
**Figure 4:** Interaction profile of *Triyaq-e-Wabai* phytocompounds and active site residues of SARS-CoV-2 main protease 3CLpro. Each color of amino acid residues and interaction markers indicates different types of interaction. Green represents conventional H-bonding, Yellow indicates  $\pi$ -SH interaction, Pink denotes  $\pi$ -amide interaction and rest of them represents weak van der Waals interaction.

Molecular dynamics simulation of SARS-CoV-2 S glycoprotein-Crocin and 3CLpro-Aloin A complexes were executed for 100ns. The 3CLpro-Aloin A complex remained stable throughout the simulation period with an

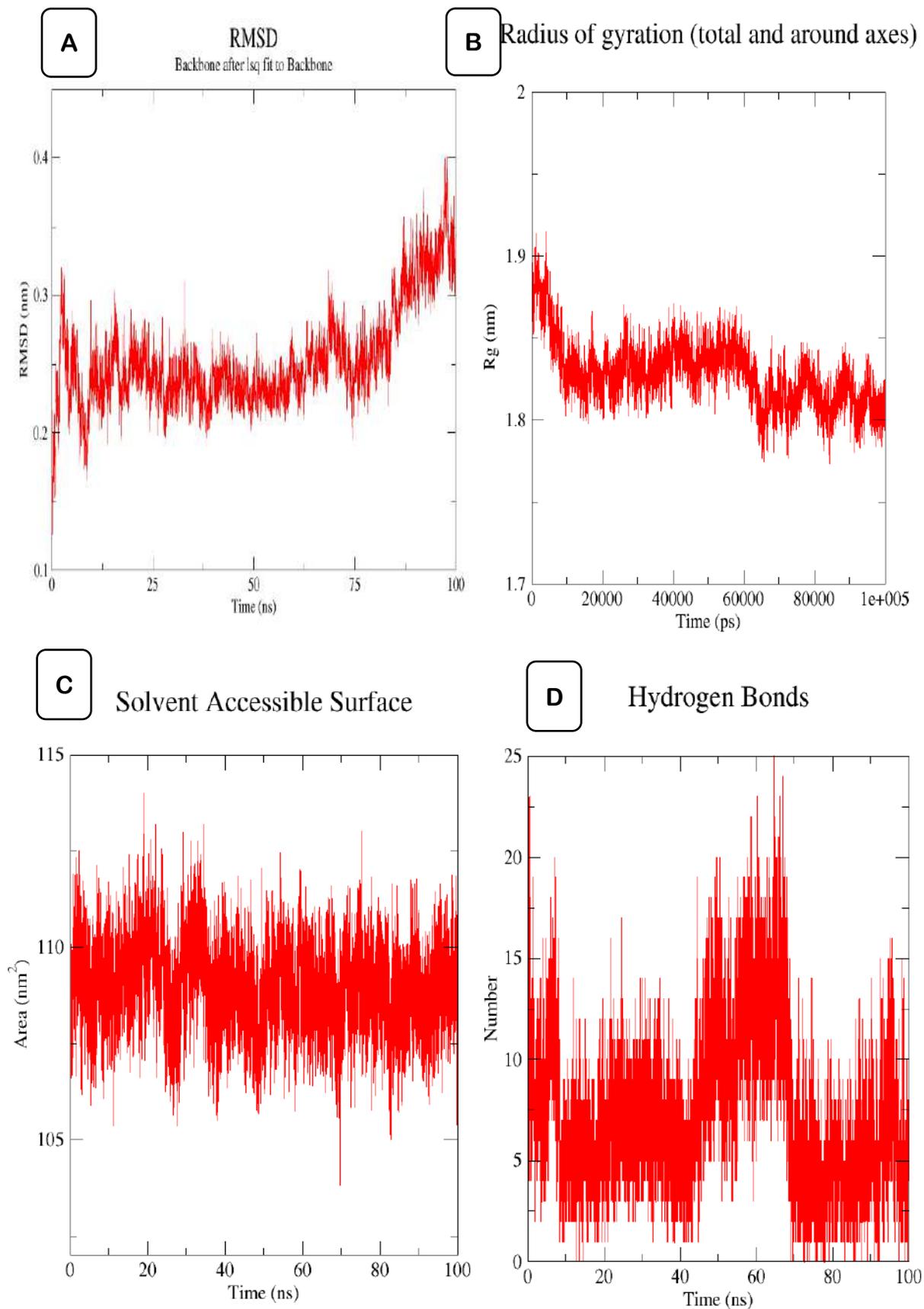
approximate RMSD value of 0.25nm (Figure 5A). The S glycoprotein-Crocin complex remained stable until 60ns and then diverged losing its stability (Figure 6A). In the gyration plot 3CLpro-Aloin A complex slightly loses its compactness

