

**Anti-COVID-19 effects of ten structurally different hydrolysable tannins through binding
with the catalytic-closed sites of COVID-19 main protease: An *in-silico* approach**

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Abstract

Coronavirus disease 2019 (COVID-19) was recently appeared all over the world. The viral main protease (3-chymotrypsin-like cysteine enzyme) controls COVID-19 duplication and manages its life cycle, making it a drug discovery target. Therefore, herein, we analyzed the theoretical approaches of 10 structurally different hydrolysable tannins as natural anti-COVID-19 through binding with the main protease of 2019-nCoV using molecular docking modelling via Molecular Operating Environment (MOE v2009) software. Our results revealed that there are top three hits may serve as potential anti-COVID-19 lead molecules for further optimization and drug development to control COVID-19. Pedunculagin, tercatain, and punicalin were found to faithfully interact with the receptor binding site and catalytic dyad (Cys145 and His41) of COVID-19 main protease, showing their successfully inhibit the protease enzyme of 2019-nCoV. We anticipated that this study would pave way for tannins based novel small molecules as more efficacious and selective anti-COVID-19 therapeutic compounds.

Keywords: COVID-19; Hydrolysable tannins; Protease; Molecular docking; Structural-relationship activity.

1. Introduction

Freshly, the novel coronavirus COVID-19 was discovered on the late of 2019 in Wuhan city, Hubei province, P.R. China [1]. COVID-19 was suddenly appeared, leading not only the health authorities, but also the scientific community to swift actions toward it. As a result, the whole-genome sequence of COVID-19 was released and subsequently the COVID-19 was scientifically spotlighted [2, 3]. COVID-19 goes to β -coronavirus group, allocation origin with bat coronavirus HKU9-1, alike to SARS-coronaviruses, and regardless of sequence variety its Spike protein strongly networks with human ACE₂ receptor [4]. Up to date, the death toll exceeded 5400, with 130 thousand confirmed cases, and 70 thousand recovered cases which was speeded all over the world.

Recent studies emphasized that COVID-19 genes stake <80% nucleotide uniqueness and 89.1% nucleotide likeness with SARS-CoV genes [5, 6]. Usually, β -coronaviruses yield a ~800 kDa polypeptide upon transcription of the genome. This polypeptide is proteolytically cleaved to create various proteins, and proteolytic processing is facilitated by papain-like protease, cleaving the polyprotein at 11 different spots to generate various non-structural proteins that are important for the viral reproduction [7]. Thus, this protease plays a key role in the duplication of virus particles, and unlike structural/accessory protein-encoding genes located at the 3' end that exhibit extreme variability, it may assist as a possible target for anti-COVID-19 inhibitors [8].

Structure-based activity analyses and high throughput studies have identified potential inhibitors of SARS-CoV and MERS-CoV 3CL_{pro} [9]. Bioactive substances, especially hydrolysable tannins, have attracted significant attention which could be used to develop drugs against diseases without side-effects [10]. Hydrolysable tannins are also considered to be useful in eradicating the adverse effects of various chemotherapeutic agents as well as in prolonging

longevity and attaining positive general health with anticancer potential and can be used as an alternative cancer drug resource [11-14].

Therefore, our study was designed to find out a potent anti-COVID-19 drug candidate from 10 structurally different hydrolysable tannins which could target the main protease of COVID-19 using *in-silico* methods (molecular docking and drug scan). The result of this study will facilitate the researchers to improve the status of anti-COVID-19 therapeutics.

2. Materials and Methods

Structure based simulated screening tactic was performed using high performing computing work station with the subsequent stipulations (Intel(R) Core(TM) i7-3210M CPU @ 2.50 GHz, 5 Core(s) processor with 4.00 GB RAM and 64-bit Windows-10 Operating System). Structure based drug screening was done with Molecular Operating Environment (MOE 09).

2.1. Ligand database arrangement

Structures of 10 hydrolysable tannins including, geraniin (PubChem CID-3001497), tellimagradin (PubChem CID-73179), punicalin (PubChem CID-5388496), castalin (PubChem CID-99973), strictinin (PubChem CID-73330), granatin A (PubChem CID-131752596), pedunculagin (PubChem CID-442688), casuarinin (PubChem CID-13834145), tercatain (PubChem CID-14411424), and bicornin (PubChem CID-71308161) were retrieved from PubChem database. The tannins structure was optimized for docking by adding partial charges and energy minimization via Protonate-3D and MMFF94X force field, separately. Optimized ligands files for the 10 tannins were stored in ligand database which was used later as an input file for docking studies.

2.2. Refinement of COVID-19 main protease structure

Likewise, three-dimensional structure of COVID-19 main protease (PDB:6lu7) was retrieved from the Protein Data Bank (<http://www.rcsb.org>) with 2.16 Å resolution. To refine the protein structure, already bound ligands and water molecules were removed from the structure, 3D protonation and energy minimization was done in MOE. The minimized structure was used for docking in the subsequent steps.

2.3. Molecular docking

MOE docking tool was used to dock the 10 structurally different tannins against allosteric ligand binding site of COVID-19 main protease. Potential binding pocket was identified using site finder tool of MOE and was further taken through docking process. Ten best docked poses were generated through applying a scoring function London dG. Refinement of docking procedure was done by applying forcefield algorithm which keeps the receptor rigid. From them, best interacting ligands and molecules were screened on the basis of RMSD (Root-Mean-Square Deviation) which is usually measured in Angstrom (Å), and docking score. Already reported co-crystallized ligand. Ligand receptor binding analysis was done through LigX tool in MOE. It is designed to show potential residues interacting with the ligand molecules graphically. It generates 2D images representing the forces stabilizing ligand molecules within binding pockets of receptors [15].

2.4. Drug scan

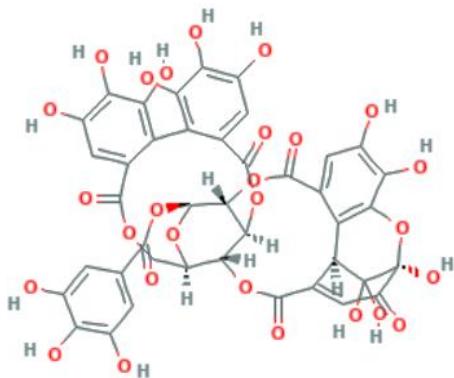
To analyze the drug likeliness of 10 tannins, all were further filtered on the basis of the bearing appropriate molecular properties to be anti-COVID-19 drug candidate.

