

Tannins inhibit SARS-CoV-2 through binding with catalytic dyad residues of 3CL^{Pro}: An in silico approach with 19 structural different hydrolysable tannins

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Abstract

Coronavirus epidemic 2019 (COVID-19), instigated by SARS-CoV-2 virus, is recently raising worldwide and inspiring global health worries. The main 3-chymotrypsin-like cysteine protease (3CL^{Pro}) enzyme of SARS-CoV-2, which operates its replication, could be used as a medication discovery point. We therefore theoretically studied and docked the effects of 19 hydrolysable tannins on SARS-CoV-2 by assembling with the catalytic dyad residues of its 3CL^{Pro} using molecular operating environment (MOE 09). Results discovered that pedunculagin, tercatin, and castalin intensely interacted with the receptor binding site and catalytic dyad (Cys145 and His41) of SARS-CoV-2. Our analyses estimated that the top three hits might serve as potential inhibitor of SARS-CoV-2 leading molecules for additional optimization and drug development process to combat COVID-19. This study unleashed that tannins with specific structure could be utilized as natural inhibitors against COVID-19.

Practical applications

The 3CL^{Pro} controls SARS-CoV-2 copying and manages its life series, which was targeted in case of SARS-CoV and MERS-CoV coronavirus. About 19 hydrolysable tannins were computed against 3CL^{Pro} of SARS-CoV-2. Pedunculagin, tercatin, and castalin interacted with Cys145 and His41 of SARS-CoV-2-3CL^{Pro}. Pedunculagin-SARS-CoV-2-3CL^{Pro} remain stable, with no obvious fluctuations. We predicted that the understandings gained in the current research may evidence valued for discovering and unindustrialized innovative natural inhibitors for COVID-19 in the nearby future.

KEYWORDS

COVID-19, hydrolysable tannins, main 3-chymotrypsin-like cysteine protease, molecular docking, structural-relationship activity

1 | INTRODUCTION

Freshly, the new coronavirus (SARS-CoV-2) was discovered on the late of 2019 (Xu et al., 2020). COVID-19 suddenly appeared, leading not only the health authorities, but also the scientific community to swift actions toward it. As a result, the entire-genome series of SARS-CoV-2 was issued and subsequently it was scientifically spotlighted (Jiang et al., 2020; Stebbing et al., 2020). The SARS-CoV-2 belongs to the β -coronavirus group, sharing ancestry with bat coronavirus HKU9-1, alike to SARS, and regardless of arrangement variety its Spike protein intensely networks with human ACE₂-receptor (Ul Qamar, Alqahtani, Alamri, & Chen, 2020). By July 13, the global death toll exceeded 574,000, with 13,200,000 confirmed cases, and 7,600,000 thousand recovered

cases in 213 countries (<https://www.worldometers.info/coronavirus/>).

Recent studies emphasized that SARS-CoV-2 genes stake <80% nucleotide uniqueness and 89.1% nucleotide likeness with SARS-genes (Kim, Kim, & Lee, 2020; Moorthy, Restrepo, Preziosi, & Swaminathan, 2020). Usually, β -coronaviruses yield a ~800 kDa polypeptide upon dictation of the genome. This polypeptide is proteolytically slashed to create numerous proteins, and the proteolytic diversion is facilitated by papain-like protease, and slicing the polyprotein at 16 different spots to create several nonoperational proteins that are vital for the viral reproduction (Macchiagodena, Pagliai, & Procacci, 2020). Thus, this protease portrays a key task in the virus duplication, and distinct structural/fitment protein-encoding genes situated at the 3' end that