at 60<sup>th</sup> ns but remain intact throughout the simulation period (Figure 5B). The S glycoprotein-Crocin complex remained tight throughout the simulation time and had become more rigid after 75<sup>th</sup> ns in the gyration plot (Figure 6B). Solvent Accessibility Surface Area (SASA) calculation

revealed that there was no significant opening or closing motions between the protein and ligand (Figure 5C and 6C). The Hydrogen bond remained in oscillation throughout the 100ns in both the complexes with major increase and decrease (Figure 5D and 6D).



**Figure 5:** Molecular dynamics of SARS-CoV-2 3CLpro and Aloin A. Variation in **(A)** RMSD (Root mean square deviation), **(B)** Radius of gyration, **(C)** Solvent accessible surface area, and **(D)** Hydrogen bonds of 3CLpro-Aloin A complex during the simulation.



**Figure 6:** Molecular dynamics of SARS-CoV-2 S glycoprotein and Crocin. Variation in **(A)** RMSD (Root mean square deviation), **(B)** Radius of gyration, **(C)** Solvent accessible surface area, and **(D)** Hydrogen bonds of S glycoprotein-Crocin complex during the simulation.

## DISCUSSION

Plants have long been used as medicine for a number of ailments including respiratory afflictions. During the last few decades, it has gained a valuable popularity across the globe as a potential source of drugs for numerous diseases<sup>5,6,35</sup>. Recent in-silico studies have predicted a number of phytochemicals from different medicinal plants as potential inhibitors of SARS-CoV-2<sup>32,35,46,47</sup>. The Unani formulation '*Tiryaq-e-Wabai*' has significantly been utilized as prophylaxis and treatment during epidemics of cholera, plague etc. The ingredients of the formulation have been reported for diverse pharmaco-biological activities including antiviral, anti-inflammatory and immunomodulatory activities.

*Aloe vera*, the main constituent of the formulation, exerts antiviral activity against different viruses via a number of mechanisms. Interference of DNA synthesis of HCMV was reported to be the major mechanism involved in the inhibitory effect of Aloe<sup>20</sup>. It is reported that emodin 'anthraquinone compound' of Aloe possess significant antiviral activity against IAV, and could inhibit virus replication and influenza viral pneumonia via activation of nuclear factor E2-related factor 2 (Nrf2) signalling and by inhibiting IAV-induced activation of Toll-like receptor 4 (TLR4), Mitogen-Activated Protein Kinase (MAPK) and Nuclear Factor Kappa B (NF- $\kappa$ B) Pathways<sup>21</sup>. Aloe extract exhibited antiviral activity against HSV-2 via inhibiting virus replication in both pre and post attachment stages of virus to host cell<sup>22</sup>. Aloe polysaccharide has been reported for significant antiviral activity against H1N1 subtype influenza virus via direct interaction with PR8 (H1N1) influenza virus particles to prevent its adsorption and replication<sup>23</sup>. Bioactive components of saffron 'crocin and picrocrocin' have been reported for significant anti-HSV and anti-HIV activity. Both the components inhibited virus replication and suppressed their penetration in the target cells<sup>29</sup>.

The present study investigated the effects of 12 phytochemicals present in *Tiryaq-e-Wabai* against the most crucial druggable targets of SARS-CoV-2 'S glycoprotein and 3CLpro' through molecular docking and molecular dynamic simulation studies. It is established that SARS-CoV-2 S glycoprotein plays a vital role in the viral entry to the host cell, and therefore considered to be the crucial targets for drug discovery<sup>30</sup>. The present study predicted that all the tested phytochemicals have good binding affinities with S glycoprotein (Table 1; Figure 2). Three phytoconstituents namely Crocin, Aloin A and Aloin B showed promising docking results comparable to nelfinavir which was used as standard drug. Interestingly, Crocin showed a binding energy of -7.3 kcal/mol (Figure 3), better than that of standard drug. It was also found that all the docked ligands have effective binding interactions with S glycoprotein and interacting well with the same amino acid residues as that of hACE2. Therefore, phytochemicals present in *Tiryaq-e-Wabai* may prove as potential inhibitors of viral interaction with hACE2 receptors resulting in slowing down or restricting the viral entry in to the host cell.