3. Results and discussion

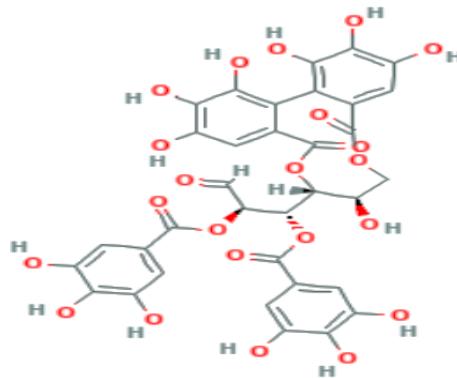
Analysis of allosteric binding of 10 structurally different tannins with COVID-19 protease initially revealed many poses and we used the best pockets with low S-score. Iterative docking of

the best docked molecules within receptor pocket originally occupied by co crystallized inhibitor.

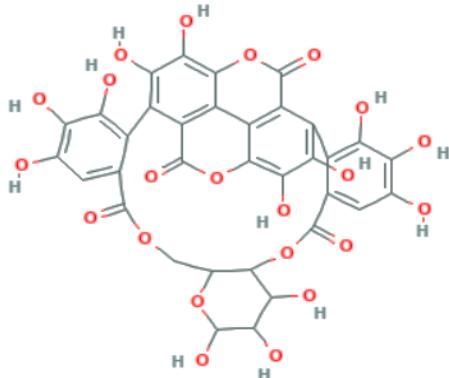
The chemical structure of our 10 structurally different tannins are shown in **Fig. 1**. The COVID-19 main protease consisted of 306 residues with chain type of polypeptide(L) as 3D-portrayed in **Fig. 2**.



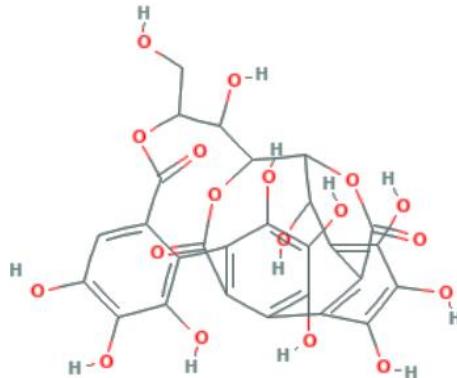
Geraniin



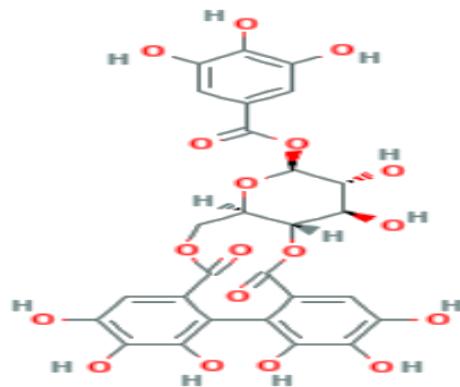
Tellimagrandin



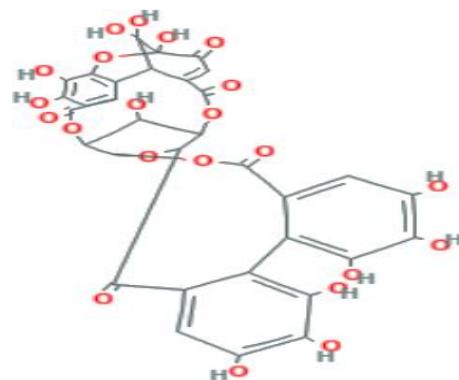
Punicalin



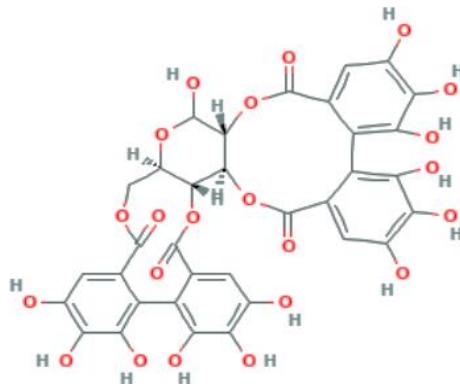
Castalin



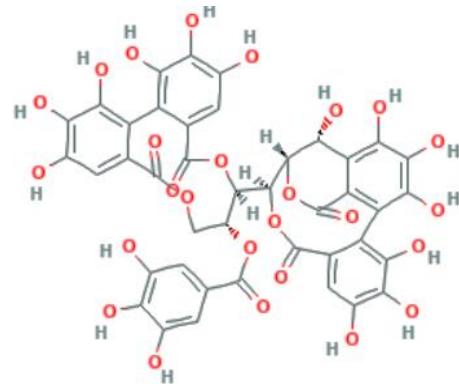
Strictinin



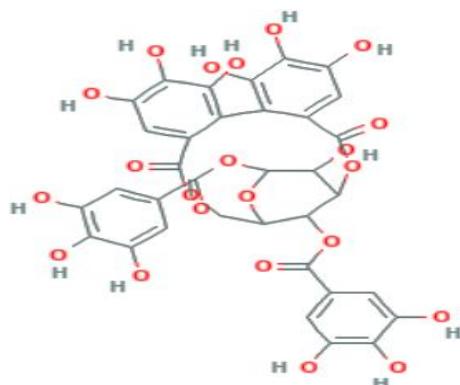
Granatin A



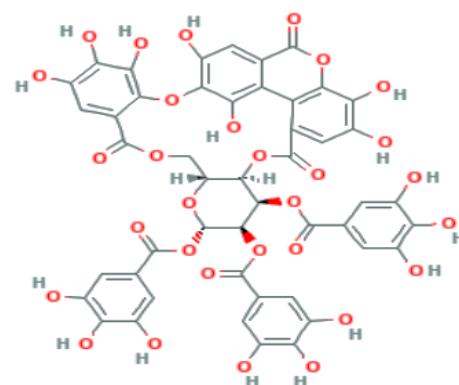
Pedunculagin



Casuarinin



Tercatain



Bicornin

Fig. 1. The 2D-chemical structures of 10 selected hydrolysable tannins.