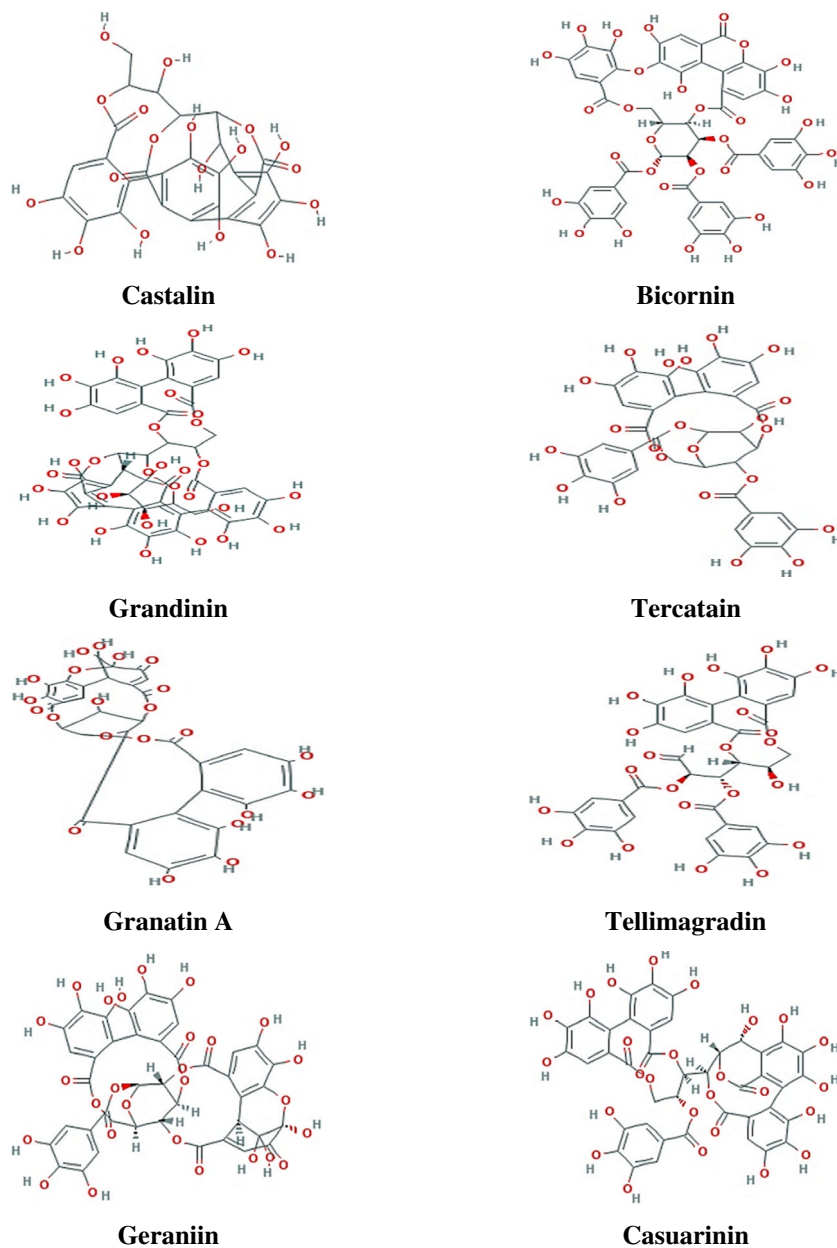


FIGURE 1 2D-chemical structures of 19 selected hydrolysable tannins

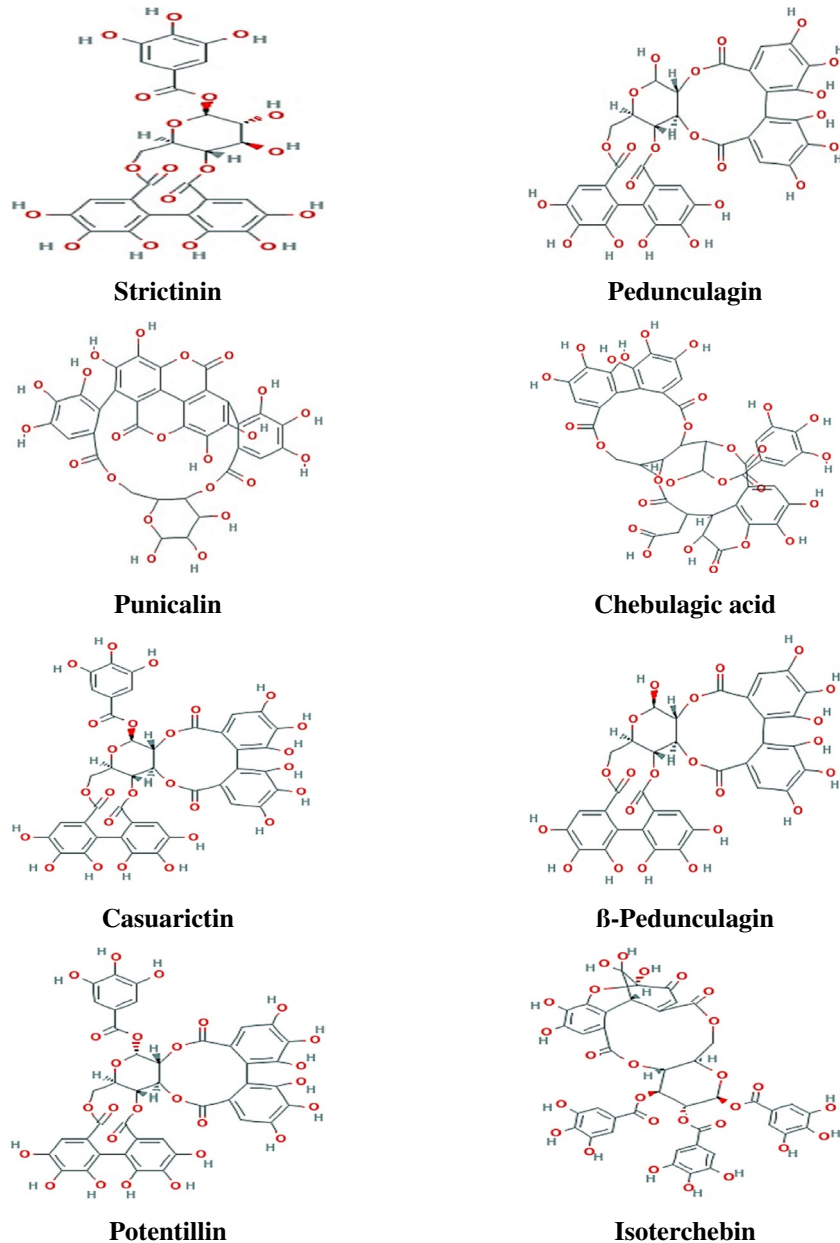
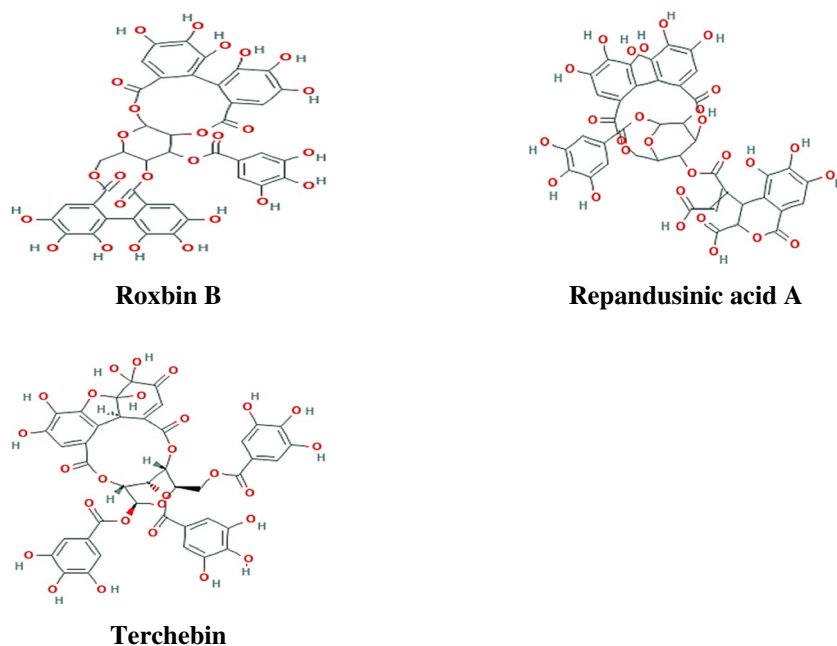


FIGURE 1 Continued

exhibit extreme inconsistency (Rut et al., 2020). Therefore, 3CL^{Pro} enzyme may assist as a possible target for natural inhibitors against COVID-19.

Structure-based effect analyses and high output findings have recognized possible inhibitors of SARS- and MERS-3CL^{Pro} (Pillaiyar, Meenakshisundaram, & Manickam, 2020). Bioactive substances, especially hydrolysable tannins, have attracted significant attention which could be valorized to develop medications without side-effects (Liu et al., 2016). Hydrolysable tannins are also measured to be valuable in eliminating the opposing effects of several chemotherapeutic stuffs as well as in extending permanency and achieving positive overall health with anticancer possible and can be utilized as a substitute cancer-drug supply (Buzzini et al., 2008; Lin et al., 2011; Aires, 2020; Jia et al., 2019;

Zhu, Khalifa, Peng, & Lic, 2018). Hydrolysable tannins, including punicalagin, punicalin, and geraniin, exerted antiviral effects toward B virus by averting the creation of cccDNA and indorsing cccDNA decline, which may aid as chief components for the expansion of novel agents to remedy HBV-infection (Liu et al., 2016). Likewise, tannin-type compounds, such as epiacutissimins A and B, castalin, vescaline, chebulagic acid, and punicalagin showed anti-herpesvirus activity via targeting viral glycoprotein-glycosaminoglycan binding to inhibit access and cell-to-cell feast (Lin et al., 2011; Aires, 2020). Most importantly, it was hypothesized that the biological activities, including antiviral effects, of tannins are structural-dependent. For example, additional reactions, such as hydroxylation, methylation, glycosylation, galloylation, and polymerization are the main factors affecting the biological activity of

**FIGURE 1** Continued

tannins. Therefore, we selected 19 different structure tannins in term of representing the structure-relationship activity of tannins.

The current study was designed to find out a potent inhibitor against COVID-19 from 19 structural different hydrolysable tannins which could target the main protease of SARS-CoV-2 using in silico approaches (molecular docking and drug-likeness scan). The findings of our study will enable the researchers to rally the status of natural therapeutics against COVID-19.

2 | MATERIALS AND METHODS

Structure-based simulated inspection 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) CPU of 4.00 GB RAM and 64-bit Windows-10 Functioning System). Structure-based medication inspection was done using Molecular Operating Environment (MOE 09).

2.1 | Ligand database arrangement

The structures of 19 hydrolysable tannins including, castalin (CID-99973), bicornin (CID-71308161), grandinin (CID-492392), tercainin (CID-14411424), granatin A (CID-131752596), tellimagradin I (PubChem CID-73179), geraniin (CID-3001497), casuarinin (CID-13834145), strictinin (CID-73330), pedunculagin (CID-442688), punicalin (CID-5388496), chebulagic acid (CID-250397), casuarictin (CID-73644), β -pedunculagin (CID-5320441), potentillin (CID-5315734), isoterchebin (CID-442685), roxbin B (CID-176131), repandusinic acid A (CID-147900), and terchebin (CID-3084341) were retrieved from PubChem

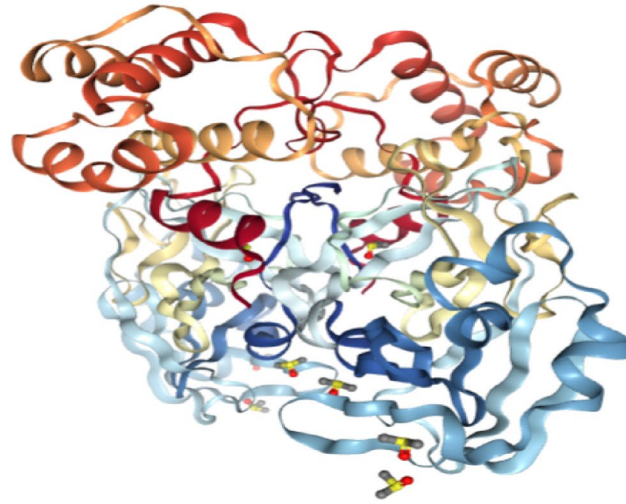
database. The tannins structure was optimized for docking by adding partial charges and energy minimization via Protonate-3D and MMFF94X strength field, separately. Adjusted ligands archives for the 19 tannins were kept in ligand catalog which was lastly utilized as an involvement file for docking investigations.

2.2 | Refinement of 3CL^{PRO} of 2019-nCoV structure

Likewise, three-dimensional structure of 3CL^{PRO} of SARS-CoV-2 (PDB: 6y84) was regained from Protein-Data-Bank (<http://www.rcsb.org>) with 2.16 Å resolution. To enhance the 3CL^{PRO}-structure, previously interacted ligands and H₂O substances were detached from 3CL^{PRO}-structure, 3D-protonation, and energy minimalization were dedicated in MOE-09. The minimalized structure was utilized for further docking operation in the following steps.