The 3CLpro of SARS-CoV-2 plays an important role in viral transcription and replication. It includes at its active site, a catalytic dyad, His41 and Cys145. Thr190, Glu166, Phe140, Gln189 and His164 are known to play an active role in the interaction<sup>31</sup>. Therefore, these residues were targeted as centre point in this study for the molecular docking calculations and binding interactions. All the tested compounds in this study showed significant interaction and binding affinities with 3CLpro (Table 2; Figure 2). Four phytochemicals 'Aloin A, Aloin B and Aloe-emodin belongs

to the class of organic compounds known as anthraquinones from *Aloe vera* and Crocin from saffron' showed significant docking results comparable to nelfinavir. It is interesting to note that Aloin A showed a binding energy of -8.1 kcal/mol (Figure 4), lower than that of standard drug nelfinavir. The interaction of these phytochemicals with 3CLpro may prove effective in inhibiting the viral transcription and replication.

Molecular dynamics simulation (MDS) analysis gives insights in to the protein-ligand binding strength. In this study 100ns molecular dynamics simulation of SARS-CoV-2 S glycoprotein-Crocin and 3CLpro-Aloin A complexes were executed, wherein the RMSD value of 3CLpro-Aloin A complex remained stable throughout the simulation period (Figure 5A), while the S glycoprotein-Crocin complex diverged losing its stability after 60ns (Figure 6A). The gyration plot shows the compactness of Aloin A and Crocin with the respective proteins, wherein both the complexes were stable during the simulation. Solvent Accessibility Surface Area (SASA) was performed to study the traceable solvent region of the protein-ligand structure, and from the plotted graph for 100ns, it was found to be stable and showed no change in SASA. The results of radius of gyration and SASA confirmed the formation of a stable S glycoprotein-Crocin and 3CLpro-Aloin A complexes. The MDS analysis confirmed that both the lead compounds i.e. Crocin and Aloin A have significant compactness and stable binding efficiency within the cavity of S glycoprotein and 3CLpro respectively.

Similar to the present study, various studies have reported promising effects of phytochemicals from different plants against SARS-CoV-2 through molecular docking studies. It is interesting to note that many phytochemicals have shown good binding affinity to different active targets. A recent study on 100 phytochemicals from 10 medicinal plant demonstrated efficient binding affinity of five phytochemicals (emodin, anthrurufin, alizarine, aloe- emodin and dantron of *Rheum emodi*) with NTD of RNA binding domain of nucleocapsid phosphoprotein of SARS-CoV-2 at three different active sites [46]. Silybin from *Silybum marianum*, Withaferin A from *Withania somnifera*, Cordioside from *Tinospora cordifolia* and Catechin & Quercetin from *Aloe barbadensis* have been reported to possess binding affinity with SARS-CoV-2 target higher than hydroxychloroquine and other repurposed drugs which are commonly used for the treatment of SARS-CoV-2 infection<sup>47</sup>. Kumar et al, 2020 investigated the binding affinity of different phytochemicals with main protease (6LU7) and demonstrated good binding affinity (-7.4 Kcal mol) of Aloe-emodin and best ADME properties<sup>48</sup>, which corroborates with the present study findings.

Emodin an anthraquinone compound derived from genus *Rheum*, *Polygonum* and other genus like *Aloe vera* has been known for inhibiting the infectivity of different viruses. Emodin exhibited significant blocking potential of interaction between S protein and ACE2 and inhibited the infectivity of S protein-pseudotyped retrovirus to Vero E6 cells<sup>49</sup>. Emodin has also been reported to inhibit the 3a ion channel of SARS-CoV and HCoV-OC43 as well as virus release from HCoV-OC43 with a  $K_{1/2}$  value of about 20  $\mu$ M<sup>50</sup>. These studies have provided a basis for in-silico studies on phytochemicals against different specific targets, which could further serve as a basis for detailed investigations to accelerate the drug discovery process for the current pandemic.