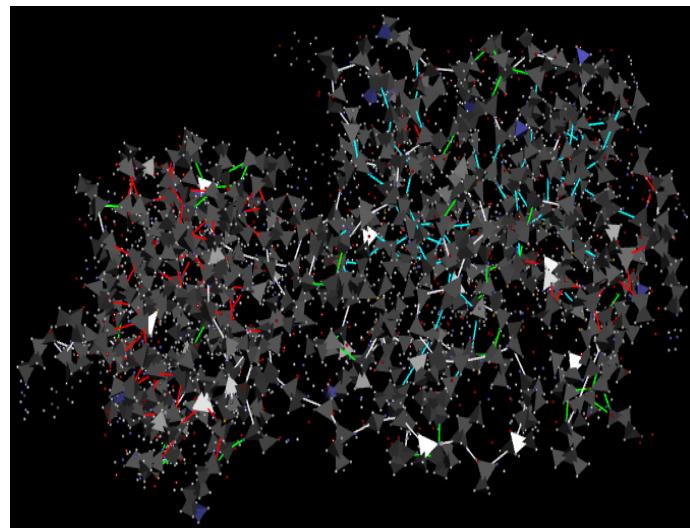


Fig. 2. The 3D-chemical structures of COVID-19 main protease.

To manage with the constant need of novel and effective small molecule as anti-COVID-19 therapeutics with negligible side effects, research is now directing more on computational drug discovery [16]. Multiple studies reported antiviral potential of tannins via computer aided drug design [11, 12]. To fasten the drug approval procedure and to discover more efficacious inhibitors with novel scaffolds that can improve the antiviral therapeutics status, computational drug discovery approaches are highly reliable. Form the ground-breaking detail of structural diversity among hydrolysable tannins, virtual screening was done to discover novel allosteric compound as anti-COVID-19. Allosteric regulation has been reported as effective strategy to attain irreversible inhibition. Spatial orientation and dock score of current reported four top-ranked leads reveals efficient binding of functional residues with maximum binding affinity. Moreover, these tannins meet the drug likelihood criteria shown in **Table. 1** and may prove excellent ready to use starting point.

Binding affinity analysis of selected tannins through LigX shown in **Fig. 3**, revealed that 10 tannins interacted with the crucial catalytic residues of pocket Spatial position of geraniin is

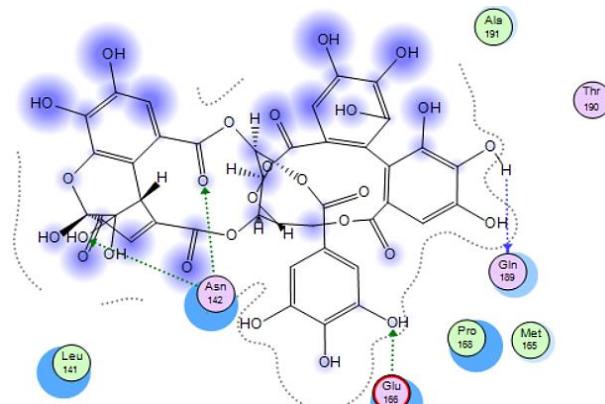
stabilized within the pocket with the lowest S-score (-9.9) and interacting through H-bonds with Gin 189, Glu 165, and Asn 142 while rest of close lying residues of Leu141, Pro158, Met155, Ala191, and Thr 190 are having weak electrostatic interactions with geraniin. Tellimagradin inlaid within the same binding pocket as shown in **Fig. 3**, lying closer to Phe294 and Gin110 via H-bonding and arene-arene forces, in addition to Thr292, lle249, Pro132, Glu240, Gly109, Pro241, Asp245, His246, Pro108, Thr243, and Gin107 residues of COVID-19 protease binding pocket with dock score of -12.7 kcal mol⁻¹. Punicalin experiences water mediated and H-bonding with Gin189, His41, and Thr25 while other closely lying 12 residues are also found. Castalin interacted with Glu166 and Thr190 via H-bonds, and Asn142, Met165, Gln189, and Arg188 were also involved. Likewise, strictinin and casuarinin networked with (Asn142 and Gln189) and (Asn142 and Glu166) through H-bonding and there are 6 amino acids residues were also participated in the interaction of strictinin and/or casuarinin with COVID-19 protease with S value of -3.9 and -10.3, respectively. Pedunculagin has been shown to in laid well within the binding pocket showing H-bonding with Ser45, His41, Thr25, and Gly143, in addition to arene-arene interactions with His41. Meanwhile, 10 amino acid residues were also involved in the interaction with COVID-19 main protease with S value of -0.6. Granatin A was only noncovalently interacted with 8 amino acid residues of COVID-19 protease with dock score of -14.7 kcal mol⁻¹. Tercatain and bicornin were assembled with (His41, Asn119, and Thr25) and (Thr24 and Gln189) with 10 and 6 residues of their interaction with COVID-19 protease, respectively.

Our analyses identified three novel non-toxic, druggable natural products that are predicted to bind to the receptor binding site and catalytic dyad (Cys145 and His41) of COVID-19 main protease (**Fig. 3**). Among these hydrolysable tannins, pedunculagin, strongly interacted with the catalytic dyad residues (Cys-145 and His-41) of COVID-19 main protease, as well as interactions

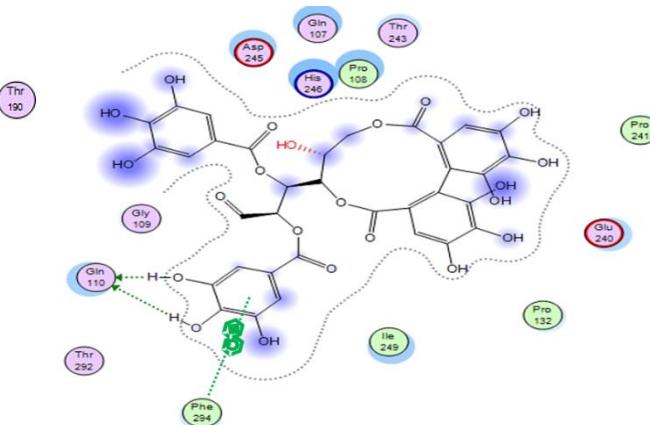
with receptor-binding residues of other 15 residues (**Fig. 3** and **4**), with sense binding affinity and docking score. Previously, pedunculagin, which could be extracted from pomegranates, walnut, and Indian gooseberry, showed antiviral activity and other biological activities. Pedunculagin was followed by tercatain and punicalin where both of them were interacted with His41 with total between 13 to 15 other residues. Likewise, tercatain and punicalin which also could be mainly extracted from pomegranates, in the leaves of *Terminalia catappa*, and *Combretum glutinosum*.