2.3 | Molecular docking and drug likeness analyses

MOE docking tool was used to dock the 19 structural different tannins toward allosteric ligand interacting spot of SARS-CoV-2-3CL^{PRO}. The possible binding compact was identified using site locator tool of MOE-09 and was then occupied through docking procedures. The 10 finest docked postures were created applying a scoring job London dG. Enhancement of docking process was ended by operating forcefield algorithm which saves the receptor firm. From them, finest relating ligands and components were selected based on Root-Mean-Square Deviation (RMSD) which is often computed in Angstrom (Å), and docking score. Ligand receptor interacting analysis was performed using LigX tool in MOE-09. It is intended



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SGFRKMAFPS GKVEGCMVQV TCGTTTLNGL WLDDVVYCPK HVICTSEDML
HHHHTTEEEE EETTEEEEEE EETTEEEEEE GGG TTGGG

NPNYEDLLIR KSNHNFLVQA GNVQLRVIGH SMQNCVLKLK VDTANPKTPK
S HHHHHHT GGEEEEEE TEEE EEEE EEETTEEEEEE ESS TT E

YKRVRIQPGQ TFSVLACYNG SPSGVYQCAM RPNFTIKGSF LNGSCGSVGF
EEE TT EEEEEEEET EEEEEEEE TTS B TT TT EEE

NIDYDCVSFC YMHHMELPTG VHAGTDLEGN FYGPFVDRQT AQAAGDTTTI
EEETTEEEEEE EEEEE TTS EEEE TTS BSTT SSSS B

TVNVLAWLYA AVINGDRWFL NRFTTTLNDF NLVAMKYNYE PLTQDHVDIL
HHHHHHHHHH HHHTT TT S HHHH HHHHHHTTB HHHHHHT

GPLSAQTGIA VLDMCASLKE LLQNGMNGRT ILGSALLEDE FTFDFVVRQC
HHHHHHHH HHHHHHHHHHH HHHH TT BTTBSS HHHHHHHH

SGVTFQ
HT

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FIGURE 2 The 3D-chemical structures of 3CL^{pro} enzyme of SARS-CoV-2 and its sequence

to display possible residues binding with each ligand realistically. It creates 2D-images signifying the forces steadying ligands within binding pouches of receptors (Khan et al., 2017). To investigate the medication likeliness of 19 tannins, all were further clarified based on behavior suitable molecular characters to be candidate inhibitor against COVID-19.

2.4 | Molecular dynamics simulations and pharmacophore analysis

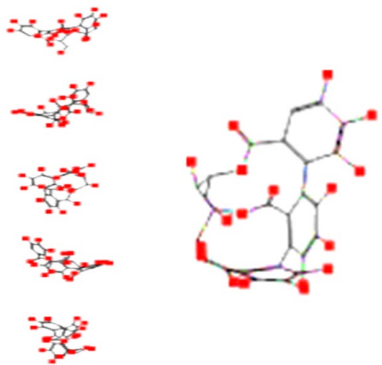
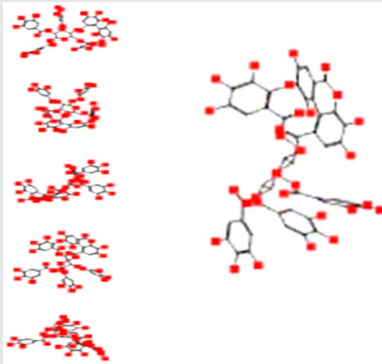
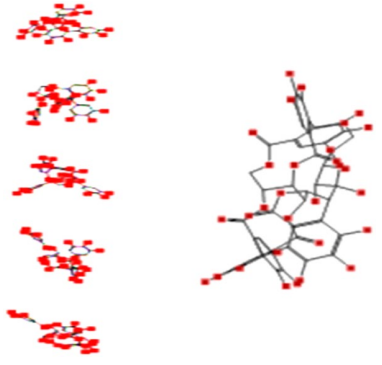
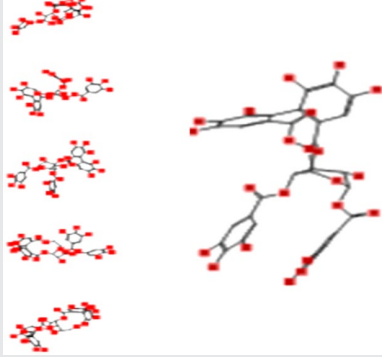
Molecular dynamics simulations (MD) were done to refine our docking results and to analyze the assembling performance and constancy of top three hydrolysable tannins using SARS-CoV-2 3CL^{pro} crystal model. GROMOS software was used to engage 80 ns MD-simulations succeeding the similar procedure as explained before (Ul-Qamar et al., 2020). The ligand topology files were built using

CHARMM force field through CGenFF server^{64,65,66}. Ligand-protein complexes were solvated in octahedron box with TIP3P water model. The predicted pharmacophore of pedunculagin was also created (Khaerunnisa, Kurniawan, Awaluddin, Suhartati, & Soetjipto, 2020).

3 | RESULTS AND DISCUSSION

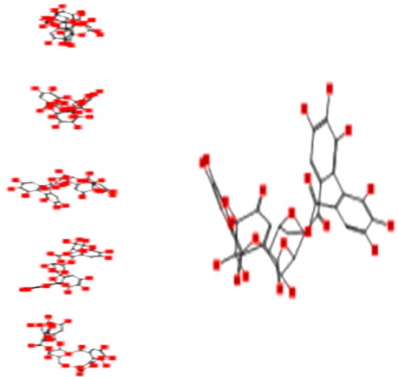
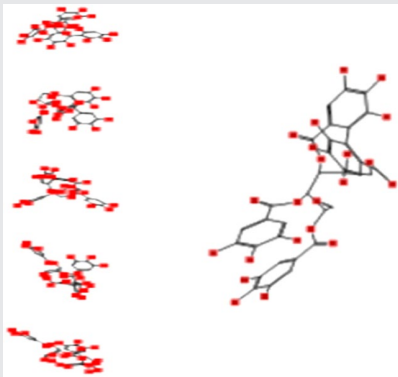
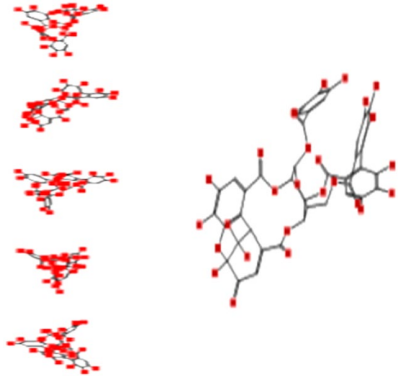
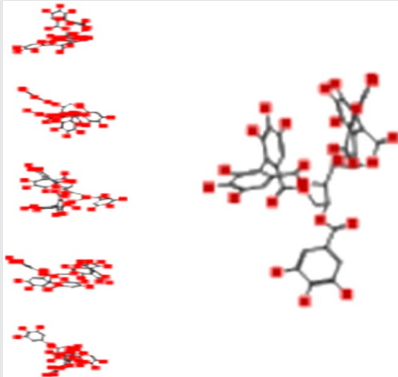
Analysis of allosteric binding of 19 structural different tannins with COVID-19 protease initially revealed many poses and we used the best pouches with small S-score. Reiterative docking of the finest docked molecules within receptor compact formerly employed by co-crystallized inhibitor. The chemical structure of our 19 structural different tannins are shown in Figure 1. By analyzing the physico-chemical parameters of SARS-CoV-2-3CL^{pro}, it was found that the enzyme consisted of 306 residues with chain type of polypeptide(L)

TABLE 1 The top five poses of the interaction between 19 hydrolysable tannins with the catalytic dyad residues of 3CL^{PRO} of SARS-CoV-2 and their docking properties

Hydrolysable tannins	Mol	S	E-conf	E-place	E-score
Castalin		-14.04	156.57	-98.32	-15.69
Bicornin		-24.36	122.35	-82.19	-14.60
Grandinin		-21.86	220.85	-127.95	-16.66
Tercatatin		-23.11	85.43	-133.94	-16.82

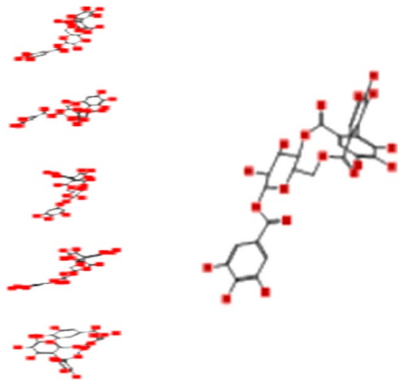
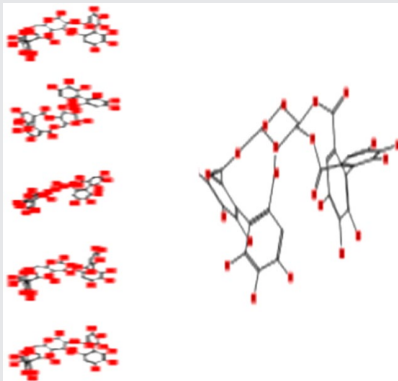
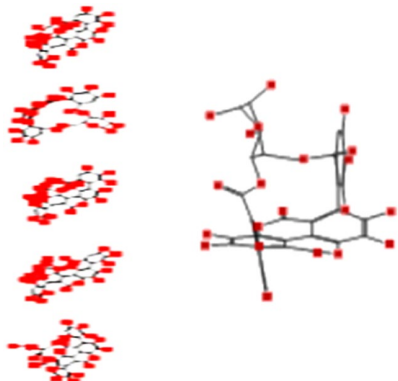
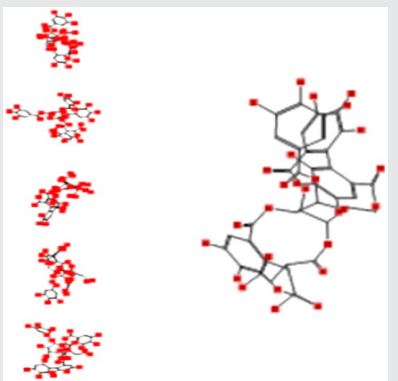
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TABLE 1 (Continued)