## CONCLUSION

In the present study, the binding affinity of 12 phytocompounds of *Tiryaaq-e-Wabai* with spike glycoprotein and 3CLpro of SARS-CoV-2 was evaluated by employing molecular docking and molecular dynamics simulation. Based on the docking scores, Crocin and Aloin A have been identified as the most promising inhibitors of Spike glycoprotein and 3CLpro respectively. These findings suggest that *Tiryaaq-e-Wabai* may have great potential to inhibit viral adsorption and replication, which have to be substantiated with further *in-vitro* and *in-vivo* studies.

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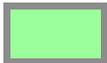
**Competing interests:** The authors declare that they have no competing interests

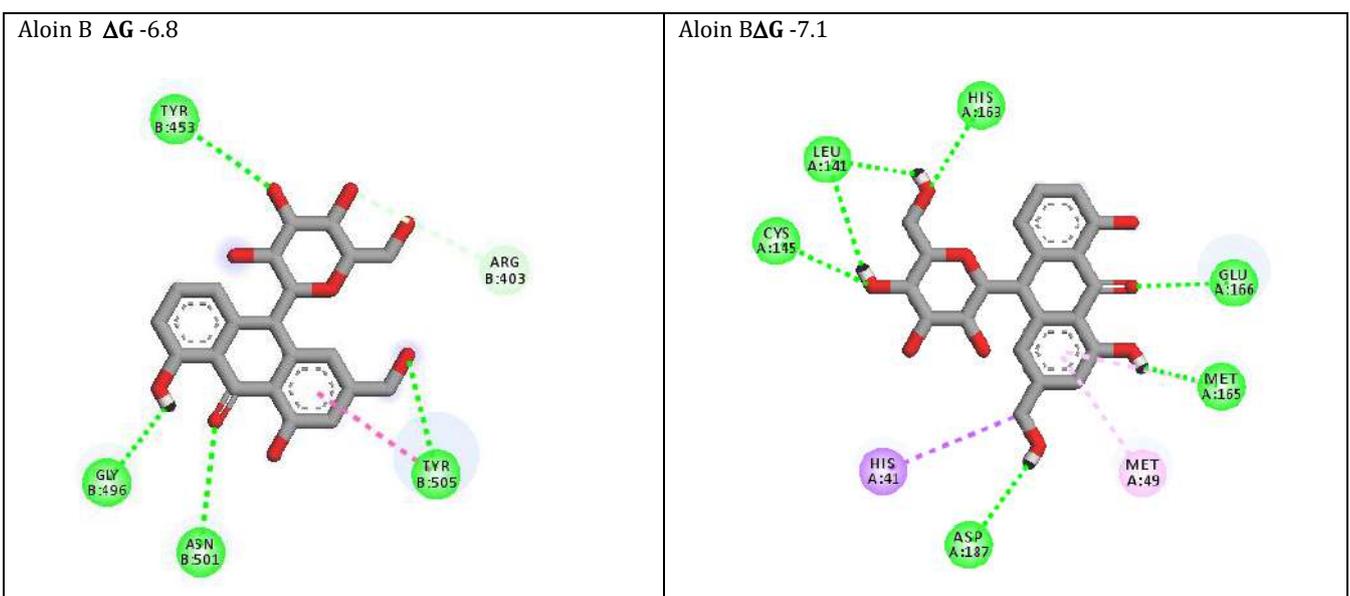
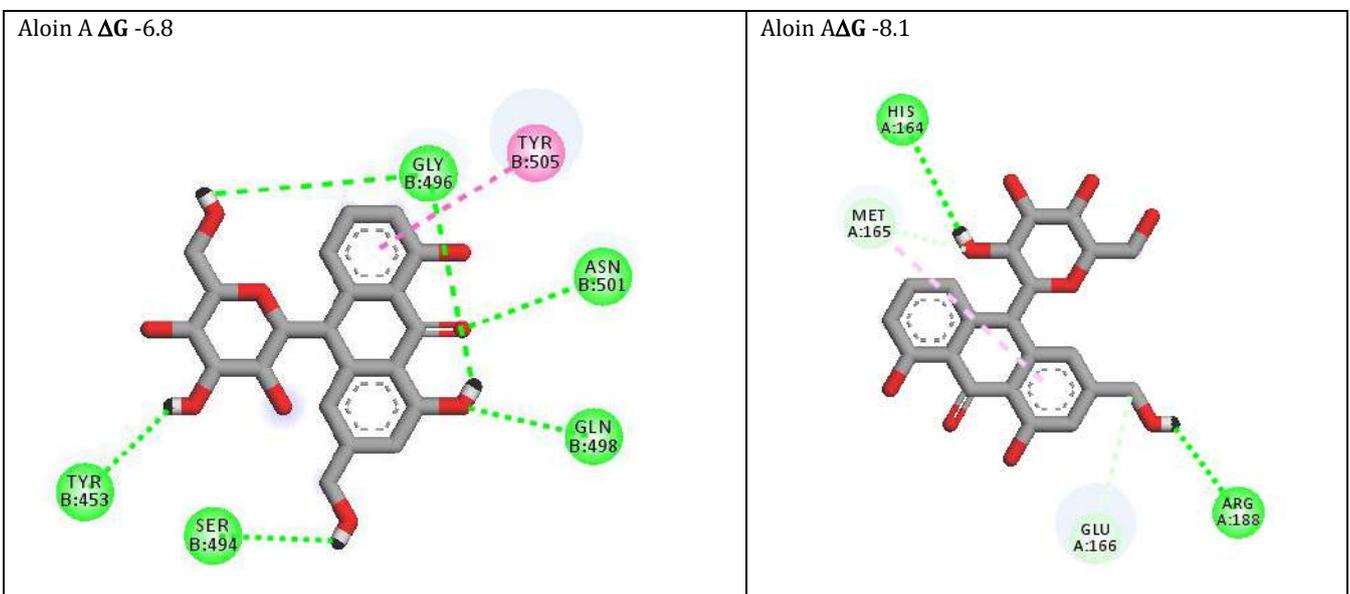
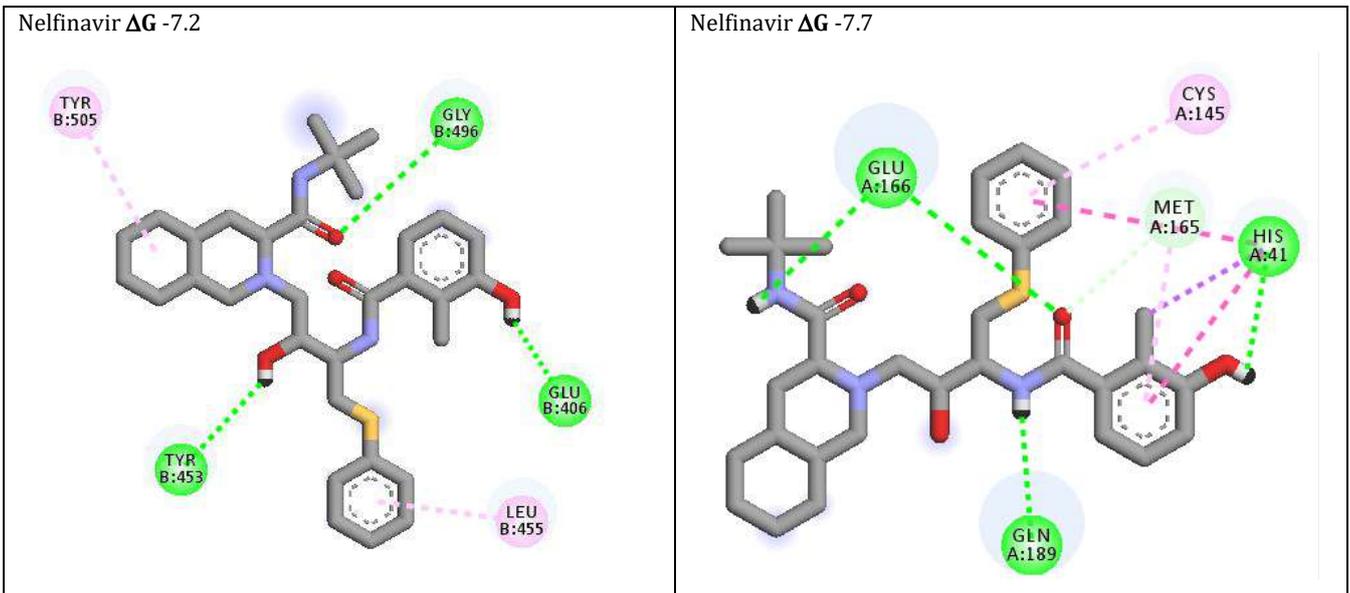
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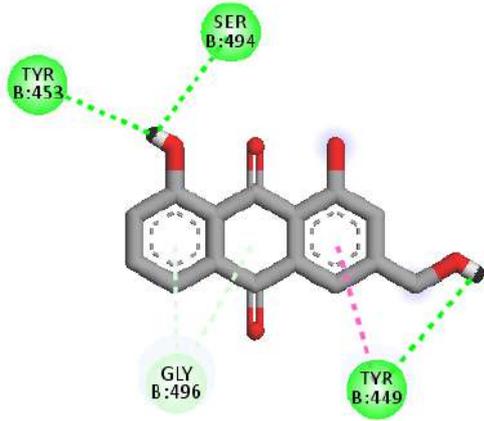
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**Table S1:** 2D representation of the interaction between *Triyaq-e-Wabai* phytochemicals and SARS-CoV-2 Spike (S) glycoprotein (6LZG) and Main Protease (7BQY). The ligand is shown in sticks and the interacting amino acid residues with their numbers are shown inside circles. Each colour of amino acid residues and interaction markers indicates different types of interaction.