Looking at the antiviral drug potential of aforementioned tannins, current study was an endeavor to exploit the chemical nature of three hydrolysable tannins as anti-COVID-19. Current molecular docking study has shown the important interactions of medicinal plant tannins with the main protease of COVID-19, which were successfully block both His41 and Cys145.

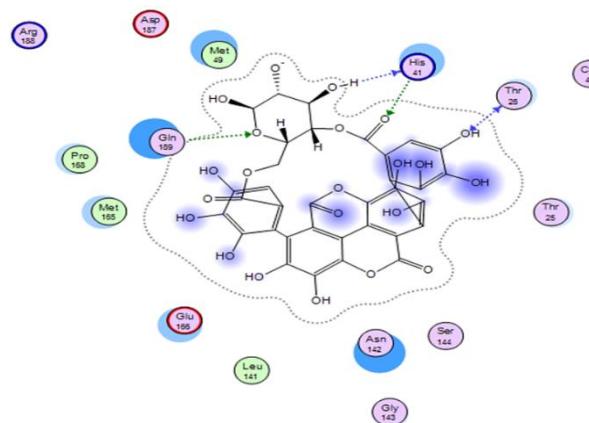
To verify the drug ability of selected tannins, ligand properties were calculated with LigX tool of MOE (**data not showed**). All selected tannins showed positive results and fulfill the criteria of the Lipinski's rule of five [15]. The rule describes that potential drug like compound should not have more than 5 hydrogen bond donors, maximum 10 hydrogen bond acceptors, and an octanol water partition coefficient log P not greater than 5. These results suggest that natural products identified in our study, specially pedunculagin, tercatain, and punicalin, may prove more useful candidates for COVID-19 drug therapy. Thus, it can be concluded from this study that these pedunculagin, tercatain, and punicalin and their sources such as pomegranates and nuts can be used as strong anti-COVID-19 in the near future.