Hydrolysable tannins	Mol	S	E-conf	E-place	E-score
Granatin A		-15.38	187.54	-98.06	-13.28
Tellimagradin I		-29.53	81.89	-107.41	-13.83
Geraniin		-22.17	152.74	-93.78	-12.09
Casuarinin		-24.15	122.36	-61.90	-15.24

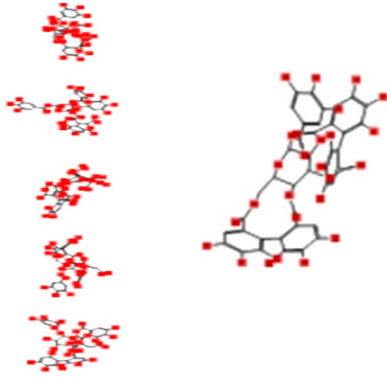
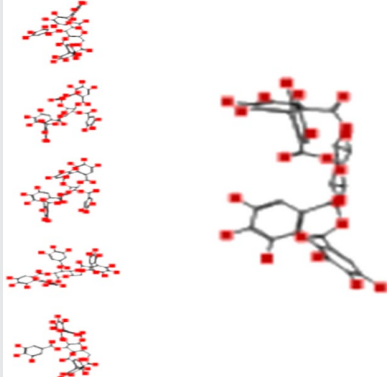
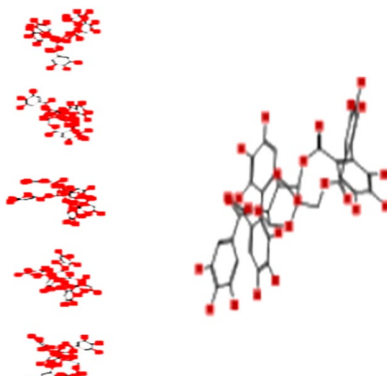
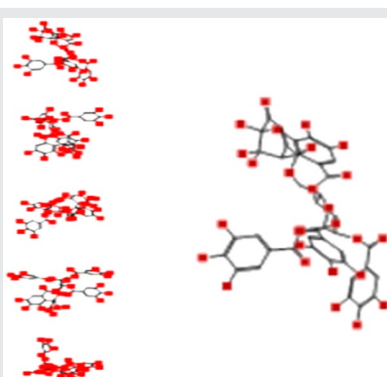
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TABLE 1 (Continued)

Hydrolysable tannins	Mol	S	E-conf	E-place	E-score
Strictinin		-23.96	98.31	-96.30	-16.82
Pedunculagin		-18.58	110.04	-70.23	-12.96
Punicalin		-18.01	81.58	-61.70	-14.78
Chebulagic acid		-22.35	132.94	-124.68	-13.74

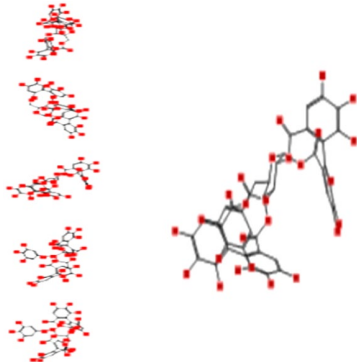
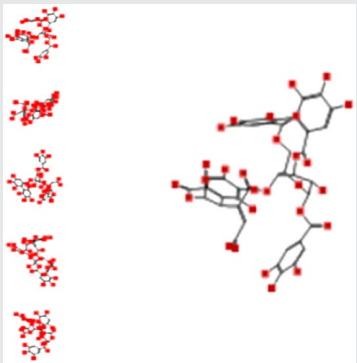
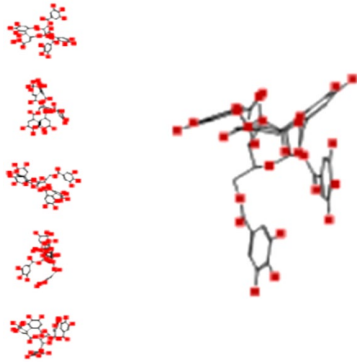
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TABLE 1 (Continued)

Hydrolysable tannins	Mol	S	E-conf	E-place	E-score
Casuarictin		-24.88	112.87	-112.35	-16.09
β -Pedunculagin		-19.05	99.45	-116.44	-15.71
Potentillin		-17.40	124.16	-44.06	-17.23
Isoterchebin		-25.38	120.42	-139.58	-16.83

(Continues)

TABLE 1 (Continued)

Hydrolysable tannins	Mol	S	E-conf	E-place	E-score
Roxbin B		-23.82	100.86	-97.63	-19.67
Repandusinic acid A		-24.15	-41.28	-167.41	-13.87
Terchebin		-29.06	119.45	-60.55	-13.21

Note: Mol: An output pose, 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.

with a molecular weight of 33.796 kDa, cataloging the protein as a steady, hydrophilic molecule able to establish H-bonds with other ligands (Figure 2). Recently, it was found that comparing the sequence of SARS-CoV-2-3CL^{pro} protein gathered with bat SARS-like, disclosing 99.02% of sequence individuality (UI Qamar et al., 2020). Furthermore, it was found that SARS-CoV-2 is much comparable to SARS than MERS, and imparts a mutual forebear with bat-based coronaviruses (Ji, Wang, Zhao, Zai, & Li, 2020; Xu et al., 2020). The findings also discovered that SARS-CoV-2 had a Cys-His catalytic dyad (Cys145 and His41), reliable with SARS-3CL^{pro} (Cys145 and His41), TGEV-3CL^{pro} (Cys144 and His41), and HCoV-3CL^{pro} (Cys144 and His41) (Yang et al., 2003). It was disclosed that SARS-CoV-2-3CL^{pro} receptor-binding concise configuration and be similar with that of

SARS-3CL^{pro} binding compact and increases the opportunity that inhibitors projected for SARS-3CL^{pro} may also mitigate the motion of SARS-CoV-2-3CL^{pro}.

To manage with the continuous essential of new and operative small molecule as natural therapeutics against COVID-19 with negligible side-effects, studies are now directing more on computational drug finding (Desai et al., 2008). Several reports testified the antiviral possible of tannins via computer-assisted medication strategy (Buzzini et al., 2008; Lin et al., 2011). To speed the drug agreement process and to realize more effectual inhibitors with novel frameworks that can progress the antiviral-based therapeutics status, computational drug discovery methods are extremely steadfast. From the innovative aspect of structural variety among hydrolysable

tannins, simulated selection was ended to realize new allosteric compounds as inhibitors against COVID-19. Allosteric directive has been described as an operative tactic to conquer irretrievable inhibition. Spatial alignment and dock score of present stated four top-ranked chiefs discloses effectual interacting of functional residues

with greatest binding affinity. These tannins fit with the drug prospect norms tabulated in Table. 1 and S1 and may attest exceptional perceptive to use as a preparatory point.

Interacting affinity analysis of 19 tannins through LigX revealed in Figure 3. It was revealed that 19 tannins differentially interacted