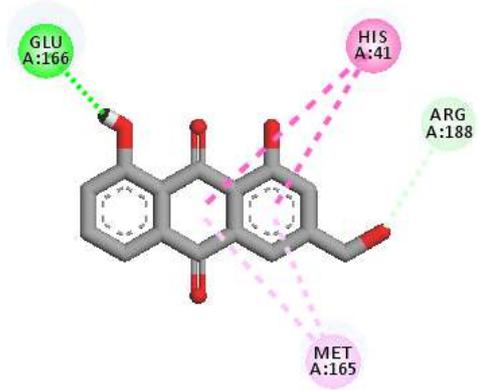
| SARS-CoV-2 Spike (S) glycoprotein<br>Binding Energy (kcal/mol)   | SARS-CoV-2 Main Protease (M <sup>pro</sup> )<br>Binding Energy (kcal/mol)                           |
|--|---|
| <b>Interactions</b>  |   |
|  van der Waals              |  Pi-Sigma       |
|  Conventional Hydrogen Bond |  Pi-Sulfur      |
|  Carbon Hydrogen Bond       |  Pi-Pi T-shaped |
|  Pi-Donor Hydrogen Bond     |  Pi-Alkyl       |



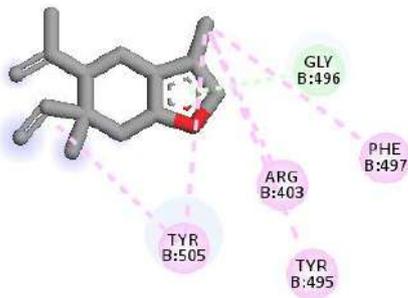
Aloe-emodin $\Delta$ G -6.1



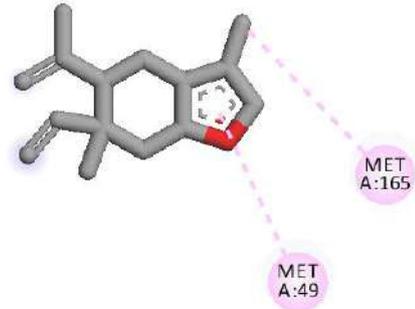
Aloe-emodin $\Delta$ G -7.3



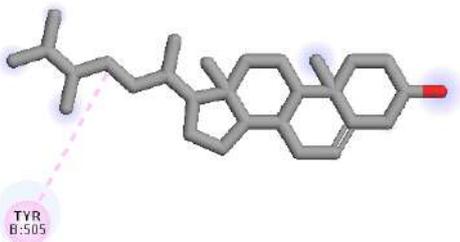
Curzerene $\Delta$ G -5.0



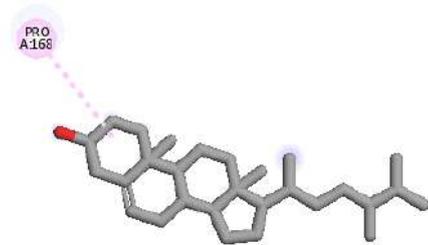
Curzerene $\Delta$ G -5.5



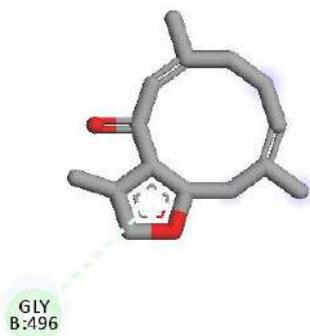
Campesterol $\Delta$ G -6.1



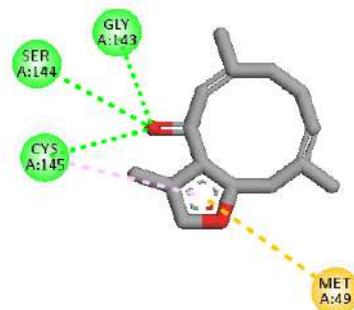
Campesterol $\Delta$ G -6.6

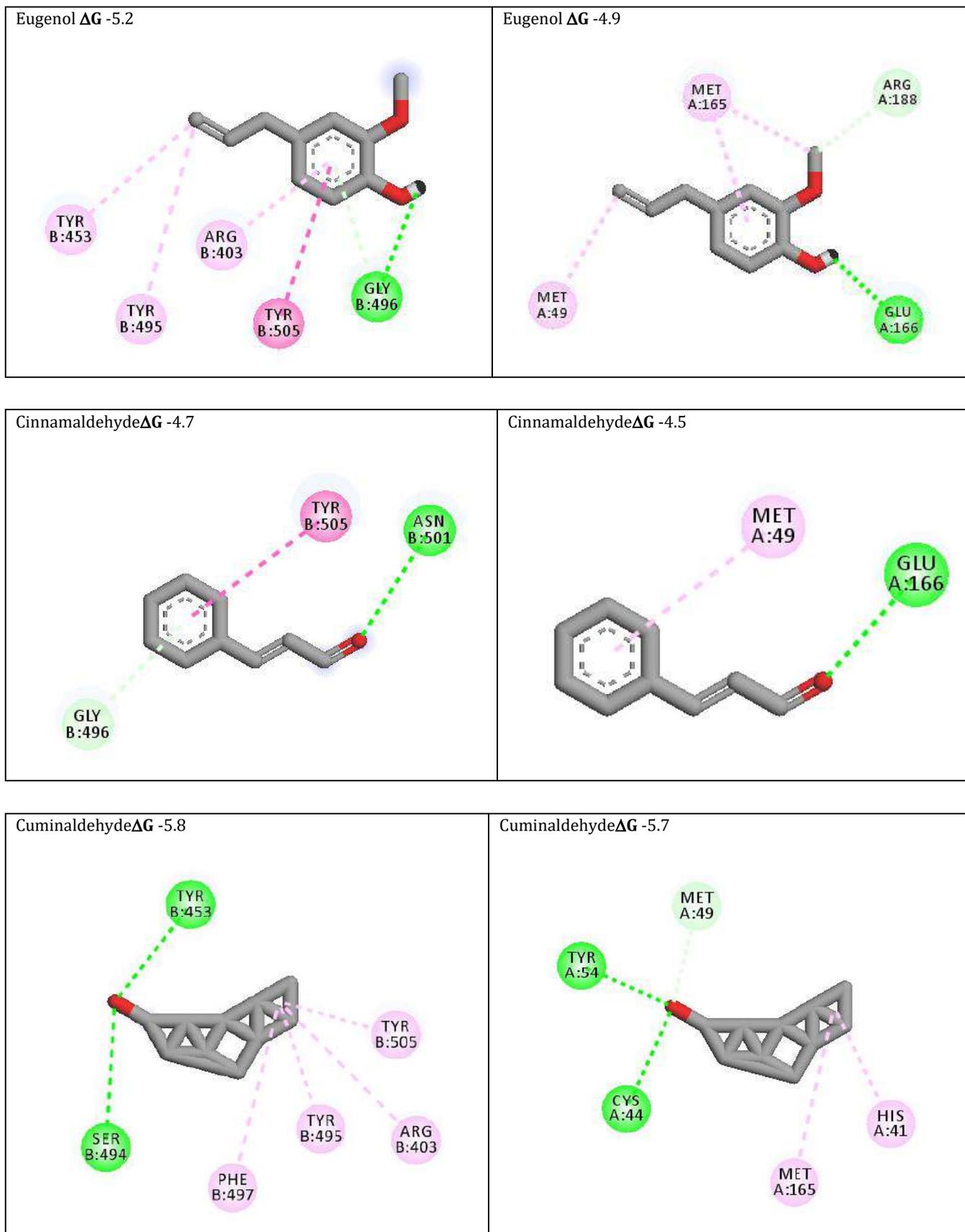