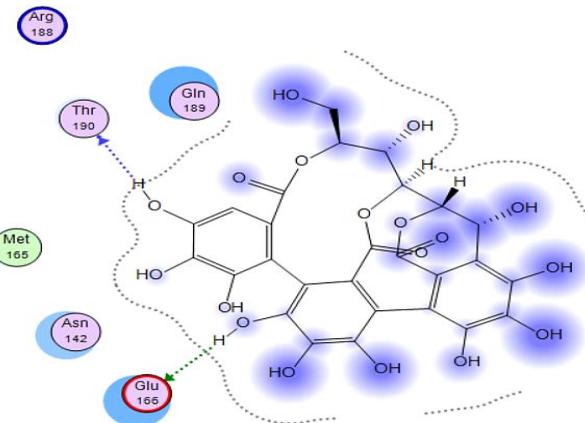
Geraniin



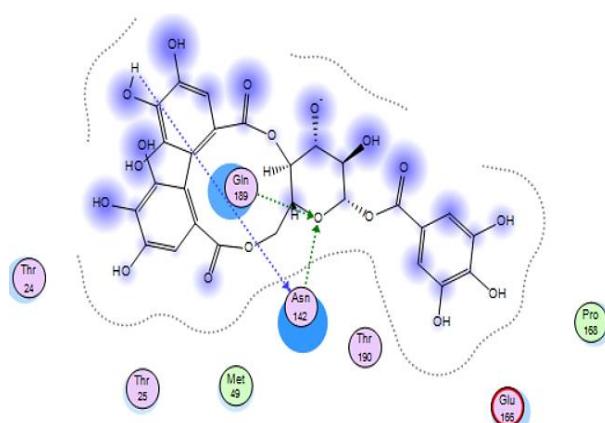
Tellimagrandin



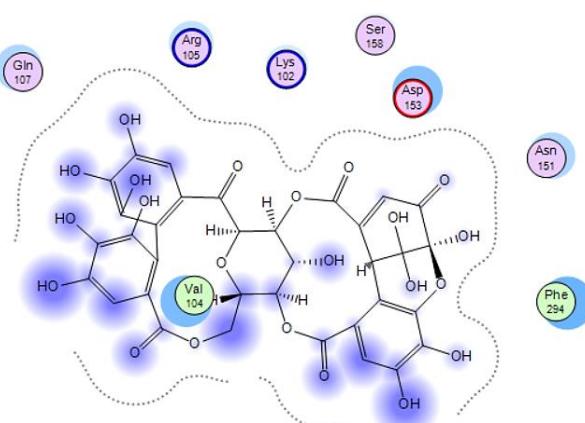
Punicalin



Castalin



Strictinin



Granatin A

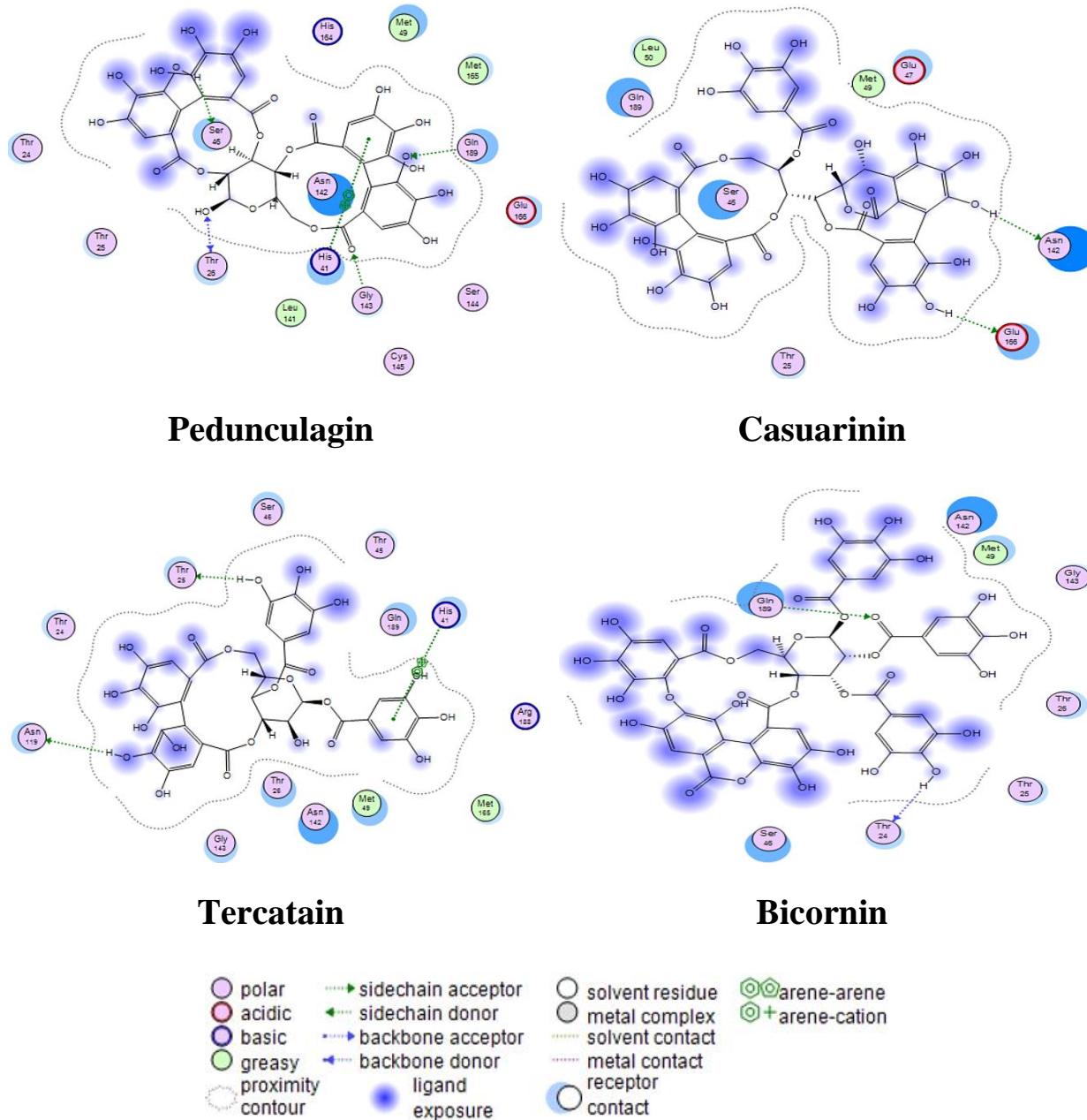
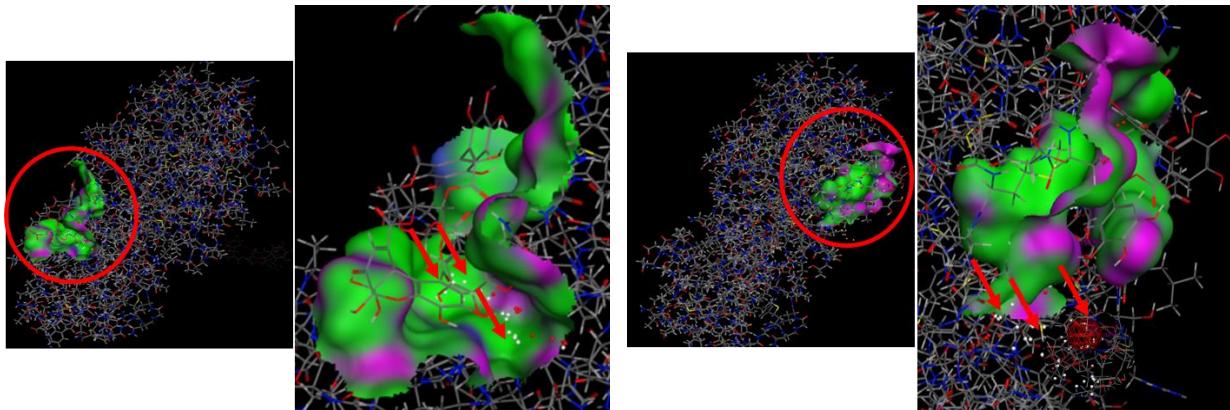
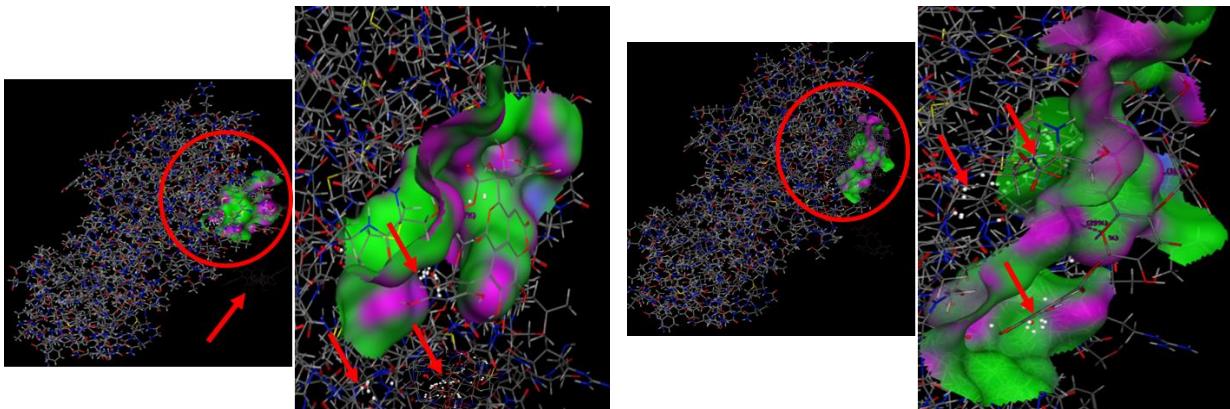
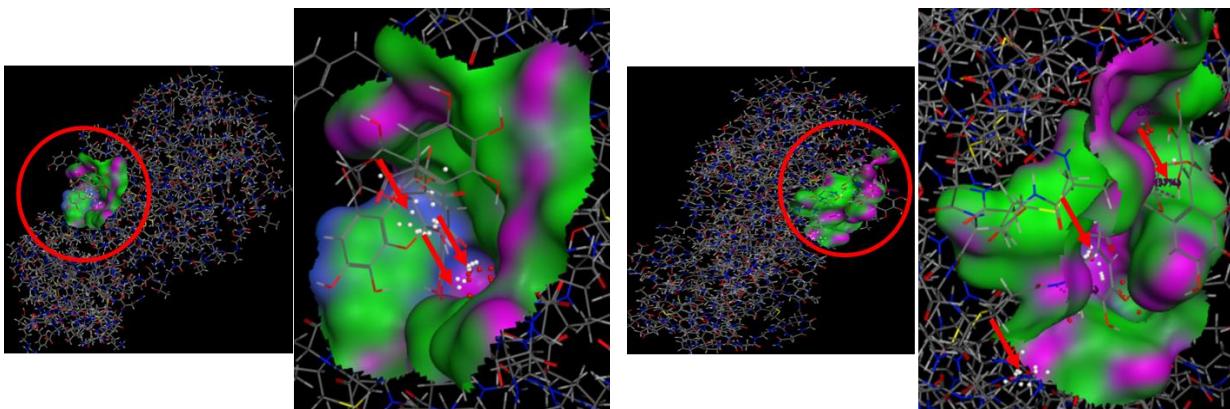