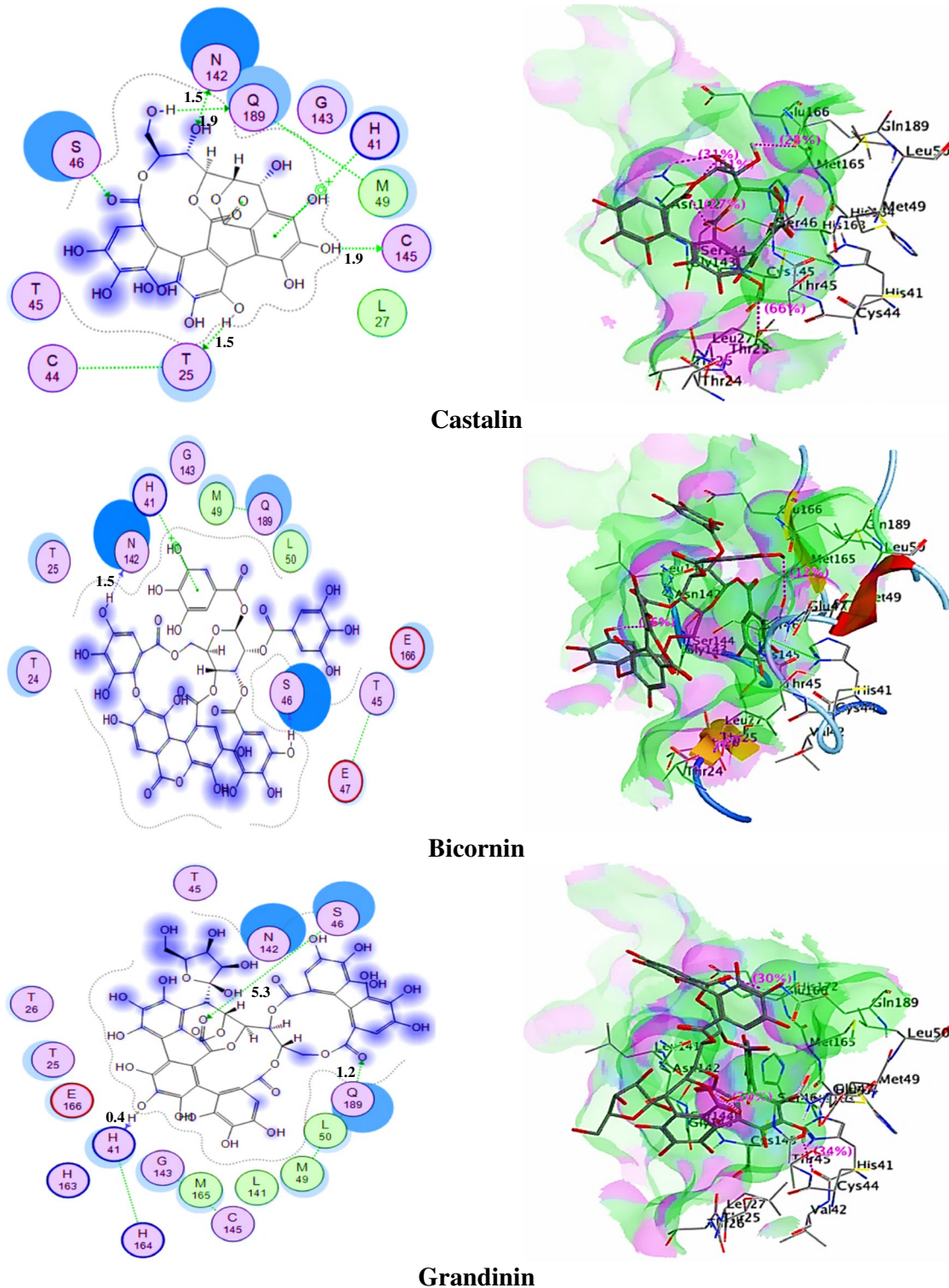


FIGURE 3 LigX interaction diagram representing binding pattern of 19 hydrolysable tannins with binding pocket residues of 3CL^{PRO} enzyme of SARS-CoV-2

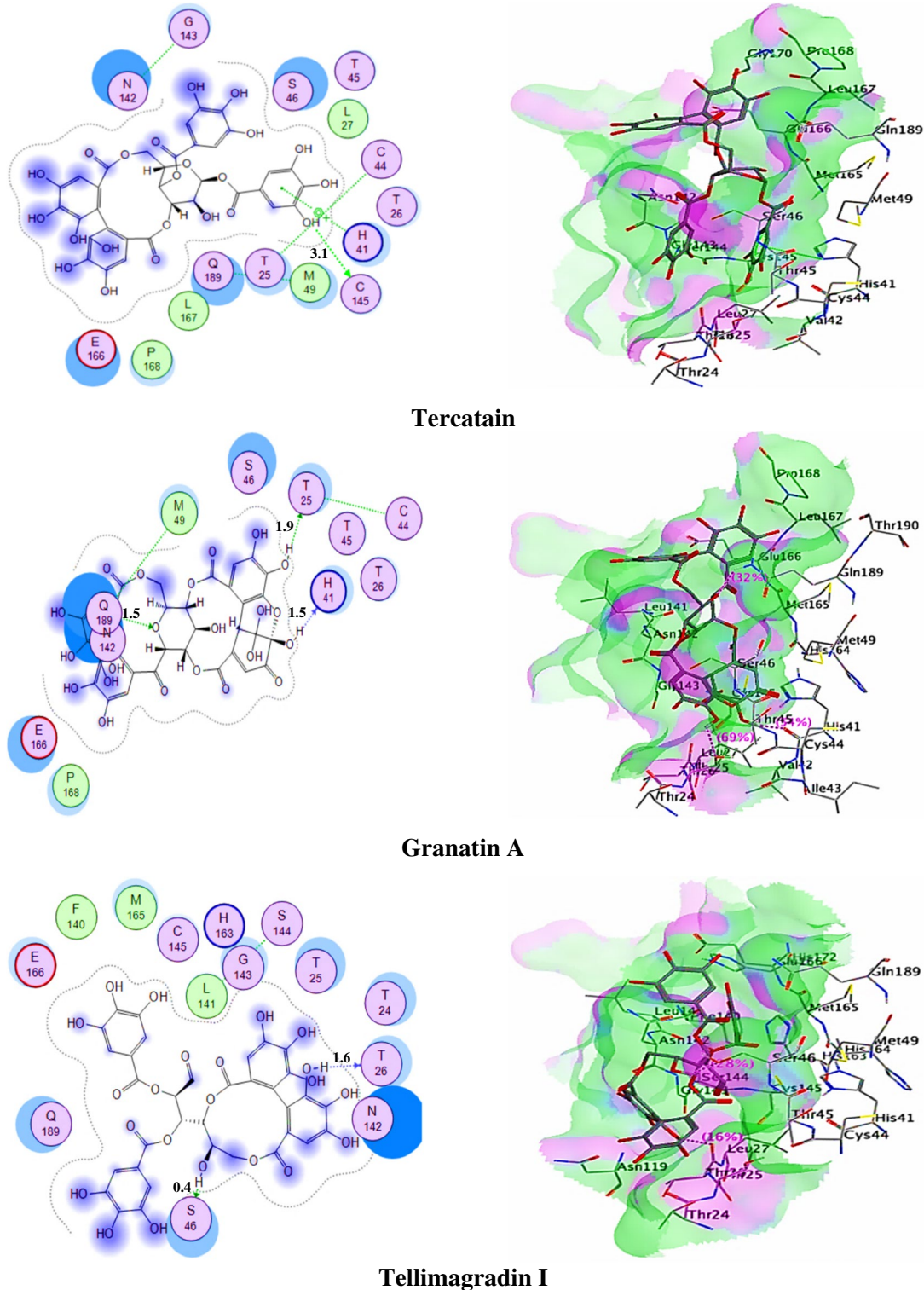


FIGURE 3 Continued

with different residues of SARS-CoV-2-3CL^{pro}. In details, pedunculagin are the best hydrolysable tannins, where it could bind with 12 amino acid residues of SARS-CoV-2-3CL^{pro} and there are 5 H-bonding forces have been occurred. Most importantly, pedunculagin directly interacted with His41 and Cys145 through H-bonding forces, showing its ability to interact with the catalytic dyad residues

of 3CL^{pro}. Likewise, castalin and tercatoin directly networked with His41 and Cys145 via arene–arene interactions and H-bonding forces. Bicornin, Repandusinic acid A, grandinin, granatin A, roxbin B, and terchebin also precisely interacted with His41 through H-bonding or arene–arene interactions, showing their influence on the catalytic dyad residues of 3CL^{pro}. Results also showed that