Furanodienone $\Delta$ G -5.7

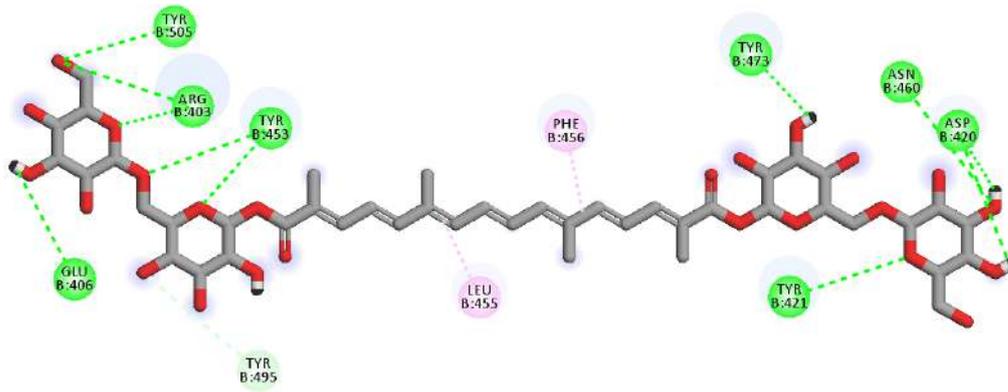


Furanodienone $\Delta$ G -6.0

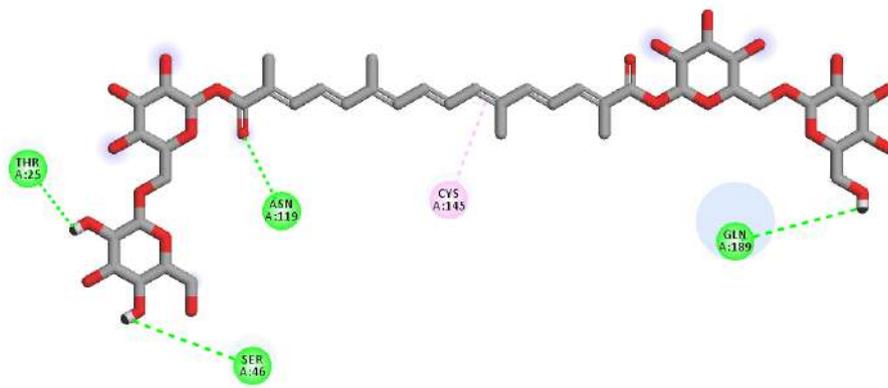




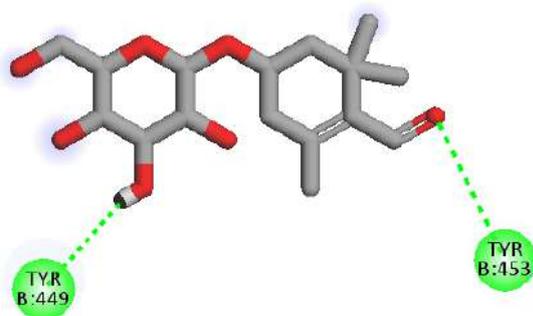
Crocin $\Delta$ G -7.3



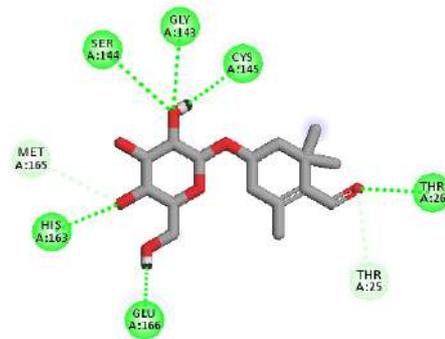
Crocin $\Delta$ G -7.0



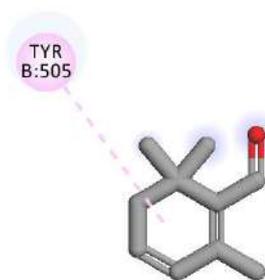
Picrocrocin $\Delta$ G -5.9



Picrocrocin $\Delta$ G -6.3



Safranal $\Delta$ G -4.8



Safranal $\Delta$ G -4.6