Fig. 3. LigX interaction diagram representing binding pattern of 10 hydrolysable tannins with binding pocket residues of the crystal structure of COVID-19 main protease.

**Geraniin****Tellimagratin****Punicalin****Castalin****Strictinin****Granatin A**

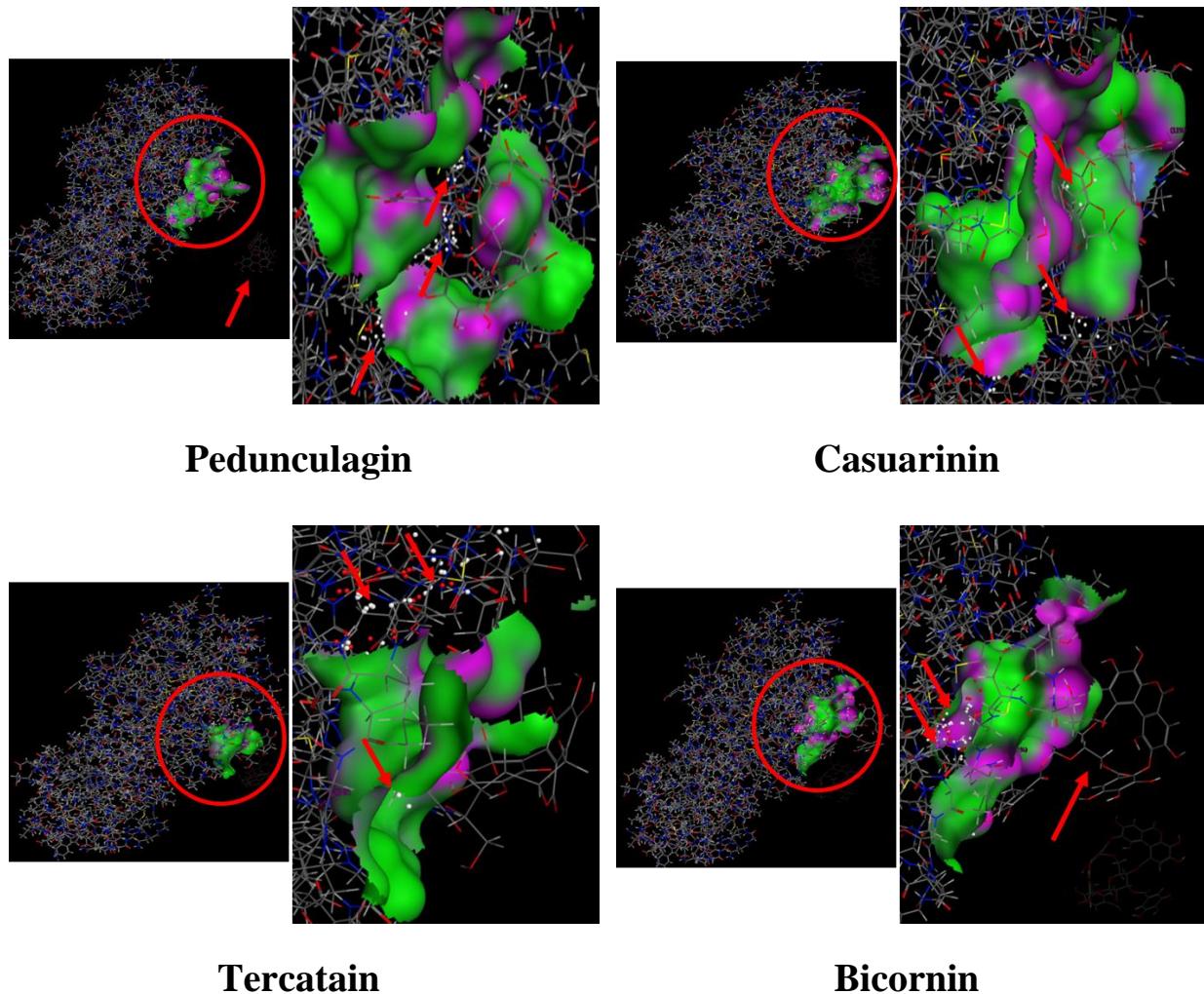
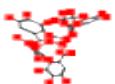