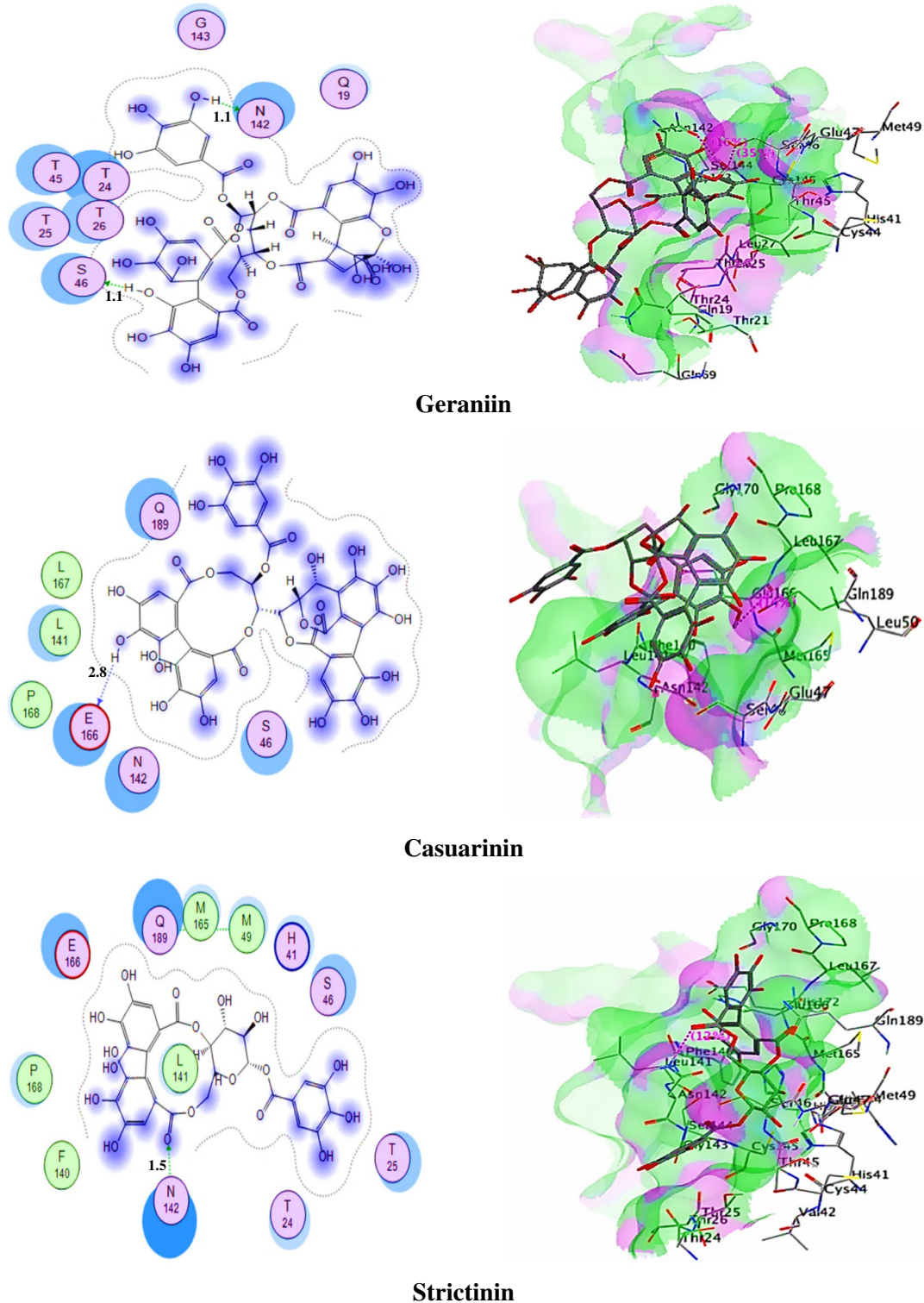
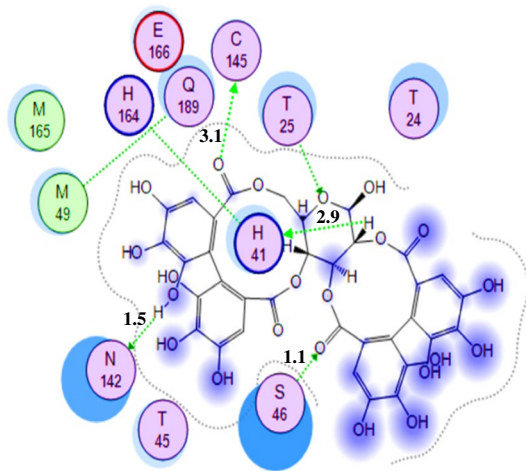


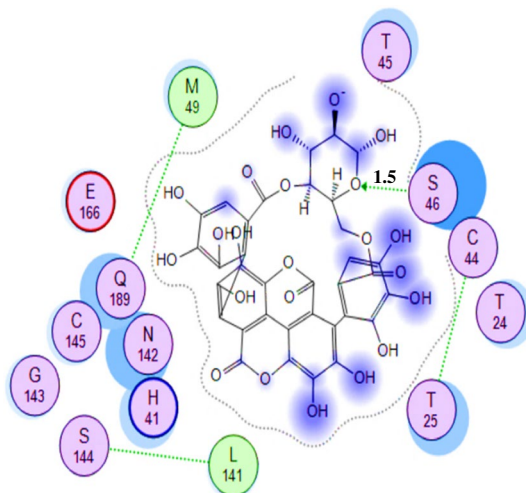
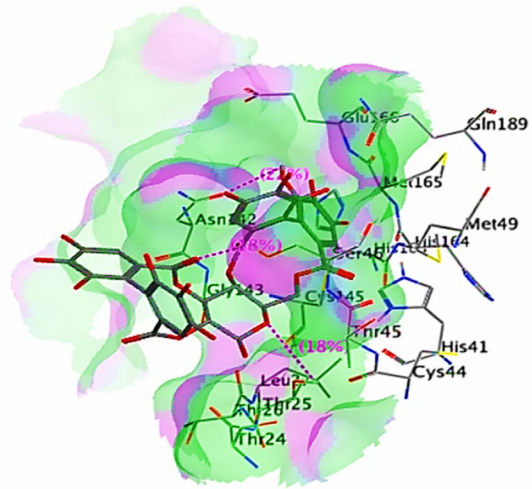
FIGURE 3 Continued

there are some of the hydrolysable tannins, including tellimagradin I, strictinin, punicalin, chebulagic acid, casuarictin, β -pedunculagin, potentillin, and isoterchebin have been secondarily interacted with both of His41 or Cys145, showing their partial influence on the catalytic dyad residues of 3CL^{pro}. On the contrary, both of geraniin and casuarinin neither interact with His41 nor Cys145; however, both

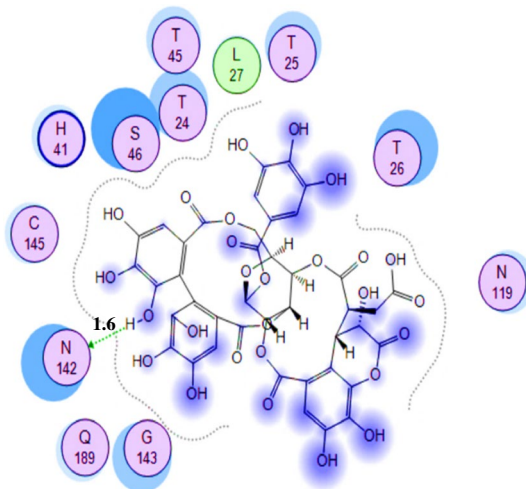
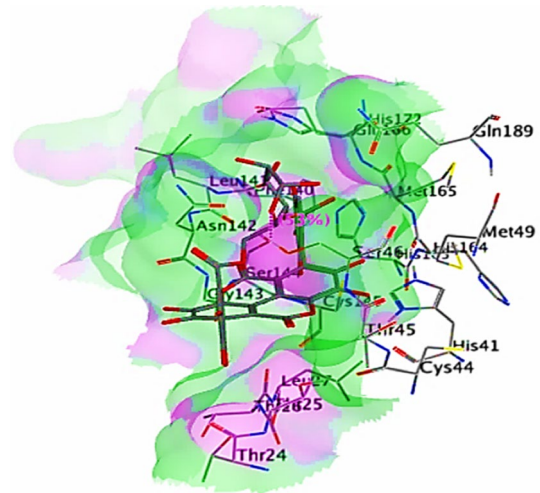
of them have been networked with eight and seven amino acid residues of 3CL^{pro} via arene–arene and H-bonding. The vital catalytic residues of compact spatial site of pedunculagin is stabilized within the pocket with the deepest S-score (–18.58) and binding with C145, H41, N142, S46, T45, T24, T25, Q189, E166, H164, M165, and M43. Castalin interacted with C145, H41, L27, M49, G143, Q189, N142,



Pedunculagin



Punicalin



Chebulagic acid

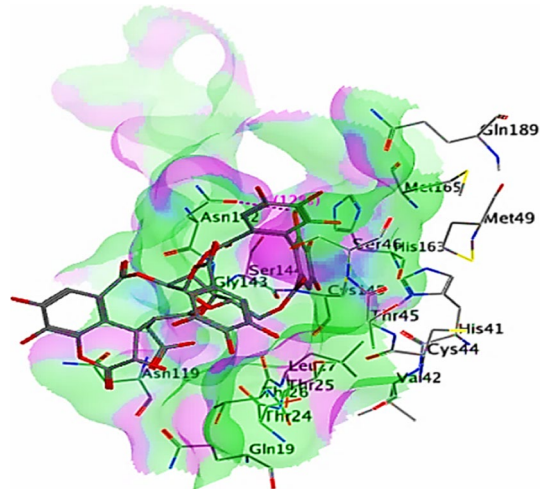


FIGURE 3 Continued

S46, T45, T25, and C44. The difference in the binding ability of each tannin with SARS-CoV-2-3CL^{pro} may be due to their structure-based relationship activity.