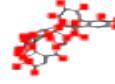
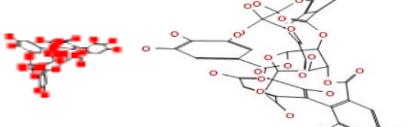
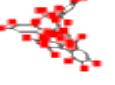
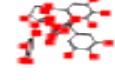
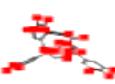
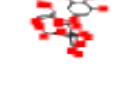
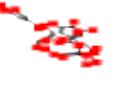
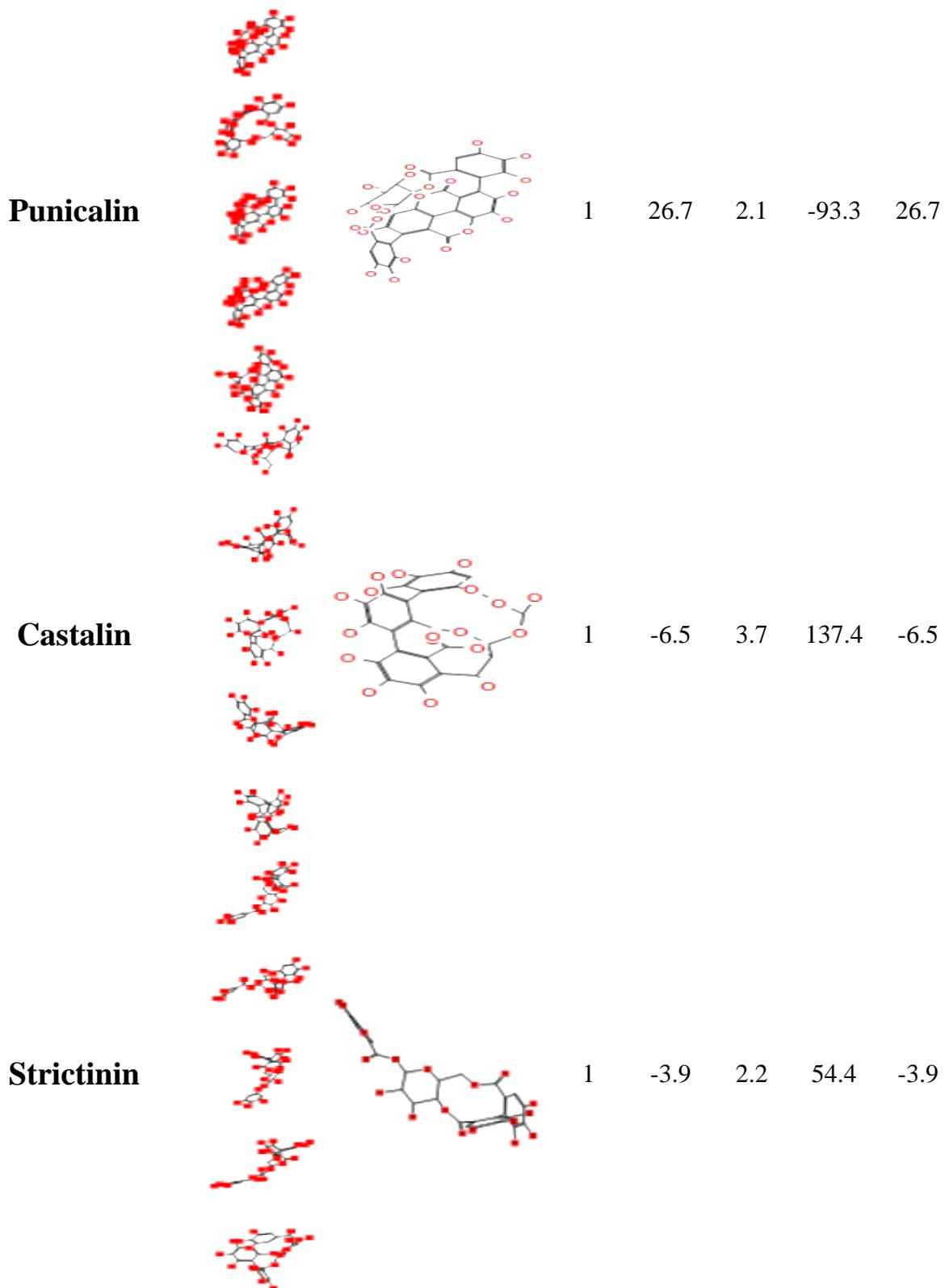
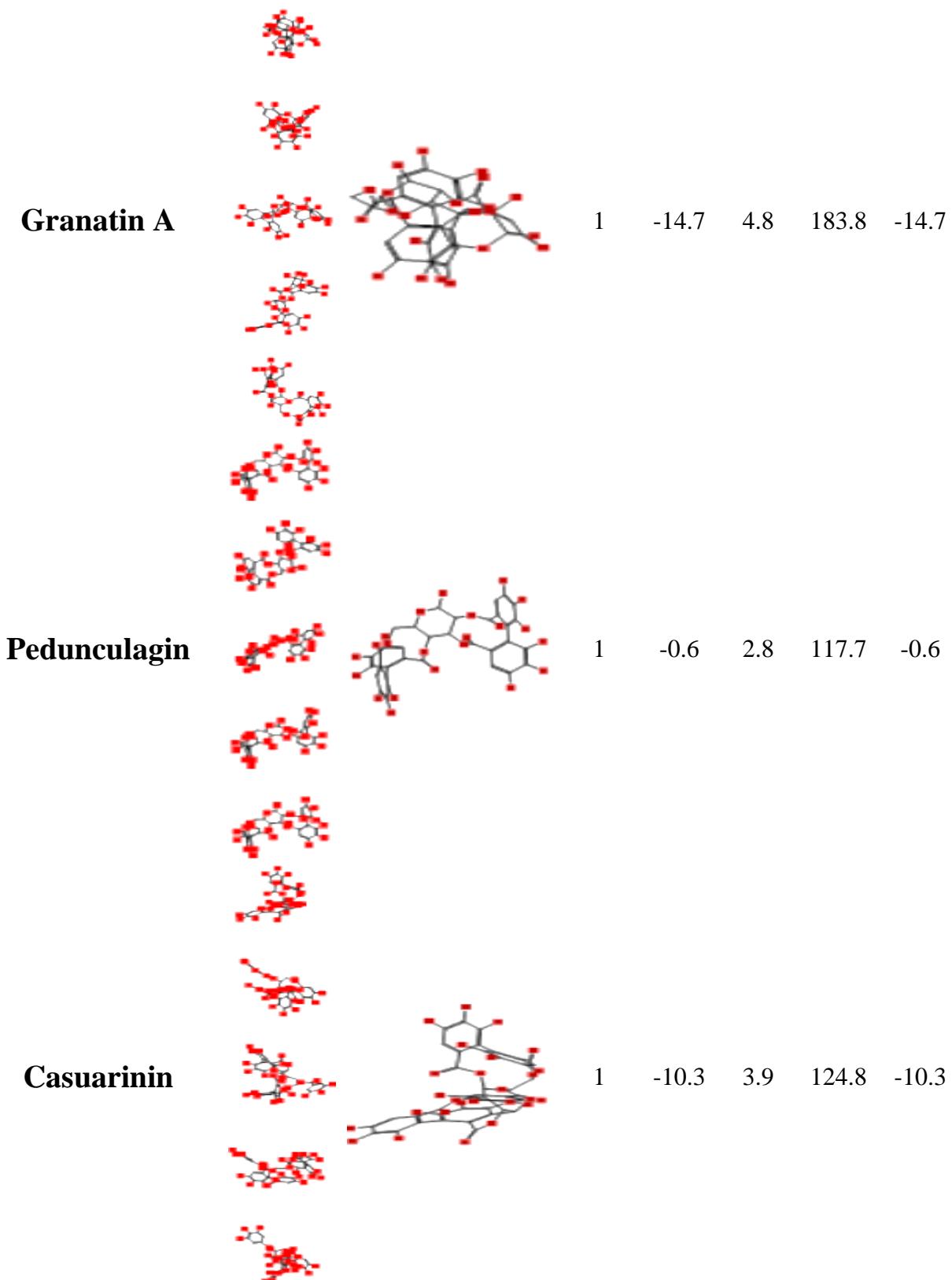