These results identified at least three novels harmless, druggable tannins that are envisaged to interact with the receptor binding spot and catalytic dyad (Cys-145 and His-41) of

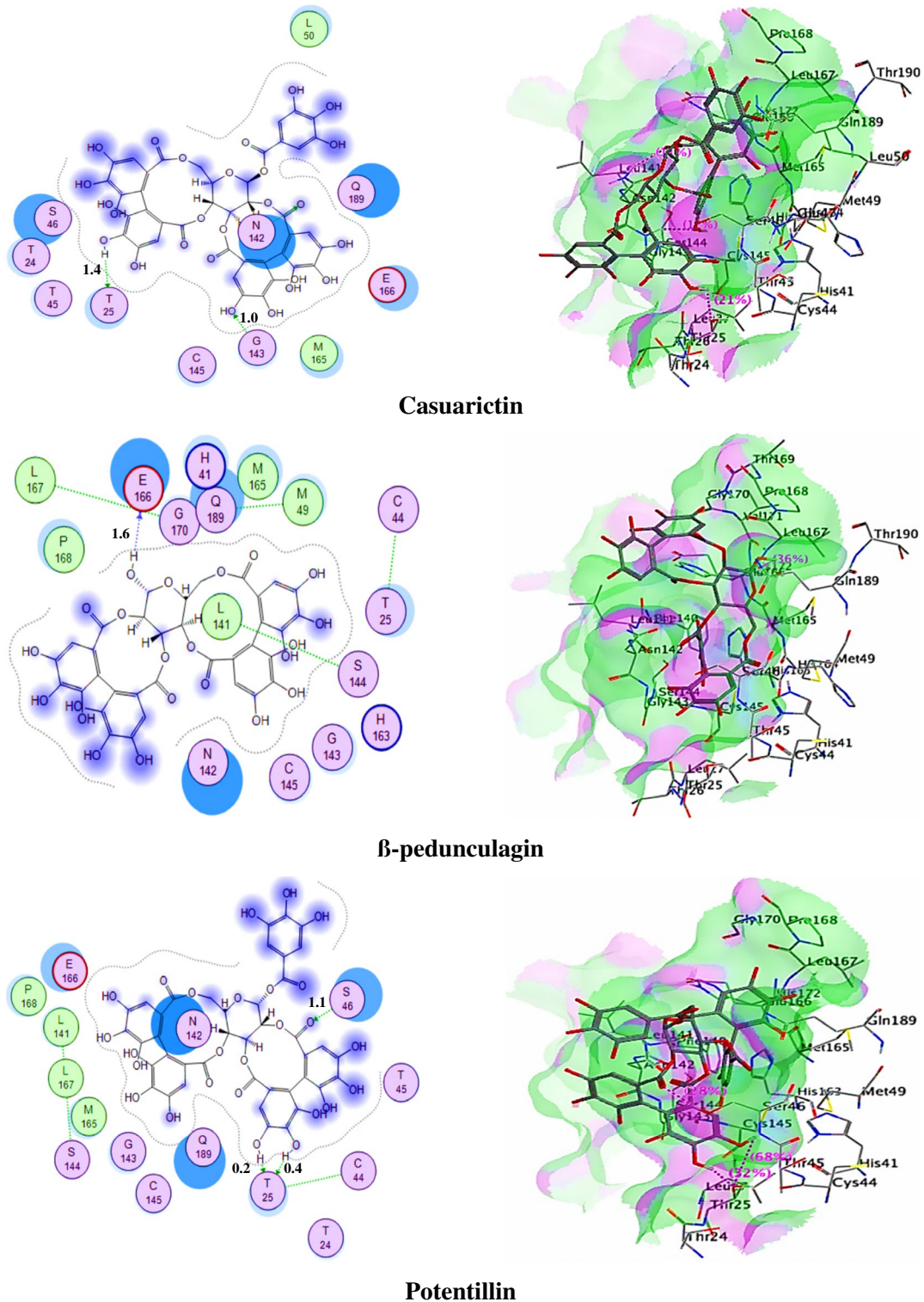


FIGURE 3 Continued

SARS-CoV-2-3CL^{pro}. Among these hydrolysable tannins, pedunculagin, strongly interacted with the catalytic dyad residues (Cys-145 and His-41) of SARS-CoV-2-3CL^{pro}, with sense binding affinity, docking score, and ADMET properties. As supplemented in Table S2, pedunculagin is an excellent compound regarding solubility, and having high intestinal absorption, and does not shows

any mutation. This molecule is not inhibitor of HERG I and HERG II that means it is not harmful to cardiac muscles (heart muscles). Previously, pedunculagin, tercatatin, and castalin which could be extracted from pomegranates, walnut, Indian gooseberry, oak wood, leaves of *Melaleuca quinquenervia*, *Terminalia catappa*, and *Combretum glutinosum* showed antiviral activity and other

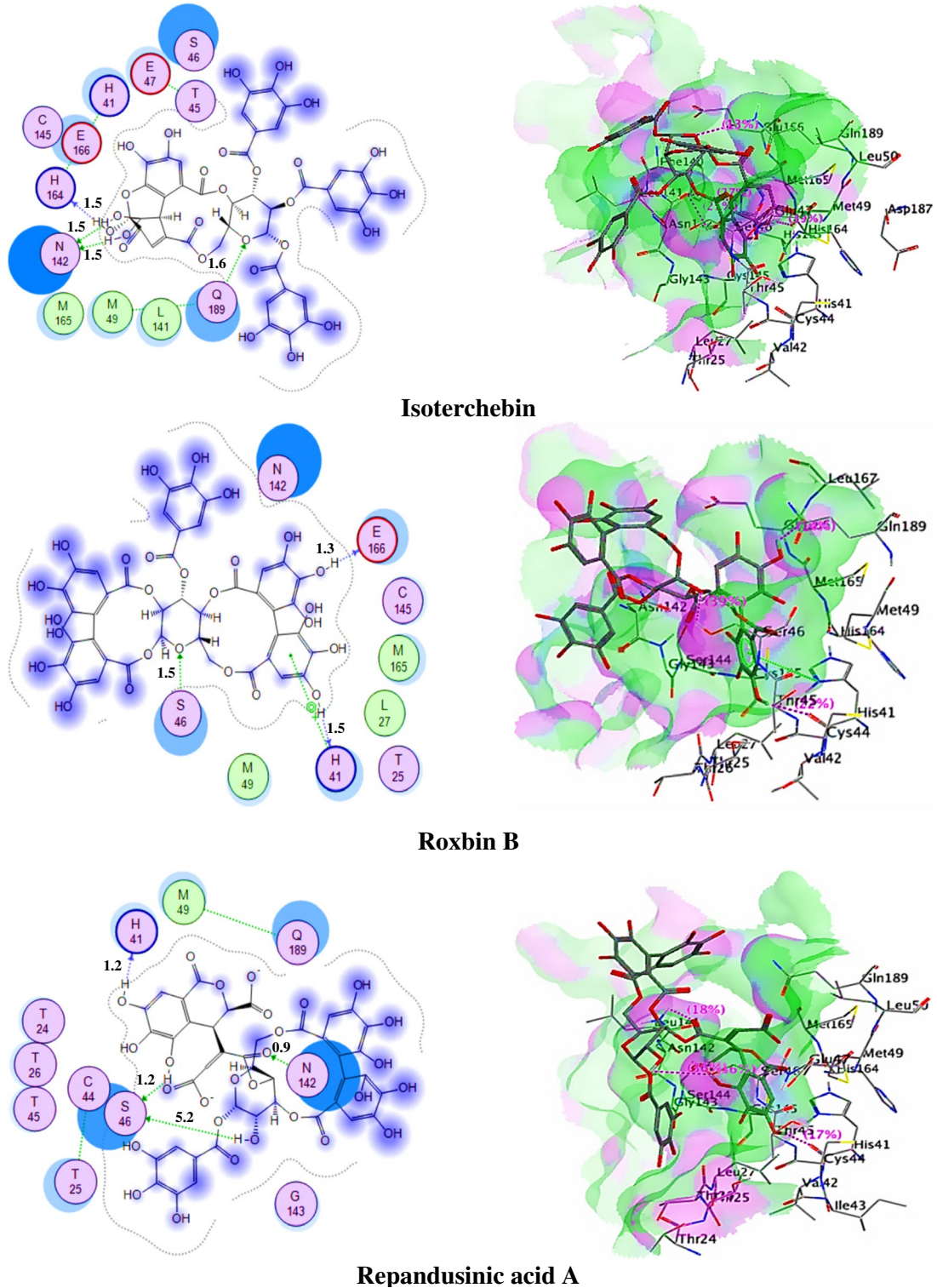


FIGURE 3 Continued

biological activities (Chang et al., 1995; Silva et al., 2016; Zuo, Li, Chen, & Xu, 2005). In a related study, Park et al. (2017) isolated 10 polyphenols from *Broussonetia papyrifera* inhibitors versus 3-chymotrypsin-like and papain-like coronavirus cysteine proteases. Jo, Kim, Kim, Shin, and Kim (2019) also examined four types of flavonoids against MERS-CoV and he reported that quercetin

3- β -d-glucoside, herbacetin, isobavachalcone, and helichrysetin blocked the enzymatic activity of MERS-3CL^{PRO}.