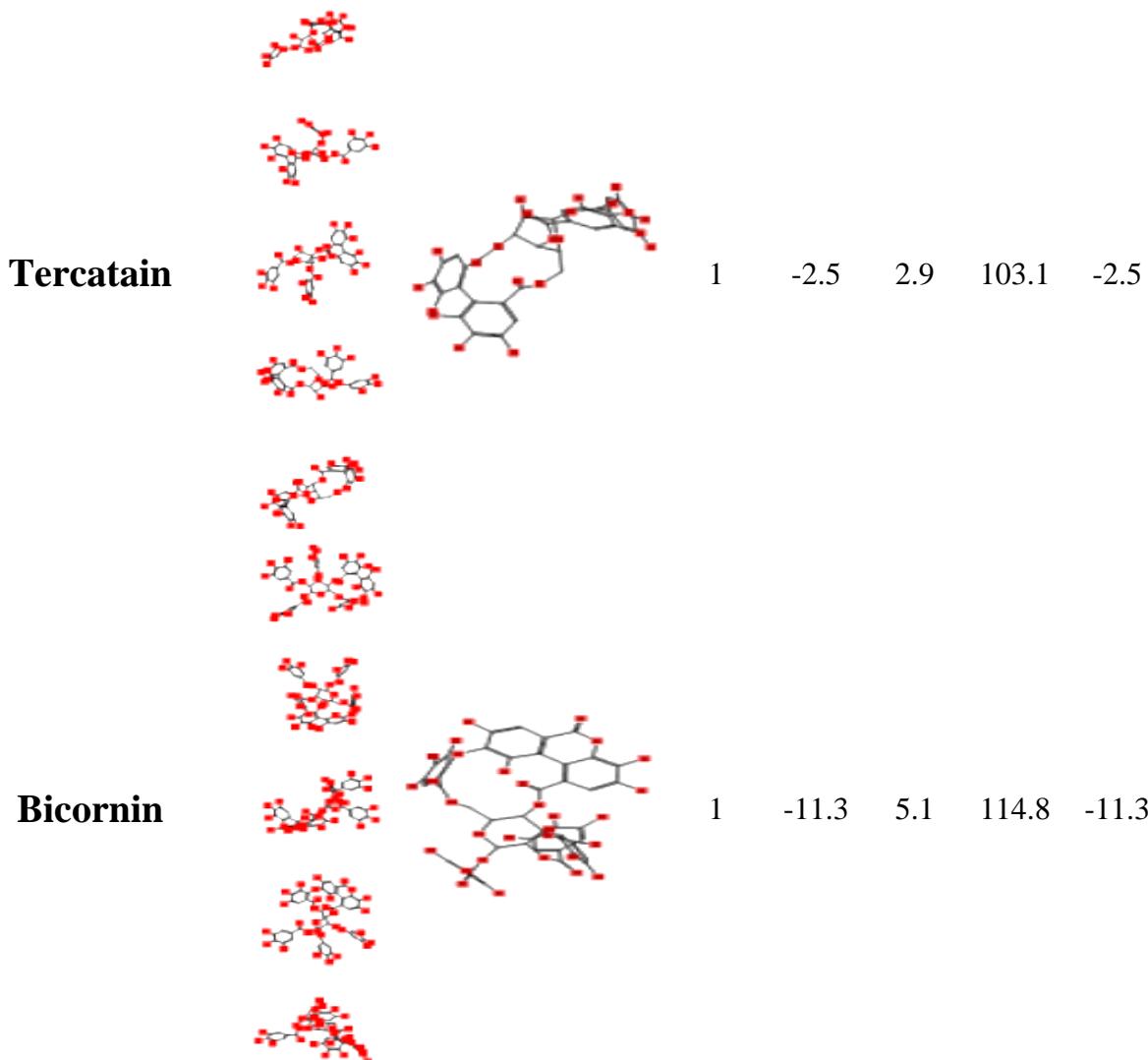
Fig. 4. Illustration of 3D spatial orientation of 10 selected hydrolysable tannins within the allaostreic binding pocket of the crystal structure of COVID-19 main protease.

Table. 1. The top five poses of the interaction between 10 hydrolysable tannins with the crystal structure of COVID-19 main protease and their docking properties.

Hydrolysable tannins	Mol	Mseq	S	E-conf	E-place	E-score
Geraniin						
						
		1	-9.9	0.0	-74.7	-9.8
						
						
Tellimagradin						
						
		1	-12.7	0.0	-91.4	-12.7
						
						







Mol: An output pose, Mseq: The molecular sequence number, S: The final score, which is the score of the last stage that was not set to None, E-conf: The energy of the conformer, E-place: Score from the placement stage, E-score: Score from the rescoring stage.

4. Conclusion

In conclusion, our study revealed that some medicinal plant rich in hydrolysable tannins, especially pedunculagin, tercatain, and punicalin, could be theoretically used to treat the outbreak of COVID-19. Herein, we screened the structural relationship activity of 10 hydrolysable tannins

plant as potential anti-viral components and we selected the top three hits that may inhibit the main protease of COVID-19 and hence virus replication. Further *in-vitro* and *in-vivo* studies are needed to transmute these potential hydrolysable tannins inhibitors into clinical drugs. We predicted that the understandings obtained in the current study may evidence valued for discovering and unindustrialized novel natural anti-COVID-19 therapeutic agents in the near future.

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Conflict of interest

The authors declare no conflict of interest.

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