On the contrary, it has been reported that hydrolysable tannins are degraded to gallic acid, pyrogallol, phloroglucinol, and finally to acetate and butyrate via sequential actions of different bacterial enzymes. Gallic, hexahydroxydiphenic, and ellagic

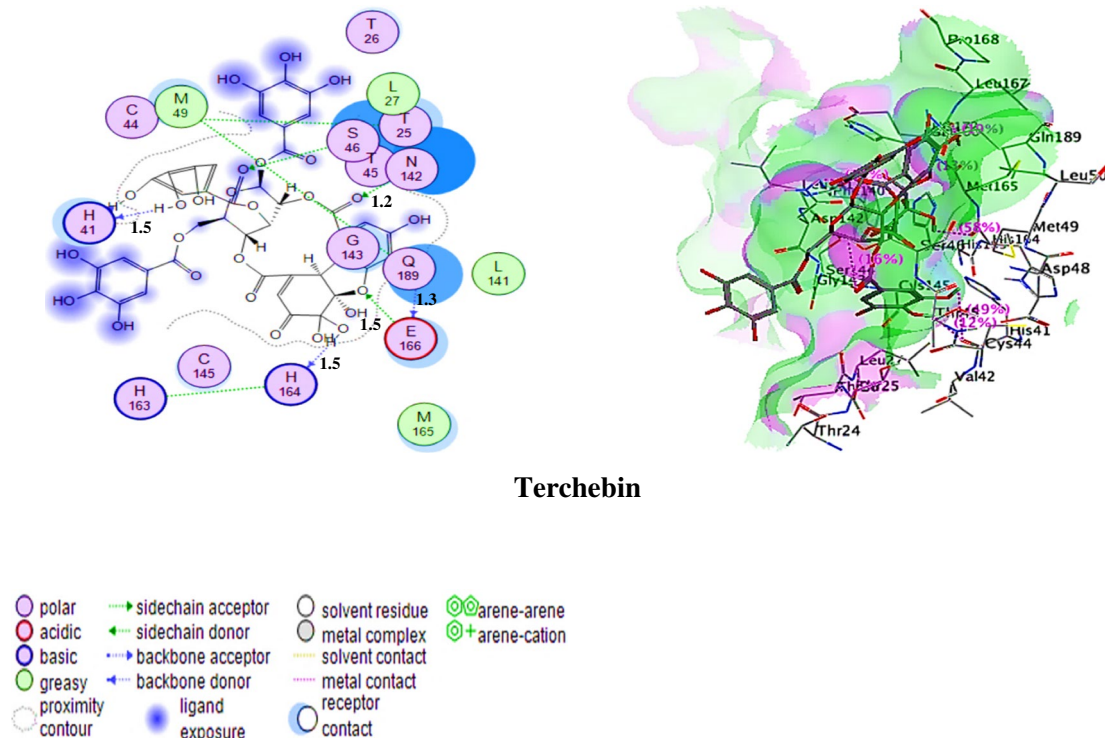


FIGURE 3 Continued

acids are also the monomer types of hydrolyzed tannins (Serrano, Puupponen-Pimiä, Dauer, Aura, & Saura-Calixto, 2009; Smeriglio, Barreca, Bellocco, & Trombetta, 2017). It was informed that ellagitannins release ellagic acid upon hydrolysis in rat's intestine. Gallic acid is infused via a paracellular route in Caco-2 cells, with t_{max} of absorption around 1.27 hr in humans. Regarding ellagic acid absorption, some authors have detected ellagic acid in human plasma between 0.5 and 3 hr after oral administration of pomegranate juice. Following absorption, ellagic acid undergoes conjugation, and conjugated forms with methyl, glucuronyl, and sulfate groups have been found in plasma and excreted in urine. Gallotannins are easily degraded by bacteria, fungi, and yeast. Tannase produced by a group of microorganisms is active in galloyl residues of galloyl esters, as well as on hexahydroxydiphenyl. Unlike proanthocyanidins, colonic bacteria are capable of metabolizing hydrolysable tannins. The presence of *Lactobacilli* with distinct tannase activity proposes that gallic acid from gallotannins may be available during colonic fermentation. For example, casuarictin incubated with caecal content increase the release of ellagic acid. In rats fed with punicalagin, ellagic acid is transformed by rat microflora to 3,8-dihydroxy-6H-dibenzo (b,d)-pyran-6-one (urolithin B) derivatives, the total urinary excretion accounting for 0.7%–52.7% of the ingested punilagin in rats. When a single dose of ellagitannin-containing foodstuff was taken by each group of human volunteers, the metabolite excretion ranged from 2.8% to 16.6% of the ingested ellagitannins. Therefore, the health effects of tannins intake are probably a consequence of the biological activity of their metabolites.

Looking at the antiviral drug possible of aforementioned tannins, current study was an endeavor to feat the chemical nature of three hydrolysable tannins as natural inhibitors against COVID-19. Present molecular docking study has publicized the significant interactions of medicinal tannins with the main protease of COVID-19, which were successfully block both His41 and Cys145.

To authenticate the drug capacity of selected tannins, ligand characters were considered with LigX tool of MOE. All chosen tannins disclosed constructive results and accomplish the standards of the Lipinski's regulation of five (Khan et al., 2017). The regulation defines that possible drug-like components should have about 5 H-bond donors, maximum 10 H-bond acceptors, and an octanol water partition coefficient $\log P$ not > 5. These results suggest that natural components identified in our study, specially pedunculagin, tercatin, and castalin, may demonstrate more valuable contenders for COVID-19 drug rehabilitation. To further examine the molecular docking results, pedunculagin was subjected to MD simulation; and RMSD, radius of gyration (RoG), and H-bond characters were expressed. Pedunculagin-SARS-CoV-2-3CL^{pro}-complex did not show any obvious fluctuations, referring to the stability of tannin-protein complexes with a typical RMSD value of 1.4 ± 0.01 Å (Figure S1a). It was also suggested normal behavior for pedunculagin-SARS-CoV-2-3CL^{pro} complex; where it was persisted dense and steady during the 80 ns simulations (Figure S1b). Likewise, H-bonds which are the key stabilizing forces in proteins, proposed that the pedunculagin-SARS-CoV-2-3CL^{pro} complex remain stable throughout the simulation, with no noticeable fluctuations (Figure S1c). It can be concluded from this study that each

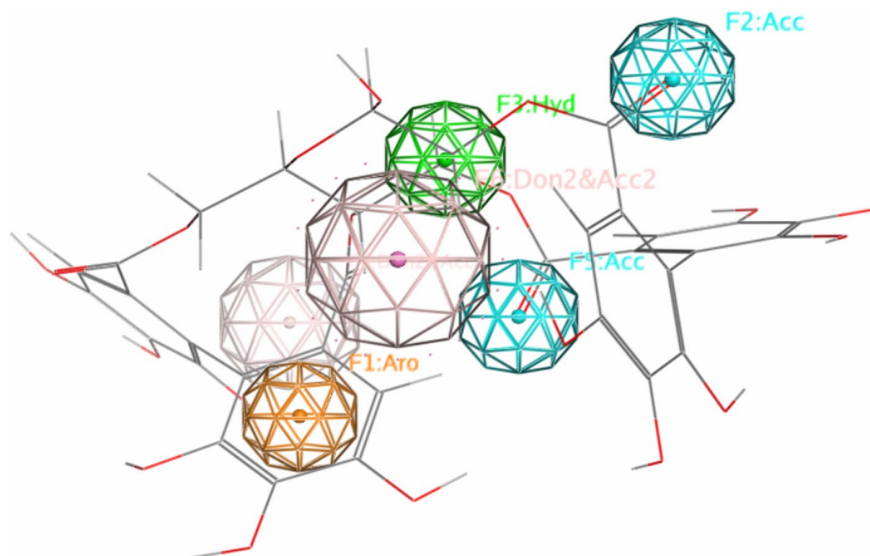


FIGURE 4 The predicated pharmacophore for pedunculagin

of pedunculagin, tercatin, and castalin and their sources such as pomegranates and nuts may assist as possible natural inhibitors against SARS-CoV-2. Pedunculagin is a hydrolysable tannin. Secondary metabolite components are regularly found in medicinal plants. We therefore predicted the pharmacophore of pedunculagin based on its active groups. Hydroxy groups ($-OH$), ketone groups ($=O$), and phenolic rings in pedunculagin are projected to play parts amino acid residue binding at the energetic spot of SARS-CoV-2-3CL^{PRO} (Figure 4). These groups were found to directly interact with the 3CL^{PRO} of SARS-CoV-2 through H-bonding and other forces.

4 | CONCLUSION

In conclusion, our study exposed that some medicinal plant rich in hydrolysable tannins, especially pedunculagin, tercatin, and castalin, could be theoretically used to treat the outbreak of COVID-19. Herein, we screened the structural relationship activity of 19 hydrolysable tannins as potential antiviral components and we chose the top three hits that may inhibit the main protease of SARS-CoV-2 and hence virus copying. Further in vitro and in vivo studies are needed to transmute these probable hydrolysable tannins inhibitors into clinical drugs. We predicted that the understandings gained in the current study may evidence valued for discovering and unindustrialized novel natural inhibitors against COVID-19 in the near future.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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